# Nutritional Contribution of Natural and Supplemental Foods for Nile Tilapia: Stable Carbon Isotope Analysis (Effect of Preservation Method on Stable Carbon Isotope Ratios of Plankton and Tilapia)

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## Introduction

Stable carbon isotope analysis is a useful technique to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds (Schroeder, 1983; Anderson et al., 1987; Lochmann and Phillips, 1996).

In the present study, stable carbon isotopic analysis will be used to obtain quantitative estimates of the contribution of natural and supplemental feeds to the nutrition of tilapia in ponds in Sagana, Kenya. This can be accomplished by comparing the carbon isotopic "signatures" of tilapia with their known and probable food sources. The assumption underlying the technique is that the fish isotopic profiles will resemble that of the food(s) they assimilate most. The results may indicate how feeding/fertilization practices can be adjusted to minimize feed costs while maximizing fish production.

Here we report the results of a pilot study that was conducted at the University of Arkansas at Pine Bluff to determine whether different methods of sample preservation (chemical versus lyophilization) affect the carbon isotope ratios of fish and plankton differently.

## Methods and Materials

Three tilapia (*Oreochromis niloticus*) and three plankton samples were collected from commercial ponds in Arkansas. Individual fish were collected by dipnet and each plankton sample was obtained by filtration of 100 ml of pond water through a glass filter (47 mm diameter, 1 micrometer pore size) (Gelman Sciences). Fish were sacrificed by a blow to the head and divided longitudinally into halves. Each half was homogenized to uniform consistency. Each plankton sample was also divided into two equal portions. One half of each fish and plankton sample was fixed in 10% buffered formalin for 48 h, soaked for 24 h in deionized water to remove formalin, then preserved in 70% isopropyl alcohol (APHA, 1995). The second half of each sample was preserved by freeze-drying. All preserved samples were sent to a commercial laboratory (Coastal Science Laboratories, Inc.) for stable carbon isotope analysis using a micromass isotope ratio mass spectrometer (Anderson et al., 1987).

## Results

The carbon isotopic ratio of plankton preserved in formalin and alcohol was significantly different to that of plankton preserved by freeze-drying (Table 1). Results did not differ for tilapia tissue preserved in formalin and alcohol versus freeze-drying (Table 1).

## Discussion

From the standpoint of isotopic analysis, either lyophilization or chemical preservation would be suitable for further use in this study because the magnitude of the preservation effect was small compared to the trophic enrichment (diet) effect expected over the experimental period. However, freeze-drying is preferred (when feasible) because noxious chemicals are not used, samples do not have to be shipped in liquid, (which reduces shipping costs), and because the variability in isotope ratios

Table 1. Results of pilot study: comparison of stable carbon isotope ratios ( $\delta^{13}C$) of tilapia and zooplankton samples preserved either chemically or by lyophilization.

| **Sample Type: Plankton** (n = 3 for each treatment) | |
|---|---|
| $\delta^{13}C$ (‰) Formalin/alcohol | $\delta^{13}C$ (‰) Lyophilized |
| -25.1 | -24.6 |
| -24.9 | -24.7 |
| -25.2 | -24.6 |
| Mean ± SD | Mean ± SD |
| -25.1 ± 0.15 | -24.6 ± 0.06 |
| (ANOVA, Fisher's LSD, $P = 0.01$) | |

| **Sample Type: Tilapia, Whole, Ground** (n = 3 for each treatment) | |
|---|---|
| $\delta^{13}C$ (‰) Formalin/alcohol | $\delta^{13}C$ (‰) Lyophilized |
| -21.9 | -21.7 |
| -21.2 | -21.6 |
| -21.9 | -20.7 |
| Mean ± SD | Mean ± SD |
| -21.7 ± 0.38 | -21.3 ± 0.58 |
| (ANOVA, Fisher's LSD, $P = 0.41$) | |

of freeze-dried samples was slightly lower than that of chemically-preserved samples.

Although lyophilization is an excellent way to prepare samples for isotope analysis, freeze-drying equipment is not always accessible at CRSP project sites. This study indicates that chemical preservation of samples would also be an appropriate method of sample preparation prior to isotope analysis.

## Anticipated Benefits

The results of this study indicate that freeze-drying is a more reliable method of preserving samples for isotope analysis than is formalin and alcohol preservation. The lyophilization technique will be used at the Sagana site, and possibly in other CRSP experiments to preserve samples to allow the estimation of the relative contribution of different food sources to tilapia nutrition.

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