

**DEVELOPING A NONLETHAL FIELD METHOD FOR DETERMINING LIPID
CONTENT OF AMERICAN EELS (*ANGUILLA ROSTRATA*)**

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

American eels (*Anguilla rostrata*) were collected from Hudson River tributary streams, and resistance was measured with a tetrapolar Bioimpedance Assessment device. Lipid content of 20 eels was then determined in the laboratory using the Folch method. Correlation between resistance corrected for distance between the electrodes and total lipid content was substantial ($R^2 = 0.84$). However, measures of lipid content were not well correlated with Fulton's K (0.25 – 0.33).

A field study showed that American eels in one Hudson River tributary (Hannacrois Creek) were significantly higher in lipid content than eels in a second tributary (Black Creek). A test of the hypothesis that eels would have a higher lipid content in upstream locations compared to eels near the tributary mouth confirmed that this was the case.

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INTRODUCTION

Research concerning the health of freshwater eels has become increasingly important as eel populations decline across the globe. In the case of the American eel (*Anguilla rostrata*), concern over eel populations has resulted in the species being brought under review for protection via the Endangered Species Act (Federal Register 2011). The task of protecting the American eel is made more difficult by the unique life-cycle of freshwater eels wherein they migrate to the Sargasso Sea in order to reproduce. There is still much to be learned about their migratory behavior, as well the physiological and environmental requirements of this migration. Any research into effective management strategies for eel populations will be enhanced by an accurate field method for quantifying eel health.

While many fisheries studies utilize weight/length equations to quantify fish health, this methodology makes cross population comparisons difficult if not impossible. When using a condition index, an individual with a greater than the average mass for a given length receives a higher condition rating. Determining an “average” mass for a population can become quite complicated, especially when a study is being conducted over time or between populations. Length to weight ratios are greatly influenced by a myriad of environmental factors that determine fish size and shape (Froese 2006).

Traditionally, a more quantitative approach to fish health is to look at lipid content. Proximate analysis of tissue was the only way to gain insight in to the actual composition of individuals. By quantifying body metrics, researchers could make judgments about an individual's energy consumption and storage. In particular, data concerning non-polar lipid content can communicate much about an organism's energy

storage and consumption (Tocher 2003). Since lipids have the highest energy to weight ratio, they allow organisms to store energy while limiting body mass and whole-fish lipid content has been positively correlated with whole-fish caloric content (Peters et al. 2007). This is especially important for *A. rostrata* given its catadromous life cycle in which they migrate from river tributaries to the Sargasso Sea. This migration requires large stores of energy and larger eels are known to have very high lipid content. The physiological aspects of the migration are still poorly understood due to the reluctance of the scientific community to sacrifice breeding eels.

More recently, however, Bioimpedance Assessment (BIA) has used non-lethal electric current to derive information about an animal's chemical composition. BIA thus offers the accuracy of proximate analysis while also providing a non-lethal, efficient, and economical field methodology. Currently, the biggest limitation to using BIA to study eels is its limited application to fish in general and eel shaped fish in particular. Although previous studies have found strong correlations between impedance measurements and lipid content, the relationship differs between organisms (Duncan 2008; Cox and Hartman 2005). Body shape plays a large role in determining how current from the BIA device is distributed through the organism. Up to this point, no studies have been published on eel shaped fish, thus BIA remains an unproven method.

The purpose of this study was examine lipid content in eels using proximate analysis and BIA to determine if there was a correlation between BIA and lipid content for the American eel. If an appropriately significant relationship was obtained, the methodology could then be applied to investigate differences in total lipid content between upstream and downstream eel populations. Machut (2006) had investigated this

question previously using condition indices and found no significant difference in condition between the upstream and downstream populations. The hypothesis was that eels in upstream habitats, where they live at relatively low densities (Machut et al. 2007), have access to more food and thus would have a higher lipid content.

METHODS

Initial Field Collection and Chemical Analysis

The first part of this study focused on determining whether or not there was a correlation between lipid content and bioelectrical impedance. For this purpose, 20 eels of varying size were caught in the Vlockie Kill, Castleton, New York, a small tributary of the Hudson River, using a Smith-Root LR 24 backpack electroshocker. The eels were brought back to the laboratory where they were over-anesthetized with clove oil.

Impedance measurements were taken using a Quantum X tetrapolar BIA device (RJL systems) fitted with 2 cm sub-dermal needles. Prior to sampling, the device was calibrated using an included 500 ohm resistor. Individuals were placed on a non-conductive surface, which consisted of a lab bench covered in burlap, and were dried with paper towels. The electrodes were inserted laterally directly behind the gill opening and at a point near the tail that was sufficiently fleshy to allow current to pass between electrodes. Each electrode consists of a positive and negative needle and each was placed horizontally with the positive needles closest to one another. The distance between the positive poles was measured with a ruler.

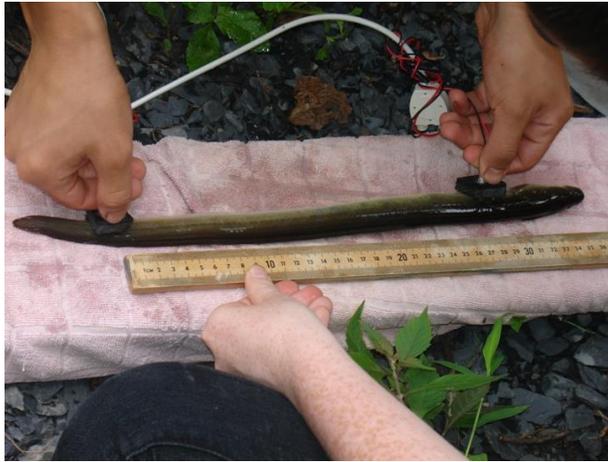


Figure 1. Electrode placement and measurement of the distance between innermost electrodes during BIA.

Outputs from the BIA device, which consisted of resistance and reactance measurements, were then recorded. After BIA analysis, each individual was weighed with a triple-beam balance and total length (TL) was taken with a measuring board. The eels were then stored in Ziploc freezer bags at -79°C for later chemical analysis.

Chemical analysis of lipid content was done to determine the correlation between lipid content and impedance measurements. Each eel was first homogenized using an industrial blender. Eel skin is extremely resistant to blending and it was necessary to score it using a razor blade prior to blending. Additionally, smaller eels ($<25\text{ g}$) often required further homogenization using a smaller handheld homogenization device. Larger eels ($>100\text{ g}$) had to be cut into smaller sections prior to blending. Once homogenized, three one-gram samples were taken from each individual and used for lipid analysis. Lipid content from the three samples was averaged for each eel.

Eel lipid content was measured using the Folch method for determining whole body lipid content of fish (Folch et al. 1957). Each sample was placed in 20 ml of 2:1

chloroform/methanol solution. The solution containing the homogenate was then manually shaken every 10 min over the course of an hour. The chloroform/methanol solution was then transferred into a separatory funnel while the homogenate was filtered out using #1 qualitative filter paper. The remaining solution was shaken with 4 ml of 0.1 M NaCl, which caused the more polar methanol layer, containing polar lipids and proteins, to separate from the less polar chloroform, which contained the non-polar lipids. The chloroform layer was then transferred into a 50 ml round bottom flask and evaporated using a rotary evaporator with a 30 °C water bath. Round-bottom flasks were then allowed to further dry under a fume hood until a stable mass was achieved. The lipid mass was then recorded and used to calculate total body lipid for each eel.

Analysis consisted of determining regressions between total body lipid and resistance and reactance measurements from the BIA device. Resistance measurements were corrected following Duncan (2008): Whole body resistance = (Distance between positive electrodes)²/Resistance. A curve was fit to the whole body resistance (ohms) to total lipid (g) relationship. Additionally, both total lipid and percent lipid were regressed against Fulton's Condition Factor (K) using both linear and power functions.

Applied Field Study

The second part of this study consisted of field observations comparing BIA measurements between different eel populations. Eels were sampled at an upstream and downstream location in two tributaries of the Hudson River, Black Creek (Esopus, NY) and Hannacrois Creek (Coeymans, NY).

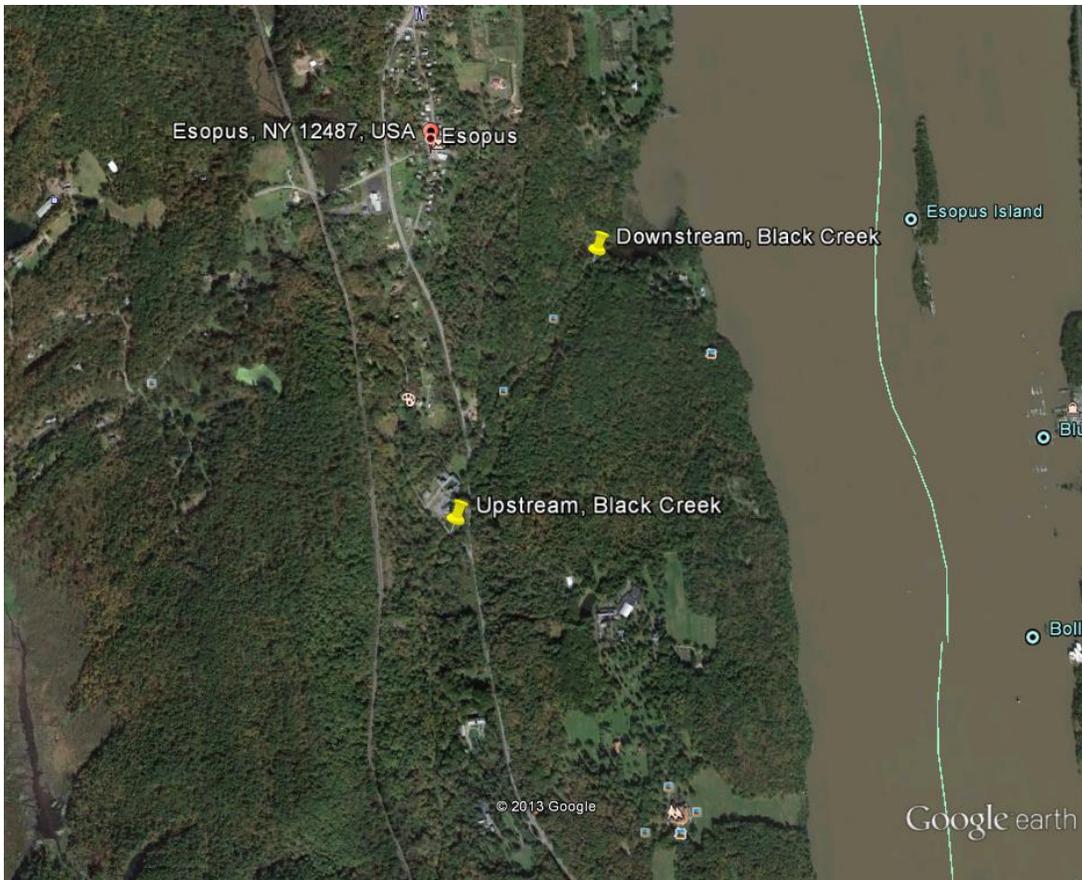


Figure 2. Map of eel sampling sites on Black Creek, Ulster County, New York. Distance between sites was 0.87 km.

In Black Creek, the downstream site was just upstream of the bridge on Winding Brook Rd., approximately 50 m upstream of the tidal Hudson River. The upstream site on Black Creek was upstream of the western bridge on Black Creek Road.



Figure 3. Map of eel sampling sites on Hannacrois Creek, Greene/Albany County, New York. Distance between sampling sites was 2.62 km.

The downstream site on Hannacrois Creek was upstream of the Rt. 144 bridge, in tidal water. The upstream site on Hannacrois Creek was downstream of the Rt. 9W overpass.

At each of the sites, 25 eels were caught for analysis after approximately an hour of electrofishing, except for the upstream Hannacrois site at which only six eels were caught over the course of an hour. Eels were anesthetized with clove oil and measured, weighed, and impedance measured according to the same procedures described above. Eels analyzed in the field were placed on a portable, non-conductive surface constructed

using two 1x4 inch pieces of wood and a cotton towel to measure impedance. Prior to using the BIA device at each site, it was calibrated using the 500 ohm resistor. The device showed very little variance when connected to the resistor and no corrections were required for the impedance data.

After measurements were taken, eels were placed in a recovery bucket filled with fresh water from the creek. Once the eels recovered from the clove oil, they were released back into the creek. Eels that did not recover from the clove oil were taken back to the lab and frozen for future analysis. Using the relationship determined in the first part of the study, total lipid content was calculated for each eel. Total lipid content was then compared between upstream and downstream populations in the respective creeks using a two-tailed T-test. Additionally, a two-tailed T-test was used to determine if there was a significant difference in lipid content between the creeks using combined data from upstream and downstream populations.

RESULTS

Initial Field Collection and Chemical Analysis

A total of 21 American eels were collected in the Vlockie Kill. The average total length was 34.4 cm (20.0-61.0) and the average weight was 99.8 g (17.5-436.5). When corrected for electrode width, resistance measurements showed a strong correlation ($R^2 = 0.84$) with total lipid content (Figure 1). The best fit regression was a power relationship with the equation:

$$Y = 31.536 X^{1.4262}$$

where Y is total lipid content in grams and X is the corrected whole body resistance.

Fulton's condition factor did not correlate well with either percent lipid or total lipid of the collected eels (Figures 2 & 3). Percent lipid ranged from 3.1-15.6, within the range of percent lipid reported for eels from the tidal Hudson River (Steinbacher 2001; Steinbacher and Baker 2002).

Applied Field Study

Percent lipid content for eels in upstream and downstream populations in Hannacrois Creek was found to be significantly different ($P = 0.0493$). In Hannacrois Creek, the average percent lipid content per eel was 21.4% downstream and 26.7% upstream (Figure 4). In Black Creek where the difference between upstream and downstream lipid content was highly significant ($P = 0.000192$) the average percent lipid content per eel downstream was 11.6% while upstream it was 16.8%. Difference in percent lipid content for eels between the two creeks was found to be highly significant ($P < 0.0001$).

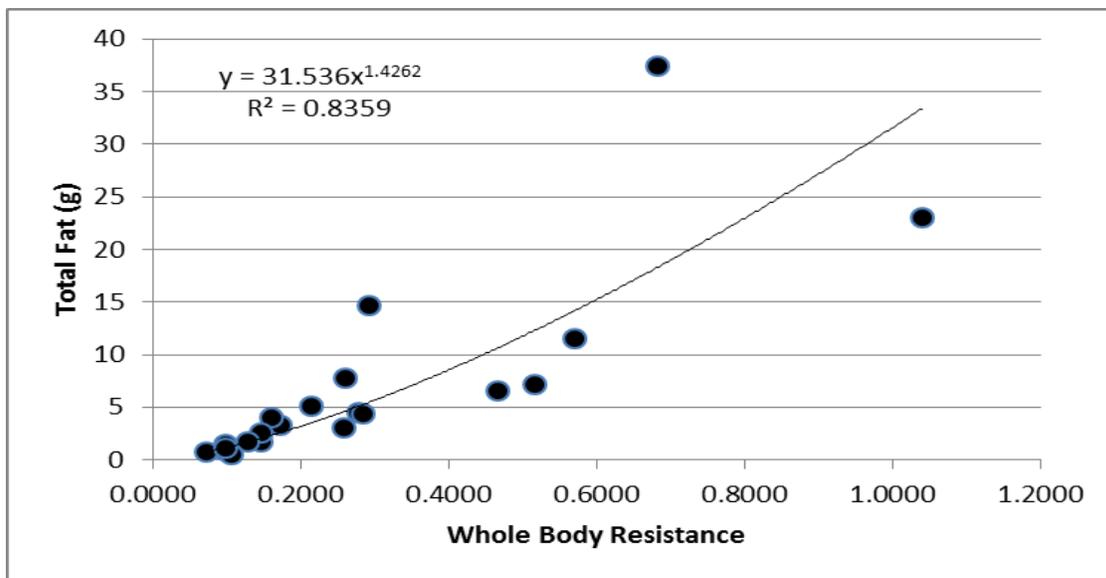


Figure 4. Regression showing correlation between total body lipid and corrected resistance.

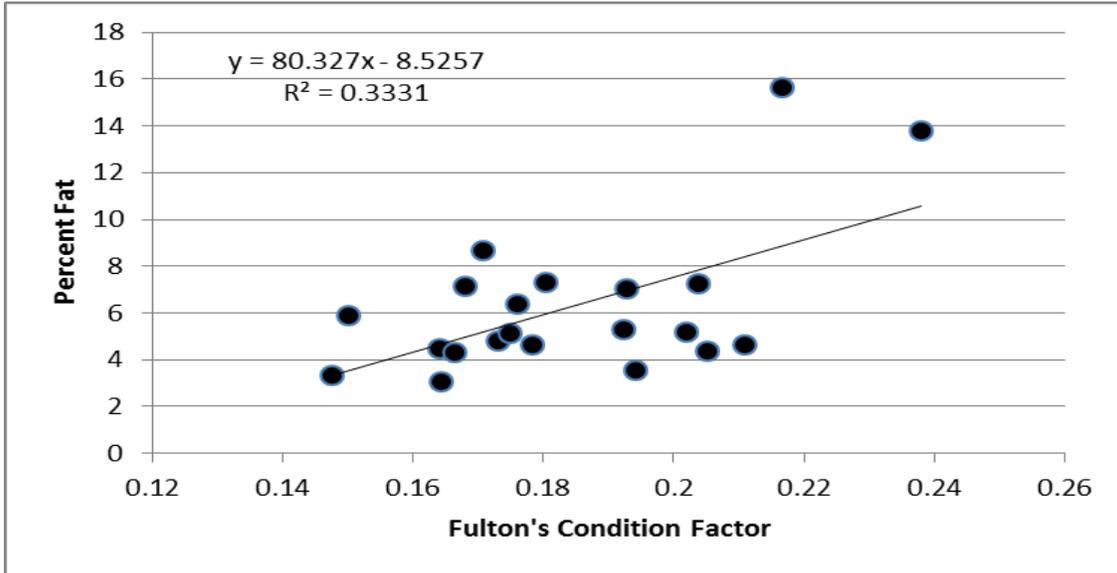


Figure 5. Linear regression showing lack of correlation between Fulton's Condition Factor (K) and percent lipid in eels.

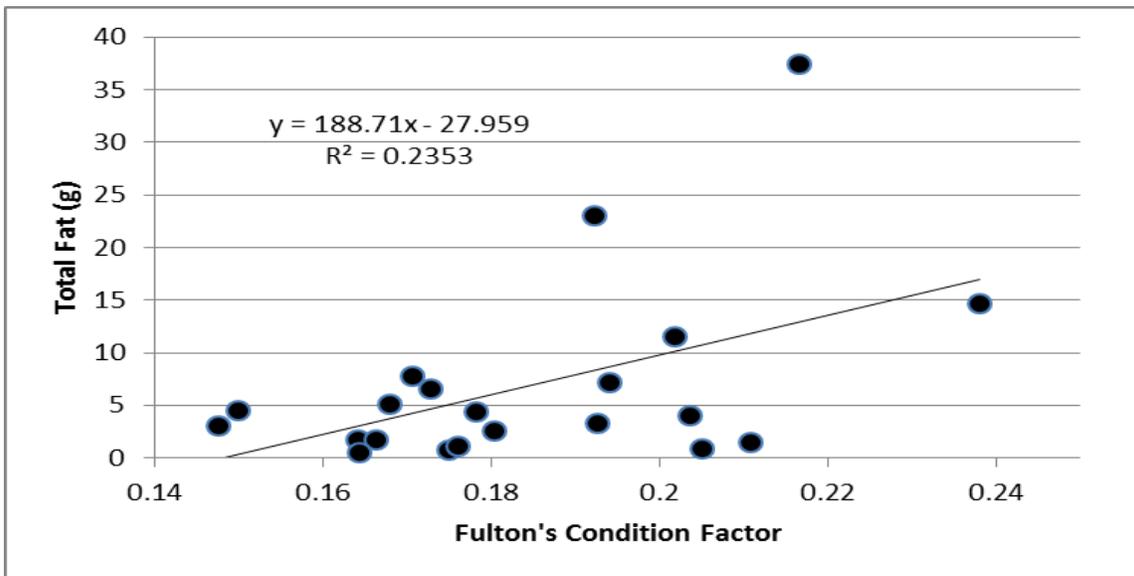


Figure 6. Linear regression showing lack of correlation between total Fulton's K and total lipid in collected eels.

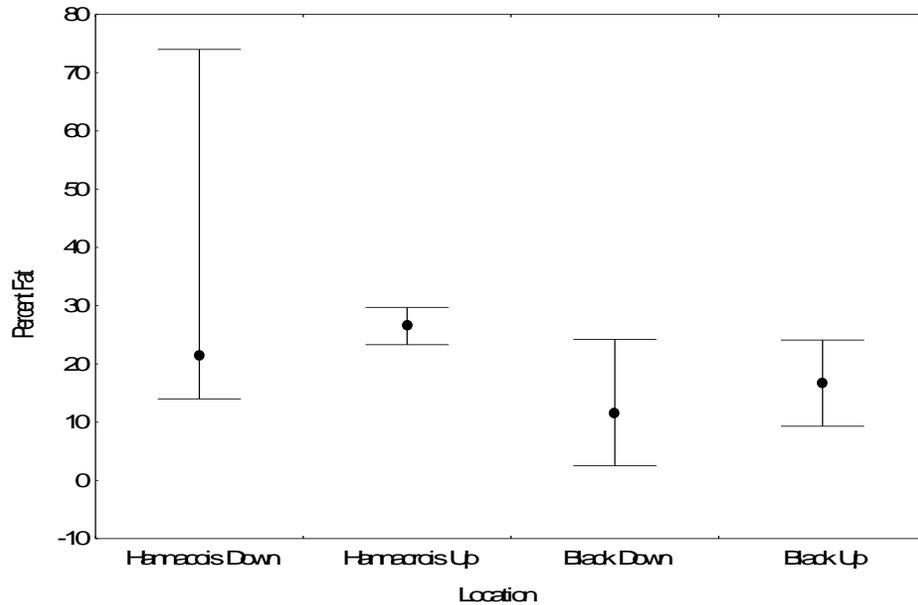


Figure 7. Mean (solid circle) and range (capped line) of percent lipid content of American eel upstream and downstream in Hannacrois Creek and upstream and downstream in Black Creek, Hudson River, NY.

DISCUSSION

Based on the results of this study, Bioimpedance Analysis appears to be a viable field method for determining lipid content in American eels. The strong statistical correlation between total lipid content and corrected resistance ($R^2 = 0.84$) indicates that lipid content could be successfully predicted using the regression developed in this study. Duncan (2008) performed a similar study on four different species of fish, and found R^2 values ranging from 0.94 to 0.75 when relating total body lipid to corrected resistance. Both extracted lipid levels from the initial sample group and predicted lipid levels were within the known range for American eels. It is worth noting that the majority of previous BIA studies on fish have used the Bligh and Dyer (1959) method for lipid extraction, which is a modified version of the Folch method (Folch et al. 1957), to determine total body lipid. According to Iverson et al. (2001), the Bligh and Dyer

method should only be used for the analysis of muscle tissue on fish with low lipid content. The decision to use the Folch method was therefore warranted, but might complicate lipid content comparisons with previous BIA studies.

Fulton's Condition Index predicted lipid content poorly, as expected. Findings of this study confirm observations that both Fulton's condition factor and the Heptosomatic Index (HSI) were a poor indication of lipid content in fish (Peters et al. 2007). Although more population specific condition indices can be derived (Froese 2006), the relative ease and effectiveness of BIA makes it a more appealing alternative. Additionally, once the eels are anesthetized for BIA, taking length and weight measurements is quite simple. Condition indices need not be sacrificed in order to perform BIA and a combination of the two methods could result in a broader understanding of the population(s) being studied.

Due to its quantitative and non-lethal nature, Bioimpedance Analysis constitutes a significant step forward when it comes to performing larger scale studies of eel health between populations. Although the method is relatively simple to perform, it should be noted that uniform electrode placement is difficult to achieve with eels due to their lack of anatomical landmarks. Though it was desirable to place the electrodes as far away from one another as possible, so the data would best represent the whole eel, the meter would read maximum when the electrode was placed too near the tail. This was solved by moving the electrodes closer to one another. Another difficulty of BIA is that it requires the eels to be completely immobile. Though this is achievable with clove oil, it requires careful application to avoid overdose. In the end the benefits of BIA clearly outweigh the few difficulties posed in its application.

The results of the field study confirmed the initial hypothesis that upstream eel populations (mean TL = 41.8 cm) would have a higher lipid content than downstream populations (mean TL = 33.1 cm) in Hannacrois Creek and in Black Creek (mean TL upstream = 28.7 cm; downstream = 31.3 cm). The second finding was that eels in Hannacrois Creek were had a higher lipid content than those in Black Creek. These data, however, are confounded by differences in average size of the eels collected. Larger eels are expected to have higher lipid content. Given the number of variables at play, it is difficult to hypothesize what other factors may be responsible for this pattern, but it certainly warrants further investigation. The qualitative observations at the two streams indicated that eel populations were much lower upstream and that crayfish, primary prey of large eels (Machut 2006), were more abundant. It has been suggested that the higher density of eels downstream, where there is greater competition between individuals, is directly responsible for reduced condition (Machut 2006) or in this case reduced lipid content. It has also been suggested that urbanization plays a broader role in eel health including direct effects such as pollution and barrier construction (Machut et al. 2007). Since these observations have thus far relied on condition indices, it would certainly be worth reinvestigating them using Bioimpedance Analysis.

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