LARGE PELAGICS RESEARCH CENTER
SEMI-ANNUAL PROGRESS REPORT – DEADLINE 07/30/2008

Report: #2 **Reporting Period: 11/01/2007 – 05/30/2008**

Project Title:

Reconstructing Diet Histories and Migration Pathways of Bluefin Tuna from Compound-Specific Stable Isotope Analyses of Scales and Bone

Principal Investigator(s):

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1. Purpose of the Project: (one paragraph):

We proposed to develop and apply isotope ratio monitoring gas chromatography mass spectrometry to the analysis of compound-specific $\delta^{13}C$ and $\delta^{15}N$ values in amino acids, and conventional isotope ratio mass spectrometry to measure δH and $\delta^{18}O$, from scales and bone of Atlantic bluefin tuna (ABT). Our focus was on scales, in particular, because they can be collected without sacrificing the fish. This is critical if we are to correlate migratory pathways determined using conventional and archival tagging approaches with independent information on natal origins of individual fish determined from geochemical signatures in tissues. We plan to develop a technique to provide information on dietary history and natal origins of an individual fish, based on δH, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$, which could then be outfitted with an archival tag and released.

2. Progress during the last six months: (one-two paragraphs including a comparison between the proposed objectives and the actual accomplishments achieved.)

We continue to develop a method for measuring $\delta^{13}C$ in amino acids from fish scales and vertebrae. We initially extracted and hydrolyzed individual amino acids from proteins that were then analyzed for $\delta^{13}C$ using irm-GC/MS. There are several issues with this approach that led us to focus our attention on the moving-wire interface that is used to introduce CO_2 gas, a product of the combustion of organic compounds, to an isotope ratio mass spectrometer. The technique is currently working for $\delta^{13}C$, and we currently have NSF funding to develop modifications to the moving wire interface that will allow the method to also measure $\delta^{15}N$ in the same amino acid samples. However, the separation of amino acids using liquid chromatography has proved more difficult than we had anticipated. We have, therefore, switched back to irm-GC/MS for $\delta^{13}C$ analyses with good results.

We have been able to determine $\delta^{13}C$ values in a total of 13 amino acids in muscle tissue and scales (alanine, glycine, threonine, serine, valine, leucine, iso-leucine, proline, asparagines, glutamine, phenyl alanine, lysine, and arginine). In a controlled feeding experiment we have demonstrated that at least some essential amino acids do not

fractionate ^{13}C trophically while other non-essential amino acids were fractionated up to 8‰ between consumer and prey (see below). These data provide further experimental evidence for the use of compound-specific stable isotopes to provide information on source C and N and trophic relationships within marine food webs.

3. Preliminary Data:

We have focused initially on determining stable carbon isotopes in specific amino acids using moving wire irm-MS. Our first step was to demonstrate that we would recover $\delta^{13}C$ values from amino acids, separated and purified using HPLC, with the moving wire interface.

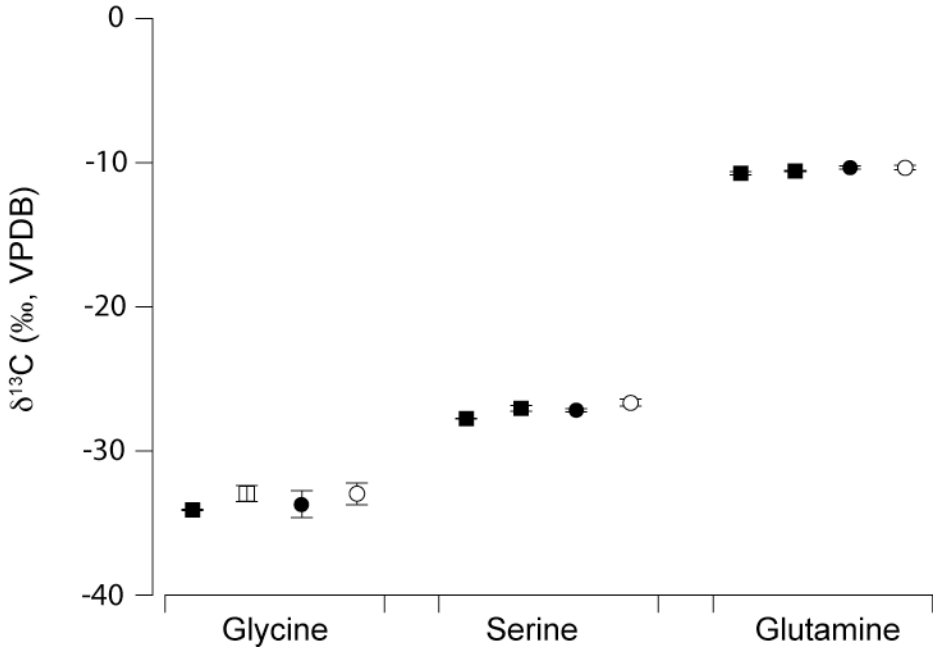

Figure 1. Compound specific $\delta^{13}C$ values for glycine, serine and glutamine standards analyzed using conventional irm-MS (squares) and moving wire irm-MS (circles) before (closed symbols) and after (open symbols) passing the compounds through a separation and purification process required for scale and vertebra samples.

Results from these assays are promising (Figure 1). We have successfully separated and purified the three most common amino acids found in ABT scales and vertebrae (glycine, serine and glutamine) using HPLC. More importantly, we have shown that this process doesn't induce significant carbon isotope fractionation. Finally, we have demonstrated that, by using the moving wire interface, we can accurately quantify $\delta^{13}C$ values from nanogram levels of carbon in the individual amino acids. Carbon isotope values for each of the three amino acid match well with values obtained by an outside laboratory at UC Davis using conventional irm-MS.

We have conducted a survey of the amino acid composition of Atlantic bluefin tuna scales and vertebrae (Figure 2). Interestingly, glycine is the dominant amino acid in both structures. Glycine is generally enriched in ^{13}C compared to other amino acids. Our data there therefore support the conclusion by Estrada et al. (2005) that enriched ^{13}C values in the scales and vertebrae of adult Atlantic bluefin tuna are not due to the presence of significant carbonate in the samples but rather due to amino acid composition.

We have conducted initial analyses of bulk $\delta^{18}O$, and compound-specific $\delta^{13}C$, analyses of adult Atlantic bluefin tuna scales collected in coastal waters around Cape Code (Figure 3). We isolated the earliest layers of material from the scales and isolated individual amino acids from one half of each of the samples using high performance liquid chromatography. The purified amino acids (glycine and glutamine [not shown]) were then assayed for $\delta^{13}C$ values using moving wire irm-MS. The remaining sample material was analyzed for $\delta^{18}O$ using elemental analysis irm-MS. The results, although clearly preliminary, hint at an interesting pattern in glycine $\delta^{13}C$ and $\delta^{18}O$ values. There may indeed be two groups, represented by relatively $\delta^{13}C$ and $\delta^{18}O$ values and relatively

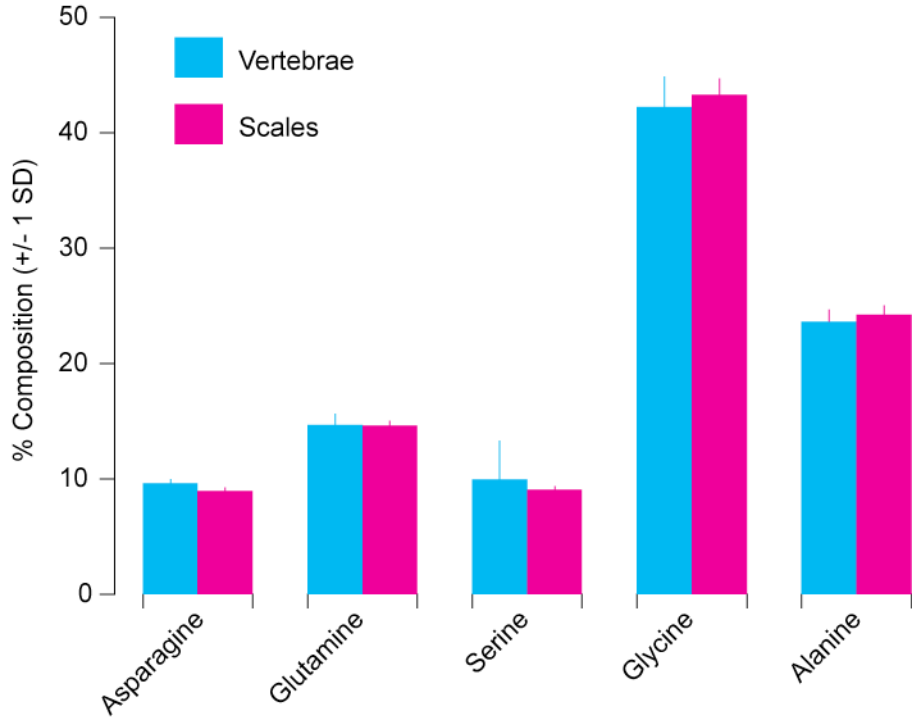

Figure 2. Amino acid composition of adult Atlantic bluefin tuna scales and vertebrae. The composition of both structures are dominated by glycine and alanine that between the two account for over 70% of the total amino acids present in the samples.

heavy $\delta^{13}C$ and $\delta^{18}O$ values, respectively. If confirmed, the data would argue for the presence of fish from at least two different natal locations in the northwest Atlantic Ocean. The obvious next step will be to analyze scales from known origin fish to ascertain baseline isotope signatures from nursery grounds in the Gulf of Mexico and in the Mediterranean Sea.

Finally, we have new data from a feeding experiment that provides more evidence for the approach that we are developing. A total of 13 amino acids in the muscle tissue from a marine fish fed three difference diets were analyzed for $\delta^{13}C$ using irm-GC/MS. Results are shown in Figure 4. The important message is that some essential amino acids (those not synthesized by the consumer) show no trophic fractionation (e.g. threonine and phenol alanine) while other non-essential amino acids show trophic fractionation of up to 8‰ (e.g. alanine and glycine). These are the first such data from a marine fish and clearly highlights the potential of the approach that we are developing.

4. Difficulties: (Provide details for any delays in meeting milestones and how they are being resolved).

We have spent a considerable amount of time on technique development. While it is possible to analyze $\delta^{13}C$ and $\delta^{15}N$ using conventional EA irm-MS, the technique requires relatively large sample sizes. We therefore continue to work on developing an approach

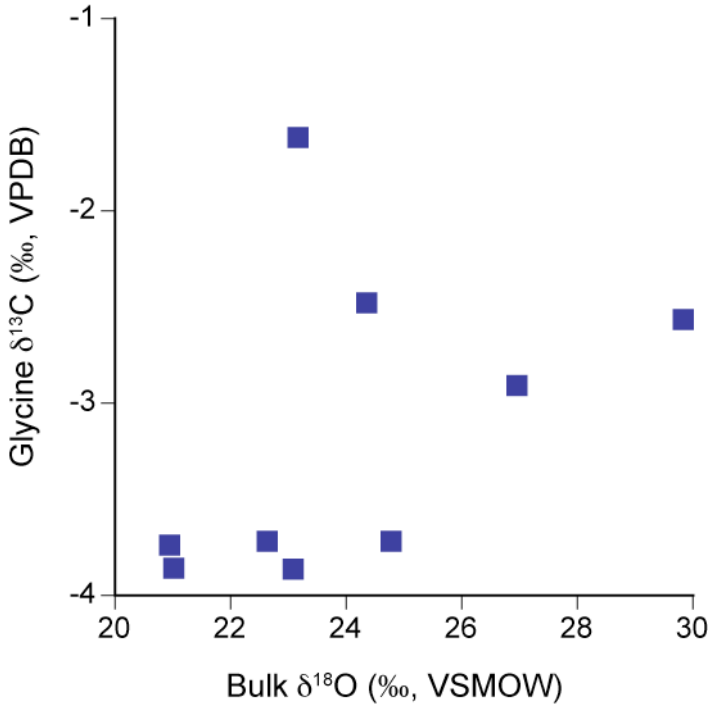

Figure 3. Isotope signatures in glycine ($\delta^{13}C$) and bulk collagen ($\delta^{18}O$) from the center portion, representing early life history, of adult Atlantic bluefin tuna scales collected in waters adjacent to Cape Cod.

that would significantly reduce sample requirements. We are working on a moving wire interface that will reduce sample requirements by at least 2 orders of magnitude, but we have had ongoing difficulties with liquid chromatography separation of amino acids. We have also been using irm-CG/MS with some success, and this is the approach that we will be using for the remaining analyses.

The second issue had been the lack of 0^+ or 1^+ Atlantic bluefin tuna, especially in the western North Atlantic. This has obviously been issue for other projects as well. However, we are happy to report that Dr. Jay Rooker has been able to get us scale samples from the eastern Atlantic Ocean. We have also just learnt that a small number of samples from the Western Altantic are available from Dr. David Secor, and these samples will be analyzed as soon as we receive them.

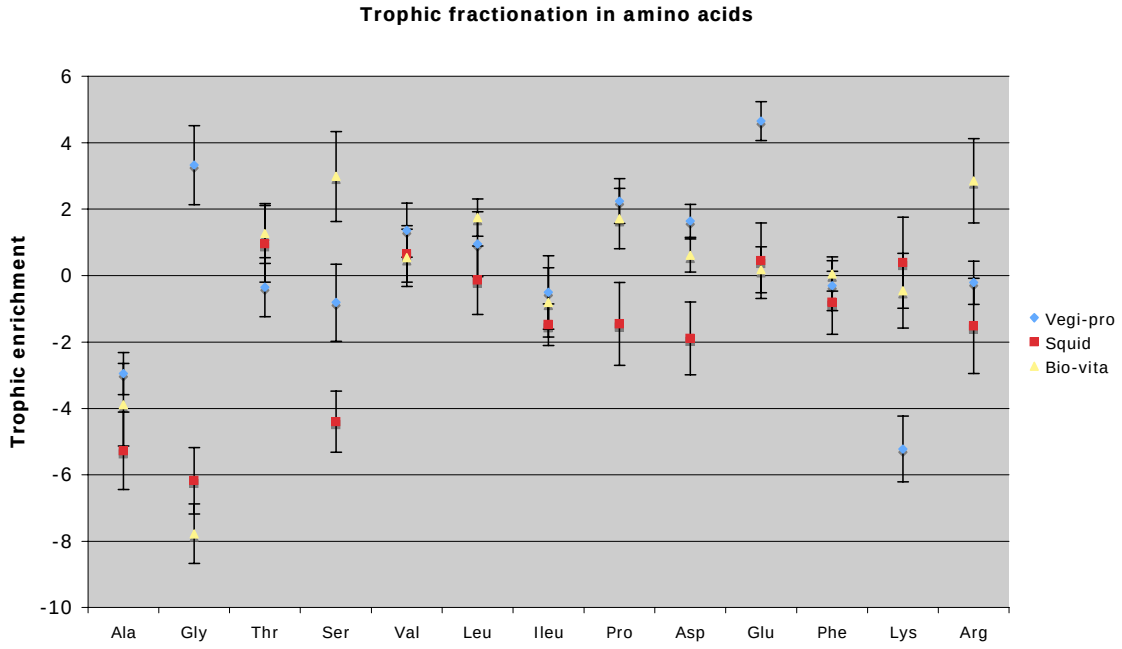

Figure 4. Mean trophic enrichment (± SD) for 13 amino acids (alanine, glycine, threonine, serine, valine, leucine, iso-leucine, proline, asparagines, glutamine, phenyl alanine, lysine, and arginine) in muscle tissues of juvenile Fundulus heteroclinus *fed diets of vegi-pro, squid, and bio-vita and analyzed using irm-GC/MS.*

5. Plans for the next six months to year: (one paragraph)

We plan to complete analyses juvenile bluefin tuna collected in the eastern Atlantic that will be provided to us soon by Dr. Rooker and analyze scales from juvenile bluefin tuna collected in the western North Atlantic in 2007. We will then complete data analysis and produce a manuscript based on the results.

6. Dissemination

Publications:

Workshops:

Conferences:

Manuals, Protocols:

Outreach Activities:

Patent, Copyright, Invention Disclosure Activity:

7. Collaborators and Personnel: (list collaborators and personnel working on this project, include terminal degree and institution not listed on the proposal).

8. Students: (list students receiving funding, degree type, anticipated graduation date, thesis or dissertation title)

Kelton McMahon, PhD student MIT/WHOI Joint Program in Oceanography, anticipated graduation date May 2010, dissertation title "Use of compound-specific stable isotope analysis to estimate connectivity in marine ecosystems".