Diet relations of juvenile Red-bellied Turtles using stable isotope analytical techniques.

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Abstract

Stable Isotope Analysis was used in a dietary study of juvenile Red-bellied Turtles at the Jug Bay Wetlands Sanctuary in Lothian, Maryland during the summer of 2001. These turtles were hoop trapped or caught by hand from a beaver pond within the sanctuary property. Tissue samples were taken from the juveniles along with samples of other organisms from the pond. In addition, samples were taken of adult Red-bellied females found nesting on the property. Samples were freeze dried and run at the Geophysical Lab of the Carnegie Institution of Washington, D.C. under the guidance of Dr. Marilyn L. Fogel. In addition, fecal samples were used in resolution of dietary items. Stable Isotope Analysis revealed a difference in nitrogen values between juvenile turtles found in the beaver pond and adult turtles from the river, consistent with the finding that the beaver pond in general was lower in nitrogen values from the river. Isotope analysis was not able to specifically clarify diet, however in conjunction with fecal analysis algae was found to be a major dietary item in juvenile turtles. Hydrilla and aquatic insects were also found to be good candidates for important dietary items in juvenile Red-bellied Turtles.

Introduction

Traditional approaches to dietary analysis in vertebrates, such as direct observation, fecal analysis, stomach flushing, and examination of gut contents can be difficult, highly invasive, or even lethal to the organism being studied A fairly new and relatively low impact alternative to traditional dietary studies is found in analysis of stable isotope ratios in living tissues. This technique involves taking small tissue samples from the living organism being studied, examining the isotopic ratios of keystone elements such as carbon, nitrogen, oxygen, or phosphorous, and comparing these ratios to suspected food items. This technique is relatively benign since it only requires such things as blood, a bit of claw, or other tissues be collected for analysis. In most cases, the animal being studied is released relatively unharmed (Drever et al, 2000, Gu et al, 1996, Hobson et al, 2000, Roth and Hobson, 2000, Peterson and Fry, 1987, Teece and Fogel).

Typically, in dietary studies ratios of the stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are examined to determine an organism's position in the food web. These ratios of stable isotopes will be different from the prevailing environmental ratios due to the fact that molecules of different weights react at slightly different rates in chemical reactions; lighter molecules typically react faster than heavier ones. Photosynthetic organisms at the level of primary productivity fix carbon isotopes at a rate determined by the photosynthetic pathways being used by that particular plant (for example, C3 vs. C4 pathways in terrestrial plants, each establishing a significantly different ratio of stable isotopes of carbon), and the ratio established changes very little as the carbon stored in plant tissues is ingested by herbivores and moves up through trophic levels. Thus, carbon can be traced through a food web from the plant that fixed it, to the herbivore that later ingests it, and in to the predators that in turn prey on the herbivores. Nitrogen is similarly useful in that it can be used to pinpoint an organism's trophic position within an ecosystem due to the fact that the ratio of $^{15}N/^{14}N$ grows heavier (more enriched with ^{15}N) at a predictable rate with each increase in trophic level. This increase is mainly caused by the chemically favorable excretion of ^{14}N in urine (Drever et al,

2000, Gu et al, 1996, Hobson et al, 2000, Roth and Hobson, 2000, Peterson and Fry, 1987).

Experimentally derived isotope ratios are expressed as parts per thousand as compared to a standard, by the formula $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$. The standard reference material used for carbon ratios is PeeDee Belemnite limestone, and atmospheric N^2 is used as a standard for nitrogen (Drever et al, 2000, Gu et al, 1996, Hobson et al, 2000, Roth and Hobson, 2000, Peterson and Fry, 1987).

Stable isotope analysis is a technique used to follow carbon and nitrogen sources through a food web. However, individual molecules are not directly marked and followed; rather ratios of the organism of interest are compared to all other relevant aspects of the food web. This requires extensive sampling of organisms from all trophic levels in the ecosystem to which the study animal belongs. By establishing a baseline value for carbon and nitrogen isotopes in an ecosystem through this sampling, carbon and nitrogen can essentially be traced through a food web from the level of primary productivity to the organism of interest (Peterson and Fry, 1987, Teece and Fogel).

Not only is stable isotope analysis useful in positioning an organism in a food web, but since it reflects all assimilated sources of carbon and nitrogen, it circumvents some of the weaknesses of traditional dietary studies. For example, underrepresented food items in fecal samples due to differential rates of digestion. It also reveals integrated diet rather than short-term diet provided by traditional analysis, and can give clues to what an animal has been eating from the recent past to its entire lifetime (Drever et al, 2000, Gu et al, 1996, Hobson et al, 2000, Roth and Hobson, 2000, Peterson and Fry, 1987). Assimilated carbon and nitrogen remains in tissues for relatively long periods of time, depending on the specific tissue types sampled. For example, ratios of stable isotopes in muscle reflects diet of the previous 1-2 months, ratios in liver tissue reflects diet of the previous week, and ratios in tissues such as fur, feathers, skin, and nails, which are not replaced as rapidly as muscle or liver can reflect diet for relatively longer periods of time, up to an entire lifetime (Drever et al, 2000, Gu et al, 1996, Hobson et al, 2000, Roth and Hobson, 2000, Peterson and Fry, 1987).

The Red-bellied Turtle (*Pseudemys rubriventris rubriventris*) is a large, secretive freshwater turtle of the eastern United States. It has a relatively limited range along the eastern seaboard states, from central New Jersey to northeastern North Carolina (Figure 3) (Ernst et. al., 1994). Very little is known about the life history of this species, with the exception of the endangered Plymouth Red-bellied Turtle (*Pseudemys rubriventris bangsi*) on which a head-starting project has been conducted by the Massachusetts Division of Fisheries and Wildlife (Haskell et. al, 1996). The diet of this species is poorly known, and most analysis has been conducted through examination of fecal materials. This species is assumed to be primarily herbivorous, but its secretive habits and affinity for deepwater habitats have made direct observation of feeding nearly impossible. It also avoids hoop traps baited with traditional baits, and is thus difficult to capture (Ernst et al, 1994). However, large numbers of female Red-bellied Turtles do come out onto land from late May to mid July to nest and are thus available for study (Ernst et al, 1994, Swarth, 1998). Taking advantage of this availability, a study of the diet of Red-bellied Turtles is being conducted at the Jug Bay Wetlands Sanctuary in Lothian, Anne Arundel County Maryland as part of continuing studies of the life history of this relatively rare animal. The sanctuary is located along the tidal Patuxent River,

which contains a large population of these turtles. Stable isotope analysis is being used in this study, in conjunction with Marilyn L. Fogel of the geophysical lab at the Carnegie Institution of Washington, D.C.

As part of this dietary study, I have attempted to analyze the diet of the juveniles of this species using stable isotopes, in particular examining differences between juvenile and adult Red-bellied Turtles. More than likely juveniles demonstrate differences in diet from the adults of this species; a common feature of reptiles is that they utilize dietary shifts during growth and development, since growing reptiles have different nutrient needs than adults. The River Cooter (*Pseudemys concinna*), a member of the same genus, is known to demonstrate such a shift, with adults being almost entirely herbivorous versus juveniles that are known to take a wide variety of animal foods as well as plant foods (Ernst et al, 1994, Lagueux et al, 1995). The Red-bellied Turtle itself is thought to follow a similar pattern.

The following patterns are expected to be observed in this study. First, a significant difference in the important isotopic ratios between juvenile and adult Red-bellied Turtles is expected to be found due to the different nutrient needs required by juvenile and adult turtles. Second, juveniles found in the beaver pond are expected to exhibit a greater degree of omnivory than adult turtles, perhaps due to a greater need for animal proteins used in growth. Finally, the important dietary items for juvenile turtles are expected to be pinpointed through isotopic analysis.

Study Site and Methods

Juvenile Red-bellied Turtles were collected from a beaver pond within the wetlands sanctuary (Figure 2). The pond is located at the mouth of, and fed by Two Run Creek, a small stream that passes through the sanctuary and empties into the Patuxent River. Six hoop traps (collapsible single funnel traps, with 2.5 cm square mesh, and 76 cm diameter hoops) were placed throughout the pond (Figure 1) and baited with a variety of fish as well as bags of corn that had been allowed to ferment for two or three days. Fish baits were changed every trapping session, but the fermented corn was not changed. Traps were run eight times from the 16th of June to the 13th of July, 2001. These traps were typically set in the early afternoon and allowed to remain open overnight, roughly twelve hours total. Three of these hoop traps were moved to areas that seemed would have higher yields during the duration of the study due to the fact that these traps were catching no turtles at all in their original positions. All turtles captured were recorded, but any Red-bellied Turtle that was caught was brought back to the lab for processing and sampling. Sampling of the turtles was done by removing a bit of shell, usually from the rear marginals, with a pair of dissecting scissors. Care was taken so that the turtles were not harmed during this process, although a bit of slight bleeding was to be expected. In addition, a bit of claw (roughly 2-5 mm) was removed from turtles whose claws were long enough to be sampled without causing bleeding. In addition, shell algal growth and feces were collected from those turtles that provided each. Also, extensive sampling of emergent plants, S.A.V.'s (submerged aquatic vegetation), and aquatic organisms including vertebrates and invertebrates was also conducted. Three to five samples of each species were collected when possible. Although by no means were all potential prey items in this pond collected, nonetheless a very broad variety of the plants and animals of the pond was collected for analysis.

Stable isotope analysis was conducted at the Carnegie Geophysical lab in Washington, D.C under the supervision of Marilyn Fogel. Samples were meticulously weighed into small tin boats, 200-500 mg of each for animal samples and 500-700 mg of each for plant samples. These boats were loaded into a machine used for isotope analysis, which consists of an elemental analyzer for processing, interfaced with a stable isotope mass spectrometer for analysis of isotope ratios. The analysis is entirely automated, and a printout containing the important information for each sample was obtained.

A large amount of data was obtained by the end of this project, and analysis of the data proved to be complicated. Since each potential prey item usually had three to five samples run, the isotope ratios for ^{15}N and ^{13}C were averaged, and a standard deviation of each was calculated. Separate graphs of $\delta^{15}N$ and $\delta^{13}C$ were generated, each including the average isotope ratio of each sample, along with its standard deviation. Regions of these graphs that fell into the expected dietary range of the turtles (3-5 per mil lighter for $\delta^{15}N$ and 1-2 per mil lighter for $\delta^{13}C$) was highlighted, and those species whose means or standard deviations fell into this range on both graphs were considered as potential diet items. Also, direct observations from fecal analysis and observations of feeding behaviors were also used in weighing the importance of items in the diet of these turtles.

Results

Capture Results - Five juvenile Red-bellied Turtles were collected for analysis; three were caught in hoop traps and two out of pure luck were captured by hand. Trapping yields of Red-bellied Turtles were very low for both juveniles and adults. In these eight trappings, only three juvenile and two adult Red-bellied Turtles were captured. In contrast, 39 Painted Turtles (*Chrysemys picta picta*), 13 Musk Turtles (*Sternotherus oderatus*), 3 Mud Turtles (*Kinosternon subrubrum*), and one very large Common Snapping Turtle (*Chelydra serpentina*) were also captured during the duration of the trapping. Based on the low yield of Red-bellied Turtles it is uncertain whether either of the baits used in the traps were effective at all in attracting these turtles. The turtles may have simply randomly stumbled into the traps, or perhaps they may have been attracted by the movements of the other species of turtles caught in the traps. Age range in the turtles was difficult to judge, so size range was considered instead. Juveniles captured ranged from a minimum plastron length of 45.40 mm and 23 g to a maximum of 166 mm and 756.4 g. These turtles were clearly still juveniles; the development of secondary sex characteristics in male turtles does not occur until a plastron length of 220 mm (roughly 11 years) and full-grown females can grow longer than 350 mm and weigh 4-5 kg (Ernst et al, 1994, Swarth, 1998).

Isotopic Analysis – A summary species sampled, mean isotopic values and standard deviations, and the number of samples of each species can be found in Tables 1 and 2. Isotopic analysis revealed a number of interesting trends among the turtles analyzed. First, there was a marked difference in the nitrogen isotope ratios between adult turtles and juvenile turtles in the beaver pond, with beaver pond juveniles having a lower mean isotope ratio than adult turtles (Figure 4). Carbon isotope ratios were relatively close between adults and juveniles from both locations (Figure 4). A single, fresh juvenile turtle found dead in the river was much closer to the adult turtles in its

nitrogen isotope signal, but was nearly 4 per mil lighter in its carbon isotope signature (Figure 4). Hatchlings and egg fragments analyzed were very close to the adults in both nitrogen and carbon ratios (Figure 4).

An interesting trend emerged in the five juvenile turtles analyzed. The smallest turtle analyzed, probably a hatchling from the previous fall or spring, had an isotope signature much closer to the hatchlings and adult river turtles, whereas the largest of the juveniles captured had an isotope signature considerably lower in nitrogen. This seemed to be a general trend among all juveniles sampled, with decreasing nitrogen values with increasing size, although it could not be statistically tested or confirmed due to small sample size.

Fecal Analysis - Fecal materials provided by two juvenile turtles was examined and found to be composed primarily of filamentous algae. In addition, leafy materials assumed to be leaves from S.A.V.'s, and various unidentified insect parts were found in fecal samples.

Dietary Analysis - Dietary analysis proved not to be as clear as what was originally hoped for, but a few interesting patterns were observed. Graphical analysis of the potential diet items revealed that only two samples, algae and Hydrilla (*Hydrilla verticillata*), fit into the expected diet range for both nitrogen and carbon (Figures 5 and 6). Neither of these items was a very good fit; algae only made it into the expected range for carbon due to a large deviation in measured samples. However, fecal samples were analyzed and demonstrated carbon and nitrogen values very close to the algae samples collected from the beaver pond.

Since none of the species that fell into the dietary range for carbon and nitrogen were a very good fit, the turtles are most likely feeding from multiple food sources. Because of this, the specifics of the diets of these animals cannot be confirmed without analysis with a third isotope for greater resolution. However, it was revealed that these animals could not be feeding on plants alone without demonstrating a higher $\delta^{15}N$ value. Since inspection of fecal materials revealed various insect exoskeletal remains, most likely the animals are consuming algae as a major food source supplemented by various insects ingested either intentionally or incidentally. Also, it is likely based on fecal analysis that the turtles are also ingesting some of the S.A.V.'s found in and around the algae mats. Mathematically, a mixed model can be constructed containing algae and some of the various aquatic insects, especially water striders and water boatmen that live among the algae mats, that fits the isotope ratios demonstrated by the turtles.

Discussion

As stated, isotopic analysis revealed some interesting trends among juvenile turtles. First, juveniles had a lower mean nitrogen ratio than river turtles. In fact, for all species found in both the beaver pond and the river, nitrogen values were considerably lower for the beaver pond. The heavier nitrogen values found in the river are probably due to nutrient loading from sewage treatment plants and also agricultural runoff, such as animal waste, from various farms along the Patuxent River. Animal waste products tend to be more enriched in heavy nitrogen, ^{15}N. The beaver pond, also a nutrient loaded system, is fed by Two Run Creek, a small freshwater creek that runs through the sanctuary and also through neighboring crop fields. The lower nitrogen values for this

system can probably be attributed to fertilizer runoff from these farm fields since common fertilizers utilize urea as a primary nitrogen source, and are much more enriched in the lighter nitrogen isotope (^{14}N). In general, it has been found that Two Run Creek, which feeds the beaver pond, has a lower concentration of nitrates and ammonia than the river, but the relationships in nitrogen isotopes are just now being revealed (Swarth and Peters, 1993). The fact that the juvenile Red-bellied Turtles caught demonstrated lighter ratios of nitrogen suggests that they live entirely in the beaver pond. Interestingly, two adult Red-bellied females were caught during the hoop trapping sessions, and analysis revealed they had a signature much closer to the river turtles. This suggests that there is movement between the beaver pond and the river by adult turtles. Personal observation suggests that there are actually very few adult Red-bellied Turtles inhabiting the beaver pond at any one time. Movement between the beaver pond and the river may somehow be related to nesting as there is a large warm season grass meadow just beyond the edge of the beaver pond, and adult females have been observed coming from the pond to this meadow to nest. The majority of hoop trappings for this study were conducted during the nesting season as well. This habitat segregation by juvenile turtles suggests that something about the beaver pond offers these turtles an advantage over living in the river during this stage of their lives. Some advantages offered by the beaver pond may be greater numbers of basking sites as compared to the river, less competition with adults for food or basking sites, some degree of protection from predators, and perhaps more abundant food sources or greater food availability.

Another interesting finding was that hatchling turtles and eggshell fragments were fairly close to the adult turtles in both nitrogen and carbon isotope ratios. Since eggshells and hatchling turtles are derived from maternal tissues, it should come as no surprise that they should have nearly the same ratios as the adult females, most of whom were collected just after nesting. Juvenile turtles collected from the beaver pond also showed an interesting trend in that the smallest, and thus youngest, of the juveniles had a higher nitrogen signal than the largest and oldest of the juveniles collected. The smallest turtle was probably a hatchling from the previous spring or fall, and more than likely its tissues were still turning over from the maternal tissues. The tissues sampled for the analysis were shell fragments and claws, composed primarily of metabolically inactive tissues that turn over very slowly, and thus this little turtle very likely still demonstrated isotope ratios closer to its mother even after a year spent living in the beaver pond. It would have been interesting to sample tissues that turn over faster, such as liver, to look for further evidence of this pattern. We would expect to see ratios much closer to the other beaver pond turtles if such pattern does indeed exist. Conversely, the larger juvenile turtles have probably spent enough time living in the beaver pond that their tissues have entirely turned over. The fact that slow metabolic tissues demonstrate a nitrogen signal consistent with the beaver pond lends further evidence to the hypothesis that these turtles live entirely within the pond during their juvenile years.

As mentioned dietary analysis revealed that the juvenile turtles have a more complex diet than was originally expected. The isotope numbers did confirm the fact that these juvenile turtles are probably omnivorous. There was no combination of plant foods alone that could possibly give the isotope ratios measured in the turtles. It was mathematically possible however to combine animal foods to give the isotope ratios measured in the turtles; however fecal analysis revealed that this could not be the case

due to the excessive amounts of algae present. We have concluded that algae is the major dietary item consumed by the turtles through fecal analysis and this was supported by the chemical data obtained in the isotope analysis. In addition, algae is super abundant in the beaver pond; mats of green algae cover almost the entire surface of the pond from midsummer on.

Since eating algae alone would give a nitrogen ratio much lighter than what was encountered in the turtles we have also concluded that the turtles are probably consuming surface insects and some S.A.V.'s that are found in and among the algal mats where these turtles are probably feeding. In particular, water striders, water boatmen, and Hydrilla combined with algae seemed to be good candidates for the combined diet of these juvenile turtles in this system. The isotope values of these items can be mathematically combined in proportions to give the expected dietary ratio of the turtles. Hydrilla seemed a very good candidate due to the fact that its measured values did show up in the expected dietary range of the turtles. However, the relative importance of Hydrilla in the diet of these turtles is still in question due to the fact that the measured isotope values were based on only a single sample, and in general Hydrilla is not all that abundant in the beaver pond. In addition, other prey such as sunfish or tadpoles, which may or may not show up in fecal samples, may also be consumed, but the amount consumed by the turtles would have to be very small to give the isotope values encountered in this study. If these turtles are consuming these items, they are more than likely simply opportunistically scavenged rather than actively pursued.

This is only the beginning of a continuing study being conducted at the Jug Bay Wetlands Sanctuary, and although this preliminary work is suggesting some very interesting things about the diet of juvenile Red-bellied Turtles, more work needs to be done before anything can be said conclusively. More extensive sampling of the beaver pond needs to be conducted to determine relative abundance of different potential diet items. Long-term sampling should be conducted to look at isotopic shifts that occur as seasons change, since time of year may be affecting how the numbers generated in this study are interpreted. A larger sample size of juvenile turtles would also be appropriate in order to further examine relationships between size and age classes of juveniles, further comparison with adult turtles, and also for further examination of the question of habitat segregation between adult and juvenile turtles. In addition, more extensive tissue sampling should be conducted in order to examine isotopic differences between tissue types. Tissues with faster turnover rates would be interesting to examine as they reflect a more short-term diet, which may be more appropriate in a habitat such as the beaver pond, which demonstrates large seasonal variations in isotope values. We sampled metabolically inactive tissues in this study, and the slow turnover rate associated with these tissues may have caused a lag in our data between the turtles and the beaver pond samples. In addition, since the beaver pond is an extremely complex system when compared to terrestrial systems, a re-examination using a third isotope may be appropriate for greater resolution of dietary items.

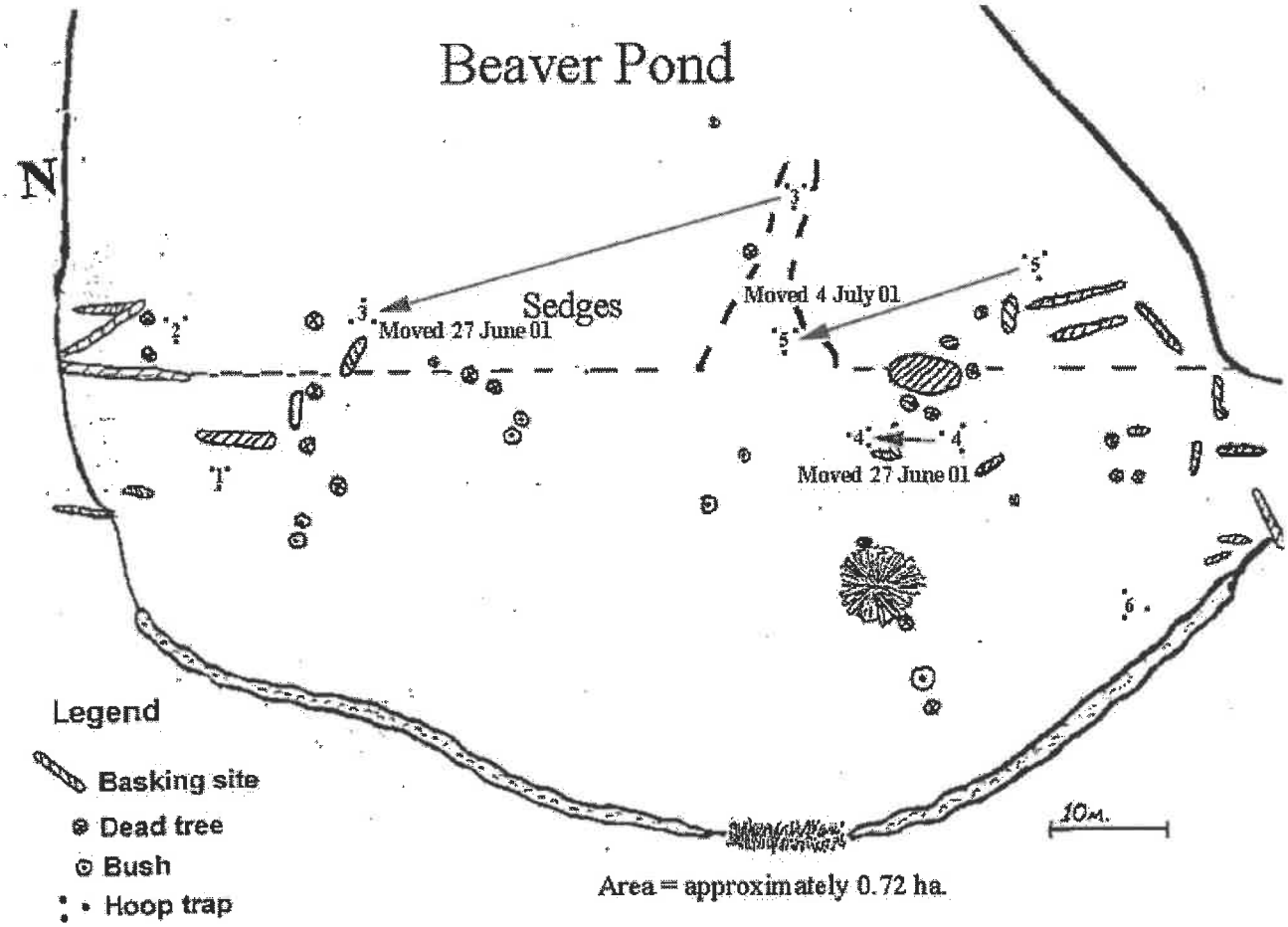

Figure 1 – Beaver Pond map, with trap locations. Arrows and dates indicate moved traps.

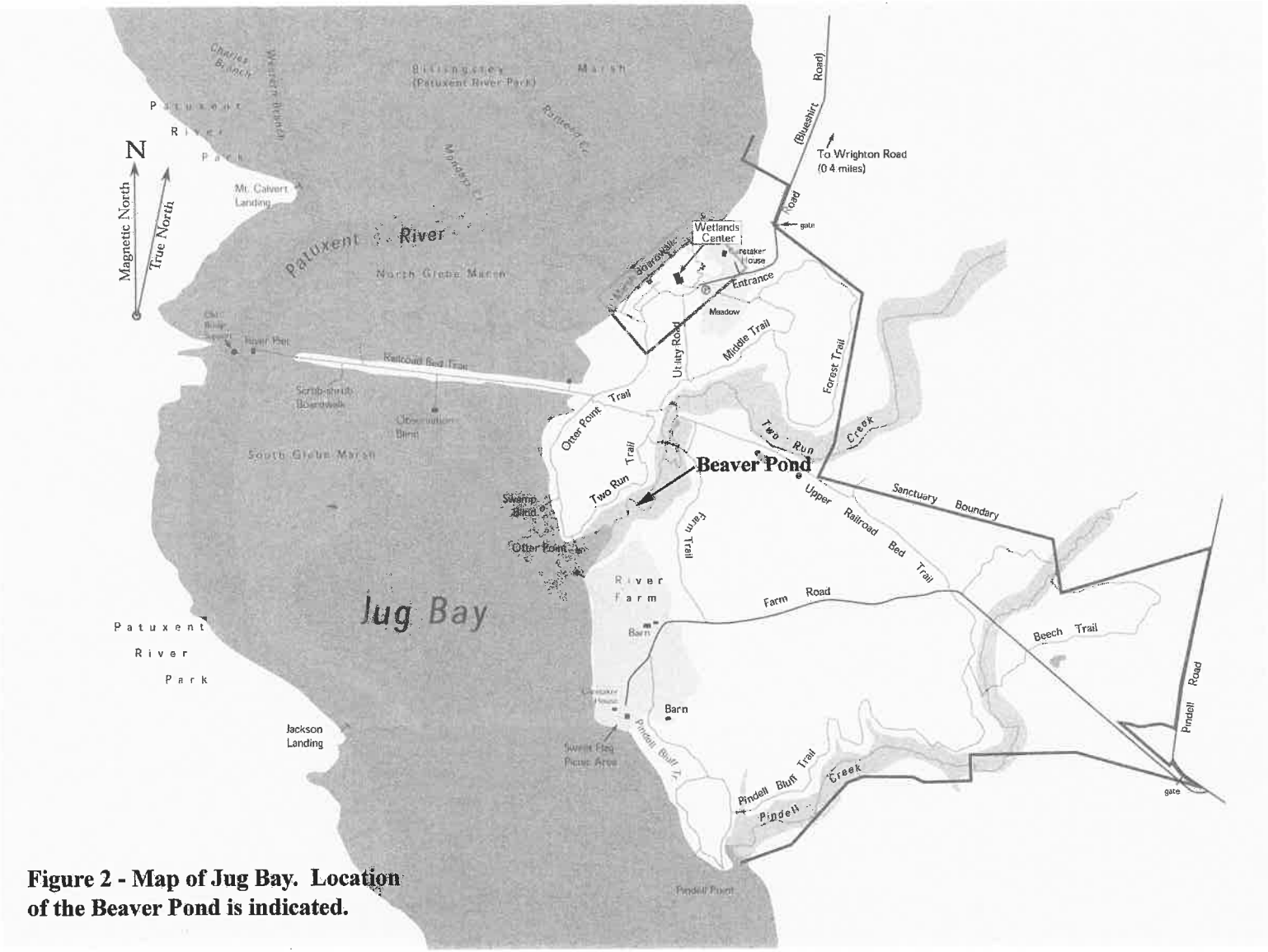

Figure 2 - Map of Jug Bay. Location of the Beaver Pond is indicated.

Figure 3 – Range of the Red-bellied Turtle (From Ernst et al, 1994).

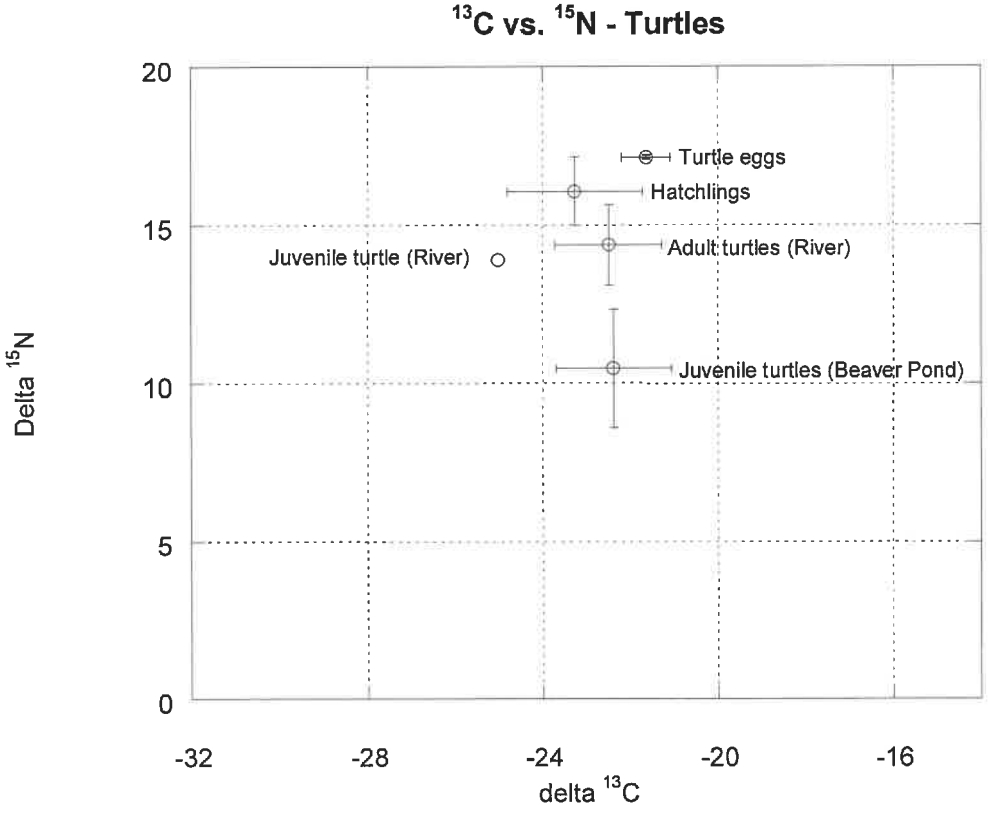

Figure 4 - δ13C vs. δ15N values of river turtles, Beaver Pond juveniles, hatchlings, and eggshells. Error bars represent standard deviations.

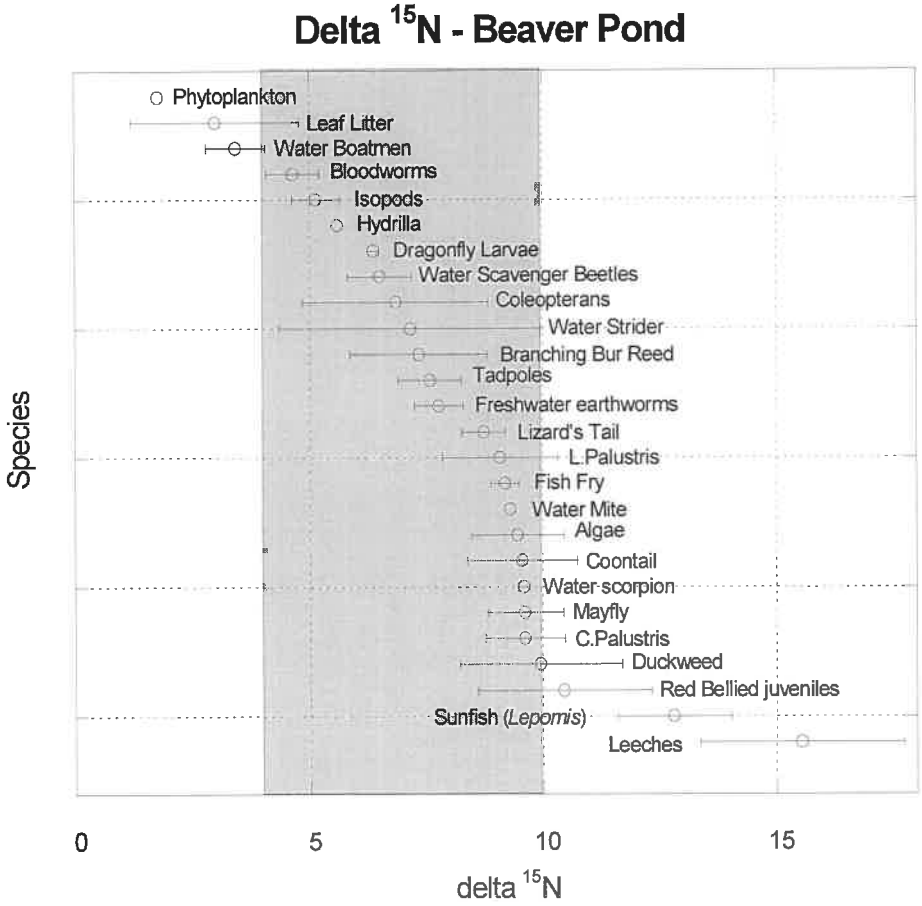

Figure 5 - $\delta^{15}N$ values for Beaver Pond samples. Error bars represent standard deviations.

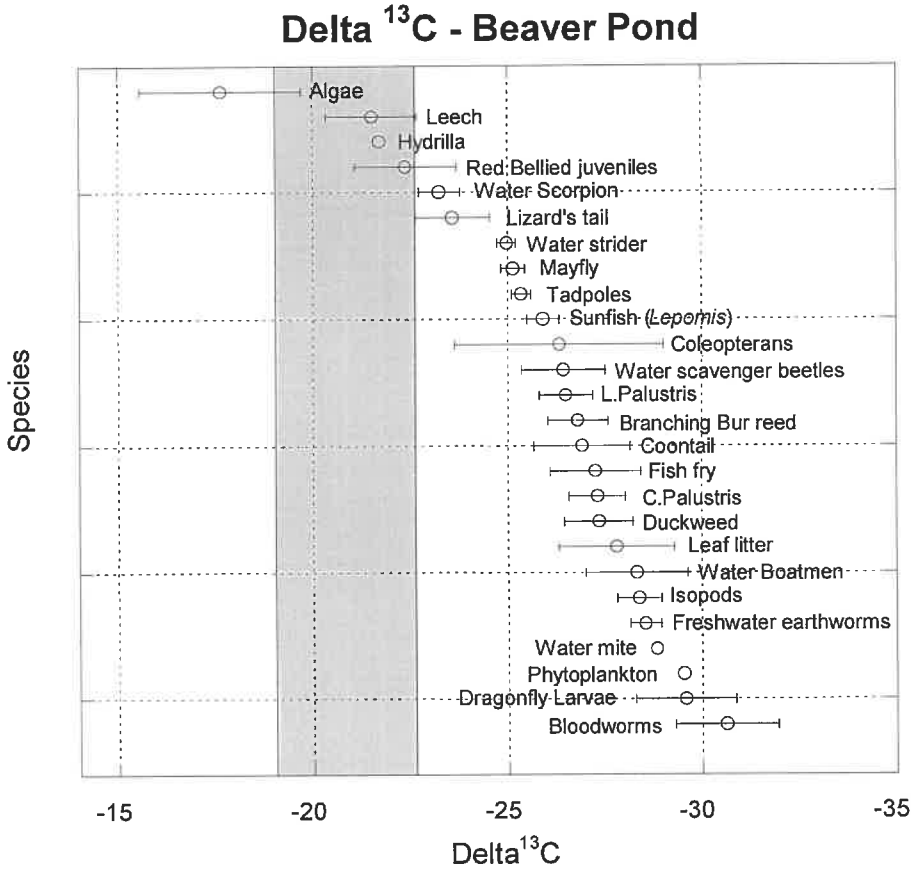

Figure 6 - $\delta^{13}C$ values for Beaver Pond samples. Error bars represent standard deviations.

Table 1 – Beaver Pond animals.

Species	delta 15N	std. Dev. 15N	delta13C	std. Dev. 13C	# of samples
Pseudemys rubriventris juv. 1 (54.45mm)*	13.26		-23.77		1
Pseudemys rubriventris juv. 2 (157mm)*	8.235	0.445477	-23.165	1.096016	2
Pseudemys rubriventris juv. 3	11.1	0.254558	-22.43	0.608112	2
Pseudemys rubriventris juv. 4 (49.40mm)*	9.83		-20.3		1
Pseudemys rubriventris juv. 5 (166mm)*	9.875	0.176777	-22.23	1.032376	2
Bloodworms (Chironomidae)	4.648	0.568217	-30.636	1.326209	5
Dragonfly larva (Odonata)	6.37	0.113137	-29.575	1.294005	3
Fish fry	9.19	0.3005	-27.253	1.172362	3
Freshwater clams (Bivalvia)	4.03	0.489524	-26.5	5.064079	3
Freshwater earthworms (Annelida)	7.76	0.533573	-28.543	0.408207	3
Isopods (Isopoda)	5.14	0.517752	-28.385	0.577437	4
Leeches (Annelida)	15.543	2.174542	-21.507	1.159756	4
Sunfish (lepomis)	12.818	1.214314	-25.905	0.414287	4
Mayflies (Ephemeroptera)	9.63	0.820244	-25.135	0.304056	2
Coleopterans	6.85	1.988131	-26.328	2.680403	4
Tadpoles	7.573	0.681567	-25.35	0.251197	3
Water boatmen (Corixidae)	3.433	0.627402	-28.313	1.33	3
Water mite (Acariformes)	9.3		-28.83		1
Water scavenger beetle (Hydrophilidae)	6.503	0.689662	-26.43	1.073918	3
Water scorpion (Nepidae)	9.61	0.127279	-23.255	0.53033	2
Water strider (Hemiptera)	7.165	2.807214	-24.975	0.233345	2

* Plastron length

Table 2 – Beaver Pond plants.

Species	delta 15N	std. Dev. 15N	delta13C	std. Dev. 13 C	# of samples
Branching Bur-reed (*Sparganium androcladum*)	7.334	1.467474	-26.804	0.781236	5
Callitriche palustris	9.63	0.855599	-27.292	0.728814	5
Coontail (*Ceratophyllum demersum*)	9.563	1.18361	-26.9067	1.241223	3
Duckweed (*Spirodela polyrhiza*)	9.962	1.75219	-27.334	0.890129	5
Algae	9.465	0.998834	-17.635	2.072098	6
Hydrilla (*Hydrilla verticillata*)	5.61		-21.71		1
Ludwigia palustris	9.09	1.248573	-26.497	0.68252	3
Lizard's Tail (*Carurus cernuus*)	8.74	0.473849	-23.593	0.962098	3
Phytoplankton	1.78		-29.54		1
Leaf Litter	3	1.788441	-27.79	1.500017	5

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