$\delta^{13}C$ AND $\delta^{15}N$ WITHIN THE LIFECYCLE OF SMALLMOUTH BASS

AN INVESTIGATION OF STABLE CARBON AND NITROGEN ISOTOPE ANALYSIS THROUGHOUT THE LIFE OF SMALL MOUTH BASS: IMPLICATIONS FOR TROPHIC LEVEL STUDIES.

By

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A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

(September) 2000

MASTER OF SCIENCE (2000) McMASTER UNIVERSITY
(Geology) Hamilton, Ontario

TITLE: An Investigation of Stable Carbon and Nitrogen Isotope Analysis Throughout the Life of Small Mouth Bass: Implications on Trophic Level Studies.

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SUPERVISOR: Professor Henry P. Schwarcz

NUMBER OF PAGES: 110

ABSTRACT

In this study $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were measured from *Micropterus dolomieu* tissue, from all stages throughout the fish's lifecycle. The different stages in the life of *Micropterus dolomieu* are represented also by shifts in the trophic level of the fish. As embryos, the $\delta^{15}N$ and $\delta^{13}C$ values for *Micropterus dolomieu* tissues are dependent on the $\delta^{15}N$ and $\delta^{13}C$ of the mother. Also until the point of metamorphosis, where the yolk sac is completely used up, the isotopes reflected in the tissues represent a combination of the $\delta^{15}N$ and $\delta^{13}C$ of the yolk and any plankton which the young fish are able to feed on. As the fish grows and its diet changes to crayfish and other smaller fish the isotopes reflected in the *Micropterus dolomieu* shift as the fish effectively increases its trophic position.

Definite trends were seen in the $\delta^{15}N$ values throughout the entire lifecycle of *Micropterus dolomieu*, and while the $\delta^{13}C$ values did not produce such definite trends it was discovered that there was an overlying spatial trend represented in the $\delta^{13}C$ values. This means that Micropterus dolomieu of similar age and size display an obvious offset in their $\delta^{13}C$ values depending on their location in the lake.

Also addressed here are the potential problems related to working with preservative chemicals and the effects they have on isotope ratios. Shifts were not only seen in both $\delta^{15}N$ and $\delta^{13}C$ (as has been addressed in the past) but also between different batches of the same preservative fluid. Different batches of formalin can potentially give different offsets to both $\delta^{15}N$ and $\delta^{13}C$, making it necessary to calibrate offsets given by all batches of formalin used throughout the entire procedure.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Dr. H. P. Schwarcz, for suggesting this topic and for allowing me this great opportunity to work under his supervision in a field I love.

Special thanks are also due to a great technician, without whom I would have been doomed, Martin Knyf. Thanks also to Dr. David Noakes, from Guelph University for steering me in the right direction and finding me an appropriate fish species to study. Also special thanks to Trevor Friesen who gave me 3 years of his life in the form of small mouth bass samples, and who also took the time out of his very busy schedule to explain them to me.

I also wish to thank John Winters and Norm Quinn from The Ministry of Natural Resources, at Algonquin Park for the permission to 'go fishing'. Thanks too, to Mark Ridgway for allowing me the use of the Harkness Laboratory at Lake Opeongo. I am grateful also to all those who helped me in the fish collection process, Gary, Trevor, and all of the Harkness staff. Special thanks to Lea, who not only helped me collect samples but also allowed me to steal a bit of tissue from each of the samples she collected for herself. Thanks to Don McQueen from York University and his biology class who made an extra plankton tow from Lake Opeongo just to get me a plankton sample. I would also like to thank Glen for a few Erie fish.

I would also like to thank my White Knight and Chris who helped me with a few computer problems, and my best friend Alia for a little bit of French translation.

Thanks also to my husband, Mike, for his patience over the last two years. Also to my parents, my aunt, and in-laws, who offered their encouragement and support, throughout the whole process.

Financial support was made available from a strategic NSERC grant provided to Dr. Henry Schwarcz.

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1. INTRODUCTION

1.1 OBJECTIVES

Stable carbon and nitrogen isotopes have been extensively applied in the studies of natural systems to determine the trophic structure of the organisms within ecological communities (Fry, 1988; Wada et al, 1987; and Minagawa and Wada, 1984).

In this study, tissue from *Micropterus dolomieu* (small mouth bass) has been analyzed for both carbon and nitrogen isotopes, in order to determine the effects of changing diets and therefore, shifting trophic levels, within an individual species. This may affect the way that stable isotope based trophic level studies are viewed in future experiments. If the age of the individual fishes must be taken into account it is obvious that this will add several levels of complexity onto many future trophic level studies. It would then be necessary to obtain samples from a large range of ages of each individual species to determine whether there are any significant isotopic changes throughout the organism's lifecycle, due to trophic level shifts. Stomach contents should also be examined isotopically to determine the differences in feeding habits at various stages of life.

1.2 TROPHIC LEVEL STUDIES

In nature, whenever two species interact, there is a link formed between them (Paine, 1988). Food webs, representing predator/prey relationships can be developed for any animal, in order to represent different trophic levels. The food web

defines any individual's position in a hierarchy of consumers with individuals which are higher up in that system feeding on the species which are lower in the hierarchy (Lawton, 1992). Any such hierarchy has a top predator, which can feed on any trophic level below it. Hastings and Conrad (1979), argue that any food web system will always collapse to the shortest possible length due to the inefficiency of energy transfer between trophic levels. There is only a relatively small transfer of energy between trophic levels with only 5% to 20% of the food energy from the prey being transferred to predator.

Paine (1988) also states that there is a fundamental problem with the basis of the food web theory. Food webs, which had up to that point, been compiled mainly on such methods as observation of the feeding habits of the relevant species and stomach content analysis, were unreliable. This is because food webs were constructed based on field data, which is undoubtedly incomplete and not representative of all of the biological processes within the system. Peters (1988) reiterates this and also comments that problems arise due to the fact that food webs were usually compiled based on the relationships, which could be observed or implied from direct observation. Even more difficult to observe would be the fact that not all species feed strictly on only one trophic level. The web then becomes extremely complex to observe and difficult to describe.

Even the simple matter of a single carnivore feeding on a herbivore becomes a complex arrangement, if all the factors are taken into account. As Pimm and Lawton argued (1978) when a fox eats a rabbit, it also eats the rabbit's parasites. This is an important omission, since the parasites are technically on the same trophic level as the fox; they too are feeding on the rabbit.

Some recent trophic level studies have used stable isotopes, namely carbon and nitrogen, to reconstruct food webs. This is possible because the cliché, 'you are what you eat' is true, but from an isotopic perspective, you are what you eat plus or minus a few per mil. (DeNiro and Epstein, 1978).

1.3 ANIMALS AND ISOTOPES

The two most useful isotopes used to study trophic levels in organic organisms are carbon and nitrogen because they are both common in all tissues of the organism. They are also useful for differentiating between trophic levels due to the fractionation that occurs between predator and prey (discussed in detail later in chapter 2).

Trophic level studies, which are conducted in the aquatic environment, are far more complex than similar studies conducted in terrestrial environments. In both the aquatic and the terrestrial systems carbon and nitrogen isotopes can be utilized to reconstruct the food chain and determine the relative trophic level of the animal. In terrestrial systems there are only three or four trophic levels. Autotrophs are considered to be the first level, herbivores the second, and carnivores the third, sometimes there will be a top carnivore that will prey on other carnivores. Aquatic systems, on the other hand, are not so clear-cut. Again autotrophs represent the first level and organisms that eat these primary producers represent the second level. From this point on things become much more complicated. Many higher trophic levels reside above this second level, with larger fish eating smaller fish, but there is always a larger fish. To complicate matters even more, the higher level fish are not restricted to eating fish on the level directly

below them. The general rule of thumb for these higher-level predators is that if the prey will fit into its mouth it will eat it.

The phenomenon of fractionation occurs between the isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) during the assimilation of food. Isotopic examination of the food web brought a whole new way of examining food webs. Early studies by DeNiro and Epstein (1978 and 1981) have shown that the stable isotopic composition of carbon and nitrogen in an animal reflects the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of its diet, respectively. They described an enrichment in ^{13}C, in the predator, of about 1 ‰ relative to the $\delta^{13}C$ of the diet. They also stated that there was an enrichment in ^{15}N, in the predator, of about 3 ‰ relative to the $\delta^{15}N$ of the diet. Figure 1.1 (DeNiro and Epstein, 1978) shows the 1 ‰ enrichment in $\delta^{13}C$ in the predator relative to the prey. Figure 1.2 (DeNiro and Epstein, 1981) details the enrichment that was observed in the $\delta^{15}N$ between predator and prey. From these two figures it is also apparent that the $\delta^{13}C$ and $\delta^{15}N$ values of different individuals can differ even if they are the same species and have the same diet.

In the aquatic system the carbon and nitrogen that are integrated into plankton and other primary producers, which represent the first trophic level (base level). All animals that feed on this plankton will display $\delta^{13}C$ and $\delta^{15}N$ values that will be related to the base level but shifted a few per mil. There is a transfer of nitrogen between predator and prey, where $\delta^{15}N$ increases 3-5 ‰ from prey to predator (France, 1995 b; Kidd et al., 1997). A similar transfer is displayed by carbon isotopes, but $\delta^{13}C$ only increases 0.5-1‰ from prey to predator (Fry, 1988). In terms of the trophic level there is a stepwise increase in the $\delta^{15}N$ and $\delta^{13}C$ values as the trophic level increases.

Delta C values of the whole bodies of animals and their diets

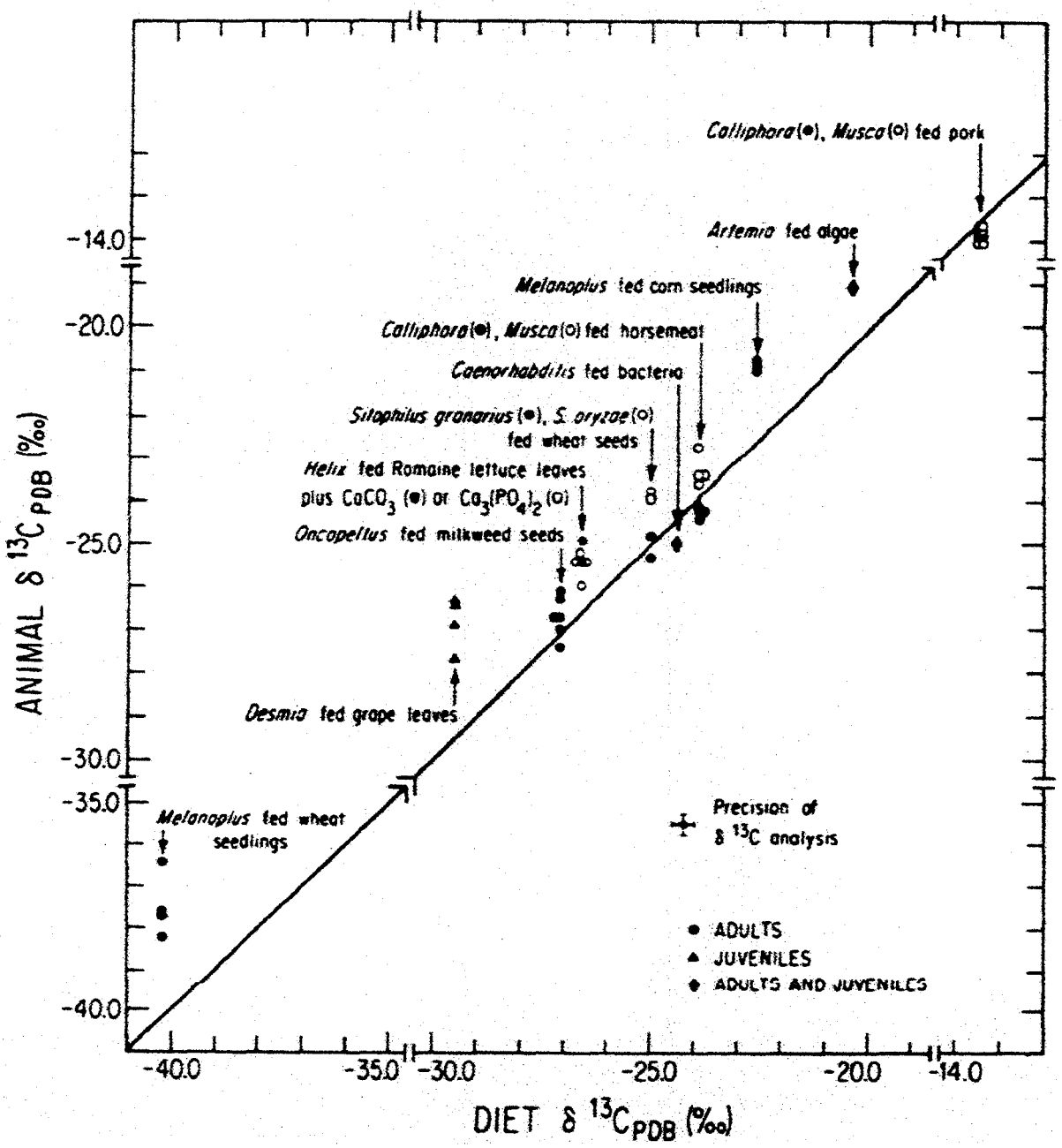


Figure 1.1
$\delta^{13}C$ values of the whole bodies of animals and their diets. In most cases the animal carbon is enriched in ^{13}C relative to the diet carbon (DeNiro and Epstein, 1978).

Delta N values of the whole bodies of animals and their diets

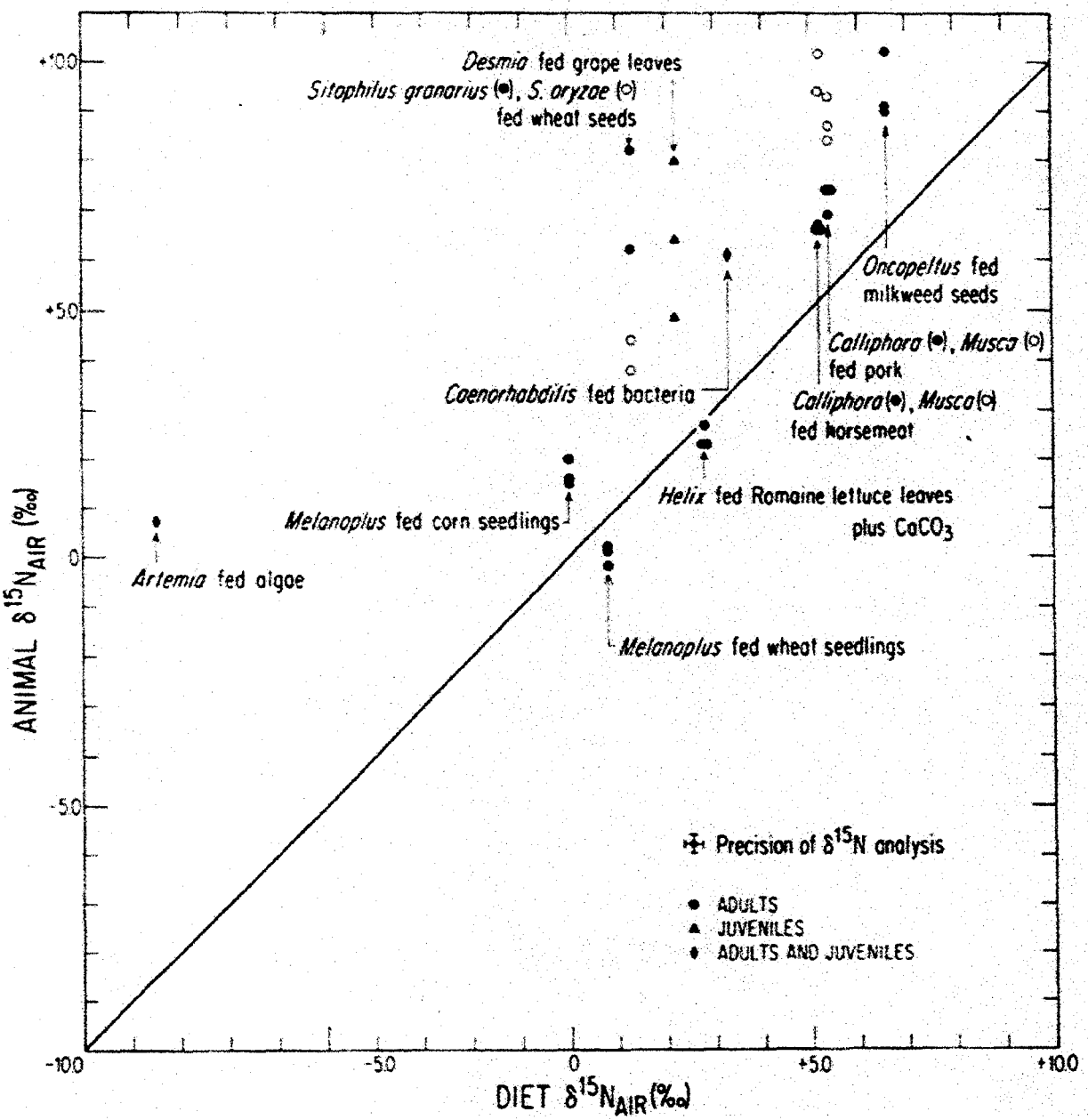


Figure 1.2
$\delta^{15}N$ values of the whole bodies of animals and their diets. In most cases the animal nitrogen is enriched in ^{15}N relative to the diet nitrogen (DeNiro and Epstein, 1981).

This gradient appears to present an excellent tool with which to reconstruct the food web of a marine or lacustrine environment. However, many of the organisms, which inhabit these systems are difficult to place into a single trophic level. This is not only because they feed on a variety of lower trophic levels but also because they are trophically inconsistent throughout their own lifecycle. Some fish start life as planktivores and remain so throughout their entire life. These fish will fit quite nicely into the established food webs previously constructed. Other fish start life as planktivores but as they grow they adopt the method of feeding on anything that will fit into their mouths, including smaller fish, crayfish, plankton, etc. Where these fish fit into the food web must then be determined by what the fish is eating which in turn is determined by the stage of life the fish is in. Species that exhibit size, age, or ontogenetic stage-related changes in diet are widespread and are not easy to position in a fixed food web (Paine, 1988).

2. ISOTOPIC THEORY

2.1 INTRODUCTION

Isotopes are atoms that have the some number of electrons and protons but differ in the number of neutrons within the nucleus. They occupy the same position in the periodic table. The first isotope to be identified was that of hydrogen, ^{1}H and ^{2}H, or deuterium. Urey et al. (1932a,b) discovered the stable isotopes of hydrogen. Since then differences in the chemical properties of other atoms, such as H, C, N, O, S, and others were observed and isotopes for these atoms were identified (Hoefs, 1997).

There are two main groups of isotopes, stable and unstable. The unstable isotopes are the radioactive ones, which decay over time, while the stable isotopes are not observed to decay but have stable nuclei.

While the electrons of an atom are responsible for the chemical properties of the element the nucleus is primarily responsible for the physical properties of the atom because that is where most of the mass is located. Isotopes of one element can be expected to have similar chemical properties to each other, since the number of electrons does not change. The addition or subtraction of a neutron changes the mass of the atom and any physicochemical properties related to mass (Hoefs, 1997). Adding neutrons can reduce the rate of any chemical reactions that the atom may undergo because the increase in mass will also increase the activation energy required to start a reaction. Figure 2.1 (Hoefs, 1997) shows that the zero-point energy level is higher for the lighter isotope, meaning that bonds formed by the lighter isotope are weaker than the bonds in the

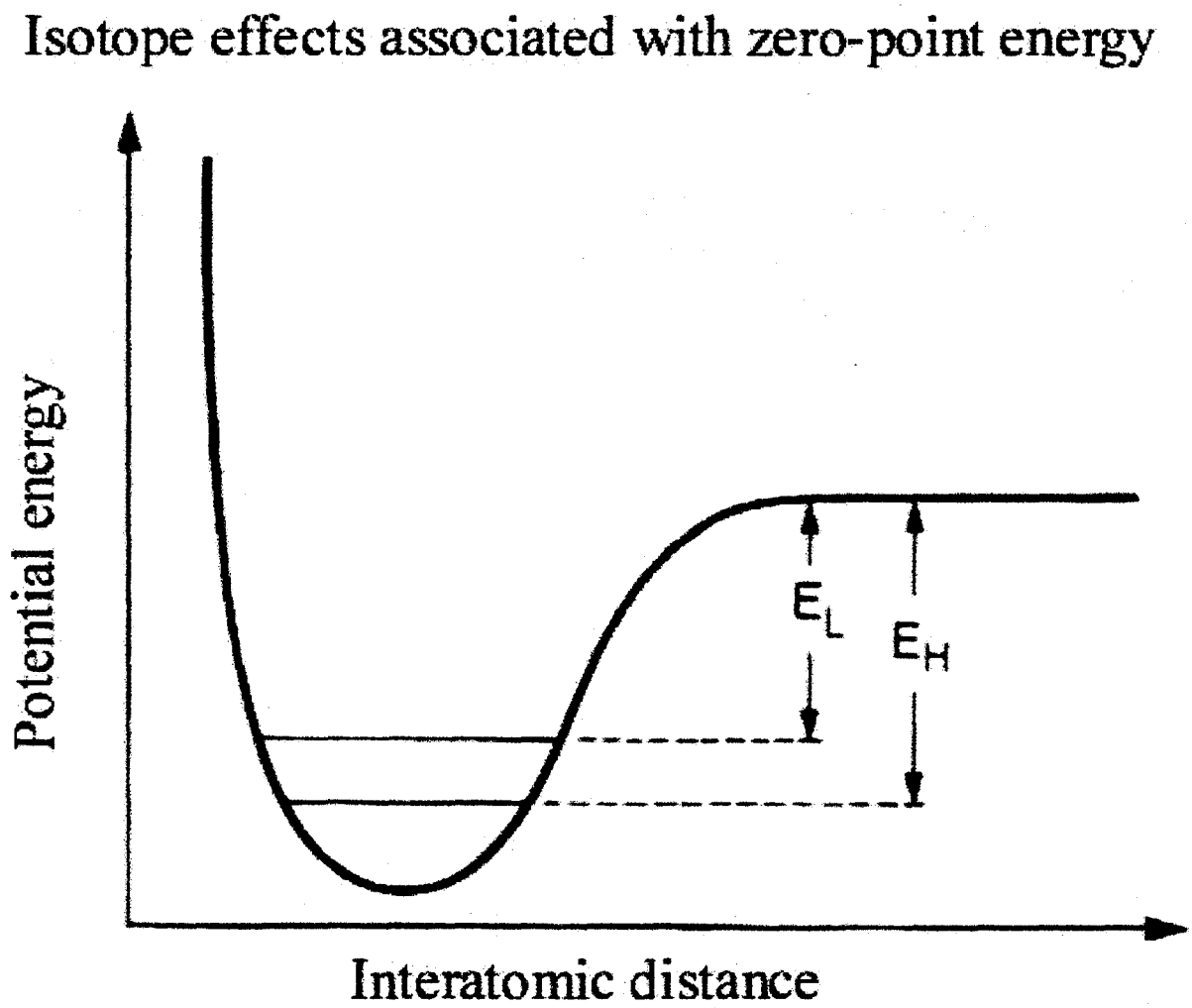


Figure 2.1
A potential energy curve, showing the interactions between two atoms in a stable molecule. E_L represents the dissociation energy for the lighter isotope and E_H represents the dissociation energy for the heavier isotope. Modified from Hoefs, 1997.

heavier isotope. This is why the lighter isotope will react more readily than the heavier isotopic component. The lighter elements are the ones in which slight mass differences can be expected to cause measurable isotopic fractionation in chemical and physical processes (Craig, 1953).

The single most important process in the study of isotope geochemistry is the phenomenon of fractionation. In nature, the stable isotopes of carbon and nitrogen are fractionated by either chemical reactions or physical processes (Faure, 1977). The main situations in which isotopes are fractionated are either: 1) isotope exchange reactions or 2) kinetic processes.

Exchange reactions occur where one isotope of an element is exchanged for a different isotope of the same element within a molecule and can be expressed as (Hoefs, 1997):

$$aA_1 + bB_2 = aA_2 + bB_1$$

Where the 1 and 2 subscripts, represent isotopic labels on molecules A and B. Kinetic effects occur in unidirectional reactions where the rate of the reaction is dependent on the isotopic composition of the elements involved (Faure, 1977). In the latter case one isotope will either react more readily or be more easily integrated into the final product of the reaction. The mass differences between the isotopes of an element are the important factor in the fractionation effects arising from physical processes. Such processes include evaporation, condensation, melting, crystallization, etc. (Faure, 1977). Fractionation

occurs within biological systems by chemical reactions, which use one isotope more readily than the other isotope.

2.2 CARBON ISOTOPES

2.2.1 GENERAL

There are two stable isotopes of carbon ^{12}C and ^{13}C (Nier, 1950). The naturally occurring abundances of each of the carbon isotopes in the atmosphere are: ^{12}C: 98.89% and ^{13}C: 1.11%

The ratio of ^{13}C to ^{12}C within any substance is expressed by $\delta^{13}C$ values, which are defined as follows:

$$\delta^{13}C = [^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1] * 1000,$$

Where the delta values for $\delta^{13}C$ are expressed in per mil (‰).

The standard used in mass spectrometric analysis of $\delta^{13}C$ is PDB. This has a higher ^{13}C content than most organic substances that are analyzed; so most organic samples will have negative $\delta^{13}C$ values when compared to PDB.

2.2.2 CARBON ISOTOPES IN FISH

It is possible to make use of carbon isotopes in the reconstruction of food webs because carbon atoms from the food source are integrated into predators' tissues.

The carbon that is integrated into the tissues is not integrated in the same $^{13}C/^{12}C$ ratios as are present in the prey. Predators tend to become enriched in ^{13}C relative to the prey. Up the food chain animals become more and more enriched in ^{13}C, that is their delta values become less negative. This is because the predators preferentially metabolize the lipid components of their prey, while they integrate the proteins into their own tissues. Since the lipids are depleted in ^{13}C the consumer becomes enriched in ^{13}C over the prey. The excess ^{12}C is excreted as CO_2.

The enrichment of ^{13}C for one trophic level, as stated earlier, is 0.5 to 1‰. Figure 2.2 (Meili, Fry, and Kling, 1993) shows progressive enrichment of ^{13}C up the food chain. The $\delta^{13}C$ of the aquatic primary production was between –35 to –38 ‰ while the zooplankton, which feeds on the plankton, had a mean around –33 ‰. Benthic predators display $\delta^{13}C$ values about 1 ‰ higher than benthic invertebrates, which feed on the zooplankton, and are likewise enriched above their food source. The fish, which feed on these benthic organisms, are likewise enriched, as are the piscivorous fish, which feed on the invertebrate-feeders.

2.3 NITROGEN ISOTOPES

2.3.1 GENERAL

There are also two stable isotopes of nitrogen ^{14}N and ^{15}N (Nier, 1950). The naturally occurring abundances of each of the nitrogen isotopes in the atmosphere are: ^{14}N: 99.64% and ^{15}N: 0.36%

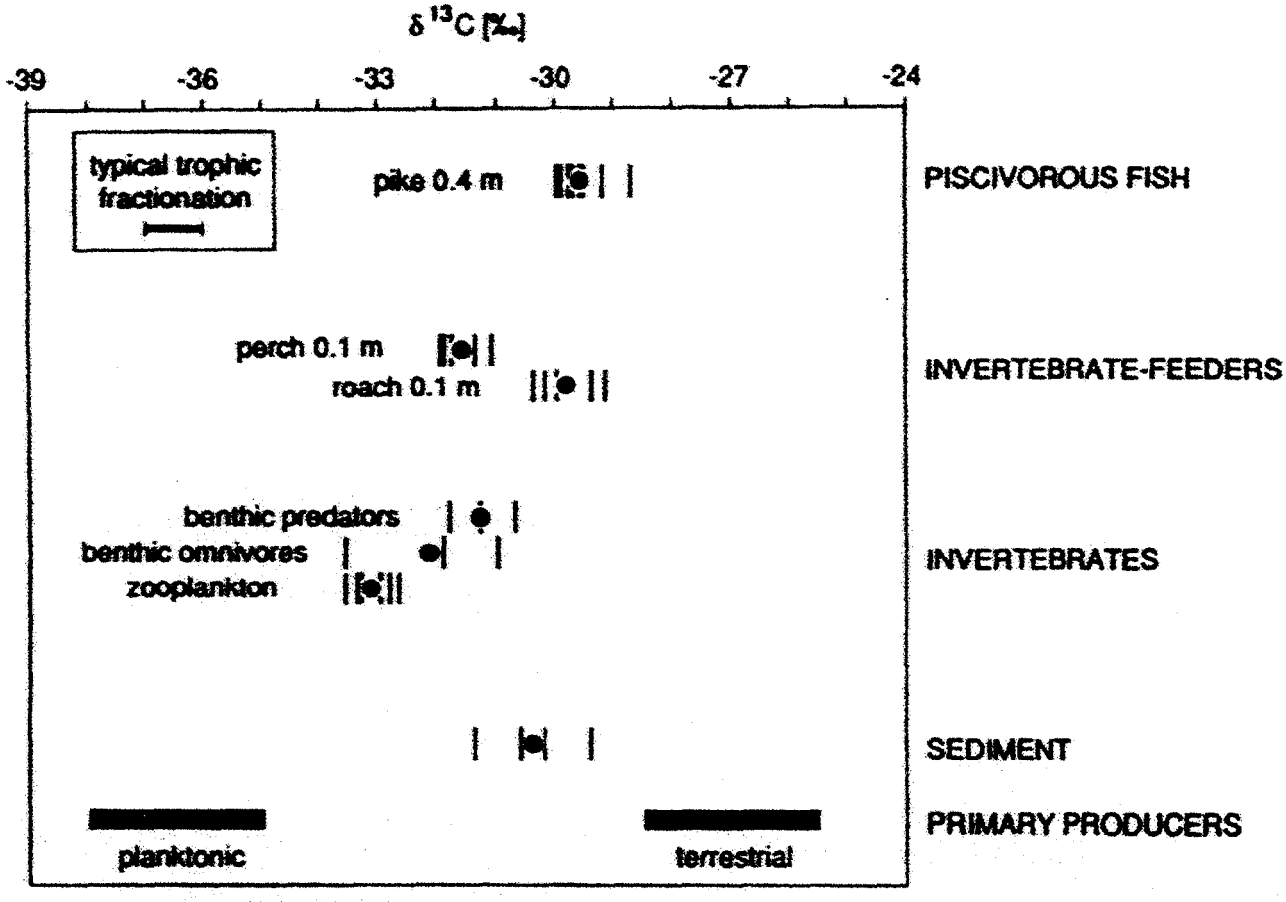


Figure 2.2
Isotopic compositions of organic carbon in the food web of Blacksastjarn, a humic lake in central Sweden. Means are represented by the solid circles and individual observations by the bars (Meili, Fry, and Kling, 1993).

The 1 ‰ enrichment of $\delta^{13}C$ between predator and prey is obvious in this graph.

The ratio of ^{15}N to ^{14}N within any given sample is expressed in $\delta^{15}N$ values defined as:

$$\delta^{15}N = [^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1] * 1000,$$

Like carbon the values for $\delta^{15}N$ are expressed in units per mil. The standard used in the case of nitrogen is atmospheric N_2 gas, which by definition has a delta value of 0 ‰ (Faure, 1986).

2.3.2 NITROGEN ISOTOPES IN FISH

As with carbon it is also possible to use nitrogen isotopes in the reconstruction of food webs because nitrogen atoms from the food source are integrated into predators' tissues. Predators tend to become enriched in ^{15}N relative to their prey. This is because urea and other nitrogenous wastes are depleted in ^{15}N, while the proteins that are integrated are enriched in ^{15}N. Therefore animals become more and more enriched in ^{15}N up the food chain. The enrichment of ^{15}N for one trophic level is approximately 3 ‰ (Wada, et al., 1987). Figure 2.3 (Meili, Fry, and Kling, 1993) shows progressive enrichment of ^{15}N up the food chain. The $\delta^{15}N$ of the aquatic primary production was between –2 and +1 ‰ while the zooplankton, had a mean around +3 ‰. The invertebrates, invertebrate-feeders, and the piscivorous fish are likewise enriched by about 3 ‰ per trophic level (Meili, Fry, and Kling, 1993).

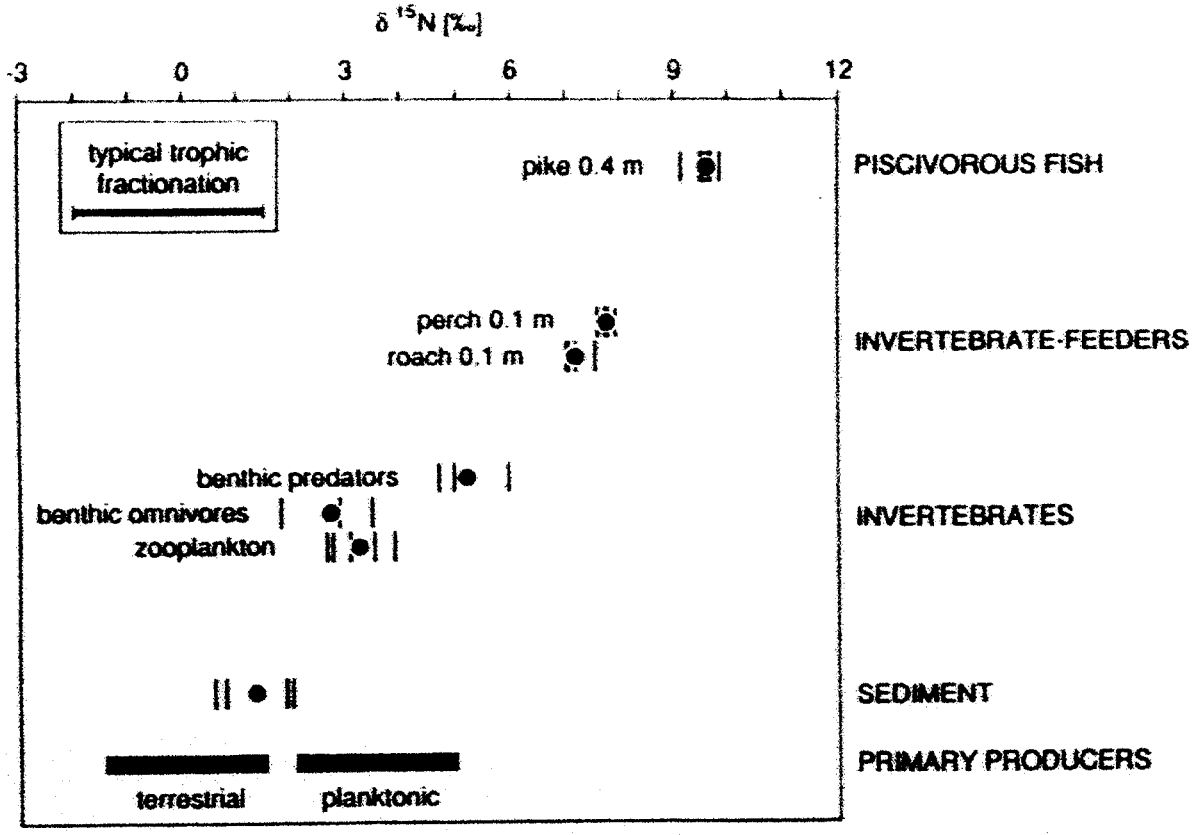


Figure 2.3
Isotopic compositions of organic nitrogen in the food web of Blacksastjarn, a humic lake in central Sweden. Means are represented by the solid circles and individual observations by the bars (Meili, Fry, and Kling, 1993).

The 3 ‰ enrichment of $\delta^{15}N$ between predator and prey is obvious in this graph.

2.4 ISOTOPES IN THE FOOD CHAIN

At each trophic level there is an increase in the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. These carbon and nitrogen fractionation effects are cumulative and so can be traced up the food chain, with higher trophic levels displaying greater enrichment of heavy isotopes (France, 1995). Once it was discovered that there was a connection between these stable isotope ratios and predator/prey relationships it was possible to imply food chain linkages from the fractionation that has occurred in the animals' tissues. Leading to the possibility of generating entire food webs from the results of isotopic analysis of the fractionation that occurs within animal tissues.

Different tissues within a single animal can display different stable isotope ratios. DeNiro and Epstein (1978) showed conclusively that no single animal tissue could be analyzed to determine the isotopic relationship between an animal and its diet. Figure 2.4 shows their results. So the analysis of several different tissues is necessary to get a better estimate of the delta value of the animal's diet.

The $\delta^{13}C$ and $\delta^{15}N$ isotopic enrichment is dependent on the food that the animal feeds on. If the same species feeds on two different trophic levels then those individuals will display different trophic levels because the starting points were different. Figure 2.5 (DeNiro and Epstein, 1978) shows two flies, *Caliphora* and *Musca*, fed on different kinds of meat. The $\delta^{13}C$ values of the two flies are much lighter when fed on horsemeat as compared to the same species of fly fed on pork. An excellent example of this in a marine environment (figure 2.6) can be found in a paper written by Cabana and Rasmussen (1994). Lake trout are one of the top predators in most small lakes

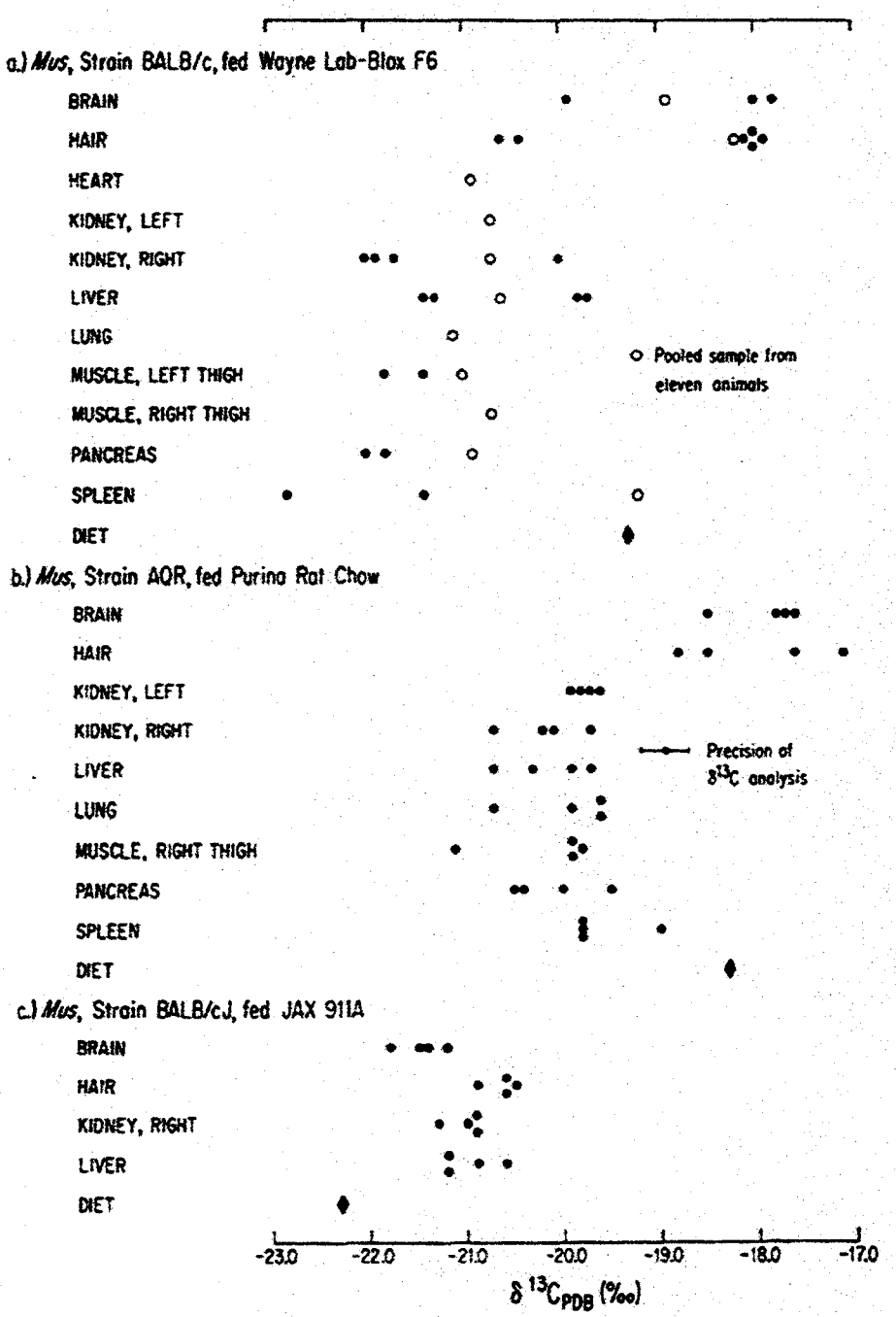


Figure 2.4
$\delta^{13}C$ values of tissues of mice and their diets. Each point represents the analysis of tissue dissected from a single mouse, except as indicated. The hollow circles represent pooled samples from eleven animals. (DeNiro and Epstein, 1978).

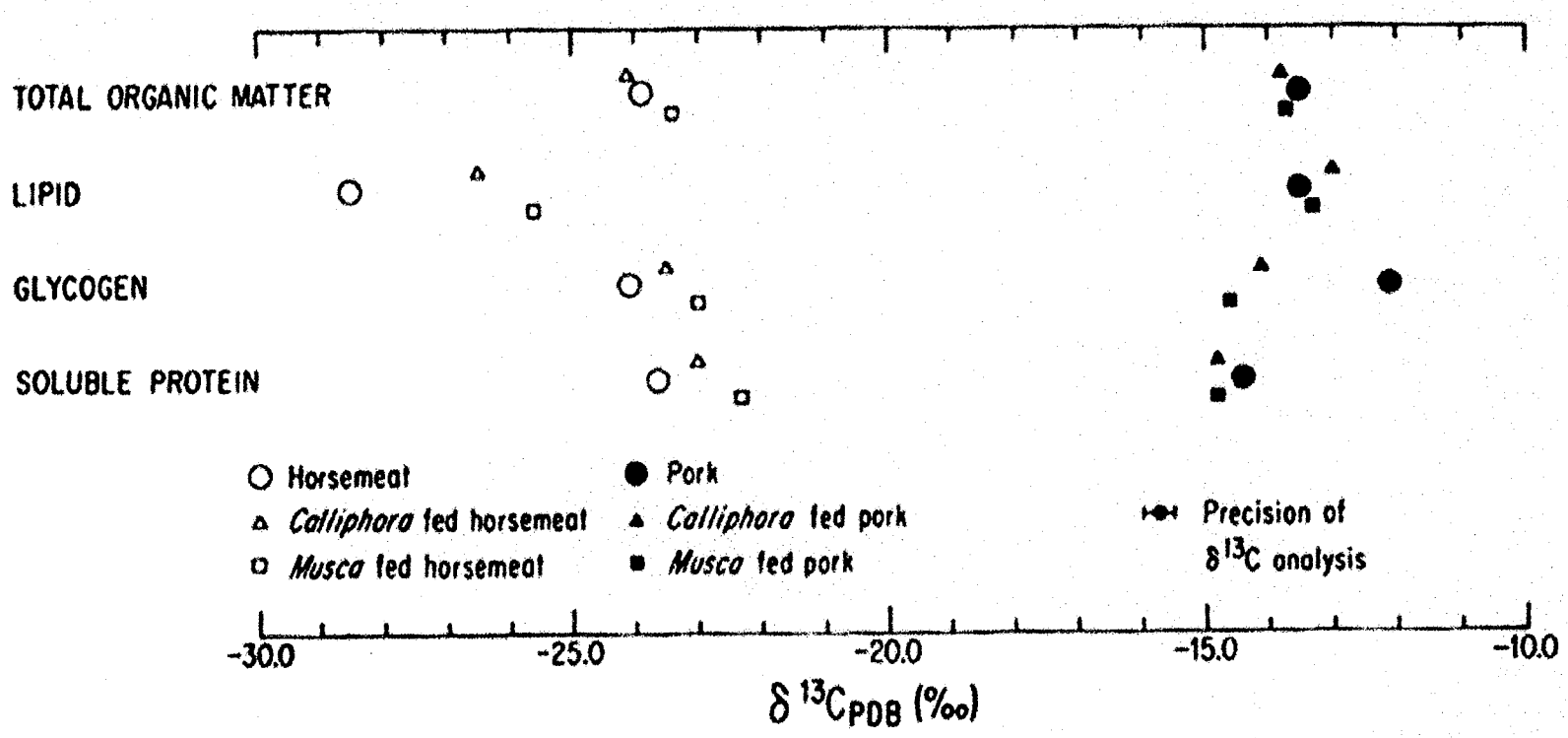


Figure 2.5
$\delta^{13}C$ values of the total organic matter and the major biochemical fractions of two species of flies and their diets. This shows that both the flies *Caliphora* and *Musca* are enriched in $\delta^{13}C$ when feed on horsemeat. These same flies display even greater enrichment when fed on pork which is more enriched than horsemeat. The flies do not appear to be enriched relative to the pork because unlike the horses the pigs were not fed a continuous diet throughout their lives, but received a more enriched diet in the later part of their lives making the pork appear more enriched than it would if the diet had remained constant for its whole life. (DeNiro and Epstein, 1978).

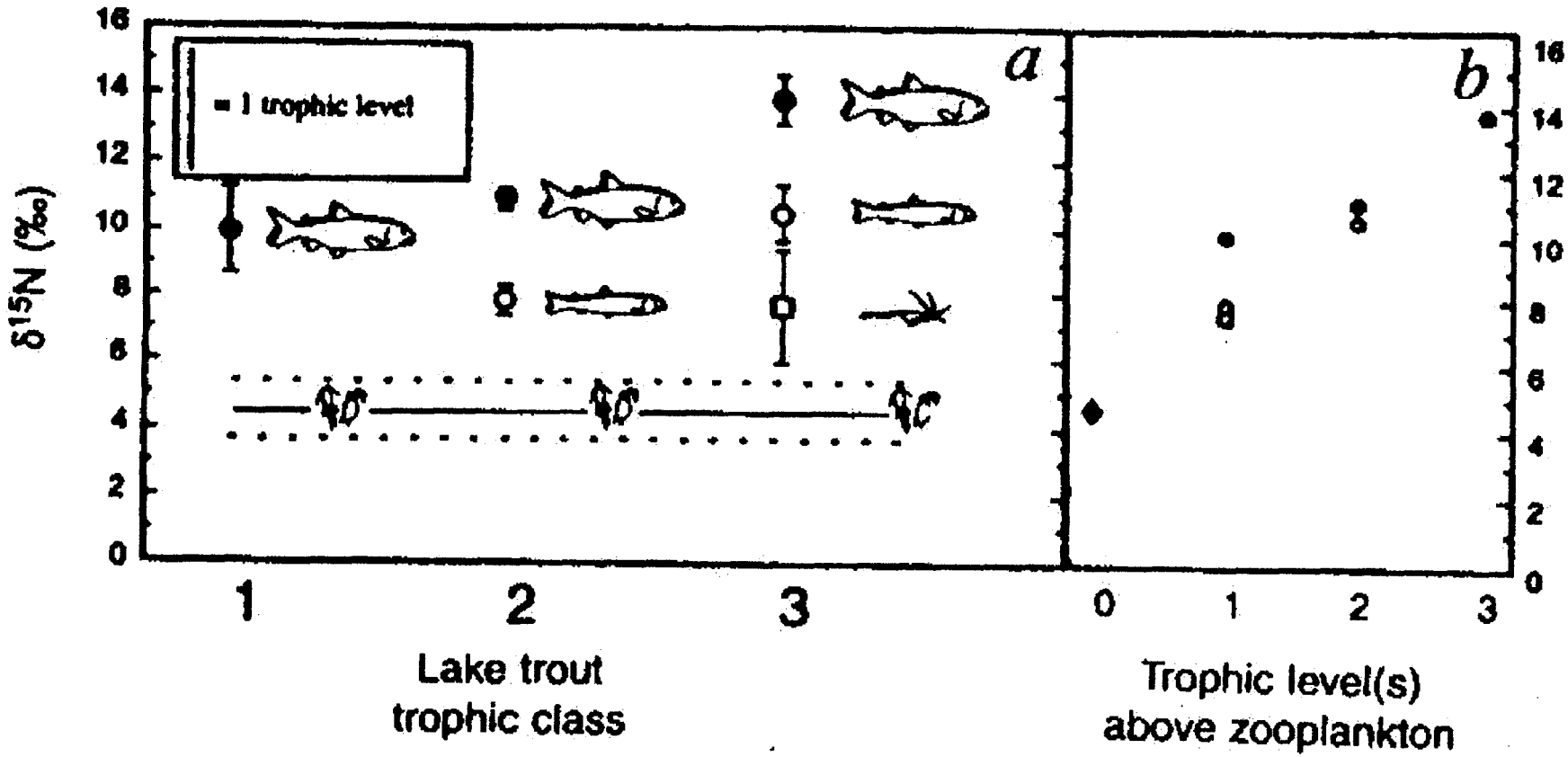


Figure 2.6
a. Class 1, 2, and 3 lakes as defined by the number of trophic levels above zooplankton. In lake trout trophic class 1 the trout feeds directly off of the zooplankton while in lake trout trophic class 2 the trout feeds primarily off of the primary consumer (a smaller foraging fish) which in turn feeds off of the zooplankton and so on...
b. Mean $\delta^{15}N$ viewed as a function of the number of trophic level(s) above that of zooplankton. Zooplankton is shown as a solid diamond, and the symbols for the other groups are as in *a.* (Cabana and Rasmussen, 1994).

but it can not be expected to have the same delta value from lake to lake. In some lakes lake trout may be considered a third level consumer if there are other species available under it in the hierarchy. In other lakes the same species of fish may be classed as a first level consumer, simply because there are no primary or secondary consumers in the lake. In their paper Cabana and Rasmussen classified Lake Opeongo as a second level lake; there are fish like lake trout and small mouth bass which feed on the fish which forage the zooplankton for food and are therefore generally at trophic level 2. Their $\delta^{15}N$ values are 6 – 7 ‰ enriched with respect to the phytoplankton in this lake.

3. METHODS AND ANALYSIS

3.1 SAMPLE COLLECTION

All of the samples of *Micropterus dolomieu* captured were obtained from Lake Opeongo, in Algonquin Park. They were obtained by permission of John Winters and Norm Quinn of The Ministry of Natural Resources (Pursuant to the provisions of the Ontario Fishery Regulations Section 60(1)). Lake Opeongo is the largest of the fresh water lakes in Algonquin Park. Figure 3.1 shows a map of Opeongo, in Algonquin Park (Algonquin Provincial Park Management Plan, 1998). Opeongo is comprised of three arms, the north, south, and east arms. The Harkness Laboratories, from which all fish collection and research was carried out, is located near the southern tip of the Southern arm.

In September 1999 large specimens of *Micropterus dolomieu*, greater than 5 centimeters total length, were obtained from Opeongo. Several methods were employed to catch the fish; trap nets were set and checked every day for seven days (Sept. 13 to Sept. 19), several Windimere and mesh traps were also submerged and checked daily. Eight sites were used for the collection of *Micropterus dolomieu* in 1999; these sites are outlined on the figure 3.1 map. The numbers represent collection locations for future reference in this study.

Fish that were under 20 centimeters were weighed and measured and taken back to the laboratory at Harkness (located at the tip of the Southern Arm of Opeongo Lake) and muscle tissue samples were taken from the fish's back just under the first dorsal fin. All of these samples were immediately placed in glass vials containing

Figure 3.1
Map of Lake Opeongo in Algonquin Park. Numbers 1-8 represent collection sites. Harkness laboratory is also on the map at the bottom of the South Arm. Modified from Algonquin Provincial Park Management Plan, 1998.

37% formalin (10% buffered formalin). Several of the whole fish were preserved in the formalin also. Most of the bass that were greater than 20 centimeters were tagged and released as part of an ongoing population/tagging study. It was possible to obtain several bass greater than 20 centimeters from ones that were dead or dying in the net as only healthy fish were being tagged. Several of the healthy fish, larger than 20 centimeters, were taken back to the Harkness laboratory alive and drugged with MS222 to knock them out. These fish were also weighed and measured. Several scales and a small amount of tissue was then removed from below the dorsal fin in the form of a biopsy and the fish were carefully treated with an antibiotic, revived, and released.

Stomach contents were removed from seven of the adult *Micropterus dolomieu* collected in 1999, after the dissection of their stomachs. Four small fish and three crayfish were obtained in this manner. Only whole prey that had just been ingested was kept for isotope analysis, such that the scales and the carapaces' of the prey had not yet been broken down by the digestive acids of the *Micropterus dolomieu*, thus ensuring that any tissue obtained from the prey was representative of that animal and not from the predator. These stomach contents were also prepared for $\delta^{15}N$ and $\delta^{13}C$ analysis in order to confirm the trophic levels within the lake.

A Ph.D. student at the University of Guelph, Trevor Friesen, obtained another set of *Micropterus dolomieu* samples from Lake Opeongo in 1992. These samples were made available for this project upon Friesen's consent. The bass captured for Friesen's thesis were all young of the year; he also collected embryos from some of the bass families he sampled. Fish from seven families were used in this analysis. All of the samples he collected were placed into 37% formalin to preserve and store them.

For this part of the collection it was possible to obtain fish which are known to be from the same families. In their first few weeks of life small mouth bass do not leave the boundaries of their nesting sites. Each nest is separate from all other bass nest sites and is vehemently guarded and protected by the male bass parent of the young in the nest. This male will also make sure that none of the young leave the site until the juvenile stage is reached. Once a nest site has been identified it is therefore assumed that all the young within that site are siblings, which are the product of only two parents, the male guarding the nest and a female (which leaves shortly after laying the eggs). The samples, which were obtained by Trevor Friesen (1992), range from eggs and larva to post-metamorphosis bass with lengths up to 5 cm. Samples of plankton were also obtained from the nesting sites, throughout the spawning season – from May 29 to early September in 1992 (Trevor Friesen, personal communication).

Mother and embryonic tissue was also obtained from *Oncorhynchus mykiss* to determine if there were any trophic level differences visible between mother and young. Carbon and nitrogen samples were prepared for isotopic analysis in the same manner as the previous samples.

3.2 AGE OF THE FISH

For the fish which were older than young of the year, that is two years or older, the scales were examined to determine the age of the individual. The scales were examined microscopically and the age determined by counting the growth rings (Casselman, 1985; Weatherley and Gill, 1987).

3.3 PROCEDURE FOR ISOTOPIC ANALYSIS

Before the formalin-treated tissue samples were weighed they were freeze-dried. A small amount of muscle tissue (about a square centimeter) which had been removed from the larger fish (bass with a total length of greater than 5 centimeters) was placed in a 10 ml test tube, which was sealed with a punctured aluminum foil cap. Fish that were under 3 centimeters and embryos were placed whole within the tubes, as these smaller fish would not yield enough muscle tissue to provide an adequate sample. The aluminum foil was punctured to permit the escape of the water/formalin mixture when the samples were dried. These tubes were then placed in a stand and set in a container with dry ice overnight or for a few hours to freeze. When frozen, the tubes were transferred to an airtight container, which was sealed and attached to a vacuum pump. The samples were left until a hard vacuum was reached indicating that all moisture had been removed from the samples. Then the apparatus was disassembled, and the samples were placed into tightly sealed plastic centrifuge tubes. A portion of each sample was removed and weighed for isotopic analysis.

In the case of both carbon and nitrogen only a small amount of organic tissue is required to perform stable isotope analysis. Since there are large amounts of carbon in organic tissue only 1 to 3 milligrams of dry tissue is required for analysis of $\delta^{13}C$. Within the same amount of tissue, there is much less nitrogen, so 5 to 10 milligrams of dry tissue is required for the analysis of $\delta^{15}N$. Plankton samples contain even less nitrogen, and 10 to 13 milligrams of tissue must be used for each analysis.

The weighed samples were then transferred to a heat-treated Pyrex tube. Each Pyrex tube was 6 millimeters in diameter and 230 millimeters long. Before the samples were loaded each tube had been heat-treated at 550 ^{0}C to eliminate any impurities on the surface of the tubing. Prior to placing the samples in the tube, heat-treated cupric oxide (CuO) was first added: a layer of about one and a half centimeters in thickness at the bottom of the tubes, which was used for the analysis of carbon isotopes. While a three-centimeter layer was used for the larger samples used to detect δ^{15}N. Then the tissue was loaded into the tube on top of the cupric oxide. Then more cupric oxide was added on top of the tissue; the same amount as in the bottom of the tube, one and a half and three centimeters, respectively. All of the tubes were then evacuated for at least two and a half-hours to eliminate any air and residual moisture and then they were sealed under vacuum with a gas-oxygen torch. The sealed tubes were placed in at oven at 550 ^{0}C for two and a half-hours. This temperature was sufficient to combust the organic tissue in the tubes and allow the cupric oxide to act as an oxidizing agent. The oxygen it releases reacts with the organic matter in the tissue to produce carbon dioxide (CO_2), nitrogen gas (N_2), and water vapour (H_20). The CO_2 and N_2 were then analyzed on a SIRA mass spectrometer. Values of δ^{13}C and δ^{15}N were obtained with respect to the standards PDB and AIR, respectively.

3.4 PRECISION OF ANALYSIS

Each day that any samples were run on the mass spectrometer several standards were also run to make sure that the mass spectrometer was performing within

the same parameters for all the samples. The standard used for carbon analysis was a gelatin that had an average $\delta^{13}C$ value of -25.4 ± 0.06 ‰. The standard used for nitrogen analysis was atmosphere, which had an average corrected $\delta^{15}N$ value of -3.7 ± 0.04 ‰. Also in order to ensure accuracy many of the samples were prepared and run in triplicate, and the standard deviations of these replicated samples was determined.

3.5 FORMALIN EXPERIMENTS

To determine the effects of formalin on the tissue a small side experiment was preformed. Fresh frozen, store bought lake trout, was sampled in the same manner as the *Micropterus dolomieu.* Some of the tissue was also freeze-dried for isotopic analysis immediately from the frozen state. Other sections of the tissue were placed into formalin and allowed to become saturated with it for several weeks. This tissue, too, was then prepared for isotopic analysis in the same manner as the above. The effects of formalin on the tissue would then cause any differences seen in the isotopic compositions of these two samples. This was prepared in triplicate for both carbon and nitrogen isotopic analysis. The reference for this experiment was a paper by Bosley and Wainright (1999).

4. GROWTH STAGES IN THE LIFE OF *MICROPTERUS DOLOMIEU*

4.1 INTRODUCTION

In their first few weeks of life small mouth bass do not leave the boundaries of their nesting sites. Each nest is separate from all other bass nest sites and is vehemently guarded and protected by the male bass parent of the young in the nest. This male will also make sure that none of the young leave the site until the juvenile stage is reached. So once a nest site has been identified it is safe to assume that all the young within that site are siblings, which are the product of only two parents, the male guarding the nest and a female (which leaves shortly after laying the eggs).

Micropterus dolomieu are fish that do not fit nicely into the food web. As juveniles they are planktivores, feeding on the plankton in their nets sites. However after the juvenile stage they disperse, from their nest sites, into the lake. At this stage they will feed on anything they can, while the young of the year will still predominantly eat plankton older fish will also eat crayfish and other smaller fish. Small mouth bass that are older than one year old will feed on anything that will fit into their mouths.

4.2 LIFECYCLE

Micropterus dolomieu eggs hatch about seven days after they are laid, figure 4.1 (Zanden et al., 1998). Upon hatching the young enter the embryo stage and are about 6 mm in length. At this stage they are endogenous feeders, living primarily off of their yolk sacs. The embryonic stage lasts until the fishes are about 32 days old.

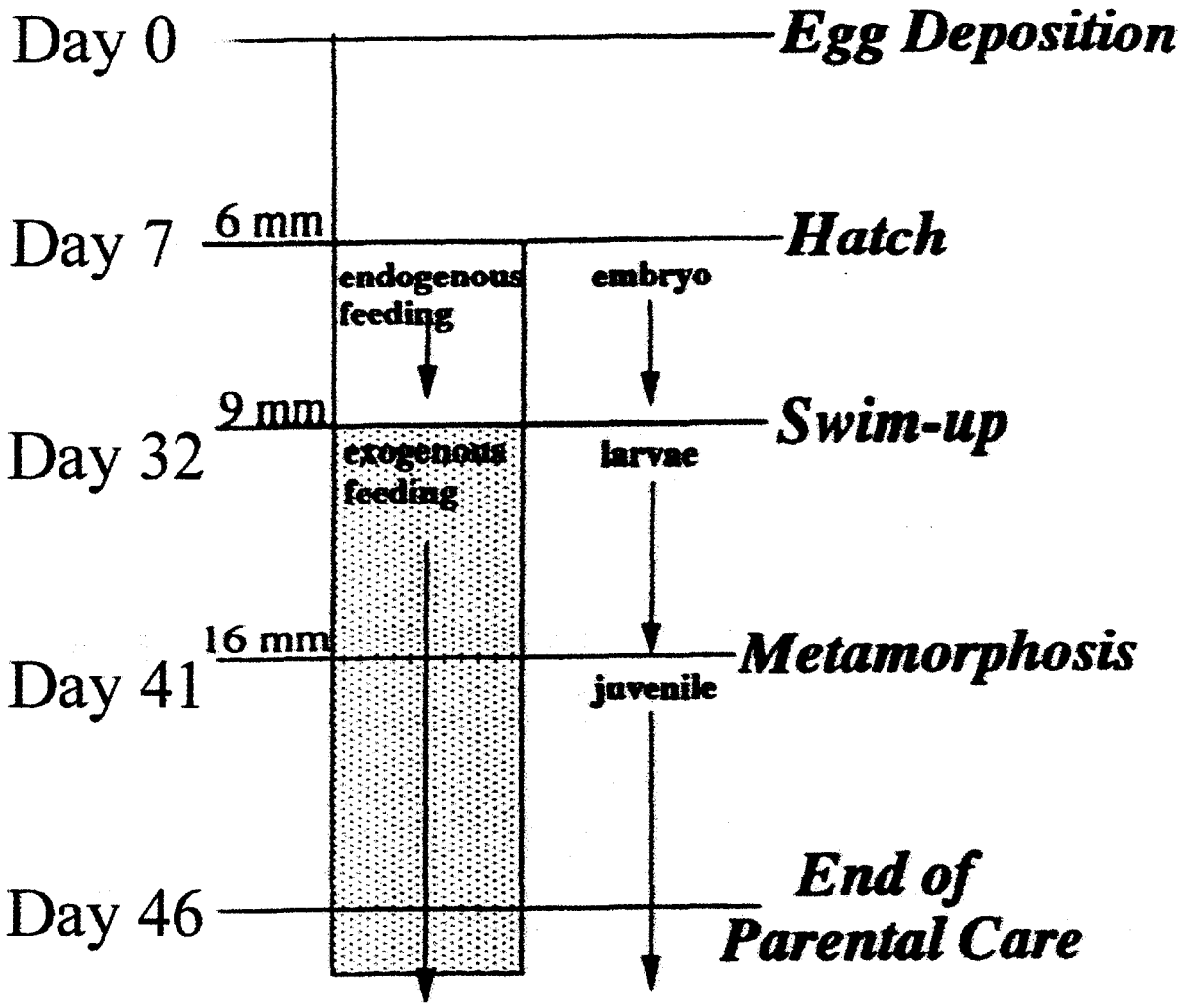


Figure 4.1

Development during the early stages of age-0 small mouth bass from Lake Opeongo, Ontario. The dates are averages based on Ridgway and Friesen (1992). Modified from Zanden et al. (1998)

The onset of exogenous feeding represents the transition from embryo to larvae. At the beginning of the larvae stage the fish can now be as large as 9 mm. Without the yolk sac the primary food source for the larvae is plankton. The larval stage lasts until the fish are about 41 days old and are 16 mm in length; after which they undergo a metamorphosis and enter the juvenile stage. The metamorphosis that the fish exhibits is a streamlining of the body and a change of colour. On day 46, about 5 days after the metamorphosis, the parental care ends and the male parent leaves the nest and the young to look after themselves.

Without the male parent there the young small mouth bass soon leave the nest and disperse into the lake. From this point on it is not possible to keep track of the family the fish came from because the young of the year now intermingle in the middle of the lake with the adults.

Adult small mouth bass are not confined to feeding on plankton; they will also feed on crayfish and other small fish. Adults will even cannibalize young of the year small mouth bass from nests other than their own, hence the need for a guardian for the first month and a half of life.

4.3 LIFECYCLE AND ISOTOPE STAGES

Small mouth bass drastically change their feeding habits throughout their lifecycle. They start by feeding on plankton and zooplankton and then progress to feeding on crayfish and then smaller fish. This represents a food source of several trophic levels, from primary producers to second and/or third level consumers. It is

reasonable to assume that throughout the lifecycle of a single small mouth bass, many isotope levels will be represented. The eggs will display isotopic ratios related to the mother. Technically the eggs develop from the mother's tissue, implying that their isotopic ratios will be similar that of the mothers'. Only the mother's isotopic values are taken into account because all the nourishment the embryo receives will be from the maternal tissues. The egg, itself however, is rich in fat, which tends to be lower in ^{13}C than other tissues. The eggs would then be expected to be lighter then the mothers' tissue but heavier than the general population. The young larvae, which would be feeding on plankton, would have the lightest isotope ratios of all the small mouth bass sampled. Also the embryos, while they are feeding on the yolk sac, are observed to be heavier with respect to $\delta^{15}N$ than the larvae but lighter than the original egg. Post-metamorphosis bass that have dispersed throughout the lake increase their trophic position and therefore their isotopic status also changes. The fish is moving up the food chain as it changes its diet and so it becomes isotopically heavier. The adults would have isotopic ratios that are heavier than that of the young and the post-metamorphosis bass. Finally, the embryos would be expected to have a large range of isotopic values, which would be dependent on the ratios of their individual mother's isotope levels, which could vary with age and size.

The age, weight, and size of the fish, as well as food availability are all important in determining the type of the food that any individual bass will be feeding on at any given point in time. In other words if the bass is large enough to eat other small fish (cisco was a common food source for the small mouth bass in Algonquin Park) and these are available then the bass will feed on them. The younger bass are not large

enough to feed on these larger fish, so their diets are confined to smaller food, like plankton and zooplankton.

5. FORMALIN AND TISSUES

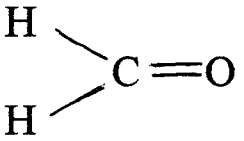
5.1 EFFECTS OF PRESERVATIVES ON TISSUES

Preservatives can affect organic tissues in several ways; the weight of fresh tissue, dry weight, and concentrations of carbon and nitrogen can all be influenced by preservatives. Based on studies on plankton, it has been noted that considerable quantities of organic matter are lost from tissues when they are stored in preservatives (Lasker, 1966; Lovegrove, 1966; Hopkins, 1968; Omori, 1970). Hopkins (1968) reported a loss of both carbon and nitrogen from animal tissues after 15 weeks of being in a Hexamine-buffered formaldehyde solution. The organic matter that is leached from the tissues is mainly proteinaceous in nature. Omori (1978) suggested that due to the rapid increase in the carbon/nitrogen ratio in the first week of preservation that it is the amino acids that are lost from the sample in the early stages of preservation. The fact that greater amounts of nitrogen are being lost than carbon further suggest that it is proteins that are being lost from the tissues (Williams and Robins, 1982).

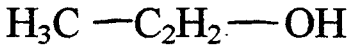
A mixture of 37% methanal in ethanol, otherwise known as formalin is used extensively for the preservation of organic tissues. Like other preservatives it is reported to have an effect on the tissues which are placed into it, especially weight loss and shrinkage of the tissue. On the molecular level, formalin effects the proteinaceous parts of the tissue and causes the loss of amino acids.

5.2 EFFECTS OF FORMALIN ON ISOTOPES

Since formalin changes the structure of proteins, during the preservation process, it also has the capacity to affect the isotopic component of the tissue. The chemical formulae of methanal and ethanol are as follows:



Methanal



Ethanol

The fact that formalin contains carbon atoms suggests that it may be able to affect the carbon isotopic ratios of tissues. Its very presence in any tissue implies that any $\delta^{13}C$ differences between the tissue and the formalin will be visible upon mass-spectrometric analysis.

Despite the fact that formalin does not contain any nitrogen in its chemical formula it is able to affect the $\delta^{15}N$ of tissues through its influence on the proteins. It is able to attack and remove the amine end of amino acids in the proteins within the tissue. There may be a kinetic isotope effect in this reaction that causes fractionation to occur. The amino acids in the protein contain both isotopes of nitrogen, ^{14}N and ^{15}N. One of these isotopes can be lost more readily from the protein than the other. As seen earlier in figure 2.1, the heavier isotope possesses stronger bonds than the lighter isotope does. When formalin reacts with the protein ^{14}N is lost more readily than ^{15}N, and thus fractionation of nitrogen occurs in the preservation process. It is the

loss of amino acids that causes the decrease in the bulk weight of the sample, but since more ^{14}N is lost than ^{15}N the tissue becomes isotopically heavier with respect to $\delta^{15}N$.

Bosley and Wainright (1999) preformed several experiments that compared the differences in the carbon and nitrogen isotopic ratios in fresh-frozen tissue and formalin treated *Pleuronectes americanus* (muscle tissue of juvenile winter flounder) and *Crangon septemspinosa* (tail tissue from mud shrimp). This demonstrates and quantifies the effects of formalin on organic tissues. Their findings (figure 5.1) conclude that treatment with formalin produces significant increases in $\delta^{15}N$ values. The tissues became isotopically heavier, by 0.5 – 1.4 ‰, with respect to nitrogen. That is, we can expect that $\delta^{15}N$ of tissues would increase by 1.0 ± 0.5 ‰ after formalin treatment. This is dependent on the loss of the amino acids containing the lighter isotope of nitrogen. Figure 5.1 also depicts Bosley and Wainright's findings for $\delta^{13}C$ values. Formalin treatment of tissues produces decreases in $\delta^{13}C$ values of 0.6 – 2.3 ‰. Bosley and Wainright also found that there was a greater variability in both the $\delta^{15}N$ and $\delta^{13}C$ values of formalin-preserved tissues over fresh-frozen tissues.

Different sources of formalin may present a problem to the researcher if they cause the tissues to display variations in the delta values of carbon and nitrogen due to differences in their own isotopic composition. Therefore, laboratory studies must be carried out to determine the effects of the particular batch of formalin used. We expect that this difference between the sources of formalin will be greatest for $\delta^{13}C$, since it is the atoms of carbon, from the formalin, that produce the effect.

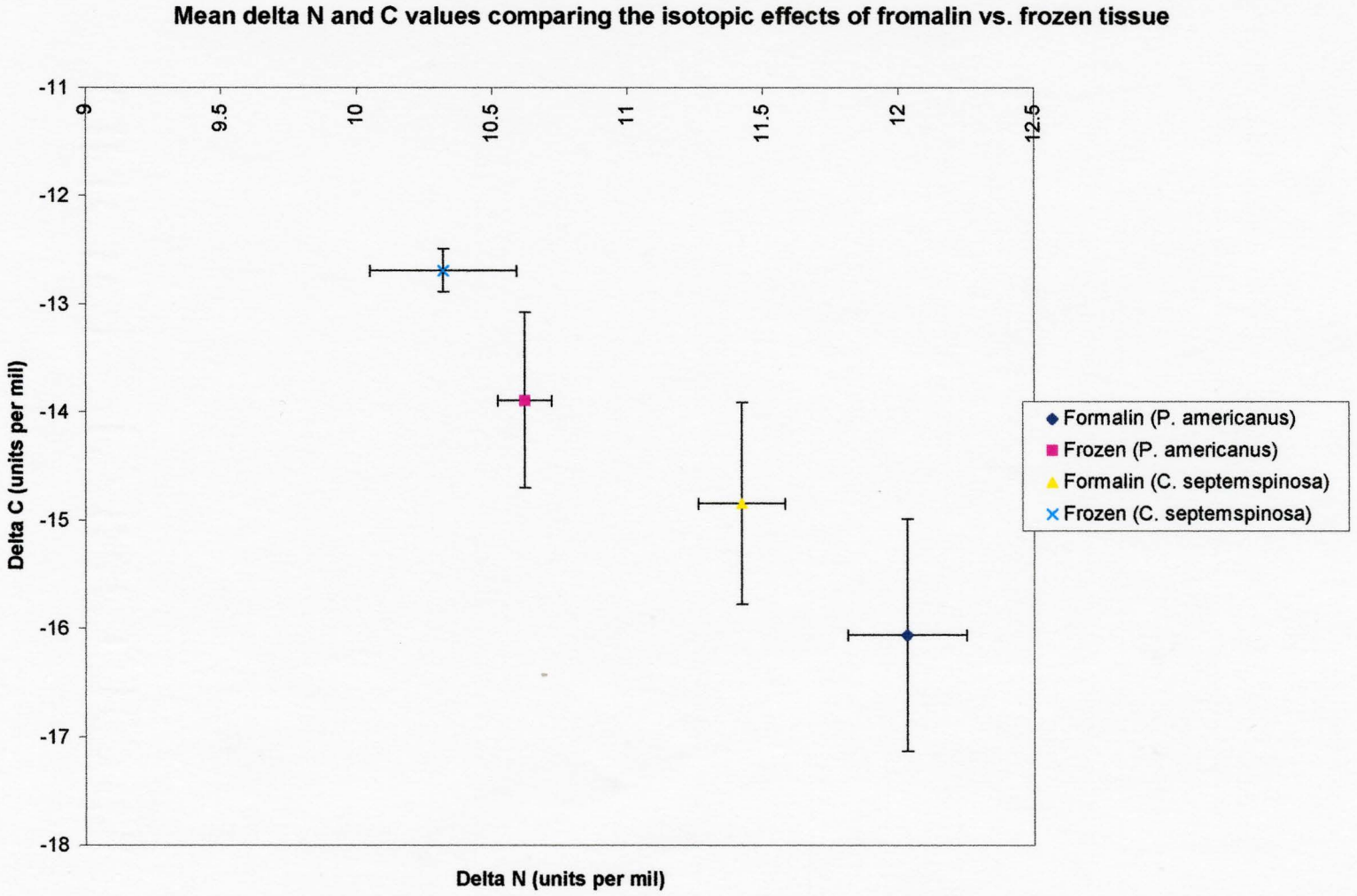


Figure 5.1
Mean $\delta^{15}N$ and $\delta^{13}C$ values for *P. americanus* and *C. septemspinosa* obtained using two preservation methods. In both cases the formalin-treated tissues became heavier with respect to $\delta^{15}N$ values. Also the formalin-treated tissues became lighter with respect to $\delta^{13}C$ values. Modified from Bosley and Wainright (1999).

5.3 FORMALIN EXPERIMENT METHODS

Since all of the *Micropterus dolomieu* samples obtained, both in 1992 and in 1999, were preserved in formalin, no fresh *Micropterus dolomieu* tissue was immediately available to compare $\delta^{13}C$ and $\delta^{15}N$ of fresh and formalin-treated tissues. Therefore, in order to determine the effects that the formalin used in the 1999 experiments had on the isotopic values for $\delta^{13}C$ and $\delta^{15}N$ two specimens of lake trout were obtained from a grocery store. These lake trout, were whole-untreated, fresh-frozen fish. The ages of the trout were not determined but both trout were measured and weighed in the same manner as the *Micropterus dolomieu* were. Two muscle tissue samples were taken from the back under the dorsal fin from each of the two fish, making a total of four samples. One sample from each trout was immediately freeze-dried and prepared for both $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis, in the manner of the previous *Micropterus dolomieu* samples. The other two samples, one from each trout, was then placed into the 1999 batch of formalin and left to preserve for 30 days time. After this time the formalin-treated tissues were also freeze-dried and prepared for isotopic analysis in the like manner as the previous samples. These samples were then analyzed by the same mass spectrometer as all of the *Micropterus dolomieu* samples. Each sample was prepared and analyzed in duplicate for accuracy purposes. The results of this experiment will allow the determination of the effect that the 1999 batch of formalin has on the $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of organic tissues, which are preserved in it.

To determine whether there is a difference between the batches of formalin used in 1999, for this study, and the formalin used in 1992, Friesen's samples, it

was necessary to look in the literature at the paper by Vander Zanden et al. (1999). This paper describes the $\delta^{13}C$ and $\delta^{15}N$ isotopic values of young *Micropterus dolomieu* from the same lake, Opeongo. In this paper, however, only fresh tissue samples were used, making it possible to compare $\delta^{13}C$ and $\delta^{15}N$ values of fresh and formalin-treated *Micropterus dolomieu* from Lake Opeongo.

5.4 FORMALIN AND *MICROPTERUS DOLOMIEU*

The *Micropterus dolomieu* samples used in this work were collected over a seven year period by Friesen (1992) and myself (1999). It was, therefore, necessary to determine whether or not there was an isotopic difference between the formalin Friesen used and the formalin used in 1999. The formalin prepared for the preservation of the fish captured in 1999 was tested to determine the isotopic effects it would have on fish tissues. The formalin-treated lake trout displayed an enrichment in $\delta^{15}N$ of between 0.5 and 0.7 ‰, over the fresh-frozen tissue. The formalin-treated lake trout was depleted in $\delta^{13}C$ by between 2.1 and 3.5 ‰, over the fresh-frozen tissue. The results for $\delta^{15}N$ can be seen in figure 5.2 and those for $\delta^{13}C$ can be seen in figure 5.3.

It is possible to see from the data from both Bosley and Wainright and this study that formalin produces a shift in both $\delta^{15}N$ and $\delta^{13}C$. The offsets recorded by these studies are shown in table 5.1. It would appear, from these two sets of data, that different batches of formalin may give different offsets in both carbon and nitrogen isotope values. In order to get some idea of the offset produced by the formalin used by Friesen, we are

	$\delta^{13}C$	$\delta^{15}N$
Bosley and Wainright	-1.5 ± 0.9 ‰	+1.0 ± 0.5 ‰
This study	-2.8 ± 0.7 ‰	+0.6 ± 0.1 ‰

Table 5.1 – Isotopic offsets between formalin-treated and fresh tissues. Bosley and Wainright (1999) determined $\delta^{13}C$ and $\delta^{15}N$ offsets between formalin-treated and fresh tissues for *Pleuronectes americanus* and *Crangon septemspinosa*. Likewise this study determined $\delta^{13}C$ and $\delta^{15}N$ offsets on formalin-treated and fresh tissue on lake trout.

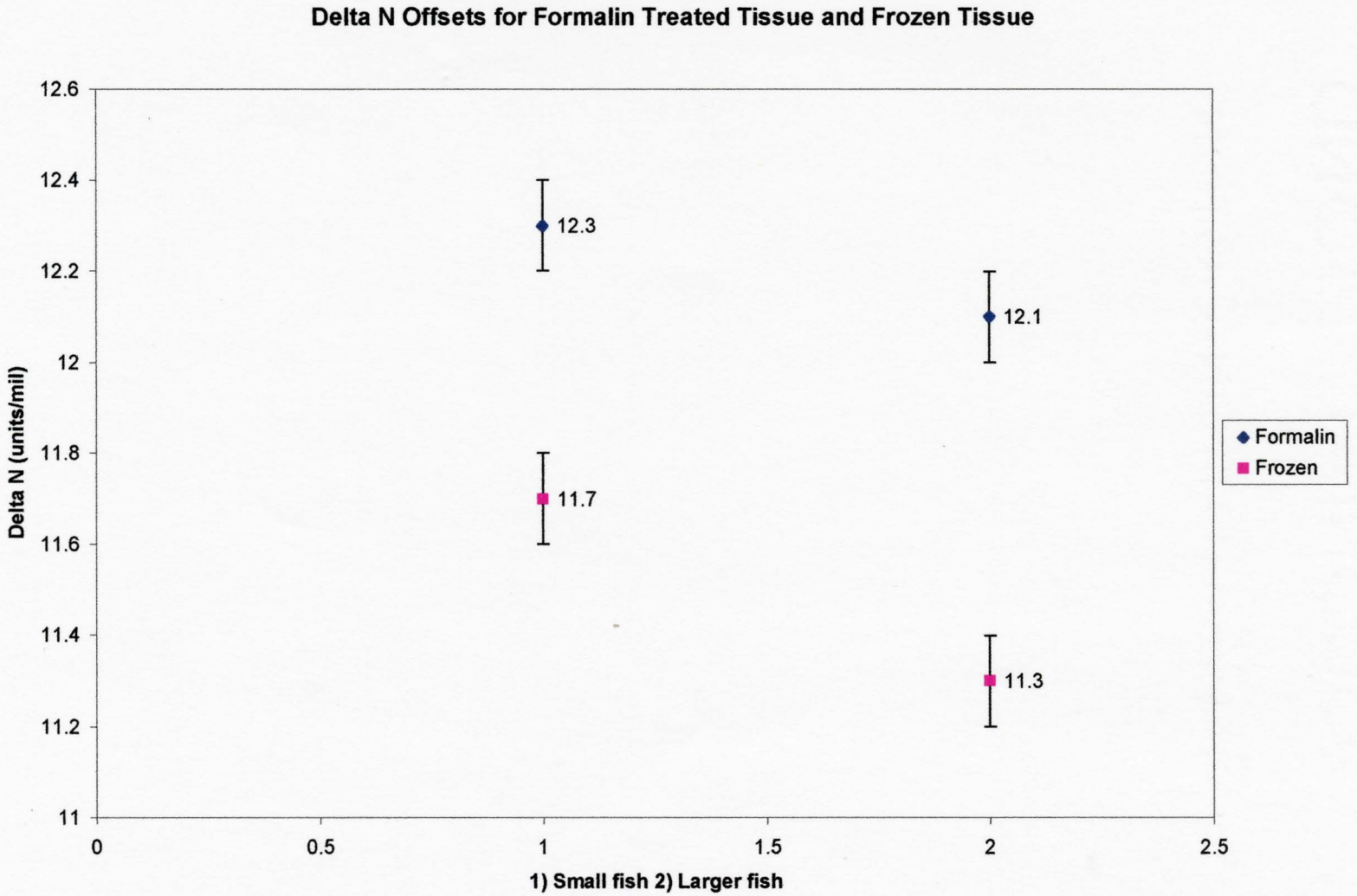


Figure 5.2
This represents the offsets for $\delta^{15}N$ for formalin-treated tissue compared to fresh-frozen tissue, for Lake Trout. Each point represents the average of three samples run in triplicate. Error bars represent standard deviation.

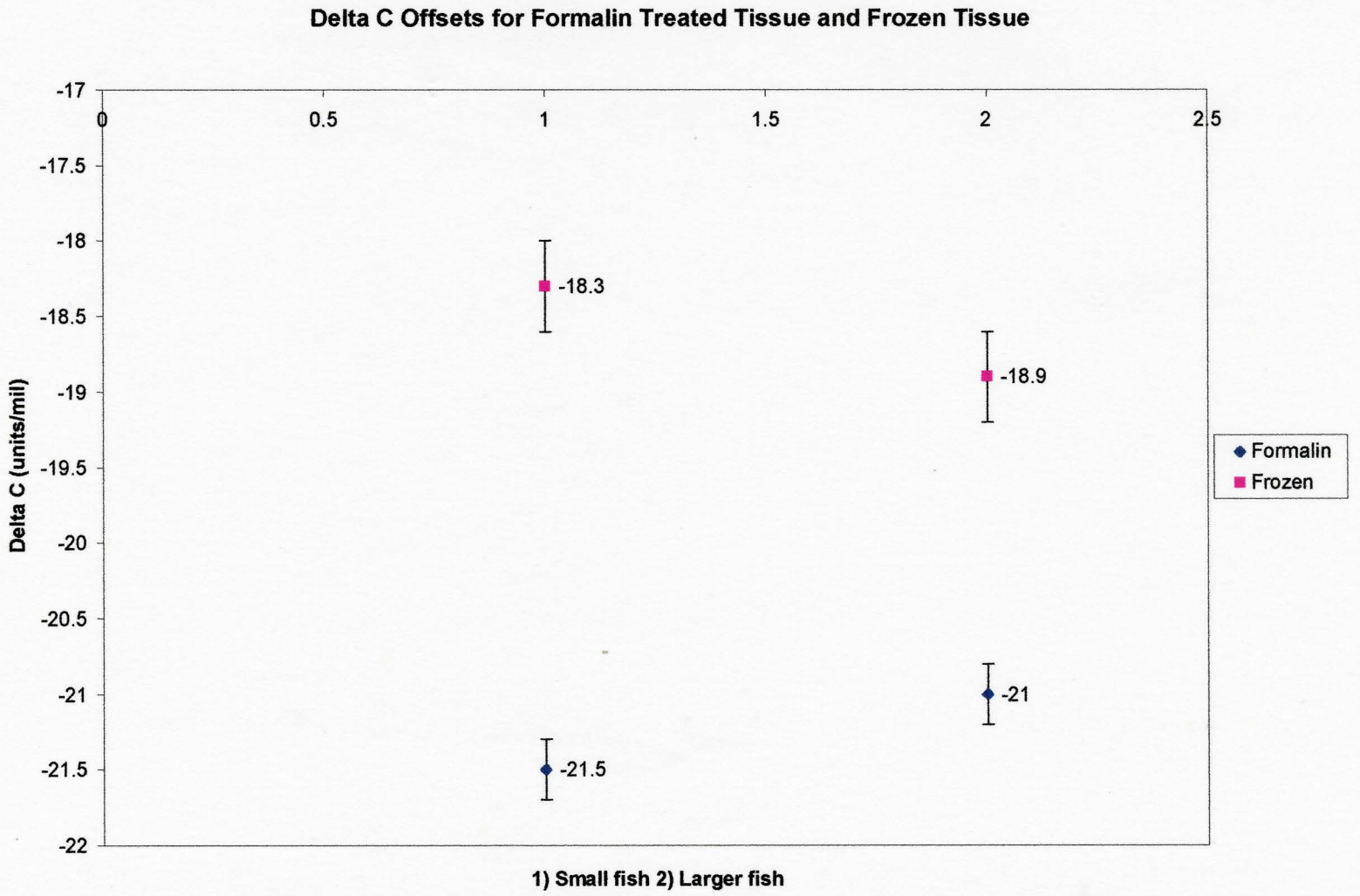


Figure 5.3
This represents the offsets for $\delta^{13}C$ for formalin-treated tissue compared to fresh-frozen tissue, for Lake Trout. Each point represents the average of three samples run in triplicate. Error bars represent standard deviation.

able to compare the data obtained by Vander Zanden et al. on *Micropterus dolomieu* from the same lake.

Around the metamorphosis stage in the lifecycle of *Micropterus dolomieu* there is a shift in the $\delta^{15}N$ values, the yolk sac is used up and no longer influences the isotopic values of the young fish (Vander Zanden et al. 1998). An isotopic plateau of 3.0 ± 0.5 ‰ can be observed in the $\delta^{15}N$ values, figure 5.4 (Vander Zanden et al., 1998), after the metamorphosis stage. Vander Zanden used fresh tissue from young of the year *Micropterus dolomieu* samples to obtain the results seen here. A similar plateau is seen in the $\delta^{15}N$ values obtained from the formalin-treated young of the year *Micropterus dolomieu* samples obtained by Friesen. This plateau is located at 4.5 ± 0.5 ‰ in the formalin-treated fish, this can be seen in figure 5.5. This represents an average offset of 1.5 ± 0.5 ‰ for Friesen's formalin-treated tissue verses fresh tissues. To correct $\delta^{15}N$ data obtained from Friesen's formalin-treated samples with respect to standard fresh samples it will be necessary to subtract 1.5 ± 0.5 ‰ from all of the $\delta^{15}N$ values obtained from Friesen's samples. Also, as seen by table 5.1, it will be necessary to shift all of $\delta^{15}N$ results obtained from the 1999 samples by -0.6 ± 0.1 ‰.

As pointed out earlier we expect a similar offset between the $\delta^{13}C$ values of fresh-tissue and formalin-treated tissue. Vander Zanden (1998) used fresh young of the year *Micropterus dolomieu* tissue from Lake Opeongo to obtain $\delta^{13}C$ values, figure 5.6. (Vander Zanden et al., 1998). The $\delta^{13}C$ values show an isotopic plateau at – 24.0 ± 1.0 ‰ for fresh-tissue. The equivalent plateau for $\delta^{13}C$, for formalin-treated young of the year *Micropterus dolomieu* collected in 1992, is – 25.0 ± 0.7 ‰, figure 5.7. This

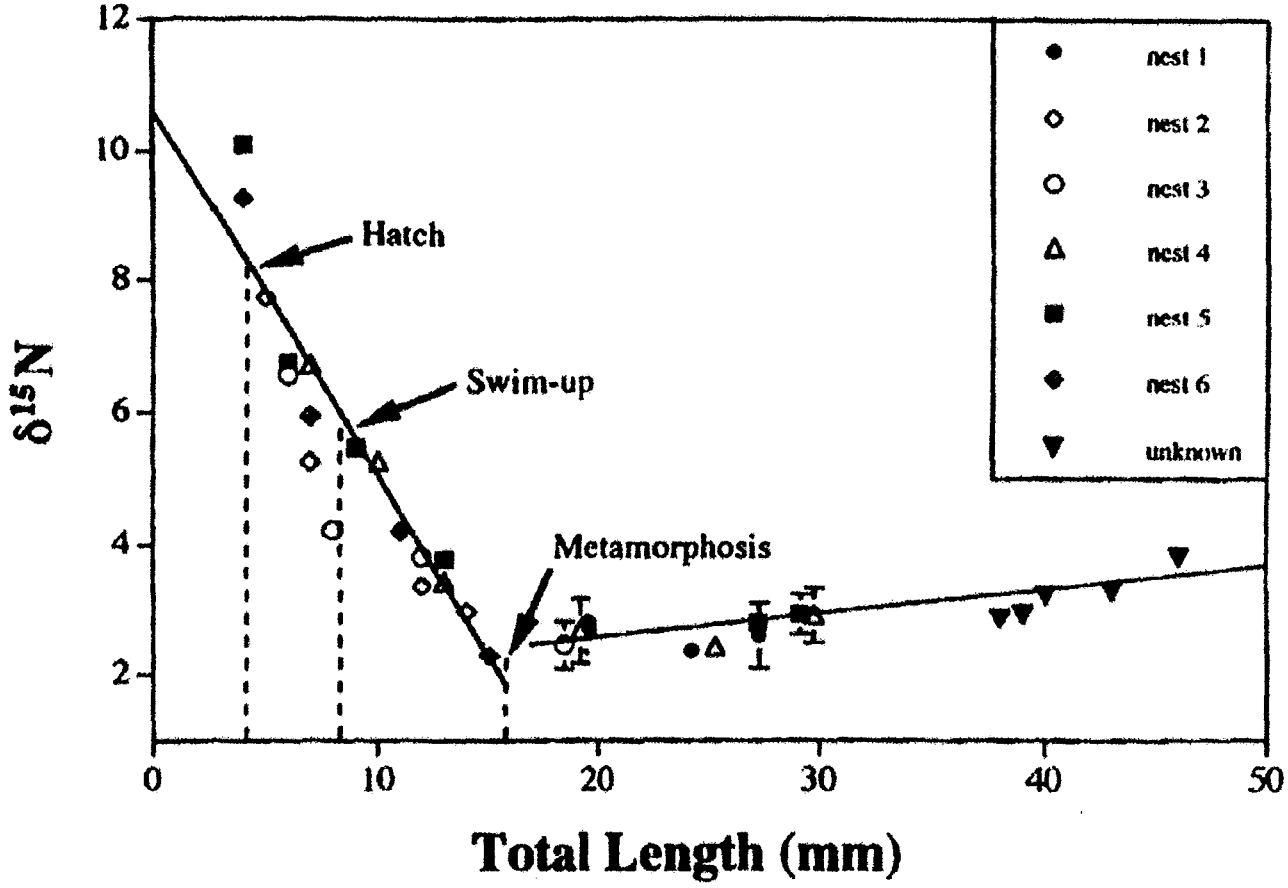


Figure 5.4
This represents nest-specific $\delta^{15}N$ plotted against total length for age-0 smallmouth bass from Lake Opeongo. (Vander Zanden et al. 1998). Note that the samples used here are taken from fresh-frozen tissue and there is an isotopic plateau around the metamorphosis stage in the lifecycle of the fish, it levels off at about 3.0 ± 0.5 ‰.

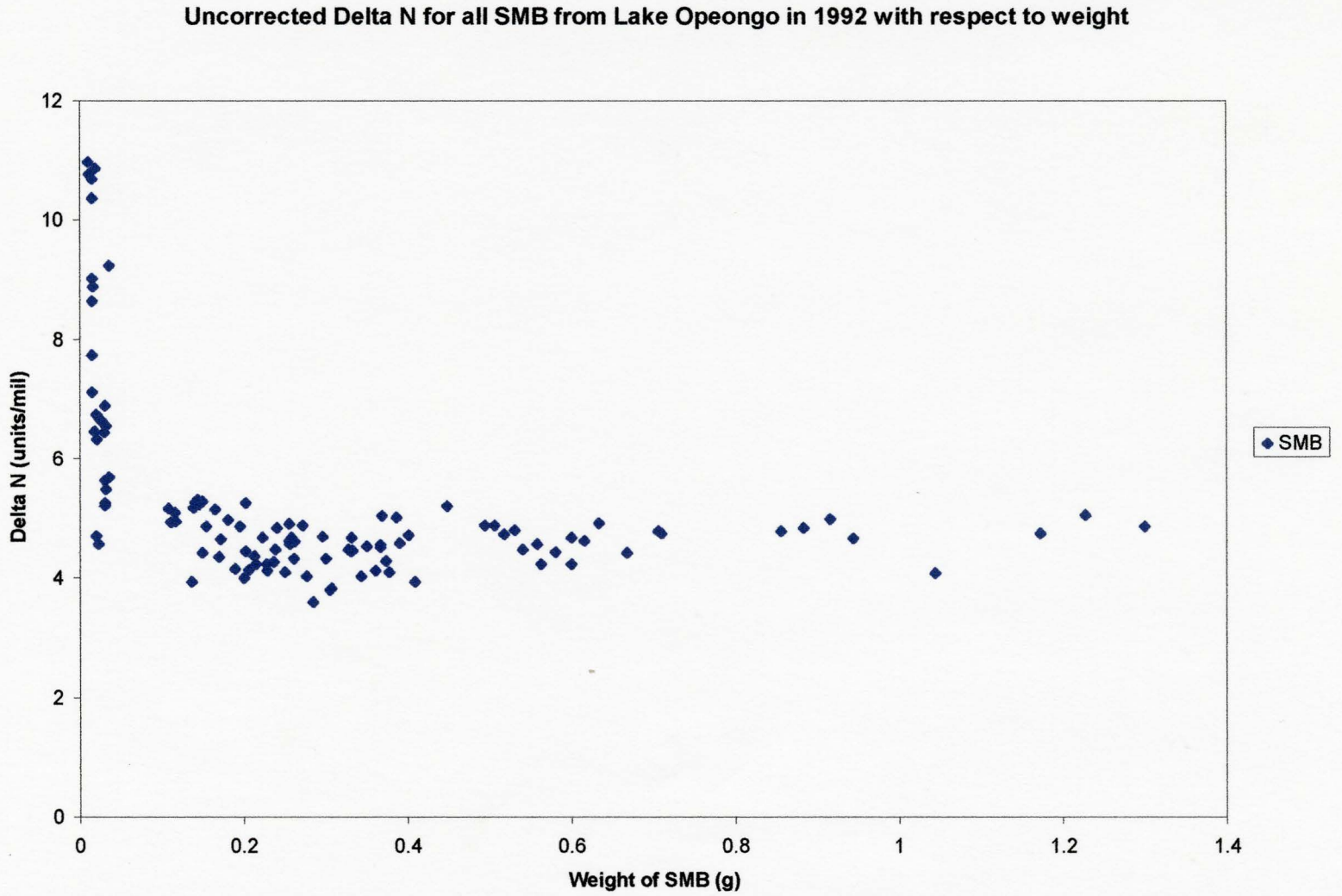


Figure 5.5
A graph showing the $\delta^{15}N$ values for all the Small Mouth Bass (SMB) with respect to weight. All samples were collected from Lake Opeongo in 1992 (Friesen) and analyzed in 1999 for this study. These values are considered to be uncorrected due to the fact that – 1.5 ± **0.6** ‰ has to be subtracted from each point in order to calibrate these $\delta^{15}N$ values with $\delta^{15}N$ values of fresh SMB samples.

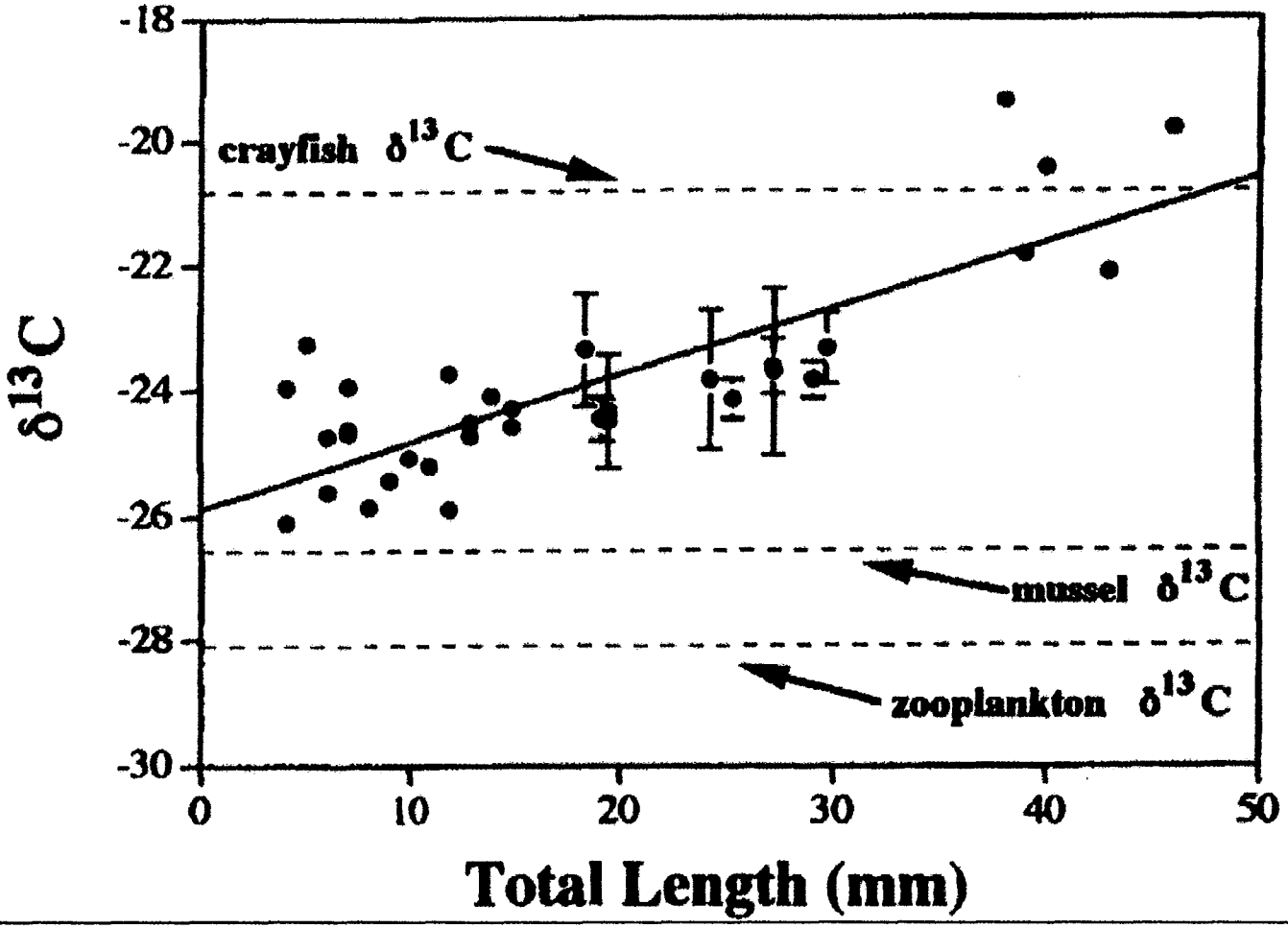


Figure 5.6
This represents the relationship between $\delta^{13}C$ and the total length for age-0 smallmouth bass from Lake Opeongo. (Vander Zanden et al. 1998). Note that the samples used here are taken from fresh-frozen tissue and there is an isotopic plateau around the metamorphosis stage in the lifecycle of the fish, it levels off at about – 24.0 ± 1.0 ‰.

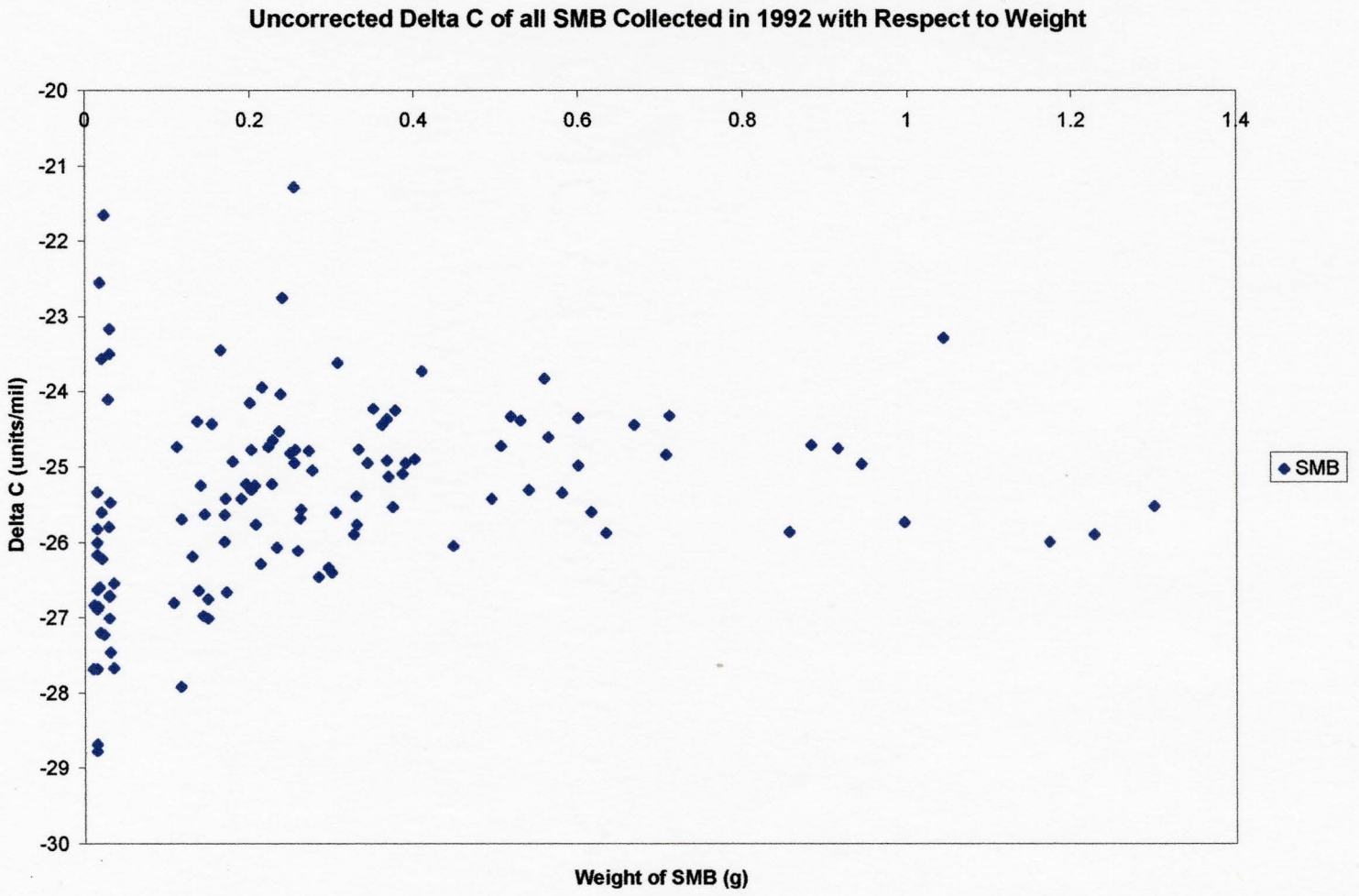


Figure 5.7
A graph showing the $\delta^{13}C$ values for all the Small Mouth Bass (SMB) with respect to weight. All samples were collected from Lake Opeongo in 1992 (Friesen) and analyzed in 1999 for this study. These values are considered to be uncorrected due to the fact that – 1.0 ± 1.0 ‰ has to be subtracted from each point in order to calibrate the $\delta^{13}C$ values with those of the 1999 SMB samples, which were preserved in a different batch of formalin.

represents an average offset of -1.0 ± 1.0 ‰ for Friesen's formalin-treated tissue verses fresh tissues. This implies that in order to correct our analysis of $\delta^{13}C$ of Friesen's formalin-treated samples with equivalent fresh tissue values it will be necessary to shift all of Friesen's $\delta^{13}C$ values by +1.0 ± 1.0 ‰. Also, as seen by table 5.1, it is necessary to shift all the 1999 $\delta^{13}C$ results by +2.8 ± 0.7 ‰ to calibrate them to equivalent fresh tissue values.

In subsequent discussions we will present the data corrected as follows:

Samples	Correction	Error
Friesen, 1992	$\delta^{15}N$ corrected = $\delta^{15}N$ observed – 1.5 ‰	± 0.6
	$\delta^{13}C$ corrected = $\delta^{13}C$ observed + 1.0 ‰	± 1.0
Fekete, 1999	$\delta^{15}N$ corrected = $\delta^{15}N$ observed – 0.6 ‰	± 0.1
	$\delta^{13}C$ corrected = $\delta^{13}C$ observed + 2.8 ‰	± 0.7

The errors in these corrected data must include the uncertainty in the offset; therefore the errors on the corrected data.

6. TROPHIC LEVELS

6.1 INTRODUCTION

As stated earlier there is an isotopic relationship between predator and prey (Epstein and DeNiro, 1978 and 1981). In the predator, both $\delta^{13}C$ and $\delta^{15}N$ values are enriched, by about 0.5 ‰ and 3 ‰ respectively, over the prey. In the aquatic environment autotrophic plankton is on the lowest rung of the trophic ladder. Other organisms within the lake feed on the plankton or other creatures, which are higher up in the food chain.

6.2 TROPHIC LEVELS IN OPEONGO

Adult *Micropterus dolomieu* are at the top of the food chain in the natural environment of Lake Opeongo. They feed on any of the three trophic levels below themselves, such as other smaller fish (like cisco), benthic animals (like crayfish), and plankton. Figure 6.1 is a graph depicting the $\delta^{15}N$ of adult *Micropterus dolomieu* (represented by the squares) and their prey (represented by the diamonds) as determined by examining the isotopic values of stomach contents. Likewise figure 6.2 is a graph depicting the $\delta^{13}C$ of the *Micropterus dolomieu* (represented by the squares) and their prey (represented by the diamonds). Chart 6.1 shows the site where each of the samples for figures 6.1 and 6.2 were obtained as well as the isotopic values of the *Micropterus dolomieu* and their stomach contents. Of the seven stomach contents analyzed, samples 1 to 4 were smaller fishes, cisco, and samples 5 to 7 were crayfish.

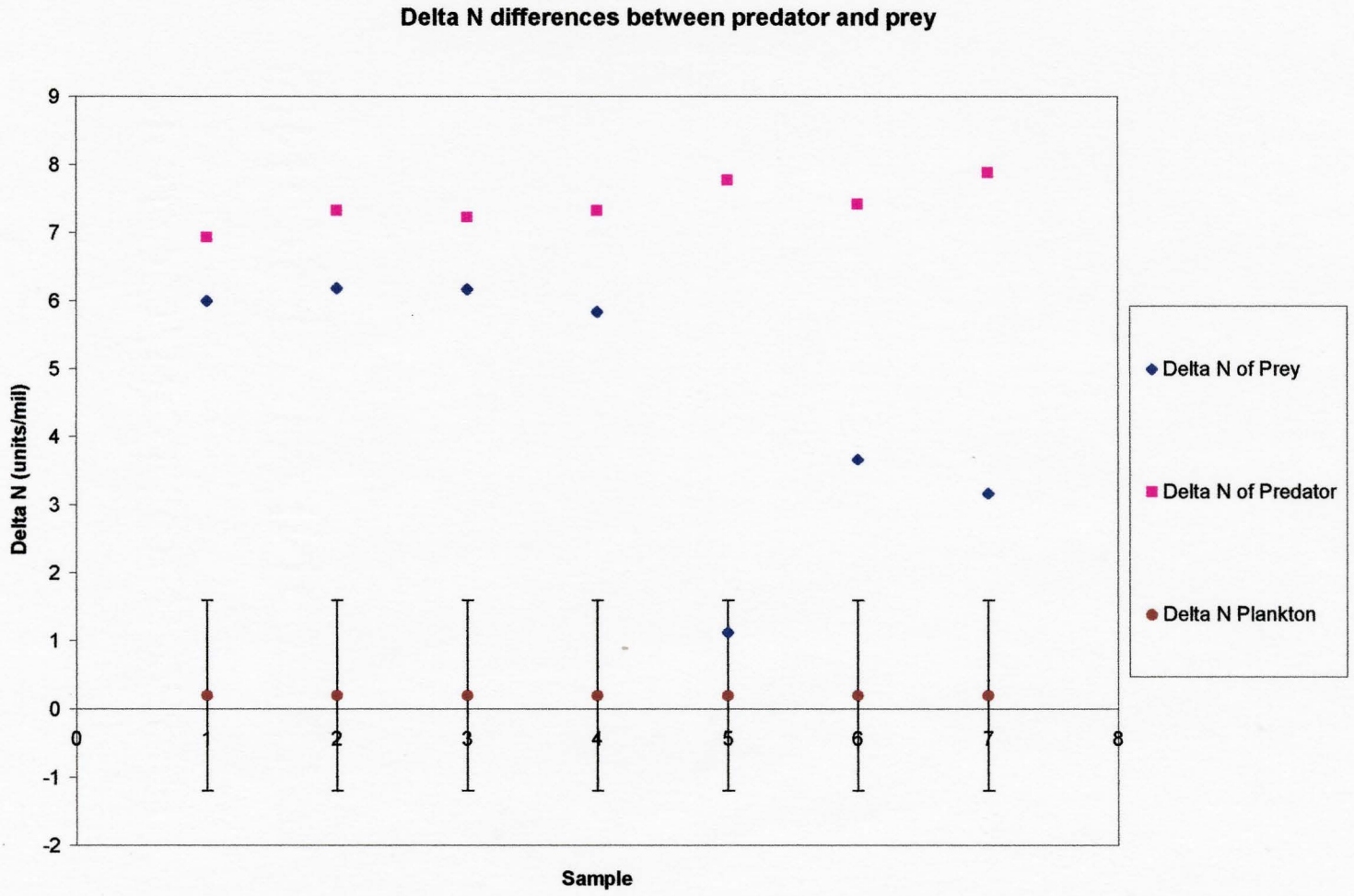


Figure 6.1 – $\delta^{15}N$ values of the *Micropterus dolomieu* (predator) and their prey, cisco in samples one to four and crayfish in samples five to seven. Error bars lay within the range of the points given for the predator and the prey. The bars around the plankton represent the standard deviation from the 9 samples used to obtain the average $\delta^{15}N$ of the plankton, here at 0.2 ‰.

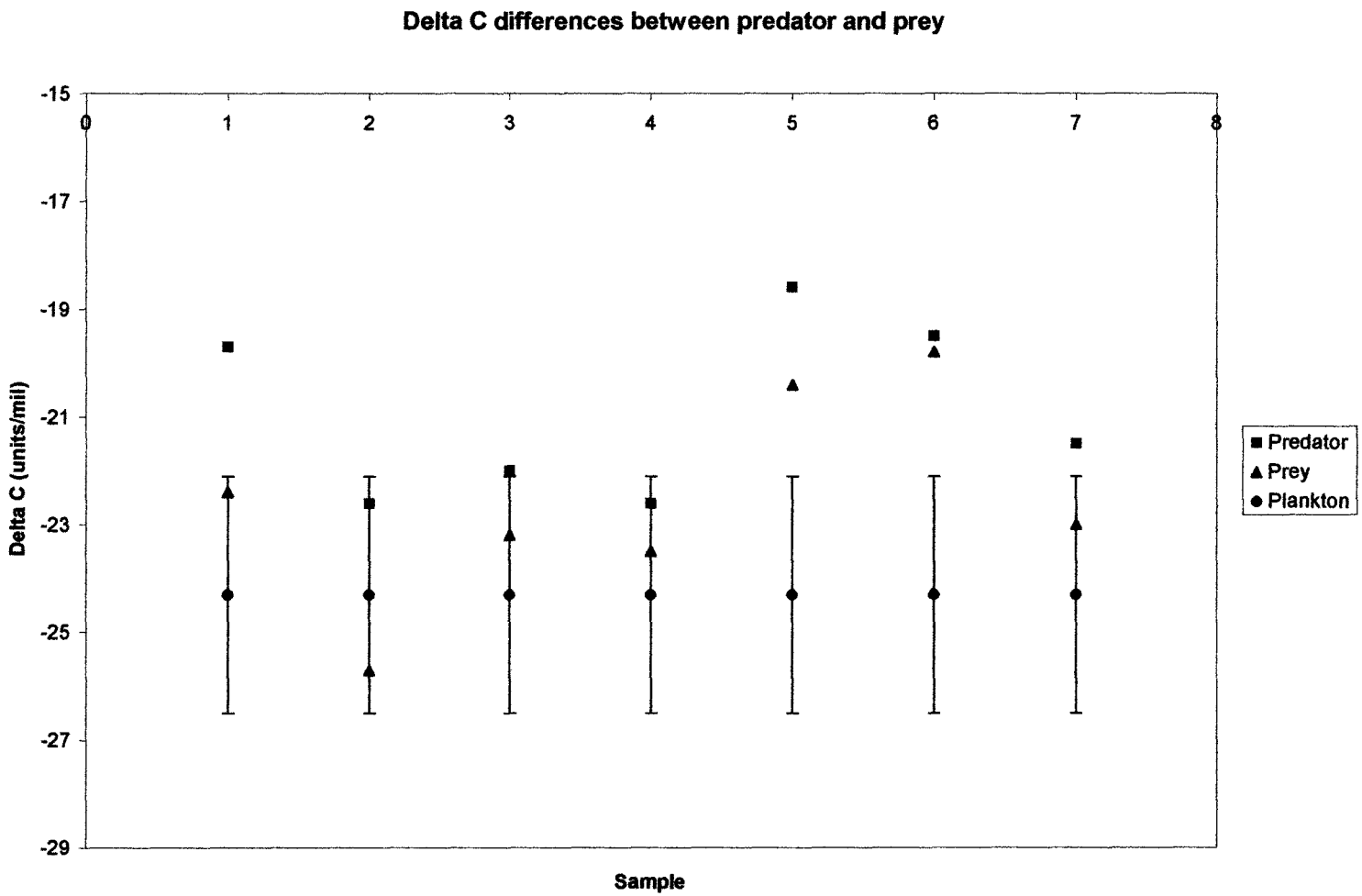


Figure 6.2 – $\delta^{13}C$ values of the *Micropterus dolomieu* (predator) and their prey, cisco in samples one to four and crayfish in samples five to seven. Error bars lay within the range of the points given for the predator and the prey. The bars around the plankton represent the standard deviation from the 9 samples used to obtain the average $\delta^{13}C$ of the plankton, here at –24.3 ‰.

Sample #	Site fish was obtained from (Numbered from figure 3.1)	$\delta^{13}C$ of Predator (‰)	$\delta^{13}C$ of Prey (‰)	$\delta^{15}N$ of Predator (‰)	$\delta^{15}N$ of Prey (‰)
1	8 (North Arm)	-19.7	-22.4	7.0	6.0
2	5 (West side of South Arm)	-22.6	-25.7	7.3	6.2
3	5 (west side of South Arm)	-22.0	-23.2	7.2	6.2
4	5 (West side of South Arm)	-22.6	-23.5	7.3	5.8
5	8 (North Arm)	-18.6	-20.4	7.8	1.1
6	7 (Jones Bay)	-19.5	-19.8	7.4	3.7
7	5 (West side of South Arm)	-21.5	-23.0	7.9	3.2

Chart 6.1
$\delta^{13}C$ and $\delta^{15}N$ for *Micropterus dolomieu* (the predator) and its stomach contents (the prey). All samples were obtained from Lake Opeongo (1999). The stomach contents for samples 1-4 were cisco (smaller fish) and crayfish for samples 5-7. These fish were obtained from different areas within Opeongo. Samples 2, 3, 4, and 7 were obtained from site 5 from the upper section of the South Arm. Sample 6 was obtained from an upper eastern section of the South Arm in Jones Bay. While samples 1 and 5 were obtained from the North Arm.

The $\delta^{15}N$ values of the adult *Micropterus dolomieu* used in this study were fairly constant around 7.4 ± 0.5 ‰, while the $\delta^{15}N$ values for the cisco also remained fairly consistent around 6.0 ± 0.2 ‰. The $\delta^{15}N$ values of the crayfish however had a wide range of $\delta^{15}N$ values from 1.1 ‰ to 3.7 ‰, with an average of 2.7 ± 1.4 ‰. The $\delta^{15}N$ values for the plankton were obtained from the average of 9 samples, with the error bars representing the standard deviation. It is possible to see that the $\delta^{15}N$ values of the *Micropterus dolomieu* are about 1.4 ‰ above that of the cisco and 4.7 ‰ above that of the crayfish and 7.1 ‰ above the plankton. This seems to confirm the results of Cabana and Rasmussen (1994) that Lake Opeongo is a second order lake as seen in figure 2.6. Cisco prey upon plankton and zooplankton and the cisco are in turn preyed upon by the smallmouth bass.

The $\delta^{13}C$ results are a lot more varied than the $\delta^{15}N$ values. There is a large range of $\delta^{13}C$ values, for adult *Micropterus dolomieu*, ranging from –18.6 ± 0.4 ‰ to –22.6 ± 0.4 ‰. The prey (the cisco and the crayfish) also display high variability in $\delta^{13}C$ values ranging from –19.8 ± 0.4 ‰ to –25.7 ± 0.4 ‰. Despite the obvious overlap between the $\delta^{13}C$ values for the predator and the prey it is still possible to see that for each sample the predator is always heavier than its corresponding prey (the stomach contents). The $\delta^{13}C$ values for the plankton were obtained from an average of 9 samples, with the error bars representing the standard deviation. The $\delta^{13}C$ differences between the *Micropterus dolomieu* and their prey range between 0.5 and 3.0 ‰. At first glance there appears to be no pattern to the $\delta^{13}C$ values. However, a significant spatial pattern is evident when the site where the fish was captured is taken into account. Three different sites, 5, 7, and 8 (chart 6.1) are represented by the seven *Micropterus dolomieu*

(and their stomach contents) sampled for figure 6.1. Looking back to figure 3.1 (the map of Lake Opeongo in Algonquin Park) it is possible to see that site 8 is located in a different arm, the North Arm, than the other two sites that were sampled. Also note that while both sites 5 and 7 are located within the South Arm they are located on different sides of the arm, several kilometers from each other.

There is much less variation between *Micropterus dolomieu* from the same sites. The four samples of *Micropterus dolomieu* obtained from site 5 and the two samples from site 8 demonstrate this, these having a standard deviations of only 0.5 ‰ and 0.8 ‰ respectively, compared to the standard deviation of 1.6 ‰ seen in the amalgamation of all the samples.

6.3 IMPLICATIONS

This fact that there are $\delta^{13}C$ variations implies that while the $\delta^{13}C$ values of the *Micropterus dolomieu* in Lake Opeongo tend to be site specific the $\delta^{15}N$ values from the same predator are not site specific, but are consistent throughout the lake. This also implies that the *Micropterus dolomieu* follow the shoreline, live, and feed within a restricted habitat range.

Despite the fact that $\delta^{13}C$ and $\delta^{15}N$ come from the same source (the prey in the case of the *Micropterus dolomieu*) the $\delta^{15}N$ values of the *Micropterus dolomieu* appear to be relatively consistent throughout Lake Opeongo it is possible to compare all samples regardless of the site from which they were collected. On the other hand, the inconsistency between the $\delta^{13}C$ values, make it impractical to directly compare $\delta^{13}C$

values of *Micropterus dolomieu* from one collection site with other $\delta^{13}C$ values from *Micropterus dolomieu* from a different site. This is because the sources of ^{13}C are different in the primary production. The ^{13}C of the primary production (and therefore everything else in the food chain) comes from dissolved carbonates, river inflows, and decomposition, which can differ from one part of the lake to another part. This is especially true in the case of Lake Opeongo since the three arms have different sources of inflow. Within the lake the major source of input for ^{15}N is by atmospheric deposition, implying that nitrogen will be much more evenly dispersed in the lake than carbon will be. In the case of Lake Opeongo, it was observed, in this study, that the ^{15}N is indeed evenly dispersed throughout the lake while ^{13}C is not. The $\delta^{13}C$ values indicate that the sources of ^{13}C in the North Arm are heavier than the sources of ^{13}C within Jones Bay, which in turn are heavier than the sources of ^{13}C on the west side of the South Arm.

7. $\delta^{15}N$ CHANGES THROUGHOUT THE LIFECYCLE OF *MICROPTERUS DOLOMIEU*

7.1 INTRODUCTION

In this study *Micropterus dolomieu* from Lake Opeongo in Algonquin Park, were examined isotopically for $\delta^{15}N$ and $\delta^{13}C$ values. A large range of *Micropterus dolomieu* samples were obtained, varying in weight, size, and age (appendix 1); making it possible to distinguish isotopic changes which may occur throughout the lifecycle of the fish. Naturally the size of the fish is directly proportional to weight of the fish, while the age is not always directly related to either weight or size. Younger fish may be larger then fish that are older if they hatched in a nest site where nutrients and food are more readily available than in the nest site where the older fish were hatched.

Indeed, large isotopic changes in $\delta^{15}N$ are visible when the complete lifecycle of the *Micropterus dolomieu* are examined in the manner stated above (appendix 2). Figure 7.1 shows $\delta^{15}N$ values obtained from *Micropterus dolomieu* when the factor of weight is varied. The *Micropterus dolomieu* $\delta^{15}N$ values ranged from as heavy as 9.5 ± 0.2 ‰ for day old hatchlings to as light as 2.1 ± 0.2 ‰ for young of the year fish, which had just used finished using up their yolk sacs, and as heavy as 8.4 ± 0.2 ‰ for adults.

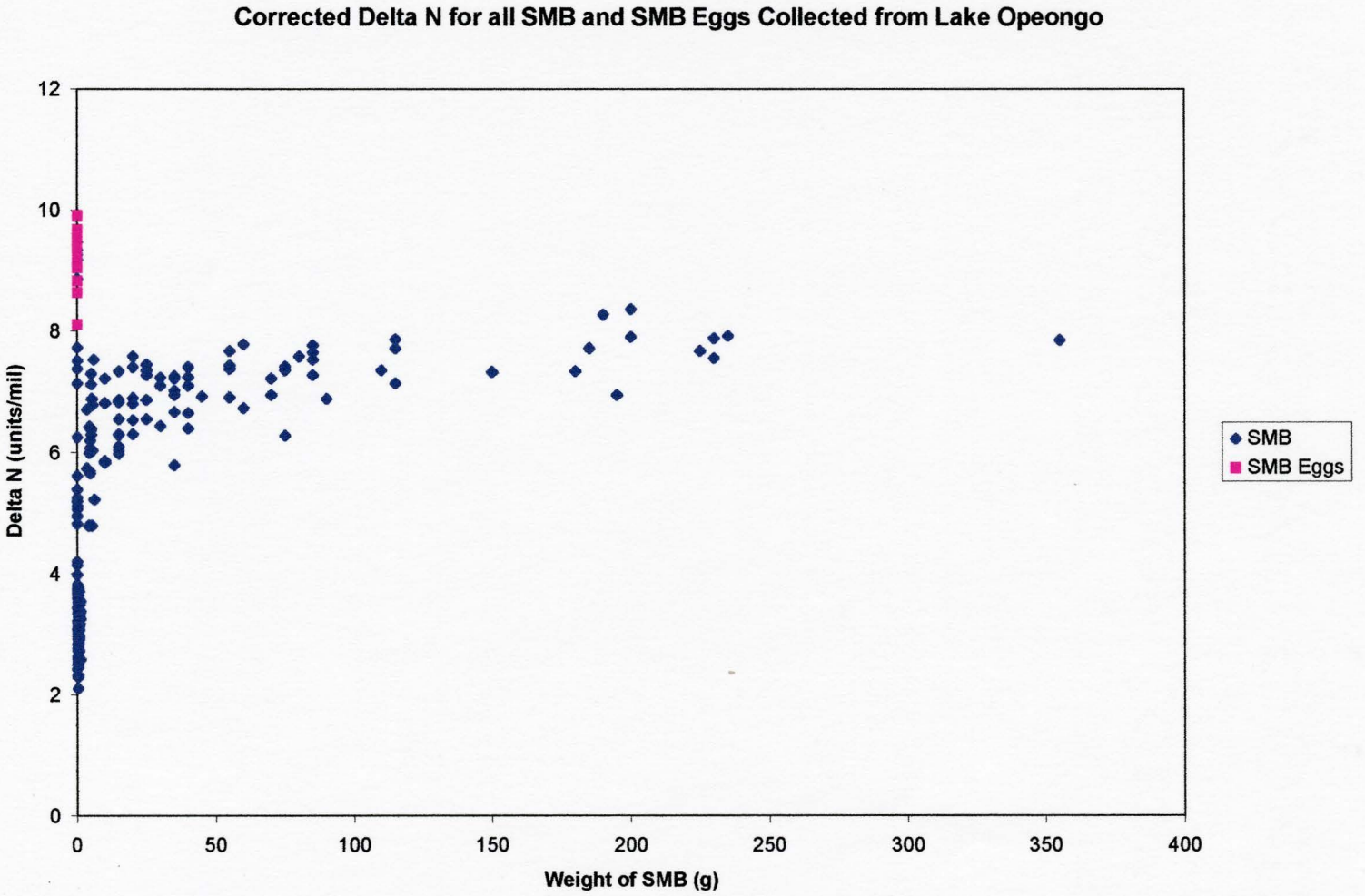


Figure 7.1
$\delta^{15}N$ of all the samples collected from Lake Opeongo in 1992 and 1999. Diamonds represent SMB samples and squares represent SMB eggs. All values have been corrected for the effect of formalin with respect to fresh tissue.

7.2 $\delta^{15}N$ TRENDS IN *MICROPTERUS DOLOMIEU*

7.2.1 GENERAL

As previously observed by Vander Zanden et al. (1999) *Micropterus dolomieu* eggs and newly hatched young of the year display $\delta^{15}N$ values, which are heavily enriched relative to the rest of the *Micropterus dolomieu* population of Lake Opeongo. The $\delta^{15}N$ values then become rapidly lighter as the yolk sac (maternal tissue) is used up. The young fishes then start to feed heterotrophically and from this point onwards their $\delta^{15}N$ values are linked to the food that they are eating. As they enlarge their diets to include higher trophic levels the $\delta^{15}N$ values of their own tissues become likewise enriched. In addition, as seen in figure 6.1 the *Micropterus dolomieu* are enriched in $\delta^{15}N$ with respect to their prey. With the exception of the *Micropterus dolomieu* eggs and embryos, the largest adults displayed the heaviest $\delta^{15}N$ values. There are two effects occurring here: 1) The $\delta^{15}N$ of the *Micropterus dolomieu* increases because they are eating prey of successively higher trophic levels. 2) The $\delta^{15}N$ values of the *Micropterus dolomieu* are 3 ‰ higher that that of its prey (figure 6.1).

While figure 7.1 shows all the $\delta^{15}N$ results obtained from the *Micropterus dolomieu* from Lake Opeongo, it is difficult to observe trends from this graph. Figure 7.2 depicts the corrected $\delta^{15}N$ values of the young of the year collected by Friesen in 1992, while figure 7.3 depicts the corrected $\delta^{15}N$ values of all the *Micropterus dolomieu* collected in 1999. The trend-line depicted in figure 7.3 demonstrates a leveling off of the $\delta^{15}N$ values as they asymptotically approach a maximum $\delta^{15}N$ value of ~ 7.9 ‰,

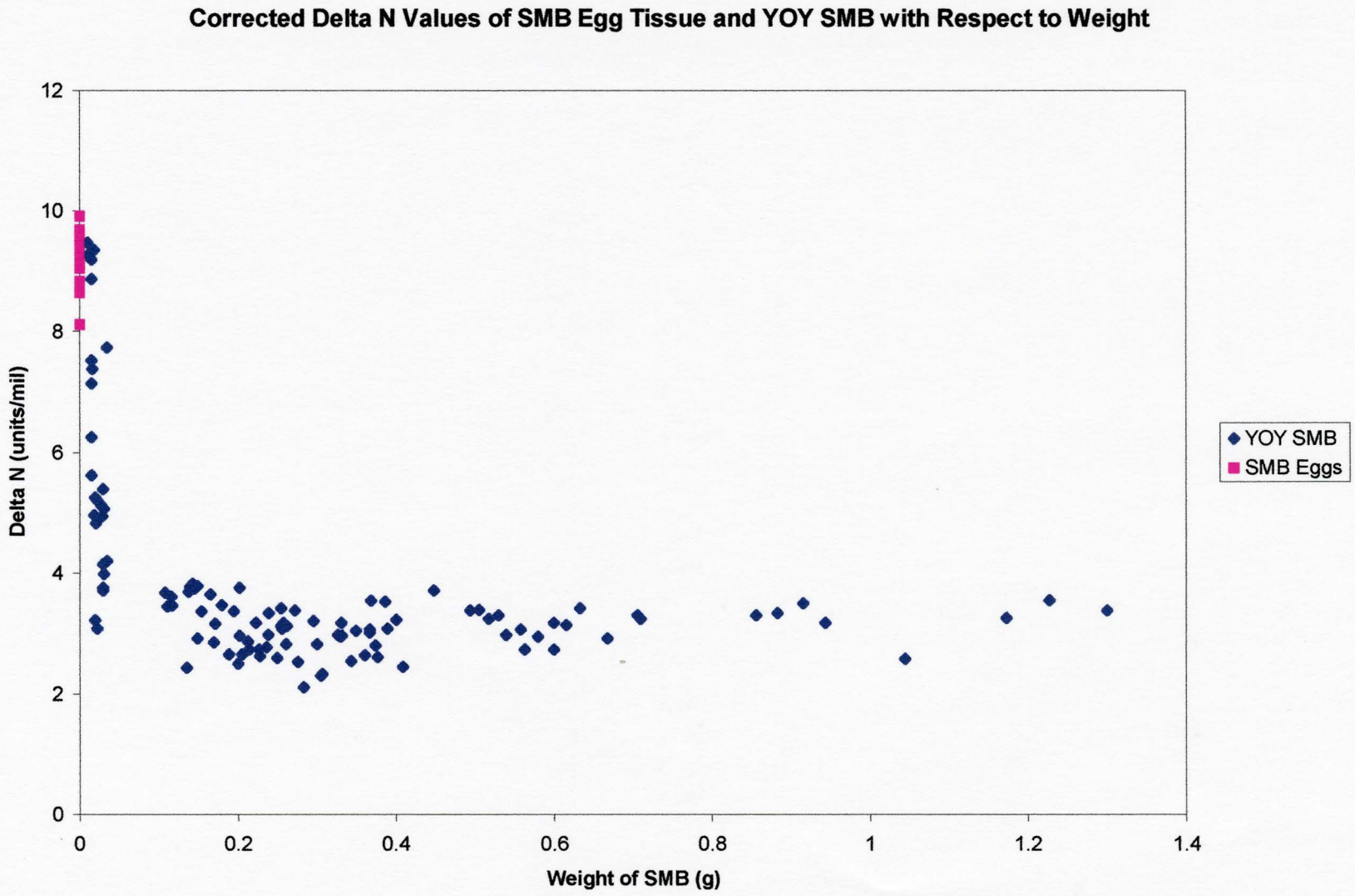


Figure 7.2
$\delta^{15}N$ of all the samples collected from Lake Opeongo in 1992. All samples are age-0 SMB except the eggs which were collected before the hatching date. Diamonds represent SMB samples squares represent SMB eggs. All values have been corrected to calibrate between differences in formalin with respect to fresh tissue.

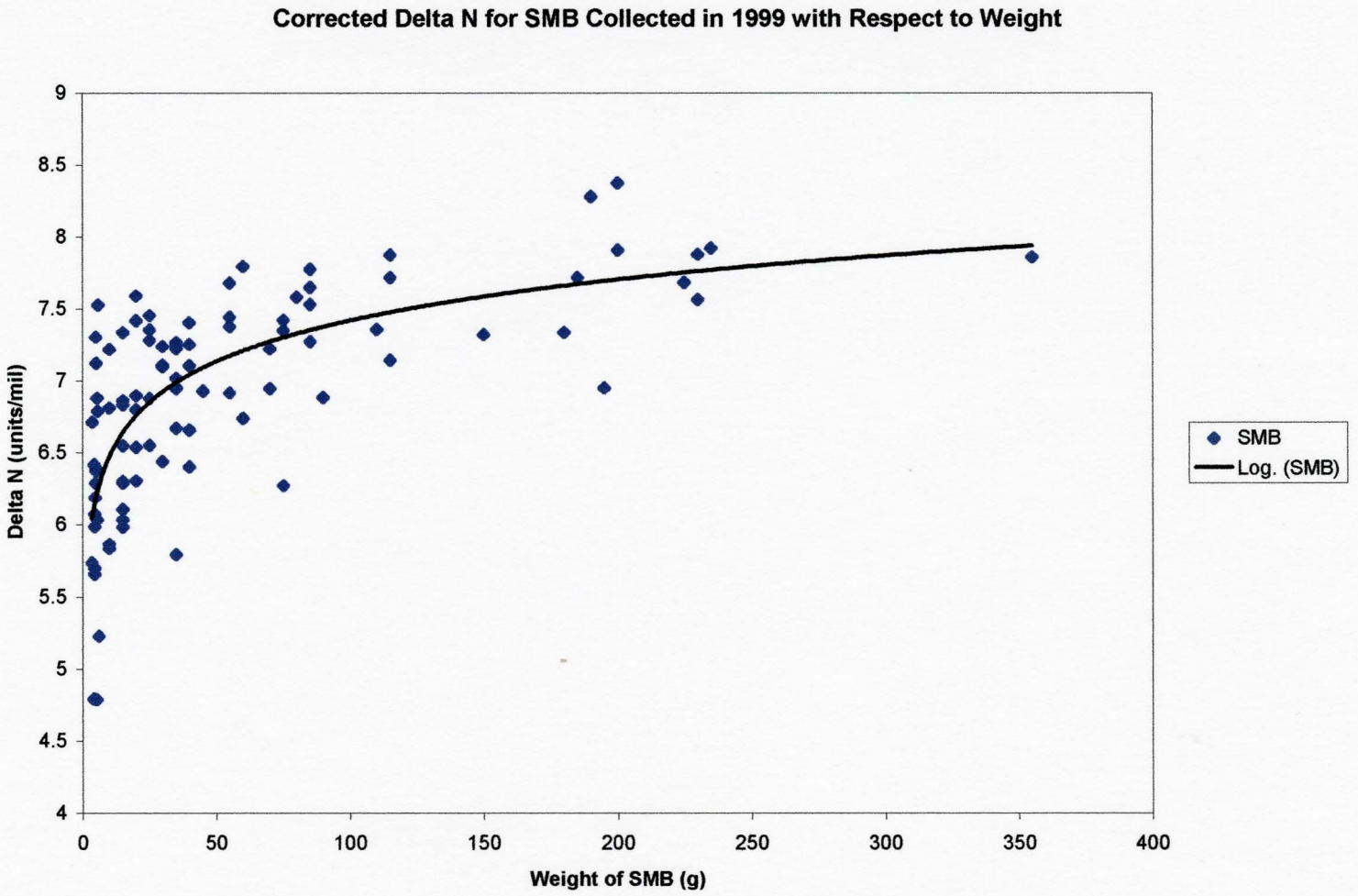


Figure 7.3
$\delta^{15}N$ of all the samples collected from Lake Opeongo in 1999. Samples range in weight from 3.5 g (age-0 SMB) to 355 g (age-8 SMB). Diamonds represent SMB samples. All values have been corrected to calibrate between differences in formalin with respect to fresh tissue.

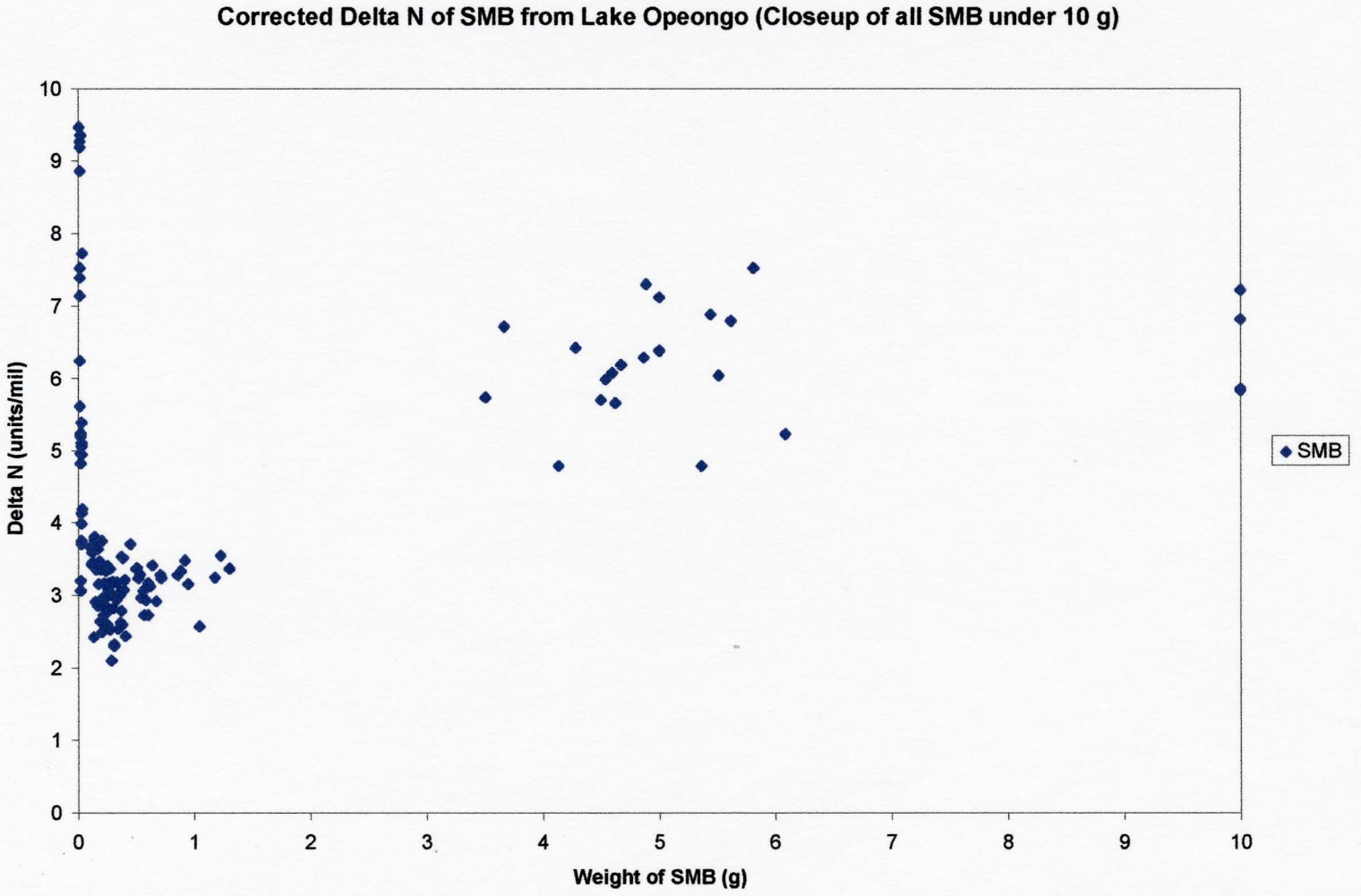


Figure 7.4
$\delta^{15}N$ of all the samples collected from Lake Opeongo in 1992 and 1999 that were under 10 g. This graph is a close up of the transition between the 1992 and the 1999 data. Diamonds represent SMB samples. All values have been corrected to calibrate between differences in formalin with respect to fresh tissue.

discussed further in section 7.2.2. Because of the differences in age and weight between the *Micropterus dolomieu* samples collected in 1992 and 1999 the $\delta^{15}N$ values seen in figure 7.2 do not overlap with those seen in figure 7.3. It is therefore, necessary, for the purposes of continuity between the two graphs, to produce figure 7.4 to envelop a close-up of all *Micropterus dolomieu* below the weight of 10 grams. This encompasses all the 1992 data and enough of the 1999 data to demonstrate continuity between the two sets of results, the 1992 $\delta^{15}N$ values have been corrected to the 1999 $\delta^{15}N$ values with respect to the formalin used. Both data sets (1992 and 1999 samples) have been corrected for the effect of formalin. Figure 7.4 shows that there is a gap between the weight range of the 1992 and the 1999 samples. There is a corresponding gap in the $\delta^{15}N$ values but when viewed in relationship to the entire population, as seen in figure 7.1, then the trend in $\delta^{15}N$ becomes more obvious and the gap is made less significant. From the continuity between the two data sets we see that the corrections for the two different batches of formalin are consistent with one another.

7.2.2 $\delta^{15}N$ TRENDS

According to DeNiro and Epstein (1981) there is a difference of 3 ‰ between one trophic level and the next. The average $\delta^{15}N$ of the metamorphosis stage *Micropterus dolomieu* was 3.1 ± 0.6 ‰, represented by the plateau. These metamorphosis stage fish were one trophic level (2.9 ‰) above that of the plankton in the lake, refer to figure 6.1. We observe a continued increase in $\delta^{15}N$ with size and age such that *Micropterus dolomieu* of age 2 have an average $\delta^{15}N$ values of 6.5 ± 0.7 ‰. This

corresponds to an apparent trophic level increase of about one trophic level. All *Micropterus dolomieu* above the age of 2 have average $\delta^{15}N$ values of 7.3 ± 0.5 ‰. This corresponds to an apparent trophic level increase of about one half a trophic level. The maximum $\delta^{15}N$ value reported was 8.4 ± 0.4 ‰ from muscle tissue taken from an age 7 individual. Also judging from figures 7.1 and 7.3 the $\delta^{15}N$ values of the adults appear to be leading towards a maximum $\delta^{15}N$ value of ~ 7.9 ‰. A third full trophic level is never actually achieved by the *Micropterus dolomieu* in Lake Opeongo because the adults do not limit their feeding to cisco (average $\delta^{15}N$ value of 6.0 ± 0.2 ‰) but also feed on crayfish (average $\delta^{15}N$ value of 2.7 ± 1.4 ‰). Being opportunistic feeders they will chose to feed on any trophic level available to them, this is the factor that limits the upper levels of the $\delta^{15}N$ values in adult *Micropterus dolomieu.*

Even though none of the adult *Micropterus dolomieu* could be considered to be two full trophic levels above the pre-dispersal young of the year their eggs could be considered for this position. *Micropterus dolomieu* eggs obtained from Lake Opeongo have an average $\delta^{15}N$ value of 9.2 ± 0.4 ‰. Further relationships between mother and egg will be discussed in chapter 9.

Figure 7.5 shows the weight and the $\delta^{15}N$ values for the *Micropterus dolomieu* samples collected in 1992, from Lake Opeongo, with respect to age. It is possible to see from this figure that the shape of the curve is quite different from that of the curve seen in figure 7.2. Within the first 25 days of life these hatchlings display large changes in the $\delta^{15}N$ values of the fishes tissues (a depletion of ^{15}N in the tissues of up to 7.8 ‰) and also large changes in the percentage weight gain (2600 % weight gain), table 7.1. There seems to be a dilution pattern in the $\delta^{15}N$ values within the first 20 - 25 days.

Figure 7.5
Weight of the SMB vs. age and $\delta^{15}N$ of all the samples collected from Lake Opeongo in 1992 with respect to age. A different pattern can be seen to the graphs, which show $\delta^{15}N$ vs. weight. Diamonds represent SMB samples. All values have been corrected to calibrate between differences in formalin with respect to fresh tissue.

Age (days)	Weight (g)	% Weight gain	$\delta^{15}N$ (‰)	Change in $\delta^{15}N$ (‰)
1	0.01	0	9.0 ± 0.7	
8 -9	0.02	100	5.7 ± 1.1	-3.3 ± 1.1
10-13	0.03	200	4.0 ± 0.2	-1.7 ± 1.1
14	0.04	300	3.0 ± 0.2	-1.0 ± 0.2
15-16	0.10	900	3.9 ± 0.9	+0.9 ± 0.9
21	0.20	1900	3.2 ± 0.4	-0.7 ± 0.9
25	0.27	2600	2.6 ± 0.4	-0.6 ± 0.4
27	0.30	2900	3.0 ± 0.4	+0.4 ± 0.4
30	0.40	3900	3.0 ± 0.4	0.0 ± 0.4
32	0.50	4900	3.2 ± 0.4	+0.2 ± 0.4
35-36	0.60	5900	3.0 ± 0.4	-0.2 ± 0.4
38	0.70	6900	3.3 ± 0.4	+0.3 ± 0.4
39	1.00	9900	2.9 ± 0.4	-0.4 ± 0.4
49	1.30	12900	3.4 ± 0.4	+0.5 ± 0.4

Table 7.1
Table showing the age in days of individual SMB collected in 1992 along with their corresponding weights, percentage weights, $\delta^{15}N$ values, and the difference between one $\delta^{15}N$ value and the next. So within the first 100 % weight gain there is a shift of –3.3 ± 1.1 ‰, and so on. The SMB at the age of 25 days is shown here because it is at this age that the metamorphosis takes place and the young fish start to feed heterotrophically.

If 9.0 ± 0.7 ‰ is the average $\delta^{15}N$ value at hatching and the 3.0 ± 0.4 ‰ is the average value for 25 day old *Micropterus dolomieu* (which feed strictly on plankton) then by the time the fish has doubled in weight a dilution factor of – 3.0 ‰ should be visible in the □^{15}N of their tissues. As seen in table 7.1 there is a □^{15}N value of 5.7 ± 1.1 ‰ seen in the tissues of *Micropterus dolomieu* that have increased in weight by 100 % (by days 8 – 9 from hatching). This dilution trend appears to continue until the fish reaches about 900 % weight gain at which point there is little fluctuation seen in the $\delta^{15}N$ values.

Conversely, over the next 25 days of growth (age 25 – 50 days) there is little or no change in the ^{15}N content of the tissues while there are huge increases in weight gain, also in table 7.1. Throughout this time-period the $\delta^{15}N$ values remain constant around 3.1 ± 0.3 ‰ while there is a huge percentage weight increase from 2600 % to 12900 %. Because of this it is important to take careful note of both the weight and the age during the first few days after hatching.

In general, during each year of growth the fish becomes progressively more and more enriched in $\delta^{15}N$. Table 7.2 shows that there appears to be an enrichment in the $\delta^{15}N$ correlated with the age of the fish. The first 25 days are left out of the calculations due to the fact that those $\delta^{15}N$ values represent embryos, which feed endogenously. These increasing $\delta^{15}N$ values seem to be asymptotically approaching a maximum $\delta^{15}N$ value of ~7.9 ± 0.4 ‰.

Figure 7.6 shows a graph modified from Dufour (1999) which appears to show similar $\delta^{15}N$ trends. This also shows an increase in $\delta^{15}N$ in relation to the length of the whitefish (*Coregonus*) from Lake Aiguebelette. However, the two heavier points from the youngest of the age-0 whitefish were rejected on the basis that they did not fit in

Age of the *Micropterus dolomieu* in Years	Average $\delta^{15}N$
0	3.9 ± 1.4
1	6.3 ± 0.4
2	7.0 ± 0.4
3	6.9 ± 0.4
4	7.4 ± 0.4
5	7.2 ± 0.4
6	7.6 ± 0.3
7	7.8 ± 0.4
8	7.9 ± 0.4

Table 7.2
$\delta^{15}N$ averages of the *Micropterus dolomieu* when separated into age groups based on years. In general, except for slight variations in the age group 3 and 5 there appears to be an enrichment of $\delta^{15}N$ with age. Age-0 fish only include $\delta^{15}N$ values over the age of 25 days, when the fish starts feeding entirely exogenously.

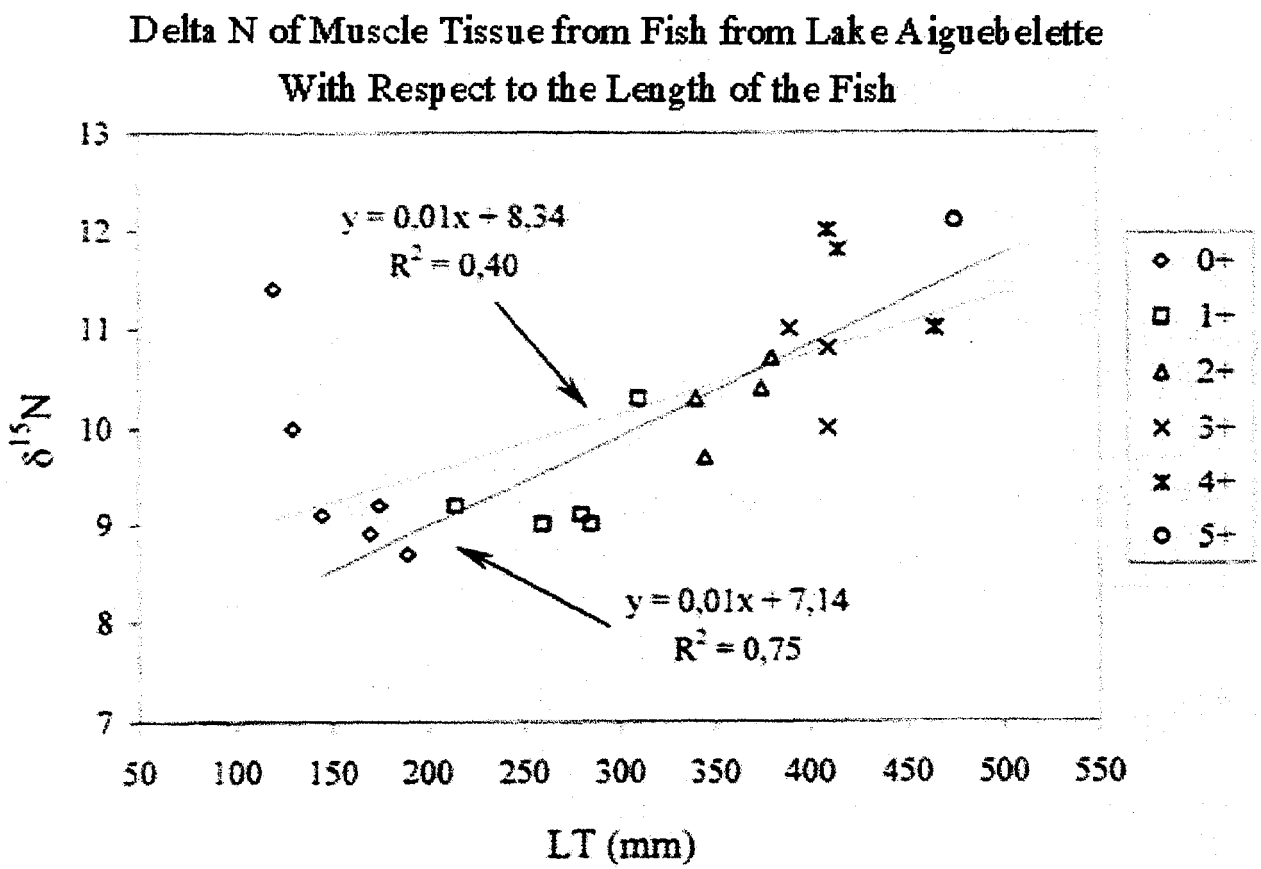


Figure 7.6
Relationship between the $\delta^{15}N$ of the muscle and the length (mm) for whitefish from Lake Aiguebelette. The two smallest individuals were not included in the relationship. (Modified from Dufour, 1999).

with the best-fit line for the rest of the data. This study shows that these two points may not be out of place but may actually be very relevant in that they may represent post-metamorphosis fish, which are still obtaining nutrients from of their yolk sacs. Also of note is the plateau in the $\delta^{15}N$ values, which occurs at 9.0 ± 0.5 ‰. After the plateau the $\delta^{15}N$ values become heavier again, just as they did in this study. Dufour (1999) did not analyze the entire lifecycle of the whitefish as seen by the fact that the $\delta^{15}N$ values do not level off at what appears to be the maximum values for that particular fish in that lake, as occurred in this study.

This pattern that has been observed in this study and in Dufour (1999) is dependent on the fact that the organism analyzed changes its diet or feeding habits throughout its lifecycle. Dufour (1999) also noted that not all species of fish show this pattern and that seals in captivity do not show these same trends. This is because the captive seals are fed a consistent diet throughout their entire lives and hence do not ever change their trophic levels. If an organism maintains a consistent diet throughout its lifetime it can be implied that it will also display consistent $\delta^{15}N$ values throughout its lifetime due to the fact that it does not undergo any trophic level shifts.

8. $\delta^{13}C$ CHANGES THROUGHOUT THE LIFECYCLE OF *MICROPTERUS DOLOMIEU*

8.1 INTRODUCTION

The $\delta^{13}C$ values obtained from the Micropterus dolomieu from Lake Opeongo do not display as clear-cut trends as the $\delta^{15}N$ values did. This is not surprising as many other studies claim that there are no determinable patterns associated with $\delta^{13}C$ and age, such as Sholto-Douglas et al. (1991) and Kidd et al. (1995).

Figure 8.1 shows $\delta^{13}C$ values obtained from *Micropterus dolomieu* with the factor of weight being varied. The *Micropterus dolomieu* $\delta^{13}C$ values (appendix 2) ranged from as light as -27.8 ± 0.4 ‰ for day old hatchlings to as heavy as -18.2 ± 0.4 ‰ for young of the year fish, which had just used finished using up their yolk sacs, and as light as -25.0 ± 0.4 ‰ for adults. Unlike with the $\delta^{15}N$ values there is a lot of overlap with the $\delta^{13}C$ values of the eggs and the young of the year. However, it is also possible to see that there is also not a lot of overlap between the adults and the eggs. Only 12 of the adults $\delta^{13}C$ values overlap with the egg $\delta^{13}C$ values. This implies that there may be a detectable pattern, at least between the eggs/young of the year and the adults, even if there are no visible stepwise increases in $\delta^{13}C$; as would be expected for trophic level studies.

A problem with using carbon in a trophic level study such as this is that carbon undergoes little (<1 ‰) further fractionation with food assimilation (DeNiro and

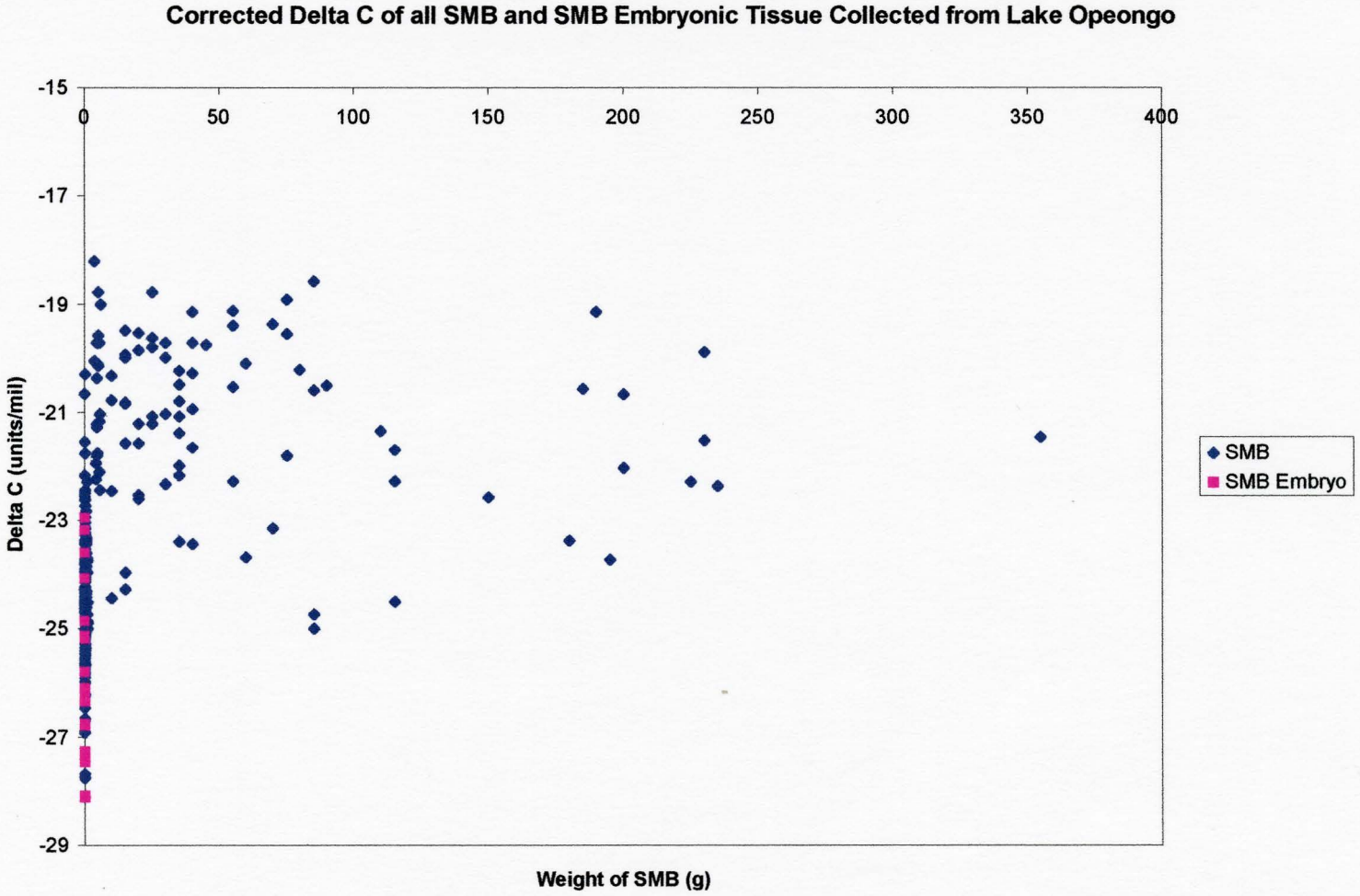


Figure 8.1
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1992 and 1999. Diamonds represent SMB samples and squares represent SMB eggs. All values have been corrected to calibrate between differences in formalin with respect to fresh tissue.

Epstein, 1987; Peterson and Fry 1987). This means that the $\delta^{13}C$ differences between benthic and planktonic algae can be sufficient to be visible in the $\delta^{13}C$ signatures of the consumers (France, 1995 a), *Micropterus dolomieu* in this case. As mentioned in chapter 6 all $\delta^{13}C$ values examined may also have to be examined spatially simultaneously with trophic level studies. So this point too will be examined.

8.2 $\delta^{13}C$ TRENDS IN *MICROPTERUS DOLOMIEU*

8.2.1 GENERAL

As predicted *Micropterus dolomieu* eggs, embryos, and young of the year are depleted in ^{13}C relative to the adult *Micropterus dolomieu* in the population of Lake Opeongo. These eggs and young fish display depleted $\delta^{13}C$ values because these samples are enriched in lipids which are depleted in ^{13}C relative to proteins, discussed further in chapter 9.

Figure 8.1 shows all the $\delta^{13}C$ results obtained from *Micropterus dolomieu* from Lake Opeongo, but as with figure 7.1 it is difficult to observe any trends from this graph. Figure 8.2 depicts the corrected $\delta^{13}C$ values of the young of the year collected by Friesen in 1992 and figure 8.3 depicts the corrected $\delta^{13}C$ values of all the *Micropterus dolomieu* collected in 1999. Again the $\delta^{13}C$ values seen in figure 8.2 do not overlap with those seen in figure 8.3. It is therefore, necessary, for the purposes of continuity between the two graphs, to produce figure 8.4 to envelop a close-up of all *Micropterus dolomieu* below the weight of 10 g. All the 1992 and 1999 $\delta^{13}C$ values have been corrected with respect to the formalin used. Note that the trend-line for the 1992 data (slope of

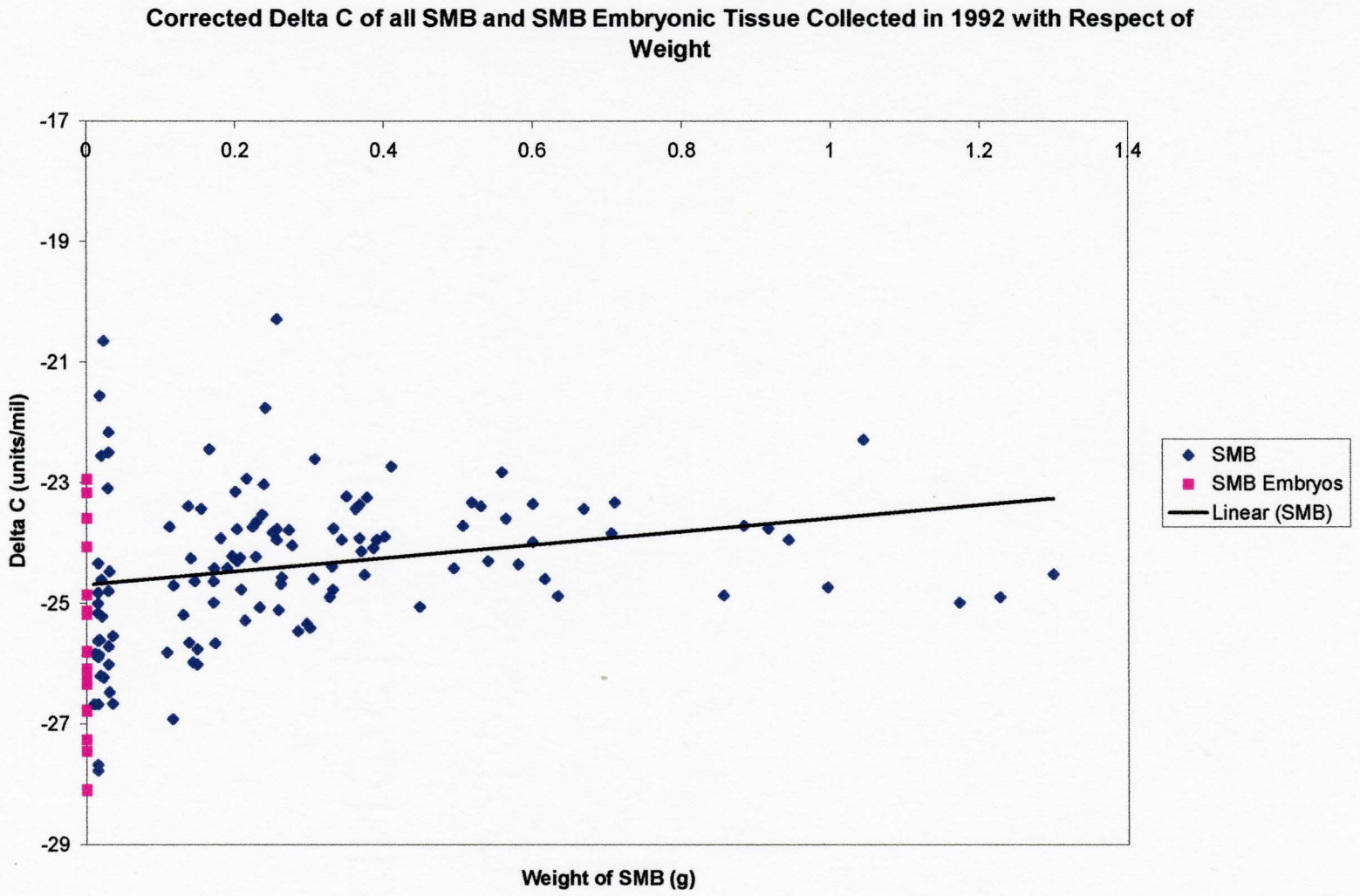


Figure 8.2
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1992. All samples are age-0 SMB except the eggs which were collected before the hatching date. Diamonds represent SMB samples squares represent SMB eggs. All values have been corrected to calibrate between differences in formalin with respect to fresh tissues. The trend-line shows the tendency towards enrichment in the embryos and young of the year.

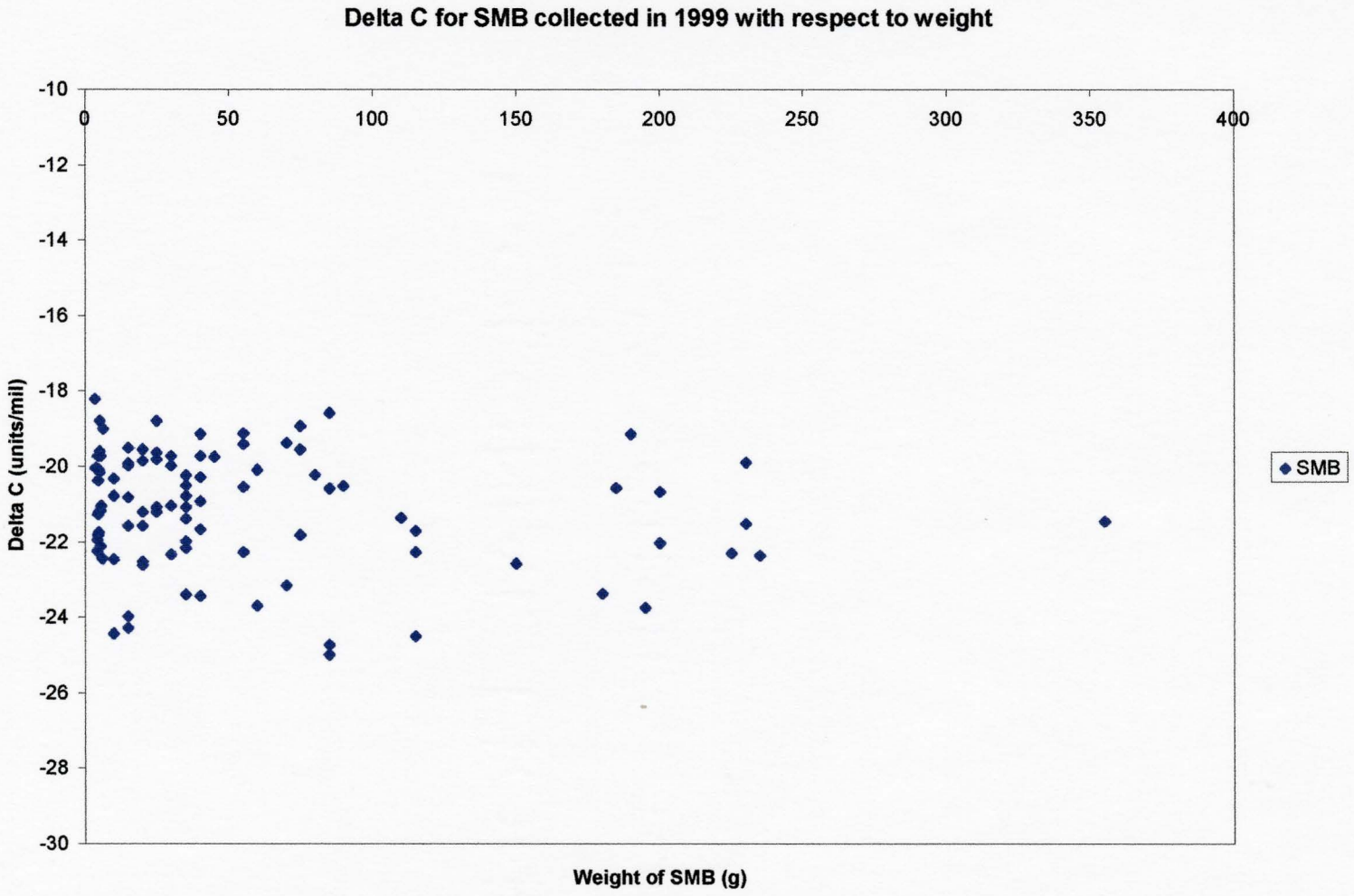


Figure 8.3
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1999. Samples range in weight from 3.5 g (age-0 SMB) to 355 g (age-8 SMB). Diamonds represent SMB samples. All values have been corrected to calibrate between differences in formalin with respect to fresh tissues.

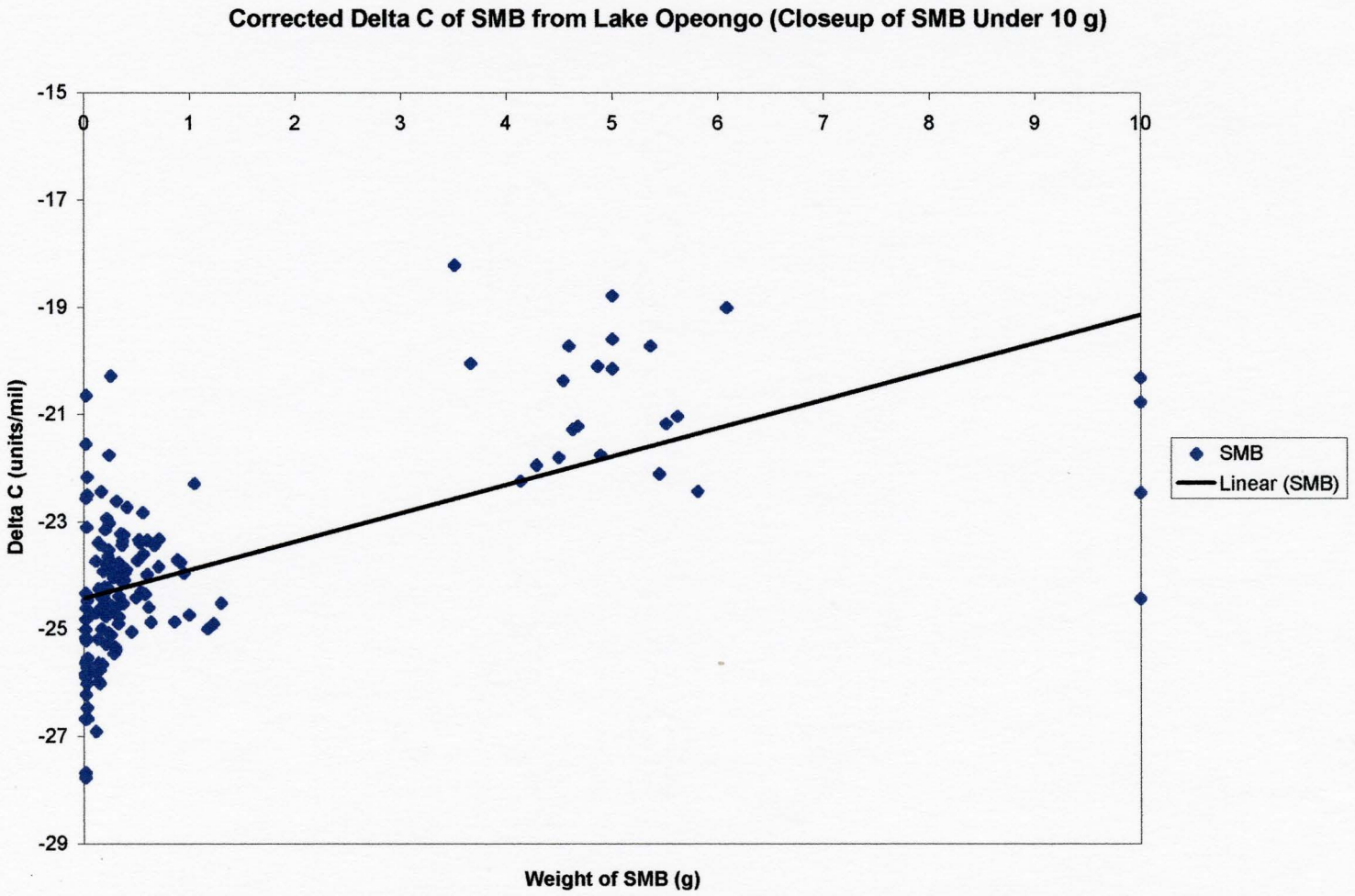


Figure 8.4
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1992 and 1999 that were under 10 g. This graph is a close up of the transition between the 1992 and the 1999 data. Diamonds represent SMB samples. All values have been corrected to calibrate between differences in formalin with respect to fresh tissues.

~0.64 ‰/g) extrapolates into the cluster of points at the low-weight end of the 1999 population, again confirming the continuity of the trend (as was done in chapter 7 for the $\delta^{15}N$ data). Thereafter, the slope that was increasing in $\delta^{13}C$ with age decreases markedly, as seen in figure 8.6.

8.2.2 $\delta^{13}C$ TRENDS

$\delta^{13}C$ values are quite variable for both the 1992 and the 1999 samples. This is likely due to the spatial variations in $\delta^{13}C$, as were already observed in chapter 6. Figures 8.2 and 8.4 clearly show that *Micropterus dolomieu* eggs and embryos are depleted in $\delta^{13}C$ but when the young start feeding heterotrophically the $\delta^{13}C$ values rapidly increase ranging from -27.8 ± 0.4 ‰ to -18.3 ± 0.4 ‰ within the first year of life. This is mainly due to the loss of the yolk sac within the first month of life. Figure 8.5 shows the same data as 8.2 but with different families represented, with trend-lines for each. It is possible to see that most of the families start life off at between -26.7 ± 0.4 ‰ and -25.7 ± 0.4 ‰ and become enriched at an average rate of 0.06 ‰/g.

The adult *Micropterus dolomieu* display $\delta^{13}C$ values ranging from -19.0 ± 0.4 ‰ to -25.0 ± 0.4 ‰. It is possible to see from figure 8.6 that when $\delta^{13}C$ values of the adults are separated into collection sites that there is a base variance between -23.0 ± 0.4 ‰ and -20.2 ± 0.4 ‰ throughout the lake. This makes it difficult to observe trends in $\delta^{13}C$ values of the adult *Micropterus dolomieu* with respect to the whole lake. However, it is also possible to see that, with the exception of site 5, the slopes of the trend-lines are

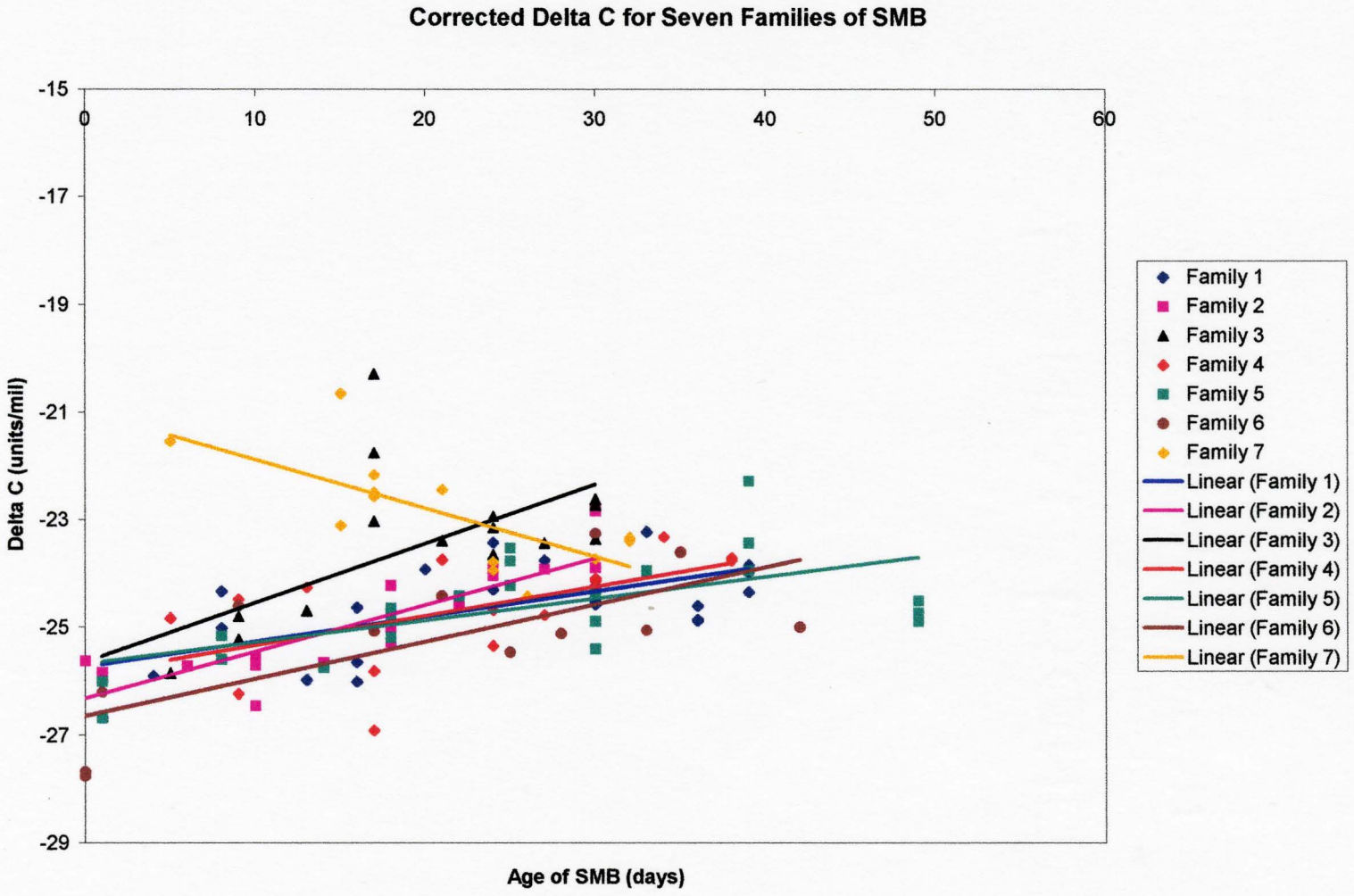


Figure 8.5
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1992 separated into individual family groups. The trend-lines represent trends in $\delta^{13}C$ for each of the families.

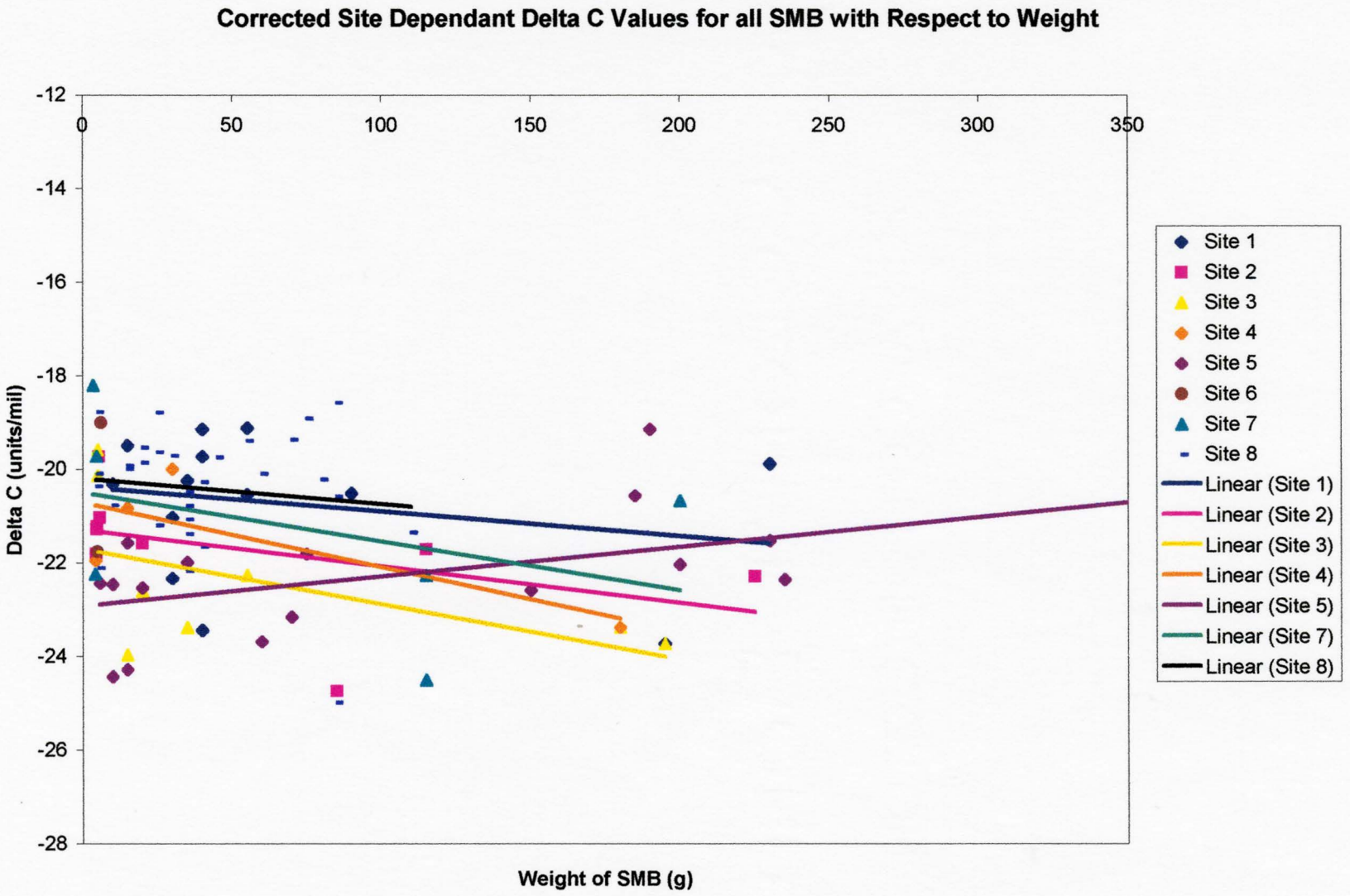


Figure 8.6
$\delta^{13}C$ of all the samples collected from Lake Opeongo in 1999 separated into collection sites as seen in figure 3.1. The trend-lines represent trends in $\delta^{13}C$ for each of the collection sites.

similar to each other, with an average rate of –0.008 ‰/g. Note that site 6 was ignored because there were only two samples from this area.

The results here appear to line up with the findings from figure 6.2 and chart 6.1. *Micropterus dolomieu* from sites 7 and 8 tend to be isotopically heavier with respect to ^{13}C while fish from site 5 tend to be isotopically lighter. This is further proof that the $\delta^{13}C$ values of the fish's tissues are site dependent.

There is also a tendency for the $\delta^{13}C$ of the *Micropterus dolomieu* tissues to become lower as the weight of the fish increases, this may be due to an increase of lipids in the tissues of the fish with age.

Without further research it is not possible to determine if the $\delta^{13}C$ of the fish is more closely related to the age or to the weight of the fish. From this study the $\delta^{13}C$ values within a population seem to be more indicative of the site from where the fish was captured and the lipid content of the tissues, rather than to changes in the trophic level with age. There is no indication that $\delta^{13}C$ values increase with trophic level for *Micropterus dolomieu* in the lake. The proposed trophic level effect of 0.5 – 1.0 ‰ per level seems to be overridden by a large inter-site difference in $\delta^{13}C$.

An anova (appendix 3) of the 1992 data shows that there is a significant difference between the starting values of $\delta^{13}C$ in the different families. The variance ranges from 0.8 and 2.5 showing that the starting values of $\delta^{13}C$ are significant and proves that there is an inter-site difference in $\delta^{13}C$. This makes it difficult to observe trends in the $\delta^{13}C$ values if the samples are taken from all over the lake. If an analysis is to be preformed on $\delta^{13}C$ trends it will be necessary to take samples from the same location in order to maintain continuity.

9. EGGS AND EMBRYOS

9.1 INTRODUCTION

It is reasonable to expect that there will be a direct and predictable relationship between the $\delta^{15}N$ and $\delta^{13}C$ of a mother and their embryos, since the eggs are formed from tissues available from the mother. This means that whatever the isotopic stage of the mother the same ratios can be expected to be reflected in the tissues of the eggs and the embryos. The isotopes of the mother are dependent on the age and life-stage of the fish, in question, as seen in chapters 7 and 8. So if there is a large range in the $\delta^{15}N$ and $\delta^{13}C$'s of the mothers there will be an equally large range in the $\delta^{15}N$ and $\delta^{13}C$'s of the eggs and embryos. As seen earlier in chapter 7, figure 7.1, the $\delta^{15}N$ values of adult *Micropterus dolomieu* range from an average of 7.3 ± 0.5 ‰ to a maximum $\delta^{15}N$ value of 8.4 ± 0.4 ‰. The $\delta^{13}C$ values of adult *Micropterus dolomieu* range from –25.0 ± 0.4 ‰ to –18.4 ± 0.4 ‰, as seen in chapter 8, figure 8.1.

There is a large range in the both the delta values of carbon and of nitrogen for the *Micropterus dolomieu* eggs. For the eggs the average $\delta^{15}N$ was 9.2 ± 0.4 ‰, while the isotopically heaviest adult sampled only reached a $\delta^{15}N$ value of 8.4 ± 0.4 ‰. Fertilized eggs are isotopically heavier, with respect to nitrogen, than even the largest and heaviest of the adults of the same species. In the case of carbon the $\delta^{13}C$ values of the eggs ranged from –28.1 ± 1.4 ‰ to –22.9 ± 1.4 ‰. The $\delta^{13}C$ values for the adults, do not display as clear-cut trends as the $\delta^{15}N$ values, there is an overlap between the adults and eggs. However, the lightest $\delta^{13}C$ value for an adult *Micropterus dolomieu*, at –25.0 ± 0.4 ‰, does not exceed that of the lightest value for the eggs.

9.2 RELATIONSHIP BETWEEN MOTHER AND EMBRYO

In an open system, like a lake, it is difficult to obtain mother and egg tissue from *Micropterus dolomieu* for isotopic analysis because the mother simply lays the eggs and departs, leaving the male to fertilize and protect the eggs. However it was possible to obtain tissues from female fish and sample their unfertilized eggs. No samples of *Micropterus dolomieu* where available for this but mother and egg samples of *Oncorhynchus mykiss* were made available by Dr. Noakes from the University of Guelph. These eggs and muscle tissue were prepared and analyzed in the same manner as all the tissue samples prepared earlier.

From the data presented in chapters 7 and 8, there appears to be an offset between the $\delta^{15}N$ and $\delta^{13}C$ of the mother and the embryo. Figure 9.1 shows the results of the analysis of the *Oncorhynchus mykiss* from this study. A distinct offset, of 0.4 ‰ between the $\delta^{13}C$ of the mother and the embryo, with $\delta^{13}C$ values of -21.9 ± 0.2 ‰ for the mother and -22.3 ± 0.2 ‰ for the egg. While the offset for $\delta^{15}N$, seen in figure 9.2, was only 0.2 ‰, with $\delta^{15}N$ values of 10.9 ± 0.02‰ for the mother and 10.7 ± 0.2‰ for the egg.

9.3 IMPLICATIONS

From chapter 7 it is possible to see that there is a distinct enrichment in the $\delta^{15}N$ of the fertilized eggs verses the $\delta^{15}N$ of the adults in the same population. Here,

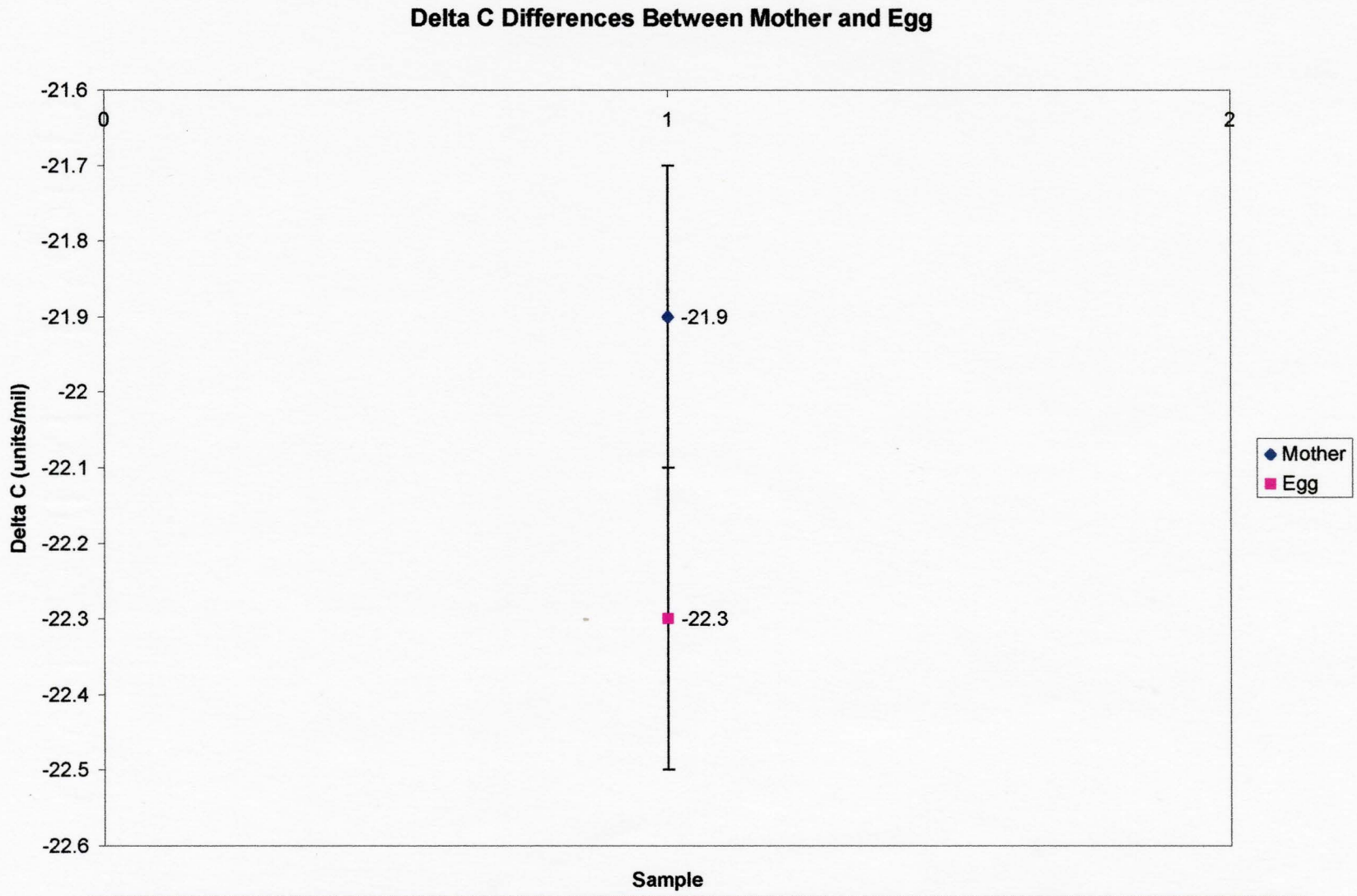


Figure 9.1
$\delta^{13}C$ offsets between mother tissue (diamond) and egg tissue (square) from the same mother for *Oncorhynchus mykiss*. Each point represents the average of each sample run in triplicate with the error bars representing standard deviation.

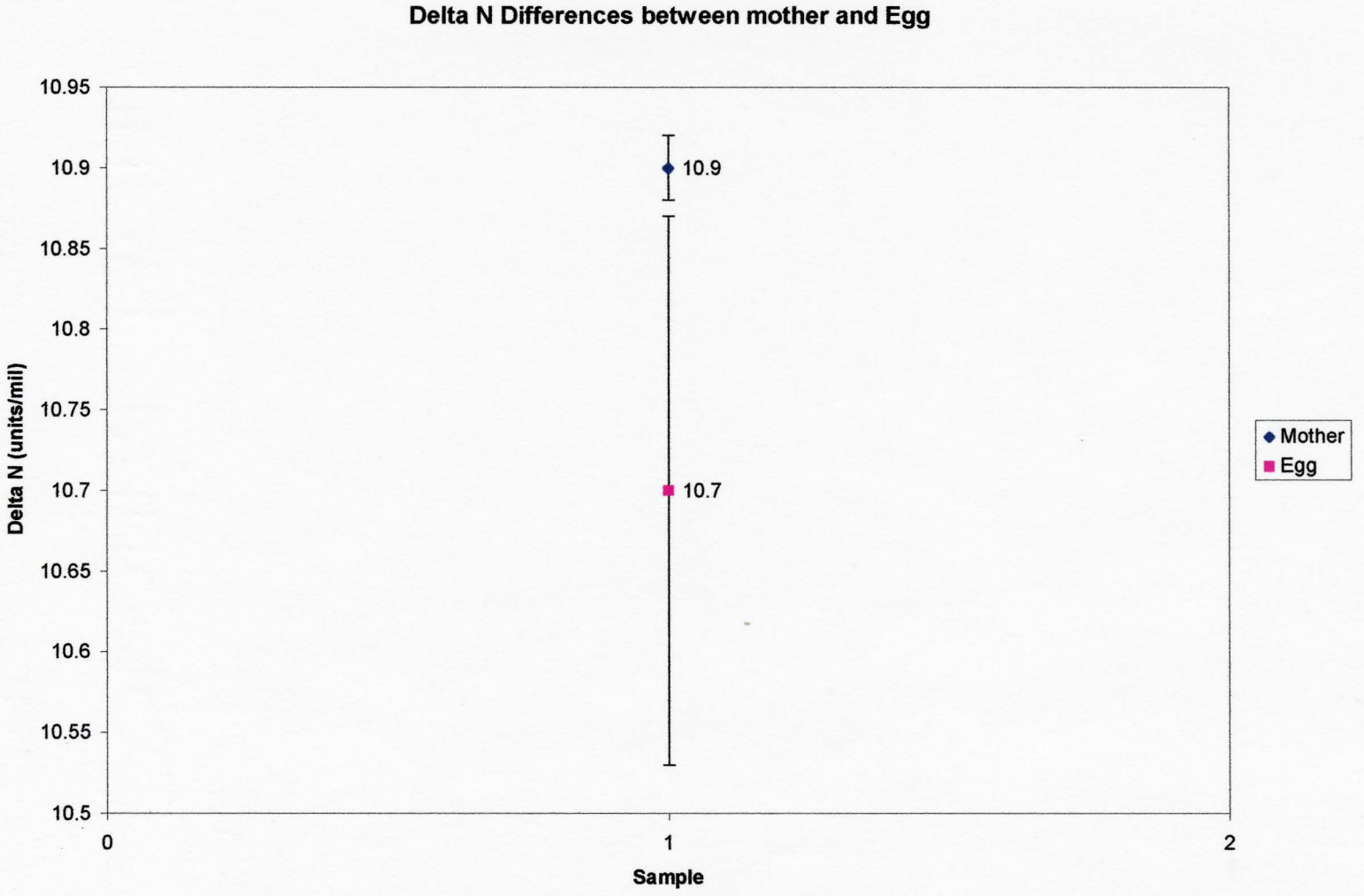


Figure 9.2
$\delta^{15}N$ offsets between mother tissue (diamond) and egg tissue (square) from the same mother for *Oncorhynchus mykiss*. Each point represents the average of each sample run in triplicate with the error bars representing standard deviation.

however, the opposite is seen, there is an enrichment in the $\delta^{15}N$ of the mother over that of her unfertilized eggs. This implies that there may be an isotopic shift in the $\delta^{15}N$ values that occurs when the eggs are introduced into the aqueous environment or upon fertilization.

The $\delta^{13}C$ of the eggs appear to be lighter than that of the adult's tissues. This is because of the fact that eggs are mainly composed of fatty acids and lipids, which are isotopically lighter, with respect to $\delta^{13}C$, than other tissues of the body. Figure 9.3 shows this phenomenon in a study by Tieszen et al. (1983). Since the major component of eggs and embryos are lipids, tissue samples from eggs and embryos are expected to be depleted in ^{13}C. Such samples are seen to do exactly that in this study.

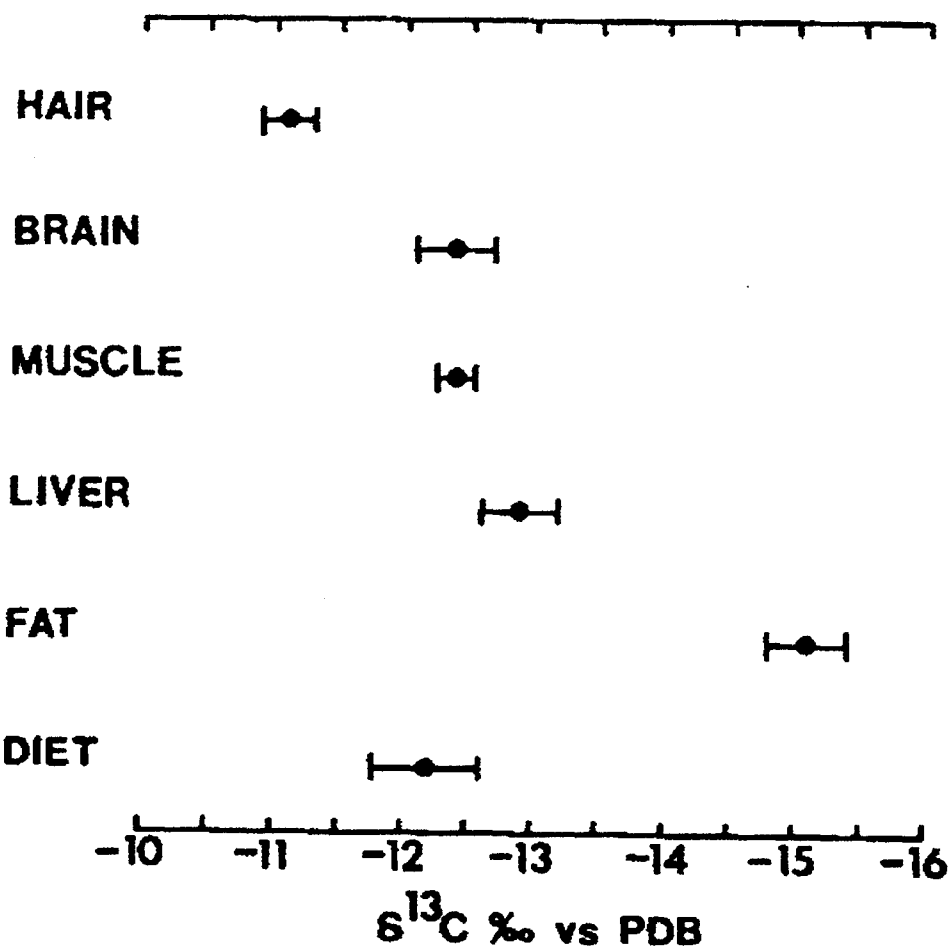


Figure 9.3
Isotope ratios of selected gerbil tissues. Values indicated are means (N=5) ± standard errors. (Tieszen et al., 1983).

10. DISCUSSION

10.1 COMMENTS

This study, preformed on *Micropterus dolomieu* (small mouth bass) from Lake Opeongo in 1992 and 1999, examined the isotopic values of carbon and nitrogen from tissues taken from the fish and some egg tissues. The majority of the fish's lifecycle was examined in the form of samples of tissue taken from Micropterus dolomieu, which ranged in age from age-0 eggs and embryos to age-8 adult fish. Both $\delta^{15}N$ and $\delta^{13}C$ values were obtained in search of a changing relationship between the age/weight of the fish and changing feeding habits, also changes in the trophic level of the fish as it grows and matures.

Trophic levels within Lake Opeongo were briefly examined. Chapter 6 shows that Opeongo is a second order lake with the adult *Micropterus dolomieu* being one of the top-level feeders within the lake.

There appears to be a direct relationship between the $\delta^{15}N$ of *Micropterus dolomieu* tissues and the life-stage of the animal. The eggs are highly enriched in ^{15}N compared to all other stages in the fishes life, even compared to the general population of adults and their own parents. Immediately after hatching, however, there is a rapid depletion in the ^{15}N content of their tissues as the highly enriched yolk sacs are used up for nutrients. From this point on the $\delta^{15}N$ of the young *Micropterus dolomieu* reflects each individual's feeding habits. The very young feed on plankton (primary producers) and so are considered to be first level trophic feeders, while the young adults change their feeding habits to include crayfish and other small organisms making them second level

trophic feeders. Even older, larger fish tend to feed on a mixture of crayfish, cisco, and even young of the year *Micropterus dolomieu*, hereby, elevating them to the highest level feeders in Lake Opeongo. The age-dependent increase in $\delta^{15}N$ levels off after an age of about 5 years. This is believed to be due to the blending into the fish's diet of fish and invertebrate fauna of wide-ranging trophic levels (crayfish to young of the year *Micropterus dolomieu*) as seen in figure 7.3. This leveling off was not expected, but is likely due to the fact that these adult fish are already at the upper limit of the trophic levels in Lake Opeongo.

Any relationship between $\delta^{13}C$ of the *Micropterus dolomieu* tissues and the life-stage of the animal was more difficult to observe. Most of the changes that were found in the $\delta^{13}C$ values of the tissues occurred within the first year of the fish's life. The eggs were found to be depleted in $\delta^{13}C$ relative to the $\delta^{13}C$ of the adults and the general population of *Micropterus dolomieu* in Lake Opeongo. There is a gradual enrichment within the first year of life but then the $\delta^{13}C$ values leveled off and little change was seen to occur after the first year of life. This is probably because the *Micropterus dolomieu* in Lake Opeongo only undergo an increase of two trophic levels throughout their lifetime. Perhaps larger differences could be seen if a higher level predator was analyzed or if the *Micropterus dolomieu* was in a lake with more trophic potential. The $\delta^{13}C$ values found within a population of this single species was found to reflect the location in the lake where the fish was captured and the lipid content of the tissues, more than reflecting trophic level changes throughout the lifecycle of the fish. The proposed trophic level effect of 0.5 – 1.0 ‰ per trophic level seems to be overridden by a large inter-site difference in $\delta^{13}C$. Also there seems to be a decrease in the $\delta^{13}C$ values (figure 8.6) with

the weight and age of the adult fish implying that there may be a physiological change in the composition of the fish with increasing age. This physiological change may represent an increase in the amount of lipids in the adult fish as they gain weight and/or become older.

Any studies that wish to correlate trophic level with the age/weight of a fish (which changes it diet throughout its lifetime) should use $\delta^{15}N$ analysis of the tissues, while using $\delta^{13}C$ values to determine variations in the carbon sources throughout the lake.

An examination of the isotopic relationship between mother and egg gave some conflicting results with the rest of the data in this study. The $\delta^{13}C$ of the egg was depleted relative to the mother in both the case of *Micropterus dolomieu* and *Oncorhynchus mykiss*, the $\delta^{15}N$ values of the mothers were not as high as those of the eggs. An examination of the *Micropterus dolomieu* in Lake Opeongo (chapter 7) showed that the eggs are highly enriched relative to the general *Micropterus dolomieu* population. However, chapter 9 suggests that the mother is more enriched than the eggs. The one difference being that all the *Micropterus dolomieu* egg specimens were fertilized and taken from the water of Lake Opeongo while the *Oncorhynchus mykiss* egg specimens were not fertilized and taken out from the mother before they were laid.

It was also discovered that different batches of the same preservative could produce different offsets in both the $\delta^{15}N$ and the $\delta^{13}C$ values. This was examined in detail, in chapter 5 and the differences between the formalin used in 1992 by Friesen and the formalin used in 1999 by this study was determined. Correction factors of -1.5 ± 0.6 ‰ and $+1.0 \pm 1.0$ ‰ were applied to Friesen's $\delta^{15}N$ and $\delta^{13}C$ results, respectively, and correction factors of -0.6 ± 0.6 ‰ and $+2.8 \pm 0.7$ ‰ were applied to the

1999 results, respectively. These correction factors were necessary in order to correlate them with the $\delta^{15}N$ and $\delta^{13}C$ results that would be obtained from fresh tissues.

10.2 RELEVANCE

Many ongoing studies and studies in the past have built food web structures based on isotope information. For example a species was positioned in a food web, which was being developed, from the average $\delta^{15}N$ and $\delta^{13}C$ values of several individuals sampled from that species. This study shows that this is not an accurate way to describe a species that changes its diet throughout its lifetime. Before such assumptions can be made it is necessary to define any isotopic changes that occur within the lifecycle of each individual species within the food web. Species that do not change their diets throughout their entire lifetime will most likely have relatively consistent $\delta^{15}N$ values throughout each individual's lifetime. However, a species such as *Micropterus dolomieu*, which does change its feeding habits will also display large, but predictable, shifts in their $\delta^{15}N$ isotopic values. In the aquatic environment, the proposed trophic level effect of 0.5 – 1.0 ‰ per level, of the $\delta^{13}C$ values of the tissues of an individual, seem to be overridden by large inter-site differences in $\delta^{13}C$.

11. SUGGESTIONS FOR FUTURE STUDIES IN THIS AREA

11.1 TOPICS

Looking back through the results obtained here it became obvious that there may be some repercussions on the future use of isotopes in the reconstruction of food webs and trophic level studies:

1. Since distinctive patterns were seen in the $\delta^{13}C$ and $\delta^{15}N$ ratios in correlation with the age of the fish, it is necessary to take this into account when producing food webs from isotope data. Trophic level studies based on isotope data should take such patterns into account in the future. While the $\delta^{15}N$ ratios seem to directly reflect the age and/or weight of the fish, the $\delta^{13}C$ values change rapidly during the first year of life (in *Micropterus dolomieu*) and level off immediately. Any spatial patterns in the $\delta^{13}C$ sources should also be examined in order to make the patterns clearer.

2. While several previous studies have encountered the relationship between the $\delta^{15}N$ of the tissues and the changes that it undergoes throughout the lifecycle of fishes that change their diets throughout their lifetime, the correlated relationship between $\delta^{13}C$ and the tissues has not been well documented. The relationship between $\delta^{13}C$ and the age and/or weight of fish has been largely ignored to this point because it is difficult to discern and is not evident in most previous cases. This is an area that may be developed in the future.

3. Different level lakes should be examined for similar patterns in the $\delta^{13}C$ and $\delta^{15}N$ ratios with the age of an individual species. Lake Opeongo was a second order lake (chapter 2, and figure 2.6) with Micropterus dolomieu at the top of the food web. Top predators in higher level lakes should be likewise examined to see if higher trophic positions could be observed throughout the lifecycle of an individual species.

Methods:

4. If more than one type of preservation method or even different batches of the same preservative are used throughout the experimental procedure then the isotopic effects of each preservative must be examined and the data correlated.

Some areas of this research left some unanswered questions, which could be addressed in the future:

5. The depletion in the $\delta^{13}C$ values of the eggs in relation to enriched values of the mother can be explained by the composition of the eggs themselves. The fact that the eggs are composed of greater, relative, quantities of lipids than the muscle tissues of the mother accounts for this. However, there is a discrepancy between the $\delta^{15}N$ values of the mother and her unfertilized eggs and that of the mother and the fertilized eggs. The mother is enriched over the unfertilized eggs

but depleted relative to the fertilized eggs. This phenomenon has not been addressed before and more research is necessary.

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Appendix 1

List of all the Micropterus dolomieu collected from Lake Opeongo. Including the type of site, the site number, the date sampled (1992 data), the age in days, length (mm), and weight (g) for the samples collected in 1992 by Friesen. For the 1999 samples, collected for this study the data listed here the date includes the site, the age (years), the fin length (mm), the total length (mm), and the weight (g).

Friesen's fish from Opeongo
in 1992

Type of Site	Site	Date Sampled	Age (Days)		L (mm)	W (g)
Protected	1	01-Aug	39		28	0.58
	1	01-Aug	39		27	0.706
	1	01-Aug	39		27	0.6
	1	29-Jul	36		28	0.856
	1	29-Jul	36		26	0.633
	1	29-Jul	36		25	0.616
	1	23-Jul	33		20	0.35
	1	20-Jul	30		23	0.374
	1	20-Jul	30		20	0.262
	1	20-Jul	30		18	0.206
	1	17-Jul	27		16	0.332
	1	14-Jul	24		14	0.202
	1	14-Jul	24		14	0.154
	1	14-Jul	24		12	0.111
	1	10-Jul	20		13	0.18
	1	06-Jul	16		9	0.138
	1	06-Jul	16		9	0.145
	1	06-Jul	16		9	0.149
	1	03-Jul	13		9	0.143
	1	28-Jun	8		7	0.015
	1	28-Jun	8		7	0.015
	1	24-Jun	4		7	0.015
	1	20-Jun	1		7	0.01
Exposed	2	25-Jul	30		25	0.558
	2	25-Jul	30		24	0.54
	2	25-Jul	30		22	0.401
	2	22-Jul	27		21	0.367

	2	19-Jul	24		17	0.276
	2	19-Jul	24		17	0.25
	2	19-Jul	24		17	0.343
	2	17-Jul	22		15	0.305
	2	13-Jul	18		13	0.213
	2	13-Jul	18		13	0.195
	2	13-Jul	18		13	0.17
	2	09-Jul	14		12	0.172
	2	05-Jul	10		12	0.035
	2	05-Jul	10		12	0.03
	2	05-Jul	10		12	0.031
	2	01-Jul	6		9	0.03
	2	26-Jun	1		7	0.011
	2	29-May	0 (Egg)			0.015
Exposed	3	23-Jul	30		25	0.409
	3	23-Jul	30		21	0.367
	3	23-Jul	30		18	0.307
	3	20-Jul	27		20	0.361
	3	17-Jul	24		14	0.215
	3	17-Jul	24		16	0.228
	3	17-Jul	24		14	0.2
	3	14-Jul	21		13	0.136
	3	10-Jul	17		13	0.255
	3	10-Jul	17		13	0.238
	3	10-Jul	17		13	0.24
	3	06-Jul	13		11	0.117
	3	02-Jul	9		10	0.029
	3	02-Jul	9		10	0.021
	3	28-Jun	5		6	0.016
	3	24-Jun	1		5	0.035
Protected	4	31-Jul	38		30	0.916
	4	31-Jul	38		26	0.883
	4	27-Jul	34		26	0.71
	4	23-Jul	30		24	0.386
	4	23-Jul	30		24	0.369
	4	20-Jul	27		20	0.331
	4	17-Jul	24		18	0.296
	4	17-Jul	24		18	0.261
	4	14-Jul	21		14	0.223
	4	10-Jul	17		12	0.108
	4	10-Jul	17		13	0.116
	4	06-Jul	13		11	0.14
	4	02-Jul	9		10	0.023
	4	02-Jul	9		10	0.031
	4	28-Jun	5		8	0.015
Semi-Protected	5	06-Aug	49		34	1.227

	5	06-Aug	49		30	0.996
	5	06-Aug	49		35	1.3
	5	27-Jul	39		26	0.944
	5	27-Jul	39		30	1.044
	5	27-Jul	39		25	0.668
	5	21-Jul	33		23	0.39
	5	18-Jul	30		17	0.3
	5	18-Jul	30		18	0.327
	5	18-Jul	30		18	0.33
	5	13-Jul	25		15	0.202
	5	13-Jul	25		17	0.236
	5	13-Jul	25		15	0.227
	5	09-Jul	14		15	0.171
	5	05-Jul	12		13	0.17
	5	05-Jul	12		13	0.207
	5	05-Jul	12		11	0.13
	5	01-Jul	9		13	0.149
	5	26-Jun	7		9	0.018
	5	26-Jun	7		9	0.015
	5	22-Jun	4		9	0.015
	5	18-Jun	1		7	0.03
	5	18-Jun	1		6	0.015
Semi-Protected	6	30-Jul	42		30	1.173
	6	24-Jul	35		25	0.563
	6	21-Jul	33		23	0.448
	6	18-Jul	30		21	0.377
	6	16-Jul	28		18	0.258
	6	13-Jul	25		18	0.284
	6	09-Jul	21		13	0.189
	6	05-Jul	17		15	0.233
	6	27-Jun	9		10	0.02
	6	19-Jun	1		8	0.019
	6	03-Jun	0 (Egg)			0.015
	6	03-Jun	0 (Egg)			0.015
protected	7	25-Jul	32		24	0.518
	7	25-Jul	32		28	0.6
	7	25-Jul	32		25	0.53
	7	23-Jul	30		24	0.506
	7	19-Jul	26		23	0.494
	7	17-Jul	24		16	0.255
	7	17-Jul	24		17	0.256
	7	17-Jul	24		16	0.272
	7	14-Jul	21		15	0.165
	7	10-Jul	17		13	0.03
	7	10-Jul	17		13	0.02
	7	10-Jul	17		13	0.03
	7	08-Jul	15		10	0.028

	7	08-Jul	15		10	0.023
	7	28-Jun	5		8	0.018
Scattered	embryos					
	a18	29-May				
	nb2	29-May				
	s68	29-May				
	a14	29-May				
	nr1	29-May				
	82	29-May				
	s55	29-May				
	s47	01-Jun				
	c7	01-Jun				
	534	03-Jun				
	644	03-Jun				
	a2	03-Jun				
	s57	03-Jun				
	95	03-Jun				
	s67	03-Jun				
	645	03-Jun				
	s59	03-Jun				
1999 Samples						
site			age	FL(mm)	TL (mm)	W(g)
1			1	108	114	15
1			1	103	110	10
1			3	150	161	40
1			3	148	152	30
1			3	152	157	35
1			3	145	150	40
1			3	136	14.2	30
1			3	140	147	40
1			3	103	110	40
1			4	146	155	55
1			5	230	240	90
1			5	152	160	55
1			7	193	211	230
2			0	72.5	76	5.6184
2			0	69.5	73	4.4949
2			0	72	75	5.3616
2			0	67	71	4.674
2			0	69	72	4.6253
2			2	119	125	20
2			5	175	184	85
2			6	210	215	115
2			7	250	256	225
3			1	95	99	5
3			1	108	112	15

3			1	93	97	5
3			1	108	114	15
3			2	121	126	20
3			2	136	143	35
3			4	152	163	55
3			5	205	213	115
3			6	223	229	115
3			6	230	236	180
3			7	240	253	195
4			0	67.5	71.5	4.2832
4			0	64	68	3.662
4			0	73	78	5.509
4			1	106	113	15
4			2	112	116	15
4			2	122	127	20
4			3	136	142	30
5			0	75	78.5	5.809
5			1	94	97	10
5			2	115	120	20
5			2	114	120	15
5			2	113	117	10
5			3	134	141	35
5			4	165	176	60
5			4	172	182	70
5			5	180	189	75
5			6	242	253	185
5			6	196	210	150
5			7	230	238	200
5			7	235	240	230
5			7	240	252	235
5			7	136	142	190
5			8	279	300	355
6			0	70	74	4.889
6			0	75	79	6.0832
7			0	63	67	3.5062
7			0	69	73	4.592
7			0	69.5	74	4.1326
7			2	115	122	25
7			5	165	176	75
7			7	152	160	200
8			0	71.5	75.5	5.4425
8			0	69	74	4.5373
8			0	68	73	4.8652
8			1	100	104	10

8			1	108	114	20
8			1	106	117	15
8			1	114	122	15
8			1	105	108	5
8			2	115	124	20
8			2	130	136	25
8			2	126	137	25
8			3	144	151	35
8			3	140	146	35
8			3	132	140	25
8			3	125	144	30
8			3	136	145	35
8			3	145	152	40
8			3	152	160	45
8			3	134	141	25
8			3	138	145	35
8			3	145	152	40
8			3	139	143	35
8			4	152	161	55
8			5	185	194	85
8			5	185	197	85
8			5	178	190	80
8			5	192	203	85
8			5	201	210	110
8			5	175	184	70
8			5	163	170	75
8			5	164	170	60

Appendix 2

List of all Micropterus dolomieu samples and their weights as well as the calculated $\delta^{13}C$ and $\delta^{15}N$ values and the $\delta^{13}C$ and $\delta^{15}N$ values corrected for the differences in formalin used between the 1992 and the 1999 samples.

Sample	Weight (g)	$\delta^{13}C$	Corrected $\delta^{13}C$		Number	Weight (g)	$\delta^{15}N$	Corrected $\delta^{15}N$
1 1	0.010	-27.7	-26.7		1 1	0.01	11.0	9.5
2 1	0.011	-26.8	-25.8		2 1	0.011	10.8	9.3
1 2	0.015	-26.9	-25.9		1 3 1	0.015	7.7	6.2
1 3 1	0.015	-26.0	-25.0		1 3 2	0.015	8.6	7.1
1 3 2	0.015	-25.3	-24.3		2 e	0.015	10.4	8.9
2 e	0.015	-26.6	-25.6		4 2	0.015	9.0	7.5
4 2	0.015	-25.8	-24.8		5 3 2	0.015	7.1	5.6
5 1 1	0.015	-27.7	-26.7		5 1 2	0.015	10.7	9.2
5 3 1	0.015	-26.2	-25.2		3 2	0.016	8.9	7.4
6 e 1	0.015	-28.7	-27.7		7 3 3	0.018	6.5	5.0
6 e 2	0.015	-28.8	-27.8		6 1	0.019	10.9	9.4
3 2	0.016	-26.9	-25.9		6 3	0.02	6.7	5.2
5 3 2	0.018	-26.6	-25.6		7 5 2	0.02	4.7	3.2
7 2	0.018	-22.5	-21.6		3 3 2	0.021	6.3	4.8
6 1	0.019	-27.2	-26.2		4 3 1	0.023	6.7	5.2
6 3	0.020	-25.6	-24.6		7 3 2	0.023	4.6	3.1
7 5 2	0.020	-23.6	-22.6		7 3 1	0.028	6.6	5.1
3 3 1	0.021	-26.2	-25.2		3 3 1	0.029	6.4	4.9
4 3 2	0.023	-27.2	-26.2		2 3 2	0.03	5.6	4.1
7 3 1	0.023	-21.6	-20.7		2 2	0.03	6.9	5.4
7 3 2	0.028	-24.1	-23.1		7 5 1	0.03	5.2	3.7
3 3 2	0.029	-25.8	-24.8		7 5 3	0.03	5.3	3.8
2 2	0.030	-26.7	-25.7		2 3 3	0.031	5.5	4.0
2 3 2	0.030	-26.7	-25.7		4 3 2	0.031	6.6	5.1
5 1 2	0.030	-27.0	-26.0		2 3 1	0.035	5.7	4.2
7 5 1	0.030	-23.2	-22.2		3 1	0.035	9.2	7.7
7 5 3	0.030	-23.5	-22.5		4 5 1	0.108	5.2	3.7
2 3 1	0.031	-27.5	-26.5		1 7 3	0.111	4.9	3.4
4 3 1	0.031	-25.5	-24.5		4 5 2	0.116	5.1	3.6
2 3 3	0.035	-26.5	-25.5		3 4	0.117	5.0	3.5
3 1	0.035	-27.7	-26.7		3 6	0.136	3.9	2.4
4 5 2	0.108	-26.8	-25.8		1 5 1	0.138	5.2	3.7
1 7 1	0.111	-24.7	-23.7		4 4	0.14	5.3	3.8
4 5 1	0.116	-27.9	-26.9		1 4	0.143	5.3	3.8

3 4	0.117	-25.7	-24.7	1 5 2	0.145	5.2	3.7
5 5 1	0.130	-26.2	-25.2	1 5 3	0.149	5.3	3.8
3 6	0.136	-24.4	-23.4	5 4	0.149	4.4	2.9
1 5 3	0.138	-26.6	-25.6	1 7 2	0.154	4.9	3.4
4 4	0.140	-25.2	-24.3	7 6	0.165	5.1	3.6
1 4	0.143	-27.0	-26.0	2 5 3	0.17	4.4	2.9
1 5 2	0.145	-25.6	-24.6	2 4	0.172	4.7	3.2
1 5 1	0.149	-27.0	-26.0	1 6	0.18	5.0	3.5
5 4	0.149	-26.7	-25.8	6 6	0.189	4.1	2.6
1 7 2	0.154	-24.4	-23.4	2 5 2	0.195	4.9	3.4
7 6	0.165	-23.4	-22.4	3 7 3	0.2	4.0	2.5
2 5 1	0.170	-26.0	-25.0	1 7 1	0.202	5.3	3.8
5 5 3	0.170	-25.6	-24.6	5 7 1	0.202	4.5	3.0
5 6	0.171	-25.4	-24.4	1 9 3	0.206	4.1	2.6
2 4	0.172	-26.6	-25.7	2 5 1	0.213	4.4	2.9
1 6	0.180	-24.9	-23.9	3 7 1	0.215	4.2	2.7
6 6	0.189	-25.4	-24.4	4 6	0.223	4.7	3.2
2 5 2	0.195	-25.2	-24.2	5 7 3	0.227	4.2	2.7
3 7 1	0.200	-24.1	-23.1	3 7 2	0.228	4.1	2.6
1 7 3	0.202	-25.3	-24.3	5 7 2	0.236	4.3	2.8
5 7 3	0.202	-24.8	-23.8	3 5 2	0.238	4.5	3.0
1 9 1	0.206	-25.2	-24.2	3 5 3	0.24	4.8	3.3
5 5 2	0.207	-25.8	-24.8	2 7 2	0.25	4.1	2.6
2 5 3	0.213	-26.3	-25.3	3 5 1	0.255	4.6	3.1
3 7 3	0.215	-23.9	-22.9	7 7 1	0.255	4.9	3.4
4 6	0.223	-24.7	-23.7	7 7 2	0.256	4.6	3.1
5 7 1	0.227	-25.2	-24.2	6 8	0.258	4.7	3.2
3 7 2	0.228	-24.6	-23.6	4 7 2	0.261	4.3	2.8
6 5	0.233	-26.1	-25.1	1 9 2	0.262	4.6	3.1
5 7 2	0.236	-24.5	-23.5	7 7 3	0.272	4.9	3.4
3 5 2	0.238	-24.0	-23.0	2 7 1	0.276	4.0	2.5
3 5 1	0.240	-22.7	-21.8	6 7	0.284	3.6	2.1
2 7 2	0.250	-24.8	-23.8	4 7 1	0.296	4.7	3.2
3 5 3	0.255	-21.3	-20.3	5 9 1	0.3	4.3	2.8
7 7 3	0.255	-24.9	-23.9	2 6	0.305	3.8	2.3
7 7 2	0.256	-24.8	-23.8	3 9 3	0.307	3.8	2.3
6 8	0.258	-26.1	-25.1	5 9 2	0.327	4.5	3.0
4 7 1	0.261	-25.7	-24.7	5 9 3	0.33	4.4	2.9
1 9 2	0.262	-25.6	-24.6	4 8	0.331	4.7	3.2
7 7 1	0.272	-24.8	-23.8	1 8	0.332	4.5	3.0
2 7 3	0.276	-25.0	-24.0	2 7 3	0.343	4.0	2.5
6 7	0.284	-26.4	-25.5	1 10	0.35	4.5	3.0
4 7 2	0.296	-26.3	-25.3	3 8	0.361	4.1	2.6
5 9 3	0.300	-26.4	-25.4	2 8	0.367	4.5	3.0
2 6	0.305	-25.6	-24.6	3 9 2	0.367	4.6	3.1
3 9 1	0.307	-23.6	-22.6	4 9 2	0.369	5.0	3.5
5 9 2	0.327	-25.9	-24.9	1 9 1	0.374	4.3	2.8
5 9 1	0.330	-25.4	-24.4	6 9	0.377	4.1	2.6
4 8	0.331	-25.8	-24.8	4 9 1	0.386	5.0	3.5

1 8	0.332	-24.8	-23.8	5 10	0.39	4.6	3.1
2 7 1	0.343	-24.9	-23.9	2 9 3	0.401	4.7	3.2
1 10	0.350	-24.2	-23.2	3 9 1	0.409	3.9	2.4
3 8	0.361	-24.4	-23.4	6 10	0.448	5.2	3.7
2 8	0.367	-24.9	-23.9	7 8	0.494	4.9	3.4
3 9 2	0.367	-24.4	-23.4	7 9	0.506	4.9	3.4
4 9 1	0.369	-25.1	-24.1	7 10 1	0.518	4.7	3.2
1 9 3	0.374	-25.5	-24.5	7 10 3	0.53	4.8	3.3
6 9	0.377	-24.2	-23.2	2 9 2	0.54	4.5	3.0
4 9 2	0.386	-25.1	-24.1	2 9 1	0.558	4.6	3.1
5 10	0.390	-24.9	-23.9	6 11	0.563	4.2	2.7
2 9 1	0.401	-24.9	-23.9	1 12 1	0.58	4.4	2.9
3 9 3	0.409	-23.7	-22.7	1 12 3	0.6	4.7	3.2
6 10	0.448	-26.1	-25.1	7 10 2	0.6	4.2	2.7
7 8	0.494	-25.4	-24.4	1 11 3	0.616	4.6	3.1
7 9	0.506	-24.7	-23.7	1 11 2	0.633	4.9	3.4
7 10 3	0.518	-24.3	-23.3	5 12 3	0.668	4.4	2.9
7 10 1	0.530	-24.4	-23.4	1 12 2	0.706	4.8	3.3
2 9 2	0.540	-25.3	-24.3	4 10	0.71	4.7	3.2
2 9 3	0.558	-23.8	-22.8	1 11 1	0.856	4.8	3.3
6 11	0.563	-24.6	-23.6	4 11 2	0.883	4.8	3.3
1 12 3	0.580	-25.3	-24.3	4 11 1	0.916	5.0	3.5
1 12 1	0.600	-25.0	-24.0	5 12 1	0.944	4.7	3.2
7 10 2	0.600	-24.3	-23.3	5 12 2	1.044	4.1	2.6
1 11 1	0.616	-25.6	-24.6	6 12	1.173	4.8	3.3
1 11 2	0.633	-25.9	-24.9	5 12 2 1	1.227	5.1	3.6
5 12 1	0.668	-24.4	-23.4	5 12 2 3	1.3	4.9	3.4
1 12 2	0.706	-24.8	-23.8	86	3.5062	6.3	5.7
4 10	0.710	-24.3	-23.3	84	3.662	7.3	6.7
1 11 3	0.856	-25.9	-24.9	88	4.1326	5.4	4.8
4 11 1	0.883	-24.7	-23.7	26	4.2832	7.0	6.4
4 11 2	0.916	-24.8	-23.8	31	4.4949	6.3	5.7
5 12 3	0.944	-25.0	-24.0	82	4.5373	6.6	6.0
5 (12)2 2	0.996	-25.7	-24.7	87	4.592	6.7	6.1
5 12 2	1.044	-23.3	-22.3	34	4.6253	6.3	5.7
6 12	1.173	-26.0	-25.0	33	4.674	6.8	6.2
5 (12)2 3	1.227	-25.9	-24.9	83	4.8652	6.9	6.3
5 (12)2 1	1.300	-25.5	-24.5	27	4.889	7.9	7.3
86	3.506	-21.0	-18.2	19	5	7.0	6.4
84	3.662	-22.8	-20.0	24	5	7.0	6.4
88	4.1326	-25.0	-22.2	80	5	7.7	7.1
26	4.2832	-24.7	-21.9	32	5.3616	5.4	4.8
31	4.4949	-24.6	-21.8	81	5.4425	7.5	6.9
82	4.5373	-23.2	-20.4	85	5.509	6.6	6.0
87	4.592	-22.5	-19.7	30	5.6184	7.4	6.8
34	4.6253	-24.1	-21.3	29	5.809	8.1	7.5
33	4.674	-24.0	-21.2	28	6.0832	5.8	5.2
83	4.8652	-22.9	-20.1	17	10	6.4	5.8
27	4.889	-24.6	-21.8	20	10	7.4	6.8

19	5	-22.9	-20.1		50	10	7.8	7.2
24	5	-22.4	-19.6		69	10	6.5	5.9
80	5	-21.6	-18.8		6	15	6.6	6.0
32	5.3616	-22.5	-19.7		12	15	6.6	6.0
81	5.4425	-24.9	-22.1		13	15	7.4	6.8
85	5.509	-24.0	-21.2		22	15	6.9	6.3
30	5.6184	-23.8	-21.0		48	15	7.9	7.3
29	5.809	-25.2	-22.4		77	15	6.7	6.1
28	6.0832	-21.8	-19.0		79	15	7.1	6.5
17	10	-23.1	-20.3		107	15	6.9	6.3
20	10	-25.3	-22.5		10	20	7.5	6.9
50	10	-27.2	-24.4		21	20	8.0	7.4
69	10	-23.6	-20.8		23	20	7.5	6.9
6	15	-22.3	-19.5		47	20	7.4	6.8
12	15	-23.6	-20.8		64	20	8.2	7.6
13	15	-23.6	-20.8		76	20	7.1	6.5
22	15	-26.8	-24.0		45	20	6.9	6.3
48	15	-24.4	-21.6		62	25	8.0	7.4
77	15	-22.7	-19.9		65	25	8.1	7.5
79	15	-22.8	-20.0		71	25	7.2	6.6
107	15	-27.1	-24.3		78	25	7.5	6.9
10	20	-24.4	-21.6		2	25	7.9	7.3
21	20	-25.3	-22.5		14	30	7.0	6.4
23	20	-25.4	-22.6		16	30	7.8	7.2
47	20	-24.0	-21.2		63	30	7.7	7.1
64	20	-22.3	-19.5		4	30	7.7	7.1
76	20	-22.7	-19.9		25	35	6.4	5.8
45	25	-22.6	-19.8		49	35	7.3	6.7
62	25	-23.9	-21.1		53	35	7.8	7.2
65	25	-21.6	-18.8		54	35	7.6	7.0
71	25	-24.0	-21.2		67	35	7.6	7.0
78	25	-22.4	-19.6		73	35	7.5	6.9
2	30	-25.1	-22.3		75	35	7.9	7.3
14	30	-22.8	-20.0		1	35	7.8	7.2
16	30	-23.8	-21.0		7	40	7.0	6.4
63	30	-22.5	-19.7		44	40	8.0	7.4
4	35	-23.0	-20.2		68	40	7.9	7.3
25	35	-26.2	-23.4		74	40	7.3	6.7
49	35	-24.8	-22.0		93	40	7.7	7.1
53	35	-23.6	-20.8		70	40	7.3	6.7
54	35	-23.3	-20.5		3	45	7.5	6.9
67	35	-23.9	-21.1		15	55	7.5	6.9
73	35	-25.0	-22.2		66	55	8.0	7.4
75	35	-24.2	-21.4		102	55	8.3	7.7
1	40	-21.9	-19.1		51	55	8.0	7.4
7	40	-26.2	-23.4		72	60	8.4	7.8
44	40	-23.7	-20.9		60	60	7.3	6.7
68	40	-23.1	-20.3		101	70	7.5	6.9
74	40	-24.5	-21.7		39	70	7.8	7.2

93	40	-22.5	-19.7		46	75	6.9	6.3
70	45	-22.5	-19.7		61	75	8.0	7.4
3	55	-23.3	-20.5		57	75	8.0	7.4
15	55	-21.9	-19.1		9	80	8.2	7.6
66	55	-22.2	-19.4		55	85	7.9	7.3
102	55	-25.1	-22.3		56	85	8.1	7.5
51	60	-26.5	-23.7		58	85	8.4	7.8
72	60	-22.9	-20.1		5	85	8.3	7.7
60	70	-22.2	-19.4		59	90	7.5	6.9
101	70	-26.0	-23.2		11	110	8.0	7.4
39	75	-24.6	-21.8		41	115	7.7	7.1
46	75	-22.4	-19.6		42	115	8.3	7.7
61	75	-21.7	-18.9		52	115	8.5	7.9
57	80	-23.0	-20.2		43	150	7.9	7.3
9	85	-27.5	-24.7		37	180	7.9	7.3
55	85	-27.8	-25.0		92	185	8.3	7.7
56	85	-21.4	-18.6		40	190	8.9	8.3
58	85	-23.4	-20.6		35	195	7.6	7.0
5	90	-23.3	-20.5		91	200	8.5	7.9
59	110	-24.2	-21.4		8	200	9.0	8.4
11	115	-24.5	-21.7		36	225	8.3	7.7
41	115	-25.1	-22.3		89	230	8.5	7.9
42	115	-27.3	-24.5		38	230	8.2	7.6
52	150	-25.4	-22.6		90	235	8.5	7.9
43	180	-26.2	-23.4			355	8.5	7.9
37	185	-23.4	-20.6					
92	190	-21.9	-19.1					
40	195	-26.5	-23.7					
35	200	-24.8	-22.0					
91	200	-23.5	-20.7					
8	225	-25.1	-22.3					
36	230	-24.3	-21.5					
89	230	-22.7	-19.9					
38	235	-25.2	-22.4					
90	355	-24.2	-21.4					

Appendix 3

Results from the Anova preformed on the 1992 $\delta^{13}C$ data to determine the relevance of the inter-site differences in the starting $\delta^{13}C$ values.

Anova: Two Way Without Replication				
Summary	Count	Sum	Average	Variance
Family 1	50	-296.6	-24.7	1.1
Family 2	50	-249.7	-25.0	0.8
Family 3	50	-216.9	-24.1	2.5
Family 4	50	-245.5	-24.5	0.8
Family 5	50	-247.4	-24.7	0.9
Family 6	50	-250.5	-25.1	1.6
Family 7	50	-208.6	-23.2	1.4
Analysis of Variance				
Source of Variation	SS	df	MS	F
Rows	12756.0	49	260.3	2.9
Columns	37914.1	7	5416.3	59.4
Error	31286.0	343	91.2	
Total	81956.2	399		