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Assessing Lipid Content in Migrating Alewife

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ASSESSING LIPID CONTENT IN MIGRATING ALEWIFE

by

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ABSTRACT

Alewife are a commercially, economically, and ecologically important fish, that expend large amounts of energy during their long migrations to spawning habitat. This energy demand can influence an individual's chances of surviving and reproducing successfully. To understand how energy use may affect fitness, we captured alewife from the Souadabscook Stream over the course of their spawning migration. Fifty fish were sampled each week from May 12th to June 10th 2019. The lipid content of each individual was measured by using i) a Distell Fatmeter and ii) gravimetric analysis by measuring muscle moisture as an indirect method to assess lipid content. Population demographics such as length, mass, and sex were recorded. Otoliths and scales were used to estimate age and spawning history respectively. Fish with higher lipid content were found during the tail end of the spawning run, and tended to be smaller than those who migrated earlier. Males were also found to have more stored lipids than females. Model selection was used to test the hypotheses that i) early migrants and ii) smaller fish have elevated lipid densities. The hypothesis that demographics such as length, mass, age, sex, and spawning history differ overtime was also tested. These findings provide information regarding the bioenergetics of alewife during migration, and may inform conservation strategies.

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INTRODUCTION

Alewife (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*) (collectively known as river herring) are a great asset to coastal regions, as they provide numerous ecological and economic benefits. These anadromous fish are considered keystone species in coastal lakes, and are used as a food source by many birds and predatory fish (Palkovacs et al. 2013). They are even hypothesized to act as a prey buffer for Atlantic salmon, who are listed as endangered under the US Endngered Species Act, as they migrate during the same time period and are more numerous (Saunders et al. 2006). The fish also impact both the freshwater ecosystem and the surrounding terrestrial landscape during and after their spawning run. Whether they die during their migration or deposit their gametes, river herring bring subsidies from the ocean to freshwater. This influences nutrient cycling and primary production in these systems (Rudstam 1988; Varpe et al. 2005). Additionally, alewife and blue backs are commonly used as lobster bait, which is a major industry in the New England region.

Before anyone reaps the benefits from this fishery, river herring undergo a long journey in order to return to their natal streams and spawn. It is known that water temperature is the primary cue for the start of their migration (Turner et al. 2015). This can begin as early as January, with the fish arriving to their respective rivers between May and June (Silva et al. 2012; Turner and Limburg 2015). Upon arrival, the spawning period can last up to two months. However, when both species of fish breed in the same river, alewife are seen arriving two to four weeks earlier than bluebacks (Turner and Limburg 2015). This is thought to be an adaptation in life history, which decreases interspecific competition between the two species. Potential “competition” would most likely be during egg incubation, as the number of eggs in an area can influence the temperature they experience and the amount of oxygen they receive. However, it is also hypothesized that the two species are segregated based on habitat preferences.

With this being said, the migration that river herring undergo is very energetically demanding. Crawford et al. 1986 found that alewife lost 18-22% of their stored lipids during a 31-32 km stretch of travel. They also found that herring who lived in waters adjacent to Newfoundland lost up to 60% of their lipid reserves in their effort to reach their spawning grounds. It is no surprise then that post spawn mortality for alewife can be anywhere from 41-91% (Saunders et al. 2006). Factors that affect the energy utilization of these fish are thought to include migration distance, water temperature, flow regime, gonad state, sex, and body size (Crawford et al. 1986 and Glebe and Leggett 1981). For example, Crawford et al. 1986 noted that males lost more of their lipid reserves than females. Additionally, fish that began their migration later in the spawning run had lower reserves than ones who ran earlier. Specifically, they found that alewife lipid content in fish that ran between May 12th-June 3rd was between 8.7 and 9.9%, while fish who ran from June 9th and later had an average of 5.7%. Silva et al. 2012 also found that smaller fish lost less energy during their migrations than larger ones.

Another factor that impacts the energy utilization of these fish is gonad development. Overtime, organisms develop adaptations that help maximize its fitness, or its survival and offspring production. Successfully doing so increases the probability that it passes on traits that made it successful into the next generation (Silva et al. 2012). Like all living things, fish have limited resources, so there are tradeoffs between its own growth and reproductive capability. Specifically, for diadromous fish, they are whether accessing optimal breeding grounds with reduced risk of predation is worth the energetic cost of migration and exposure to different environmental conditions (Quinn et al. 2016). As stated before, temperature is the key cue for river herring to begin their migration. However, this also plays a part in the spawning behavior that the fish exhibit (Turner and Limburg 2015).

Observable differences in life history have been found in anadromous fish along latitudinal gradients. With warmer and more stable temperatures in the south, fish are able to feed more, tend to grow larger, and reach maturity faster than those in the north (Leggett and Carscadden 1978). In these "favorable" conditions, congeneric fish such as American shad (*Alosa sapidissima*) focus more on gonad development during their migrations, and usually die after spawning (semelparity). Fish in the north experience colder and more variable conditions, and as a result attempt to spawn over multiple years to compensate for this (iteroparity) (Leggett and Carscadden 1978). However, this strategy has been found to have lower fecundity per year than those southern fish use.

Timing of migration is another aspect in the life history of anadromous fish. This is hypothesized to be an adaptation to environmental conditions the adults face, such as water temperature and flow regime (Hodgson and Quinn 2002). It is also thought that the timing of migration is influenced by the needs of their offspring. These include reproducing where a large portion of their offspring will successfully emerge and enjoy favorable feeding conditions. Hodgson and Quinn 2002 go on to describe how the water temperature experienced by migrating adult sockeye salmon (*Oncorhynchus nerka*) and incubating eggs may have resulted in three different migration strategies.

GOAL

To assess the lipid content of migrating alewife.

HYPOTHESES

1- Demographics such as length, mass, age, sex and spawning history of returning fish differ over time.

2- The use of a Distell Fat Meter and gravimetric analysis are both sufficient ways of evaluating the lipid content of alewife.

3- Early migrants and smaller individuals have elevated lipid densities.

METHODS

Collection

Sampling was conducted on a tributary of the Penobscot River. Alewife were collected on the Souadabscook Stream, just north of the Paper Mill Recreation Area at Great Falls from May 12th to June 10th of 2019. Fish were collected with the help of recreational dip netters, and euthanized in a concentrated solution of MS-222 (250mg/L).

From a power analysis, a sample size of forty alewife was anticipated to detect a 20% difference in lipid content (assuming a standard deviation of 4% and a desired power of 0.8). Given that we expected sex and age differences among the fish, fifty alewife were collected from the stream each week using dip nets. The alewife were then stored on ice until the following morning before processing.

Stream Temperature

Water temperature was recorded beginning on May 13th using a HOBO temp logger. This was left in the stream until the last day of sampling on June 10th (Figure I).

Alewife Parameters

The fork length (FL) and total length (TL) of each alewife was measured to the nearest 0.1 centimeter, while mass was measured to the nearest 0.1 gram. Somatic fat content was measured via fat meter and through gravimetric analysis. A Distell fat meter was used to estimate lipid content of the carcass. Four readings were taken just above the lateral line along the dorsal surface of each fish and were averaged (Crossin and Hinch 2005). In addition, a cube (approximately 1 g) of white muscle was removed from the left side of the fish behind the dorsal fin. The mass of these samples was measured before and after drying for one day in an oven at 60° C (for the first week, samples were weighed after 24 and 48 hours to confirm that a fairly constant mass was achieved after 24 hours of drying). Using the wet and dry tissue weights, an estimate of muscle moisture was then calculated (an inverse of lipid content). The gonads were then removed and weighed to calculate the gonadosomatic index (GSI):

$$\frac{mass\ of\ gonads}{mass\ of\ fish}$$

In order to assess spawning history, scales were used to estimate how many times each fish has previously spawned. These were removed anterior of the dorsal fin and above the lateral line. Up to ten scales from each fish were cleaned and mounted onto microscope slides for aging. Readings were conducted by two people, and both readers convened to solve any discrepancies. Both the left and right otoliths were also removed, cleaned, mounted to age each alewife by counting annuli. The same aging process for scales was used here.

Statistical Analysis

Model selection based on AIC was used to find best fit models for muscle moisture, fatmeter readings, and GSI considering the demographics of the alewife run. Program *R* was used to run regressions (linear, multiple, and quadratic) and AIC tables were constructed to display the results. One way ANOVA's were used to determine whether muscle moisture, average fat meter measurements, GSI, and the size of the fish (TL and mass) differed significantly between sample dates. Tukey tests were then used to see where these differences occurred. Chi-square testes were also conducted to determine whether the number of each age class, as well as the sex ratio differed between weeks.

RESULTS

A total of 250 fish were caught throughout the sampling period. The size of the fish in our sample decreased as the spawning run continued. Average male TL remained around 28.5cm during the first three weeks of sampling, then dropped to about 27.5cm during week four (June 2nd) and to 27cm in week five (June 10th) (Figure 3). Their average mass was between 201 and 203 grams for the first three weeks of sampling, and decreased to about 182 grams in week four (June 2nd), and 168 grams in week five (Figure 4). Significant differences in both TL and mass were detected between the first three weeks of sampling and the last two. Average female TL dropped from about 30.1cm to 29.1cm from week one (May 12th/13th) to week two (May 19th). This slightly decreased over the next few weeks, and was about 28cm by week five (Figure III). The average mass of females decreased during every week of sampling, starting at about 242 grams in week one and ending at around 192 grams in week five (Figure IV). Significant differences between weeks was also detected for female TL and mass.

Of the 250 alewife caught, otoliths from 235 were successfully extracted and aged (Table I). A chi-square analysis determined that there was a significant difference in the number of fish in each age class between sample dates. However, there was no difference in the number of males and females found between weeks. Sex distributions for each age class were relatively even for five and six year old fish. However, four year old males were three times more common than females (19 to 6). From our scales, we concluded that approximately 75% of the fish had previously spawned. The majority of five and six year old's had previously spawned once, while more than half of the seven year old's had spawned twice before.

Males had larger average fat meter readings (~3.5%) than females (~3.1%). Figure IV shows that this pattern remained constant throughout the sampling period. Average fat meter measurements for both sexes was also higher during the last two weeks of sampling, as a significant difference between weeks three and four was seen in females. Contrary to fat meter measurements, females had slightly larger calculated muscle moisture (~0.790) than males (~0.787). Again, this pattern was seen in each of the five sampling weeks (Figure V), but average muscle moisture decreased during the last two weeks in both sexes. Significant differences were seen between week three and weeks four and five again only in females. Female average calculated GSI was almost double that of males, which Figure VI shows to be consistent throughout the sampling period with no noticeable change (~0.107 to ~0.055 respectively). A significant difference between weeks four and five was seen in males.

Sorting our data by age rather than by sex yielded no consistent patterns. Four year old fish had the highest average fat meter reading during three of the five weeks of sampling (Figure VII). However, there were no observable patterns between age classes when looking at muscle moisture (Figure VIII). The highest average calculated GSI was seen in six year old's in all but one of the sampling weeks (Figure IX). No apparent differences were seen between sampling dates.

The top model testing average fat meter measurement indicated that many variables such as sex, spawning history, age, and TL are collectively good predictors (Table III). Coefficients for this model indicated that fish with higher fat meter readings are younger males who have not previously spawned. A competing model also suggests that collection date also may be a good indicator. The top model testing muscle moisture yielded that a quadratic relationship in length was the best predictor (Table IV). With gravimetric analysis measuring the water content of one gram of tissue while the Distell fat meter measured the lipid content of the entire fish, this result is not concerning. The top model testing GSI indicated that sex was the best predictor, with females having larger values than males (Table V).

DISCUSSION

Migrating from the ocean into freshwater rivers and streams in an effort to spawn is a daunting task. The distance traveled, obstacles, and conditions river herring and other anadromous fish species face during their journey all influence the energy stores they built up before running this gauntlet. This preparation ultimately determines their likelihood of surviving the migration, successfully spawning, and potentially returning to sea to repeat the process in the future.

Hypothesis 1

As stated previously, the sex ratio of fish in our sample was relatively even. Counts of each sex by week also did not differ much, with 32 females and 18 males caught on June 10^{th} being the greatest difference. Also noted previously was the fact that the average length and mass of alewife both decreased as migration timing became later. From this, we can conclude that larger fish were seen at the beginning of the spawning run. One noticeable difference in the count of each age class can be seen in weeks two and three of sampling (May 19^{th} and 27^{th}). During these two weeks, twice the number of six year old's for both sexes were found, as well as the only two seven year old alewife. There were also more five year old's found during the last week of sampling (42), compared to the previous weeks having around 30 fish in that age class. However, this may be influenced by the larger number of five year old females sampled in that week than previous dates.

Hypothesis 2

Both methods were found to be a viable way of evaluating somatic lipids. Lower calculated muscle moisture is expected with higher fat meter readings, as there is more fat in proportion to water within the tissue. Figure II plots the average fat meter measurement and calculated muscle moisture of each alewife. An R^2 of 0.58 was found when plotting the best fit line of the data (two outliers of muscle moisture were removed). A regression also yielded a p-value of less than 0.01. These values indicates a strong correlation between the readings of the two methods.

Hypotheses 3

Our data from the 2019 alewife run on the Souadabscook Stream suggests that fish with higher lipid content were found during the tail end of the spawning run, and were smaller than individuals who had started their migration earlier. Males were also found to have more stored energy than females. Figures revealed no definitive patterns when considering age, while model selection considered it a good predictor of average fat meter measurements.

Previous publications such as Crawford et al. 1986 have found that fish which migrate later during the spawning run have lower lipid levels. They also reported that along with lipid content being size and sex specific, it also was dependent on reproductive maturity. Younger fish were found to have a higher visceral lipid content (surrounding the organs) than older individuals. If this is strictly referring to age, which is correlated with the stage and condition of gonad development, our results may support these findings. In three of the five sample weeks (the first, third and fourth), four year old fish had higher fat meter readings than older fish. Along with the variables included in our top model, phase of gonad development may be correlated with a fish's energy reserves. Different life strategies concerning this are seen between anadromous fish that spawn in northern latitudes compared to southern ones.

Females were expected to have lower fat meter readings than males because they invest more energy into their gonads. Glebe and Leggett 1981 published similar results in working with American shad. They found that male viscera fat levels were anywhere from 40-300% greater than females, despite the males being of smaller size. No size-dependent differences in their viscera energy use was found as they migrated up river. Males also had higher somatic energy reserves (body fat), while females had higher gonadic energy reserves. All of these findings were consistent across latitudes, as they sampled on many rivers throughout the east coast. This is also supported by our calculated GSI for each sex, and that model selection included sex in the top model.

Abiotic factors may have influenced the lipid content of the fish we sampled. The water temperature during our first week of sampling was between 10.5 and 13 C° (Figure II), which for the most part is within an adult alewife's optimal temperature range of 11 to 19 C°. This optimal temperature range was maintained through the first four weeks of sampling, with the highest fat meter readings being seen in fish that were collected in water around 16 C°. However, water temperatures during the last week of sampling were approximately 22.5 C°, yet the fat meters readings of the fish sampled were on average the second highest only behind the previous week's fish.

Stream flow may also have impacted our fat meter readings. Though we did not collect such data on our stream, this dictates whether fish can pass through seasonally shallow sections of river, or stream flow can create impassible sections of fast current. In the case of Souadabscook stream, although higher flows may cause the fish to use more energy swimming upstream, it could create more habitat for the fish to rest between long stretches. It also may provide enough water to a side channel that can be used to pass

Great Falls. Higher flow would also slow the warming of the water later during the spawning run.

Considering that we do not know how much time our alewife spent in the stream prior to being caught, it is hard to determine the impact abiotic factors may have had on the alewifes' lipid content. Some individuals may have shot straight up the Penobscot River into Souadabscook Stream where we netted them, while others may have spent more time to get around obstacles or to home in on their natal spawning grounds. This means that we have only sampled a subsection of the alewife population that runs up Souadabascook Stream, and may explain why we did not detect decreases in lipid content as the spawning run continued. Our sample only consisted of the "winners" or those who had enough energy to make it that far upstream. Including the "losers" , or those less capable of making it as far, may have resulted in different findings.

Another possibility as to why we did not detect higher lipid levels during the beginning of the alewife spawning run may be due to a phenomena known as premature migration. This occurs when diadromous fish migrate to their spawning grounds, but do not immediately spawn upon arriving (Quinn et al. 2016). Premature migration may be an adaptation to the tradeoffs of diadromy mentioned earlier. This may be favored when water temperatures and flow are moderate, or when the freshwater environment offers a lower risk of predation and energy expenditure of the adult. Premature migration is usually characteristic of larger, older fish, who would benefit less from more time at sea to grow than smaller individuals. In our sample of alewife, smaller individuals had the highest somatic lipid levels and came later in the migration. Considering we also found that larger individuals came in sooner, it is possible that these fish are focusing more energy toward gonad development, though calculated GSI did not vary much over the spawning run.

Fatty acids, or the lipid content of fish, play a large role in physiological activities of fish including egg development, ovulation, and spawning behavior (Snyder et. al 2011). They also provide a source of stored energy that can strongly influence survival, future growth, and reproductive success. As a result, through natural selection, these fish have adapted behavioral and physiological traits that increase the probability of successful migration and reproduction (Hodgson and Quinn 2002). Therefore, it is imperative that we understand the bioenergetics of anadromous fishes such as alewife, as they play a crucial role in the maintenance of freshwater ecosystems and our way of life.

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APPENDICES

APPENDIX 1 - TABLES AND FIGURES

Table I. Shows the count of ages of the sampled fish (otolith) by sex and collection date.

	4	5	6	7	Grand Total
5/12-13	**8**	**30**	**8**		**46**
Female	2	15	5		22
Male	6	15	3		24
5/19	**3**	**29**	**12**	**1**	**45**
Female	1	13	6		20
Male	2	16	6	1	25
5/27	**1**	**28**	**16**	**1**	**46**
Female		15	9		24
Male	1	13	7	1	22
6/2	**9**	**33**	**6**		**48**
Female	2	15	3		20
Male	7	18	3		28
6/10	**4**	**42**	**4**		**50**
Female	1	29	2		32
Male	3	13	2		18
Grand Total	**25**	**162**	**46**	**2**	**235**

Table II. Shows the average calculated muscle moisture, GSI, and fat meter readings of the sampled fish by age and collection date.

	Count of Fish	Average Fat Meter Measurement (%)	Average MOIST	Average GSI
5/12-13	**46**	**3.2551**	**0.7892**	**0.0785**
4	8	4.6094	0.7869	0.0718
5	30	3.0200	0.7890	0.0811
6	8	2.6781	0.7924	0.0837
5/19	**45**	**2.9220**	**0.7887**	**0.0755**
4	3	2.8417	0.7895	0.0629
5	29	2.9595	0.7886	0.0786
6	12	2.7729	0.7889	0.0826
7	1	3.8500	0.7809	0.0532
5/27	**46**	**2.7525**	**0.7926**	**0.0831**
4	1	7.6500	0.7662	0.0624
5	28	2.3107	0.7961	0.0808
6	16	2.7906	0.7897	0.0885
7	1	1.3250	0.8183	0.0416
6/2	**48**	**4.0692**	**0.7857**	**0.0818**
4	9	4.5778	0.7827	0.0749
5	33	3.9805	0.7863	0.0822
6	6	3.4333	0.7884	0.0850
6/10	**50**	**3.6290**	**0.7873**	**0.0834**
4	4	3.5313	0.7951	0.0649
5	42	3.6202	0.7867	0.0860
6	4	3.8188	0.7863	0.0742
Grand Total	**235**	**3.3235**	**0.7887**	**0.0805**

Table III. Shows AIC results of regressions run on average fat meter measurement.

Model	AICc	Delta AICc	df	AICwt
Sex + Previous Spawner + Age + TL ^2	891.5	0.0	7	0.56
Sex + Previous Spawner + Age + TL ^2 + Date	892.8	1.3	8	0.29
Sex + Previous Spawner + Age + TL	895.1	3.6	6	0.09
Sex + Previous Spawner + Age + TL + Date	896.6	5.1	7	0.04
Sex + Previous Spawner + Age	897.6	6.1	5	0.02
Age	940.5	49.0	3	0.00
Sex + Age	940.8	49.3	4	0.00
Sex + Previous Spawner + TL^2	945.8	54.3	6	0.00
Sex + Previous Spawner + TL	949.2	57.7	5	0.00
Sex + Previous Spawner + Date	953.8	62.3	5	0.00
Sex + Previous Spawner	955.1	63.6	4	0.00
First Time Spawner	955.8	64.3	3	0.00
Repeat Spawner	959.7	68.2	3	0.00
TL^2	991.6	100.1	4	0.00
TL	994.9	103.4	3	0.00
Date	1006.3	114.8	3	0.00
Sex	1008.7	117.2	3	0.00

Table IV. Shows AIC results of regressions run on muscle moisture.

Model	AICc	Delta AICc	df	AICwt
TL^2	-1617.8	0.0	4	0.77
TL	-1615.3	2.5	3	0.23
Sex	-1605.7	12.1	3	0.00
Date	-1602.4	15.4	3	0.00
Sex + Previous Spawner + TL^2	-1598.1	19.7	6	0.00
Sex + Previous Spawner + TL	-1595.6	22.2	5	0.00
Sex + Previous Spawner + Date	-1591.3	26.5	5	0.00
Sex + Previous Spawner	-1589.0	28.8	4	0.00
Repeat Spawner	-1584.6	33.2	3	0.00
Previous Spawner	-1583.6	34.2	3	0.00
Sex + Age	-1508.8	109.0	4	0.00
Age	-1504.8	113.0	3	0.00
Sex + Previous Spawner + Age + TL^2	-1493.4	124.4	7	0.00
Sex + Previous Spawner + Age + TL^2 + Date	-1492.4	125.4	8	0.00
Sex + Previous Spawner + Age + TL	-1491.0	126.8	6	0.00
Sex + Previous Spawner + Age + TL + Date	-1489.8	128.0	7	0.00
Sex + Previous Spawner + Age	-1487.3	130.4	5	0.00

Table V. Shows AIC Results of regressions run on GSI.

Model	AICc	Delta AICc	df	AICwt
Sex	-1393.0	0.0	4	1.00
Sex + Previous Spawner	-1358.8	34.2	3	0.00
Sex + Previous Spawner + TL	-1357.0	36.0	5	0.00
Sex + Previous Spawner + Date	-1357.0	36.0	5	0.00
Sex + Previous Spawner + TL^2	-1355.6	37.4	6	0.00
Sex + Age	-1303.8	89.2	4	0.00
Sex + Previous Spawner + Age	-1270.7	122.3	5	0.00
Sex + Previous Spawner + Age + TL	-1268.7	124.3	6	0.00
Sex + Previous Spawner + Age + TL + Date	-1267.2	125.8	7	0.00
Sex + Previous Spawner + Age + TL^2	-1267.0	126.0	7	0.00
Sex + Previous Spawner + Age + TL^2 + Date	-1265.6	127.4	8	0.00
TL	-1058.4	334.6	3	0.00
TL^2	-1056.3	336.7	4	0.00
Date	-1045.1	347.9	3	0.00
Previous Spawner	-1021.2	371.8	3	0.00
Repeat Spawner	-1021.1	371.9	3	0.00
Age	-985.8	407.2	3	0.00

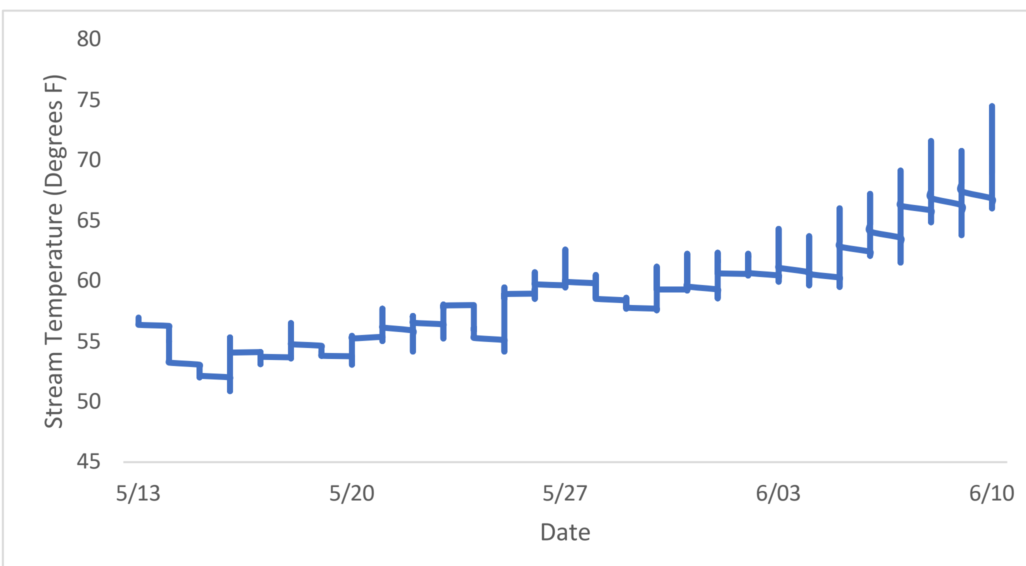

Figure I. Shows temperature readings from the Souadabascook Stream during the sample period between May 13th and June 10th.

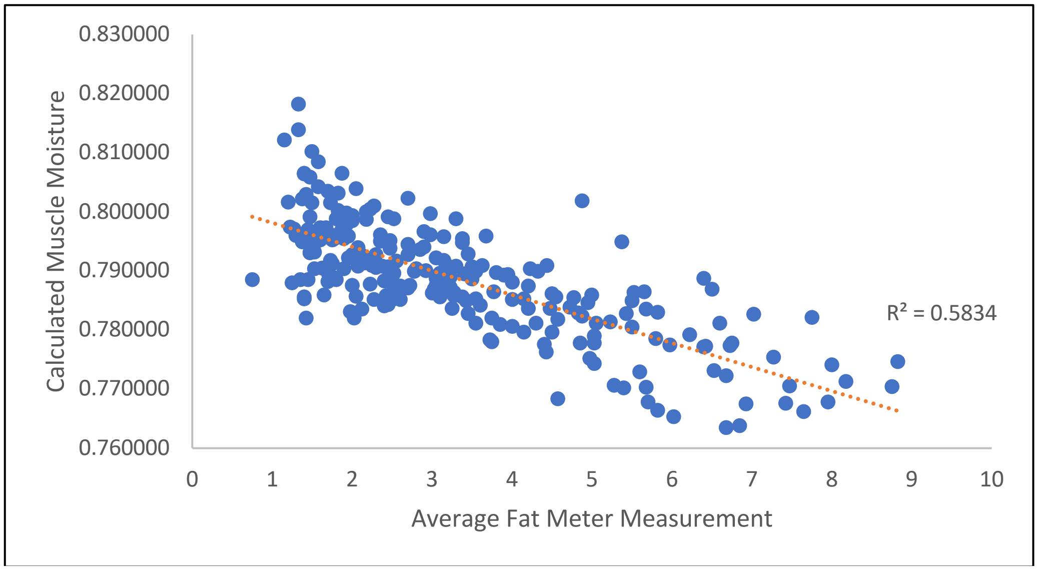

Figure II. Average fat Meter measurements plotted against calculated muscle moisture. Regression yielded a p-value of less than 0.01.

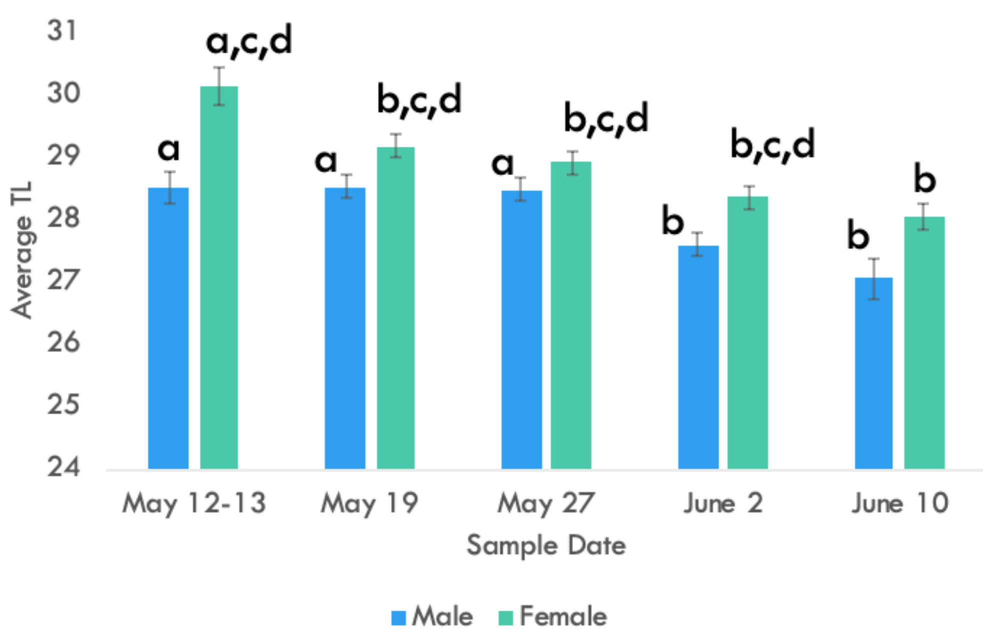

Figure III. Shows average TL by week and sex throughout the sampling period. Error bars show standard error. Letters indicate where significant differences occur. For example, differences were detected between the first three weeks and weeks four and five.

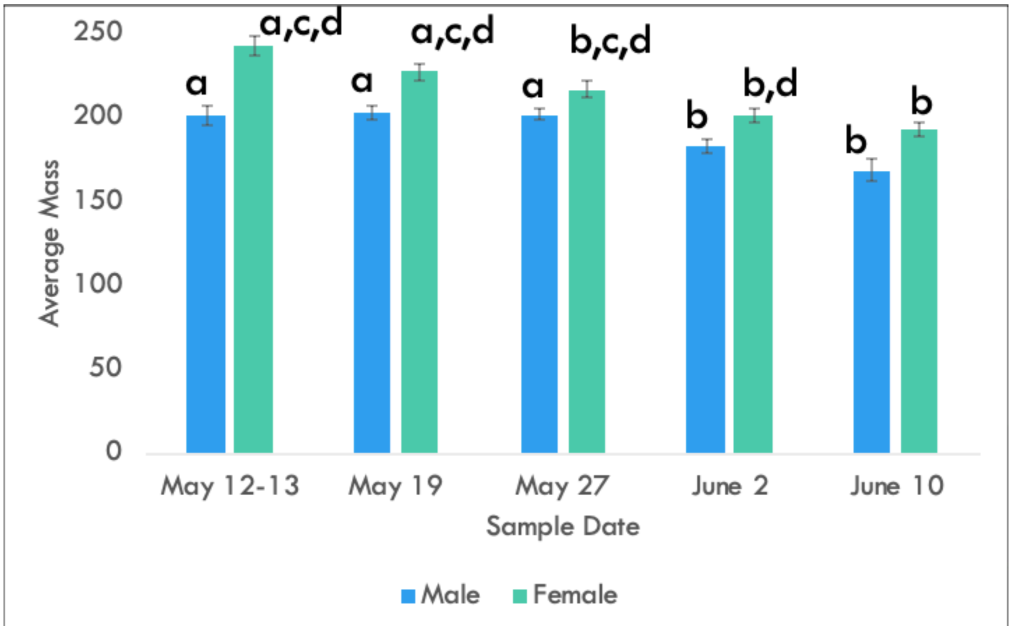

Figure IV. Shows average mass by week and sex throughout the sampling period. Error bars show standard error. Letters indicate where significant differences occur. For example, differences were detected between the first three weeks and weeks four and five.

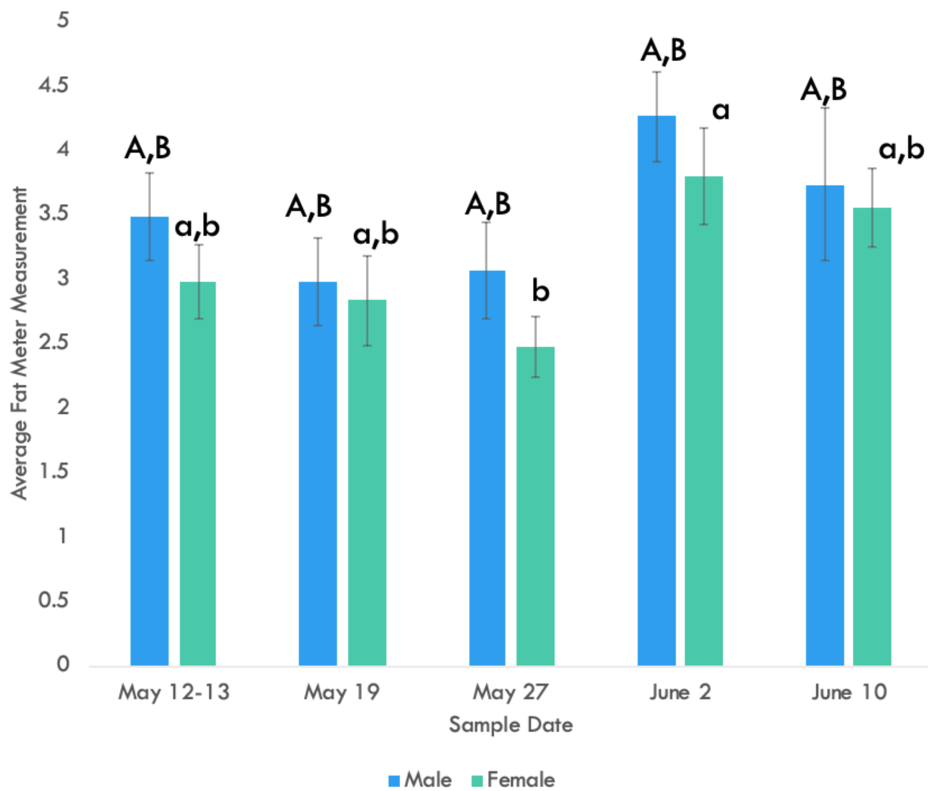

Figure V. Shows average fat meter measurements by week and sex throughout the sampling period. Error bars show standard error. Lowercase letters represent significant differences, here detected between weeks three and four in females.

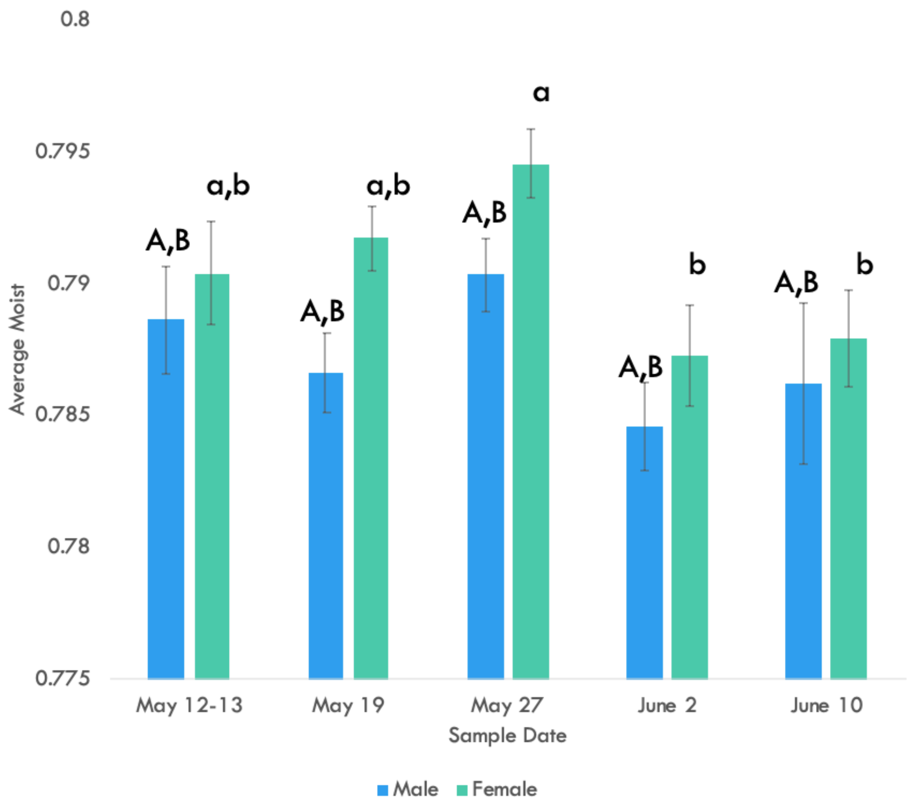

Figure VI. Shows average muscle moisture by week and sex throughout the sampling period. Error bars show standard error. Lowercase letters represent significant differences, here detected between weeks three and four, and weeks three and five in females.

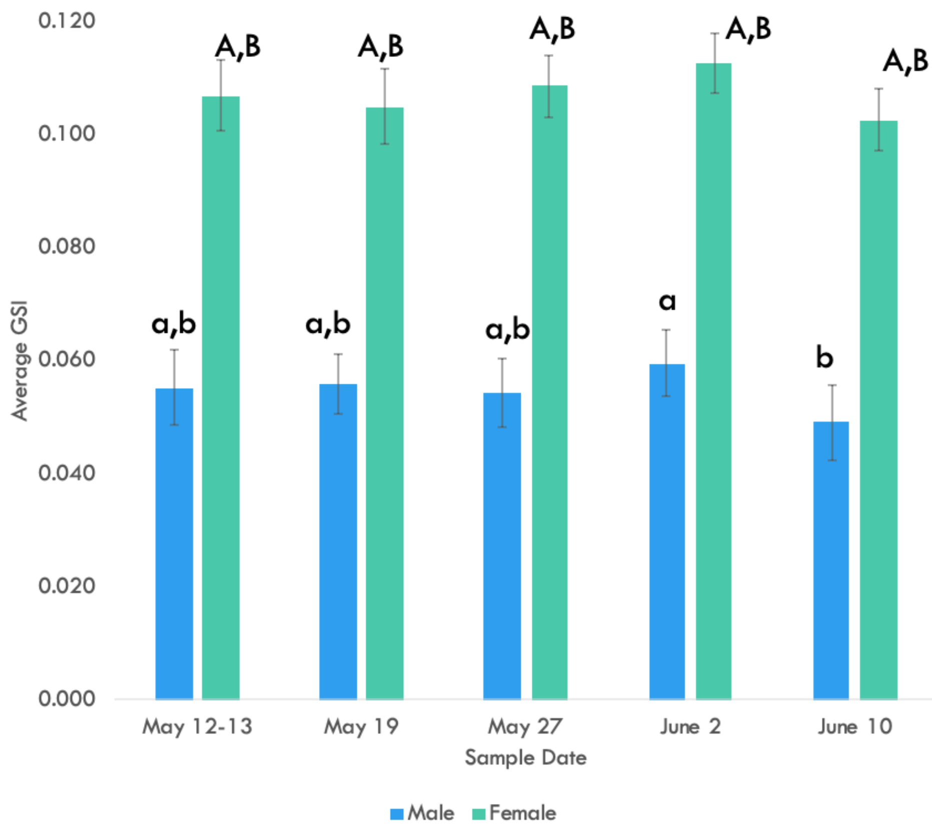

Figure VII. Shows average GSI by week and sex throughout the sampling period. Error bars show standard error. Error bars show standard error. Lowercase letters represent significant differences, here detected between weeks four and five in males.

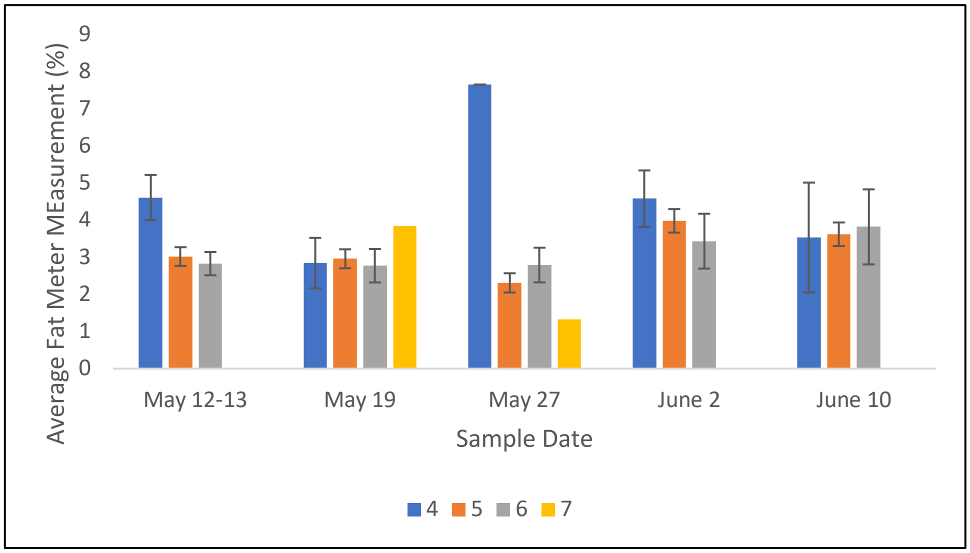

Figure VIII. Shows average fat meter measurements by week and age throughout the sampling period. Error bars show standard error.

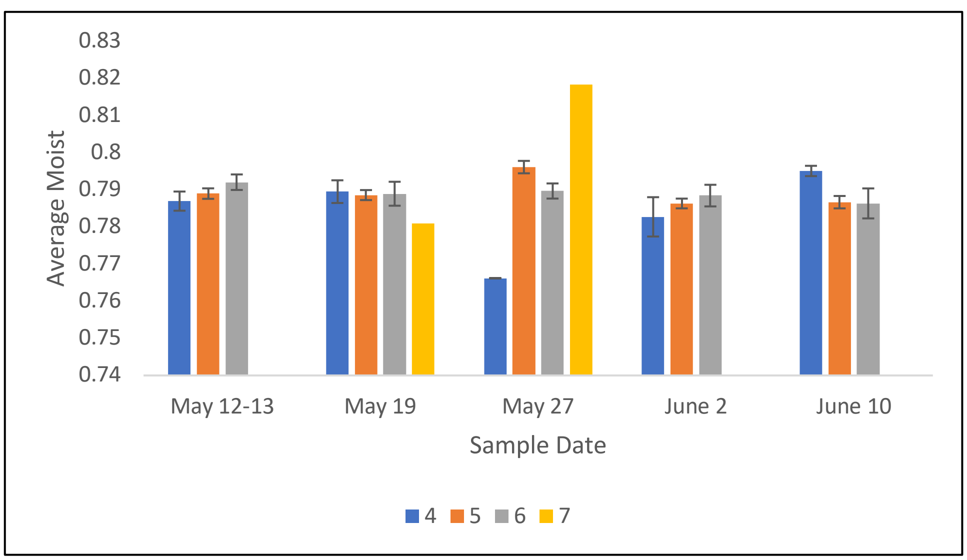

Figure IX. Shows average muscle moisture by week and age throughout the sampling period. Error bars show standard error.

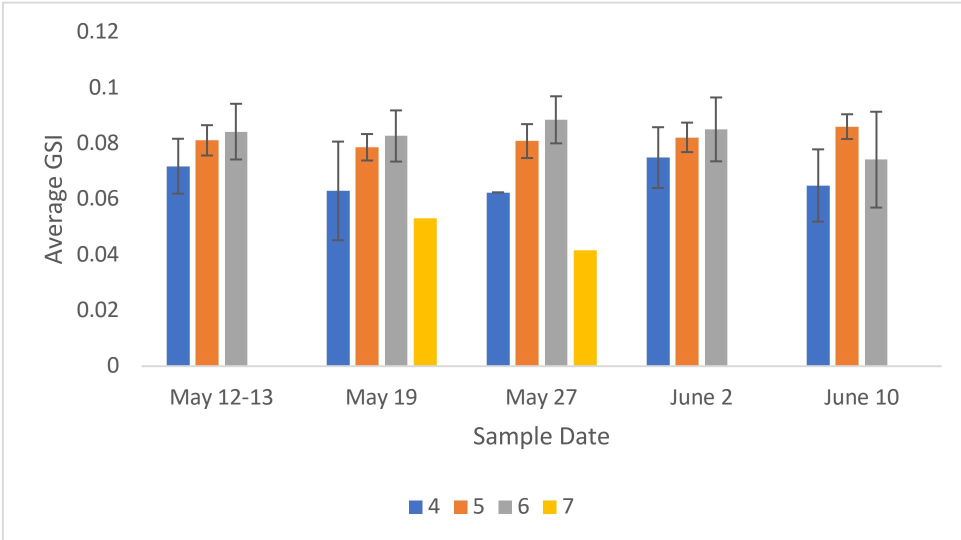

Figure X. Shows average GSI by week and age throughout the sampling period. Error bars show standard error.

OFFICE OF RESEARCH COMPLIANCE
INSTITUTIONAL ANIMAL CARE AND USE
COMMITTEE PROTOCOL REVIEW FORM FOR
RESEARCH, TEACHING, OR PILOT STUDIES

2. Title and number of course/Title of project:

Energy loss during river herring migration

3. Funding agency for project, if applicable: **None**

Please attach the vertebrate animal (VA) section/methods section from the proposal. If multiple agencies are involved, please send only the VA sections that specifically relate to this protocol.

4. Briefly describe the (check appropriate category) research, teaching, or pilot study

objectives **(not procedures)** that involve use of animals. Describe these objectives in non- technical language. Do not paste in sections of grant proposals.

The migration that alewife undergo from the ocean to freshwater lakes is very energy demanding. These fish may lose up to 60% of their lipid reserves before reaching the spawning grounds (Crawford et. al 1986). It is no surprise then that post spawn mortality for alewife can be anywhere from 41-91% (Saunders et. al 2006). Factors that may affect the energy utilization of these fish include migration distance, water velocity and temperature, gonad state, sex, and body size. In general, males lose more of their lipid reserves than females, and fish that begin their migration later in the spawning run tend to have lower reserves than ones who ran earlier.

Energy utilization of these fish is closely related to gonad development, and thus biological fitness. Because these fish have limited resources, there are tradeoffs between reproduction and other metabolic demands (Silva et. al 2012). The goal of this work is to better understand the energetics of river herring in relation to fitness. This will be accomplished by collecting migrating alewife over the course of their migration at the Souadabscook Stream and

characterizing somatic and gonadal energy stores. These data will be analyzed with regards to fish length, age, sex, and spawning history.

5. Describe how this use of animals contributes to the advancement of knowledge that may
eventually benefit humankind
and/or animals.

Alewife have returned in record numbers to many rivers in Maine, including the Penobscot River, with estimates of several million fish in this river in 2018. Much of this recovery is the result of active stocking of adult fish by DMR into likely spawning habitat. With recent changes being made to the river system, characterizing the temporal patterns of spawning and repeat spawning of these fish is important for long term planning of supplementation efforts and management. This fish is highly sought after commercially for lobster bait so that information on population demographics and energy content are relevant for sound management.

7. State the rationale for use of this/these species and life stages. Address the issue of
replacement by explaining why educational or research objectives cannot be met by the use of nonvertebrate animals, cell or tissue cultures, or non-animal systems. (Please note: the IACUC does not consider "hands-on experience" to be in and of itself an adequate educational objective, unless the course serves students whose anticipated educational and professional futures will require the skills imparted through such hands-on experience. If that is true in this instance, please describe the student population that typically enrolls in the course.)

Since the objectives of this study are linked to the restoration and management of alewife in the Penobscot River, it is essential to use this species for the study.

8. Justify the number of animals with respect to your overall project design:

a. Study Groups (e.g., treatments and replicates): Briefly outline the specific groups or treatment types that comprise your project. Describe the role each of these groups performs with respect to your specific project objectives/hypotheses (e.g., control or comparison to another treatment). Indicate whether and how these groups would be replicated. **Alewife are anticipated to be migrating from May through June, spanning an 8-10 week period. We hypothesize that the age, sex and spawning history of fish returning to the sample site (Souadabscook Stream) will differ over time. Additionally, we expect the energy content of fish returning to**

differ with respect to migration timing b. Sample Sizes: Provide a rationale for the number of individuals (per study group or replicate) based on the specific inferential methods to be used. Address the issue of reduction by explaining why the proposed number individuals is sufficient, but not excessive. A simple statement that the number proposed is required for statistical significance is not an adequate response. Formal power analyses often provide the most direct and informative rationale, and are useful in assessing sample sufficiency even when numbers are logistically limited by captures, space etc. See "power analysis guidance" regarding doing a power analysis. If a rationale is based on comparison to prior studies, or specific recommendations for a field, provide relevant citations and justify how the current design compares to those contexts. In the case of pilot studies, meaning investigations conducted for the express purpose of determining suitable approaches and sample sizes for future research, justify your numbers in terms of those objectives.

In order to detect a 20% difference in body lipid (e.g., a decrease from 25 to 20%) then a sample size of 40 would be required (assuming a SD of 4% and a desired power of 0.8 for a comparison of means, two-sided equality). While the data will be statistically analyzed via model selection (using time, age, size,sex, and spawning history) to predict lipid content, this provides a rudimentary guide for desired sample size. Given that we expect sex differences, our target weekly collection is 50 individuals.

5

c. Summary: Provide summary formula(s) that clearly depict how the numbers of
individuals listed in #6 above are obtained as a product of the number of study groups, replicates and sample sizes presented in 10a and 10b. (Example: 500 adult zebrafish = 5 exposure ***10 weeks x 50 fish =500***

9. The Committee does not wish to receive copies of research proposals or laboratory manuals. The Principal Investigator or Instructor is asked to address succinctly the following questions, as applicable. Special care should be taken to justify any procedures generally discouraged by the University's code of ethics and policy.

collection or capture **– fish will be dip netted directly out of the stream using a long handled, fine mesh net. Often, they can be captured by hand. Immediately these fish will be transferred to a bucket for euthanasia.**
planned euthanasia: **All fish will be sacrificed in a concentrated solution of MS-222 (250 mg/l). Fish will be left in the euthanasia solution for 10 minutes following cessation of opercular movement.**

All fish will be sacrificed in a concentrated solution of MS-222 (250 mg/l). Fish will be left in the euthanasia solution for 10 minutes following cessation of opercular movement.

2. If euthanasia becomes necessary due to unplanned injury or illness to the animal(s), how will it be accomplished (include dosages/duration if applicable) and verified? *

Any individual sacrificed due to unplanned injury or illness will be euthanized in a concentrated solution of MS-222 (250 mg/l). Fish will be left in the euthanasia solution for 10 minutes following cessation of opercular movement.

b) Captured from the wild (please answer the following)

1. Where and when will the animals be captured? **Soudabscook Stream (Hampden Maine) from May through June.** 2. What specific capture gear will be employed (nets, traps, electrofishing etc.) and
how will it be operated (e.g., frequency of net or trap checks). Include information how often traps checked, how long animal(s) will be in trap, how non-target species will be avoided or handled if captured (e.g., immediately released, euthanize, weigh/measure).

Dip net; any non-target fish will be immediately released (though none are anticipated with the exception of blueback herring that may mix into the run and overlap in time)

3. What steps will be taken to protect animals from exposure or other danger during
collection? **Rapid handling**

In compliance with our Public Health Service Animal Welfare Assurance, we have implemented an Occupational Health/Medical Surveillance Program. The first step will be for investigators to identify potential hazards with tasks involved with the study, so the IACUC veterinarian and Safety and Environmental Management (SEM) can assess the risks to determine if further information will be required from everyone named in the protocol (i.e., a health questionnaire). **NOTE:** In evaluating this risk assessment statement, we will be looking for animal care tasks that increase the risk of illness (such as a zoonotic disease), physical injury (such as animal bites), and/or allergic reactions to those handling the animals. Also consider hazards of animal excrement/hazards to workers handling the animals' bedding that may be important to an accurate risk assessment.

a) Provide a brief description of the protocol (cut and paste response from question 6 of the

protocol). (NOTE: Only this page, not the whole protocol, goes to SEM and the Occupational Health Physician, thus the request for duplication of the answer to question 6.)

The migration that alewife undergo from the ocean to freshwater lakes is very energy demanding. These fish may lose up to 60% of their lipid reserves before reaching the spawning grounds (Crawford et. al 1986). It is no surprise then that post spawn mortality for alewife can be anywhere from 41-91% (Saunders et. al 2006). Factors that may affect the energy utilization of these fish include migration distance, water velocity and temperature, gonad state, sex, and body size. In general, males lose more of their lipid reserves than females, and fish that begin their migration later in the spawning run tend to have lower reserves than ones who ran earlier.

Energy utilization of these fish is closely related to gonad development, and thus biological fitness. Because these fish have limited resources, there are tradeoffs between reproduction and other metabolic demands (Silva et. al 2012). The goal of this work is to better understand the energetics of river herring in relation to fitness. This will be accomplished by collecting migrating alewife over the course of their migration at the Souadabscook Stream and characterizing somatic and gonadal energy stores. These data will be analyzed with regards to fish length, age, sex, and spawning history.

b) List the tasks required. Add additional numbers as needed. (Examples: handling animals,

administering drugs, euthanasia; field work could include driving, operating watercraft.)

1. Driving 2. Walking on rocks and uneven ground 3. Handling of fish

c) For each of the tasks described in b) above, list the associated hazards. (Examples;

exposure to allergens, needle stick.)

1. **Accidental injury or death** 2. **Slipping and falling/accidental injury** 3. **Accidental cuts or abrasions** 4.

Exposure to mosquito and tick-borne pathogens

d) For each of the hazards described in c) above list how the hazards will be managed.

(Examples: use of gloves and goggles, field work training.)

1**. Driver's license and mandatory driver's safety training (University of Maine) and**

Federal safety training for operators of Federal Vehicles 2. PI will discuss risks associated with working on rocks and footwear will be

appropriate. 3. All field workers are trained in first aid and ability to conduct work will be

evaluated by the PI. 4. Participants will be instructed to wear long pants tucked into their socks and check daily for ticks and tick bites. Insect spray will also be made available for use on the face and exposed skin. Participants will be encouraged to wear permethrin-infused clothing. Participants will be given a brief training on the risks of mosquito and tick-borne diseases and the typical signs of the diseases. Participants will also be directed to the Maine CDC Website to obtain additional information: http://www.maine.gov/dhhs/mecdc/infectious-disease/epi/vector-borne/.

After this risk assessment is reviewed, everyone named in the protocol may be required to complete a health questionnaire. The health questionnaire may require review by the Occupational Health Physician. If so, there is a charge for this review (~$45). Researchers are asked to budget for these costs in proposals for outside funding. For unfunded studies, the cost will be covered by the Office of the Vice President for Research and Dean of the Graduate School. If you have any questions regarding the completion of this page, please contact, Safety and Environmental Management (SEM), 1-4055, SEM@maine.edu.

AUTHOR'S BIOGRAPHY

Anthony M. Zenga was born in New Brunswick, New Jersey on February 26, 1998. He was raised in Easton, Pennsylvania and graduated from Easton Area High School in June of 2016. After finding out that his education at the University of Maine would be taught outdoors as well as indoors, Anthony enrolled. A wildlife ecology major concentrating in fisheries, he is a member of Xi Sigma Pi and the student chapter of the American Fisheries Society. Anthony also plays the trumpet in many bands on campus, and has achieved the rank of Eagle Scout in the Boy Scouts of America. Upon graduation, he plans on finding a job closer to home working with fish.