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The Evolution of Size in Fish: Evaluation of Natural Selection for Size and Evidence for a Prolonged Cost of Rapid Growth

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Abstract of the Dissertation

The Evolution of Size in Fish: Evaluation of Natural Selection for Size and Evidence for a Prolonged Cost of Rapid Growth

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Having a larger body size tends to be positively associated with fitness, as it often leads to increased survival. For my dissertation I evaluated the strength of natural selection and the presence of prolonged trade-offs from an early period of fast growth in order to better understand the evolution of size. I began by reviewing the literature to assess natural selection pressures for size in the early life history of fishes, and calculated standardized selection differentials. I found that the majority, 77%, of standardized selection differentials were positive indicating that larger size was being favored. Because this literature review only focused on the early life history, I then used the Atlantic silverside (*Menidia menidia*) as my model species to evaluate how selection varies over the life history. Before measuring selection in the field, however, I conducted a lab validation experiment using Atlantic silversides to determine accuracy in otolith back-calculation, a method that can be used to track the traits of survivors. I then collected Atlantic silversides from the field, over their growing season, and found that selection fluctuated considerably between favoring larger size and smaller size. Lastly, I measured a prolonged growth trade-off which has the potential to counteract positive selection for size. Despite

evidence for growth costs on some timescales (i. e. immediate, end of life), there has been little evidence of prolonged growth trade-offs which occur in-between these two timescale extremes. I measured 3 traits (swimming ability, muscle morphology, lipid mass) and found that both instantaneous as well as prolonged costs of growth manifested. Interestingly, fish were able to recover their swimming ability after 36-37 days on limited rations. This dissertation has shown that overall selection intensity for size was much weaker when measuring selection over the growing season of Atlantic silversides than when solely looking at the early life history. This result has important implications for better predicting the evolution of size in fish populations due to intense harvesting. Furthermore, I have demonstrated that trade-offs can continue to manifest over a prolonged period and a new life history theory should be developed to take this into account.

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Publications

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Introduction

The majority of world fish stocks currently face heavy fishing pressure. Fifty-three percent of all fish stocks are considered fully exploited, and 32% are over-exploited or becoming depleted (FAO 2010). In addition, fishing practices have degraded natural ecosystems that act as critical nursery or spawning grounds (Auster and Langton 1999). A review of the detrimental effects caused by benthic trawling showed remarkable similarity to the impacts of terrestrial clear-cutting (Watling 2005).

Typically, fishing is intrinsically selective for fish of a specific phenotype. The selectivity of fisheries is due to gear and season restrictions enforced by management, the fisher's economic interest, mesh sizes, and even how fish are marketed (Law and Stokes 2005, Law 2000). This selectivity has been found to vary through space and time. For example, various habitats can be opened for fishing, and then closed, and regulations on mesh sizes increased from 80mm to 95mm over a 4 year span in the North Sea cod fishery (Law 2000). Fishing often selects for the biggest and thus fastest growing fish, as these are most valuable. Pacific salmon used to be sold per individual, however starting in 1945, they were sold by the pound, thus encouraging the fishery to increase harvest pressure on the largest fish (Ricker 1981, Law 2000, Law and Stokes 2005). Myers and Hoenig (1997) determined how the intensity of gear selectivity changed with size in Atlantic cod (*Gadus morhua*) using mark-recapture data from 127 tagging studies over a roughly 50 year period. Different gear types caused very different levels of fishing mortality, where cod traps were most efficient catching fish between 40-60cm, gill nets between 65-75cm, and long-lines were best between 80-90+cm (Myers and Hoenig 1997). The combination of high fishing rates, modest additive genetic variation, and the

intrinsic selectivity that often occurs with fishing could result in fisheries induced evolution. Although heritability in life history traits tends to be lower than heritability in morphological traits, adequate additive genetic variance is expected to be present (Mousseau and Roff 1987, Vandeputte et al. 2004, Tosh et al. 2010).

A 10-generation laboratory experiment tested whether size would evolve in response to fishing pressure, and if so, once fishing selection ceased whether size had the potential to recover to pre-fishing values (Conover and Munch 2002, Conover et al. 2009). In the controlled lab fishery of Atlantic silversides (*Menidia menidia)* when the smallest 90% of individuals were removed the fish evolved to be larger at 190 days and have faster growth rates, whereas the opposite trend was seen for the fishery where the largest 90% of individuals were removed (Conover and Munch 2002). The evolution of size has also been observed in wild fish populations and the patterns are consistent to what is expected from fisheries induced evolution (Sinclair et al. 2002, Swain et al. 2007, Edeline et al. 2007).

The question of whether size has the potential for rebound has been more recently addressed. When selective harvest on the fished Atlantic silverside population was stopped, size and growth rate showed signs of rebounding to pre-fishing levels, although the recovery was slow (Conover et al. 2009). However, in the field, Atlantic cod stocks, which have not been exposed to fishing pressure for the past 15 years, show no sign of recovery in their age or size at maturity (Swain 2010).

It is also of great importance to determine the effects that size-selective fishing has on traits correlated with size. Other life-history traits, including larval viability, size-at-hatch, and growth efficiency, that were not directly selected upon were also found to have evolved after 5

generations of exposure to either the small-harvest fishery or the large-harvest fishery (Walsh et al. 2006). The population under the large-harvest fishery regime evolved life-history traits that likely would be maladaptive in the field (Walsh et al. 2006). These same traits were evaluated after 5 generations of non-size biased fishing mortality in the Atlantic silverside selection experiment to test whether traits correlated to size had the potential for recovery (Salinas et al. submitted). The pattern of recovery varied from trait to trait, where some traits showed evidence of full recovery (i.e. 10-day post-hatch survival) and other traits showed no sign of recovery (Salinas et al. submitted).

When trying to form predictions about the evolution of size in fished populations and evaluate whether size can recover if fishing pressure lessens, it is critical to better understand the landscape of natural selection for size, particularly the strength and direction of natural selection over an extended period of the life history. It is also valuable to evaluate factors that may promote evolutionary stasis, such as growth trade-offs (Merila et al. 2001). Although I will briefly introduce each chapter below, more background information is provided at the outset of each chapter.

A review of selection intensity in the literature has shown that larger body size is generally favored in a wide range of taxa (Kingsolver and Pfennig 2004). Despite several paradigms that have been developed and tested to explain the relationship between body size and survival (i.e. 'bigger is better') and the numerous studies measuring size-selective mortality, selection differentials for fish were not included in the previous selection reviews. In this dissertation, I first calculate standardized selection differentials to evaluate the strength and direction of natural selection for size in the early life history of fishes (Chapter 1). I then

compare the strength of natural selection in the early life in fishes to natural selection in terrestrial taxa.

As the next step in studying size selection in fishes, it is important to understand whether selection for size remains constant when longer periods of the life history are evaluated. To address this issue, I first tested the accuracy of otolith back-calculation, which is a way to estimate size at previous ages, and a common method for measuring size-selective mortality (Meekan and Fortier 1996). I determined the accuracy in back-calculating the population mean for Atlantic silversides of known age (Chapter 2) and tested 4 back-calculation models to determine which works best for back-calculating the observed moments of the initial population distribution. Next, I measured whether size selection fluctuates over the growing season in field collected Atlantic silversides (Chapter 3). I followed a cohort of Atlantic silversides in 2006 and 2007 and repeatedly measured size selection. If selection fluctuates between favoring larger and smaller size, then overall, selection pressure may balance out.

As the final chapter in my dissertation, I asked whether prolonged growth trade-offs have the potential to promote evolutionary stasis of fish size. A growth trade-off occurs where a change in growth that is beneficial for fitness results in a change in another trait that has a negative impact on fitness (Stearns 1989). Although both immediate growth trade-offs and end-of- life growth trade-offs have been demonstrated empirically, prolonged growth costs that continue to manifest between these two periods have had much less attention. I evaluated whether poorer swimming ability, decreased lipid mass, and muscle development are prolonged costs of rapid growth in the Atlantic silverside, and if they are, specifically ask whether they can recover following return to normal growth (Chapter 4).

Chapter 1: Extreme selection on size in the early lives of fish

Abstract

Although fitness typically increases with body size and selection gradients on size are generally positive, much of this information comes from terrestrial taxa. In the early life history of fishes, there is evidence of selection both for and against larger size, leaving open the question of whether the general pattern for terrestrial taxa is valid for fishes. We reviewed studies of size-dependent survival in the early life history of fishes and obtained estimates of standardized selection differentials from 40 studies. We found that 77% of estimated selection differentials favored larger size and that the strength of selection was more than 5 times that seen in terrestrial taxa. Selection decreased with study period duration and initial length, and disruptive selection occurred significantly more frequently than stabilizing selection. Contrary to expectations from Bergmann's rule, selection on size did not increase with latitude.

Introduction

In many species, larger size results in apparent fitness advantages due to higher fecundity (Fleming & Gross 1994), increased survival (Kissner & Weatherhead 2005), and superior mate and territory acquisition (Fleming & Gross 1994). Concomitantly, estimates of selection clearly favor larger size: 79% of selection estimates for size are positive whereas selection estimates for other morphological traits are centered around zero (Kingsolver & Pfennig 2004). However, the vast majority of what we know about selection on size comes from terrestrial taxa.

Mortality rates experienced in the early life history of fishes greatly exceed those experienced by terrestrial vertebrates. It is not uncommon for only 0.01 to 0.1 percent of fish that hatch to survive to age 1 (Houde 1987). As a consequence of this extreme mortality, the opportunity for selection is much greater in fishes than in the majority of terrestrial taxa on which previous reviews were focused. Although, it is generally believed that larger larval and juvenile fish have higher survival rates (Meekan & Fortier 1996, Sogard 1997), demonstrations of size selective mortality in fishes are not commonly reported in terms of selection differentials or gradients, making it unclear how the magnitude of selection in the early life history of fishes compares with that of terrestrial taxa. In this study, we compile data on size-dependent mortality in the early life history of fishes, calculate standardized selection differentials from these data, and compare them to previously published estimates for terrestrial taxa.

Larger body size is associated with higher latitudes and elevations where temperatures are cooler (review by Meiri & Dayan 2003, Guillaumet et al. 2008), a pattern first noted by Bergmann (1847) and referred to as Bergmann's rule. Although several explanations for Bergman size clines have been offered (Lindstet & Boyce 1985, Belk & Houston 2002, Ashton

& Feldman 2003), a mechanistic understanding of the factors generating Bergman's rule remains elusive. A plausible, though untested, proximate mechanism for Bergman's rule is that selection on body size varies with latitude. We test this hypothesis by examining how selection varies with latitude in the early life history of fishes.

Methods

Since Lande and Arnold published their seminal paper (Lande and Arnold 1983) there has been a long history of using longitudinal data to estimate the strength of selection. The most salient feature of these methods is that they allow selection on a trait to be partitioned into direct and indirect effects resulting from correlations with other characters. The study of selection has been further improved upon by methods which explicitly account for the binary nature of survival data, allow more flexible relationships between phenotypes and fitness, and account for differences in viability (e.g. Mitchell-Olds and Shaw 1987, Schluter 1988, Janzen and Stern 1998, Hadfield 2008). Of particular relevance here is the fact that selection gradients estimated from linear regression on binary survival data will tend to be underestimated in absolute value (Janzen and Stern 1998)

Unfortunately, there are relatively few studies on fish that have used these methods (but see Vigliola et al. 2007 and Gagliano et al. 2007), which perhaps accounts for their absence from prior reviews (Hoekstra et al. 2001, Kingsolver & Pfennig 2004). Most typically, the data available for fish consists of cross-sectional studies in which a cohort of individuals is sampled sequentially and otoliths are used to reconstruct growth histories. These studies provide data on size but no additional information on other traits. As a consequence, the widely used longitudinal and multivariate methods can't be applied. Nevertheless, there are many such

studies on size selection in the early life history of fish and we believe that it is worthwhile to compile this data and translate it into a format that allows for comparison with estimates of selection in terrestrial taxa.

In order to interpret the selection differentials obtained from these cross-sectional studies, a number of assumptions must be made (Lande & Arnold 1983, Arnold & Wade 1984). Principal among these are that the trait does not change over time and that the same cohort is measured each time. The daily rings laid down in otoliths provide a permanent record of an individual's growth history (Stevenson & Campana 1992), and so differences in mean size at a given age from one sample to the next reflect differences in survivorship and sampling variability, but not growth. All of the studies included in this review made every effort to ensure that they sampled the same cohort repeatedly since their primary purpose was to estimate mortality.

We assume that variability in repeatedly sampling a cohort results in individuals being missing at random (e.g. Hadfield 2008) and that other changes in the size-at-age distribution are the result of selection, allowing us to estimate selection differentials on size-at-age. Of course, selection differentials estimated for a single character from cross-sectional data also include the influence of selection on correlated characters (Lande & Arnold 1983). On average, however, selection differentials and selection gradients are quite strongly correlated (Kingsolver et al. 2001) suggesting that a reasonable picture of the importance of selection on size in fishes may be obtained from the differentials we estimate.

We searched the literature for all studies that had reported differential survival in the early life stages in both marine and freshwater fish. In the majority of the studies we reviewed,

otoliths (n=33) or scales (n=1) had been used to estimate the size of surviving individuals in a previous time period. Tag, release, and recapture studies, in which a known size range of fish was tagged and released to investigate the size dependence of mortality (e.g. Leber 1995) were also included in our review (n=6). For each study included in the database, mean and variance in length of initial and survivor populations, the sample sizes from which these were estimated, and the time interval between samples were recorded. Data were collected either from the text whenever possible or digitized from figures. To minimize the error introduced in data extraction, means of three independent digitizations were used. Studies that reported anthropogenic size selective mortality (e.g. from fisheries) were excluded from our analysis.

As in previous studies (Kingsolver & Pfennig 2004), we evaluated the strength of directional selection on size. Directional selection is typically defined as the regression or covariance of a trait on fitness (Lande & Arnold 1983, Rice 2004). Unfortunately, individual fitness estimates were not available in the vast majority of studies we reviewed. Nevertheless, Price's (1971) theorem guarantees that the change in the mean due to selection is equivalent to the covariance between the trait and fitness. We therefore used standardized selection differentials to index the strength of selection on size. Specifically, we calculated the intensity of selection, *i*, as

$$i = \frac{\bar{z}_t^* - \bar{z}_t}{\sqrt{v_t}}$$

where $\bar{z}_t^*$ is the mean length at age t of the surviving population, $\bar{z}_t$ is the mean length at age t in the initial population, and v_t is the variance in length at age t in the initial population (Falconer & Mackay 1996). Note that *i* is dimensionless, permitting meaningful comparison across species. Because data for other traits were unavailable, we were unable to control for selection acting on other characters. Consequently these standardized selection differentials include both the direct effect of selection on size and indirect selection on size resulting from selection on correlated characters (Lande & Arnold 1983). To account for sampling variability, each of the unstandardized selection differentials were tested for significance using t-tests (Sokal and Rohlf 2001).

In addition to directional selection, it is of interest to determine whether selection is disruptive or stabilizing. Several methods for calculating whether selection is disruptive or stabilizing have been developed. According to Price's (1971) theorem, the covariance between fitness and the squared deviation from the mean is equal to the change in variance among the selected and initial populations plus the squared selection differential. If fitness decreases with the squared deviation from the mean then this covariance is negative and selection is stabilizing. Lande and Arnold (1983) showed that, as for directional selection, the effects of other traits could be accounted for by using the quadratic selection gradient, γ. For a scalar trait, γ is equivalent to the covariance between fitness and the squared deviation from the mean divided by the initial population variance. Neither of these indices require assumptions about the shape of the fitness function or the trait distribution for their derivation. Estes and Arnold (2007) developed another measure of stabilizing selection, *H*, based on the second derivative of the fitness landscape with respect to a change in the trait mean, assuming that the trait is normally distributed before selection. It is straightforward to show that this index reduces to the change in

the variance before and after selection, divided by the initial variance squared. Schulter (1988) suggests using regression splines to visualize the fitness surface directly.

Thus, in keeping with these previous studies, we calculated the covariance between fitness and the squared deviation from the mean,

$$c = v_t^* - v_t \ + (\bar{z}_t^* - \bar{z}_t \ )^2$$

where v_t^* is the variance in length at age t after selection. Selection is stabilizing if c, the covariance between fitness and the squared deviation from the mean, is negative. Again, *c* calculated in this manner includes both direct effects and the indirect effects of selection on other traits. Note, however that *c* is not dimensionless (units are length^2). To arrive at a dimensionless quantity for comparison across species, we used a standardized index of stabilizing selection,

$$j = \left[v_t^* - v_t \ + \left(\bar{z}_t^* - \bar{z}_t \ \right)^2\right] / v_t$$

While significance of the change in variance may be determined by a straightforward F-test, we know of no appropriate null model for *j*. To test the significance of *j*, we therefore constructed a null distribution by simulation, i.e. drawing each sample (before and after selection) from the same underlying normal distribution and calculating *j*. Significance levels were determined from 10^6 Monte Carlo replicates. For completeness, we also estimated *H* (Estes and Arnold, 2007). The results were qualitatively identical to the results for *j*: 60% of the

values indicated disruptive selection. We note, in keeping with Schluter (1988), that these indices do not necessarily mean that the fitness surface has a valley within the range of the data, only that the mean curvature is sufficiently positive to offset the decrease in variance resulting from directional selection. That said, in the three studies with sufficient data to allow visualization of the fitness surface (Schluter 1988), we found that *j* was positive and the fitness function was bimodal. Given the consistency between these measures of stabilizing selection, we focus on *j* for the remainder of the analysis.

Many factors may influence the magnitudes of *i* and *j*. For instance, it is likely that the intensity of selection varies with size and age as well as the duration of time over which selection was estimated (Hoekstra et al. 2001, Meekan et al. 2007). To evaluate the influence of these factors, we used least-squares and quantile regressions to analyze effects of size, age, and study duration. Quantile regressions for the 25^{th} and 75^{th} percentiles were calculated (Scharf et al. 1998) and confidence intervals were obtained from 1000 bootstrap samples. To evaluate the hypothesis that Bergman's rule results from selection, we used least-squares linear regression and quantile regressions to determine if selection, or the range of selection, varied predictably with latitude. We also examined whether the propensity for stabilizing or disruptive selection varied with latitude using quantile regressions on *j*.

Many studies in this review provided multiple estimates of selection. Some of these were from independent cohorts and were treated as independent observations. However, other studies provided multiple estimates of selection on a single cohort. Because these are not independent, all regressions were tested for significance by bootstrap at the cohort level. Specifically, we generated 1000 bootstrap data sets by sampling cohorts with replacement and using all observations from each selected cohort.

Results:

We calculated standardized selection differentials from 40 studies where estimates of initial population standard deviation were available (Fig. 1a). Each study used in this review yielded from 1 to 69 estimates of standardized selection differentials. Seventy-seven percent of standardized selection differentials (334 of 435) were positive indicating that selection generally favors larger size. Using a two-tailed t-test and $p<0.05$ confidence level, we found that 39% (129 of 334) of the positive differentials and 50% (50 of 101) of the negative differentials were significant. Since 129 out of 179 significant selection differentials were positive, it appears that selection overwhelming favors larger size in the early life history of fishes.

On average, the standardized selection differential for fish was 1.12, which is more than 5 times the mean found by (Kingsolver et al. 2001) for size in terrestrial taxa (t value =9.50, $p<0.001$, df = 495). The median for fish is also twice as large as that found in terrestrial taxa (0.37 v. 0.18). To estimate the mode we constructed histograms for fish and terrestrial taxa using 'optimal' bin widths (Friedman & Diaconis 1981) equal to 2 IQR $n^{-1/3}$, where IQR is the inter-quartile range. Using this approach, the modal values were roughly the same (0.24). Thus, the most frequently occurring selection intensity in fishes is about the same as that for terrestrial taxa, but the distribution is skewed toward considerably higher values.

We found evidence of both stabilizing and disruptive selection (Fig. 1b). Of the 266 observations for which variances could be estimated, 77% (n=204) showed evidence of disruptive selection (j >0) whereas 23% (n=62) showed evidence of stabilizing selection. After restricting attention to the 234 estimates that were statistically significant, we found that 44%

(n=117) indicated disruptive selection, while 6% (n=17) were stabilizing. Disruptive selection appears to be significantly more common than stabilizing selection in the early lives of fishes.

In keeping with previous research on the early life history of fish (Lorenzen 1996), we found that the standardized selection differential decreased with mean initial length ($p=0.004$ $R^2=0.03$, $N= 243$) with a slope (95% confidence intervals) of -0.69 (-1.10, -0.21). The 25^{th} and 75^{th} quantiles also decreased with initial mean length with slopes of -0.29 (-0.41, 0.03) and -0.49 (-1.18, 0.05), respectively, though neither is significantly different from zero at the 0.05 level (Fig. 2a).

The linear regressions of the standardized selection differentials on initial population age and study duration were not significant (Fig 2b), indicating that the mean does not change with either of these variables ($p=0.74$, $p=0.15$ respectively). Neither did the 25^{th} quantile depend on study duration ($p=0.24$). However, the 75^{th} quantile significantly decreased ($p=0.02$, $N=382$) with study duration with a slope of -0.29 (-1.15, -0.02). Thus, although the mean and lower bounds remain constant, the most extreme selection differentials decrease with study duration. The slopes for the 25^{th} and 75^{th} quantiles for selection gradients versus initial age were also not significantly different from zero (Fig. 2c).

The stabilizing selection index *j* did not vary significantly with mean initial age or length. We found that selection tended to become more stabilizing as study duration increased (slope = -3.3, $N = 282$, $p=0.008$). However, this was due to a single outlying datum ($j=329.9$, study duration = 14 days); after this point was removed, neither the linear regressions nor the quantile regressions indicated any significant dependence of *j* on study duration.

To test our hypothesis that Bergmann's rule is maintained by selection, we regressed *i* and *j* on latitude. Neither the mean intensity of selection ($P=0.58$, $R^2=0.0007$, $N= 38$), nor the 25^{th} and 75^{th} quantiles, varied significantly with latitude (Fig. 2d). The standardized stabilizing selection index also did not vary significantly with latitude.

Discussion

Selection clearly favors larger size in the early life history of fishes and appears to be exceedingly strong when compared to selection on size in terrestrial taxa (Kingsolver et al. 2001). We expect that this is a consequence of the exceedingly high mortality in the early life history of fish. In support of this, we found that the range of selection strengths is greatest among smaller fish, which is consistent with the fact that the mortality rate generally decreases with size (Houde 1997). We hypothesize that selection is commensurately strong in the early lives of plants and other taxa with comparable mortality rates but we do not, as yet, have data with which to test this hypothesis.

Longer study durations produced a smaller range of selection strengths, although the mean intensity of selection did not vary. This result is analogous, though not identical, to the observation in terrestrial taxa that linear selection gradients decreased on average as the time in-between selection increased (Hoekstra et al. 2001). This may simply result from the fact that individuals grow more over longer periods reducing the opportunity for selection as size increases. However, it may also indicate that selection fluctuates through time (Hoekstra et al. 2001). For example, Gagliano et al. (2007) found that selection pressure changed from favoring slower growth to favoring faster growth during the early life of a coral reef fish, highlighting the importance of looking at selection over the entire life history (Merila et al. 2001).

There was no evidence for latitudinal gradients in either the mean or range of selection. Nor was there a relationship between latitude and the degree of stabilizing selection. Thus, it seems unlikely that Bergmann's rule is the result of latitudinal gradients in selection. However, it is worth noting this observation is based on pooling gradients in selection across species, rather than looking at variation in selection within species as would be needed to ideally test this hypothesis.

Some of our selection gradients were exceptionally large. Since our review is based on studies that were not intended to estimate selection directly, it is worthwhile to estimate the range of standardized selection values that are biologically plausible. To evaluate the feasibility of observed standardized selection differentials, we calculated the maximum possible values associated with a given overall level of mortality. Regardless of the shape of the size distribution, the maximum selection differential for a given level of mortality is generated by truncation selection in which all individuals above (or below) a threshold survive. Assuming the initial size distribution is standard normal, the maximum possible standardized selection differential, *i*, and the fraction of the population surviving, S, are both functions of the point of truncation, c. These are given by

$$i(\mathrm{c}) = \frac{1}{\mathrm{S(c)}\sqrt{2\pi}}\mathrm{e}^{\frac{-\mathrm{c}^2}{2}}$$

$$\mathrm{S(c)} = \frac{1}{2}\left[1 - \mathrm{erf}\left(\frac{\mathrm{c}}{\sqrt{2}}\right)\right]$$

where erf is the error function (Abromowitz & Stegun 1972).

A similar argument can be used to construct an upper bound for the standardized stabilizing selection index, *j*. Regardless of the shape of the distribution, the maximum disruptive selection occurs under symmetrical truncation selection, i.e. no individuals within c standard deviations of the mean survive, while all individuals outside this interval do. Assuming again a standard normal distribution, the maximum value for *j* is given by

$$j(\mathrm{c}) = \frac{2\mathrm{c}}{\mathrm{S}(\mathrm{c})\sqrt{2\pi}}\mathrm{e}^{\frac{-\mathrm{c}^2}{2}}$$

$$\mathrm{S}(\mathrm{c}) = [1 - \mathrm{erf}\left(\frac{\mathrm{c}}{\sqrt{2}}\right)]$$

Given that it is not uncommon for survival in the early life history to be as low as 0.1% (Houde 1997), standardized selection differentials as large as ± 3 and stabilizing selection indices of about 12 are not implausible under a normal distribution. Approximately 8% (n=34 of 435) of the standardized selection differentials and 7% (n=19 of 282) of the stabilizing selection indices we calculated exceeded these bounds. We re-ran the statistics excluding any data outside these ranges. The results were qualitatively the same except that the range of selection differentials no

longer decreased with study duration and selection was no longer dependent on initial mean length.

Although we found evidence of both stabilizing and disruptive selection, disruptive selection was considerably more frequent. One possible explanation for this is purely artifactual. Because otoliths were used to estimate the size distribution of survivors, the variance in length among survivors will be inflated by errors in back-calculation (e.g. Wilson et al. 2009). However, since the back-calculation variance increases with study duration (Secor and Dean 1992), we would expect 'apparent disruptive selection' to increase as well, if it is due primarily to an otolith back-calculation artifact. We found no significant change in *j* with study duration. Therefore, although some of the additional variance among survivors is almost certainly back-calculation error, the prevalence of disruptive selection in the early life history of fishes can't be explained solely as a sampling artifact. Several alternative explanations are plausible. One interesting possibility is that the disruptive selection we observed results from changes in selection pressures associated with ontogenetic niche shifts (Gagliano et al. 2007). Another, simpler, explanation for the prevalence of disruptive selection is that survival is not strictly U-shaped within the range of data, but merely has positive curvature. This would be consistent with numerous observations that the length dependence of mortality decreases inversely (Lorenzen 1996) or exponentially (Munch et al. 2003) with length in fishes.

As with any study of this type, there is the possibility that our results are influenced by publication bias. Hersch & Phillips (2004) suggested that in current studies sample size is often too small to detect slight selection pressure and thus publication bias may inflate apparent selection, though not its direction. The size dependence of survival in fishes is a contentious issue in which several authors have put forward hypotheses claiming that bigger or faster

growing juveniles have higher survival (Leggett & Deblois 1994, Houde 1997, Sogard 1997) and these have been repeatedly criticized by others demonstrating situations in which smaller body size or slower growth is favored (Lankford et al 2001, Connolly & Petersen 2003, Pepin et al 2003**,** Dibattista et al 2007). In light of this, we suspect that publication bias will be somewhat mitigated by the ongoing debate regarding the size dependence of early survival in fishes.

Given the high intensity of selection that appears to exist in fish early life history, and the ubiquity of heritable genetic variation (Gjerde et al. 2004, Shimada et al. 2007), we should expect to see rapid evolution towards larger body sizes. Assuming a typical heritability value for a life history trait of 0.262 (0.012 SE) (Mousseau & Roff 1987), size in the early life history of fishes should evolve at a rate of ~ 0.29 haldanes. This is considerably higher than many evolutionary rates that have been observed (Hendry & Kinnison 1999). Despite this extreme evolutionary potential there is little evidence for rapid evolution in the early life history in fishes.

Four mechanisms may explain a lack of a response to selection: low heritability, fluctuating selection, environmentally driven covariance between fitness and size, and tradeoffs (both short term and long term). As significant heritability for growth and size are common in fishes (Gjerde et al. 2004, Shimada et al. 2007), it is unlikely that low heritability impedes evolutionary response. Variation in environments can cause covariance between size and fitness (Rasher 1992, Stinchcombe et al. 2002) generating biased estimates of selection (Stinchcombe et al. 2002). In the majority of studies in this review, a single cohort is tracked over time through a highly fluid environment making it unlikely that differences in individual environments are persistent enough to generate bias.

Selection may fluctuate through space and time either across or within cohorts. The intensity of size-selective mortality has been shown to be dependent on both biotic and abiotic factors including condition, predation rate, and winter severity (Garvey et al. 2004, Hurst and Conover 1998). However, given the preponderance of evidence for strong selection in the early life history of fishes, it seems unlikely that selection varies across cohorts enough to prevent evolution.

Growth and development rates at different ages tend to be highly correlated (Kavanagh & Alford 2003) and selection may act in different directions on different life stages. Ambon damselfish underwent a selection shift from 'smaller is better' to 'faster is better' following reef settlement (Gagliano et al. 2007b). At hatching, smaller size was favorable over larger size because the former was associated with more yolk sac reserves than the latter. Whereas upon settlement on the reef; faster growth in fish may increase survival from predators (Gagliano et al. 2007b). This is consistent with our finding that the strength of selection dampens over longer study intervals and may be a primary cause of evolutionary stasis. Moreover, although there have been many documented cases of short-term trade-offs (Lankford et al. 2001, Billerbeck et al. 2001), evidence for long-term growth costs are just beginning to appear (Olsson & Shine 2002, Metcalfe & Monaghan 2003, Innes & Metcalfe 2008). When long-term costs exist, rapid growth that increases survival during the early life history results in decreased performance later in life (Royle et al. 2006). The net effect of such costs is balancing selection that is only manifest when looked at across the entire life history. Ontogenetic changes in selection pressures and long-term trade-offs highlight the need to study selection over the entire life history (Merila 2001).

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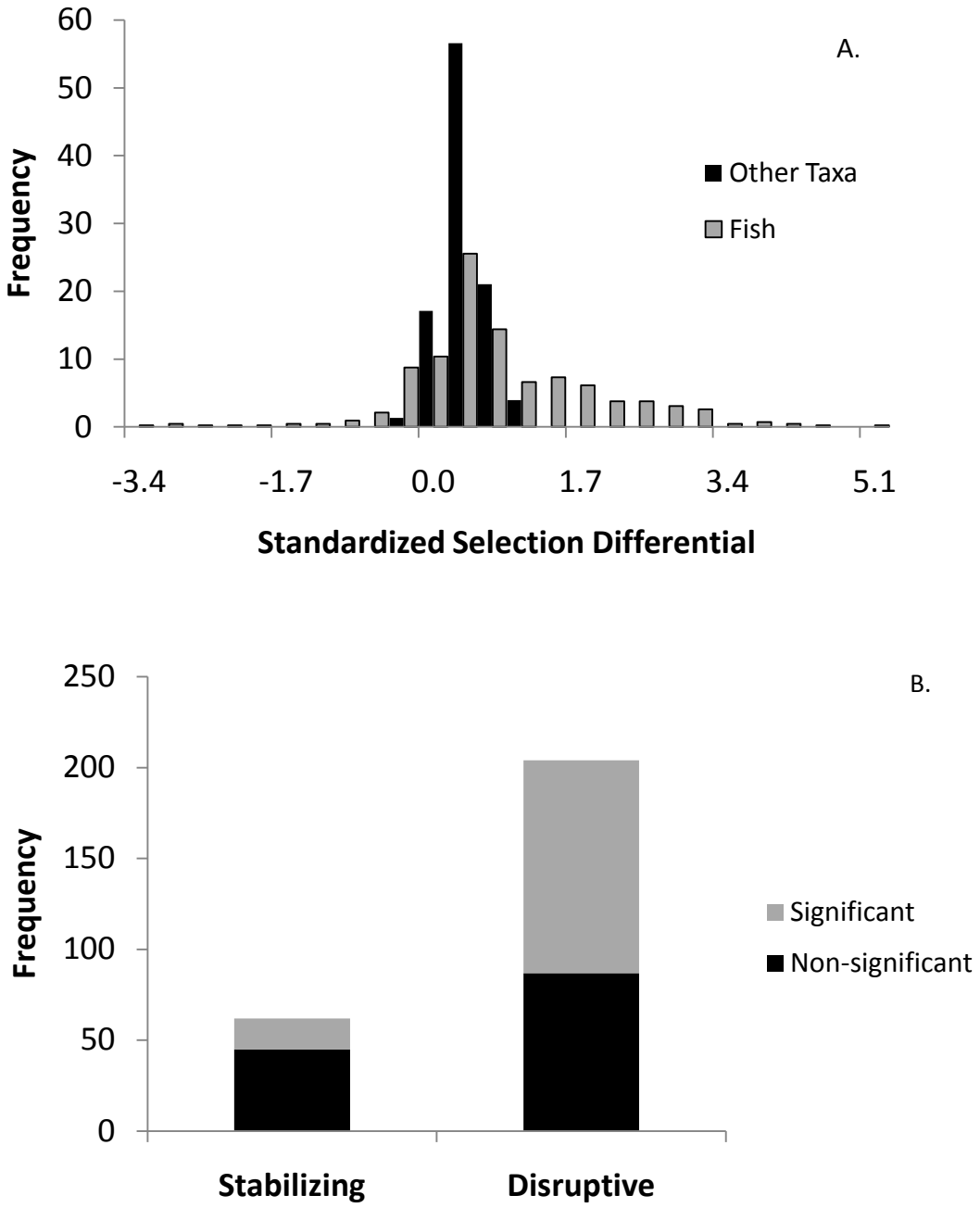

Figure 1a. Comparison of standardized selection differentials for fish and other taxa. The grey bars give the frequency of standardized selection differentials for fish, the black bars show frequencies for terrestrial taxa as extracted from Kingsolver et al. (2001). Figure 1b. Bars indicate frequencies for disruptive v. stabilizing selection. 44% of the disruptive selection estimates were significant whereas only 6% of the stabilizing selection estimates were significant.

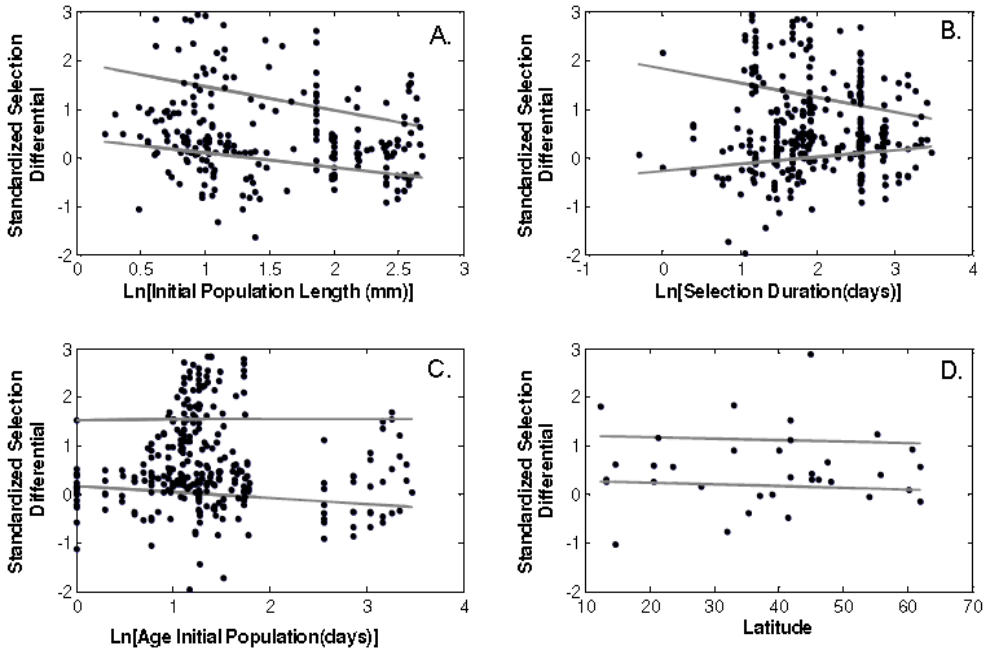

Figure 2. In each panel, points indicate the standardized selection differential for each observation in our study and the solid lines give the 25th and 75th quantiles as linear functions of the independent variable. Independent variables: A. Natural log of mean length in initial population, B. Natural log of the time interval between initial and final length estimates, C. Natural log of mean initial population age, D. Latitude at which data were collected.

Chapter 2: Validating back-calculation models using population data

Abstract

Otolith back-calculation, an estimation of size and growth at some previous age, is a valuable method of tracking the traits of surviving individuals. Numerous back-calculation models have been developed, however validation of the accuracy of model prediction does not always occur before they are used. Using the Atlantic silverside (*Menidia mendia)*, which is an ideal species for studying size selective mortality; I tested the precision of four commonly used back-calculation models (body proportional hypothesis, modified Fry, biological intercept, and time varying growth). I calculated the moments of the observed length frequency distribution (mean, variance, skewness, and kurtosis) and compared them to the moments of the back-calculated length frequency distribution. I found that the BPH model had the highest overall accuracy in predicting the moments of the observed length frequency distribution.

Introduction

Otoliths have long been a fundamental part of fisheries research. Otoliths were initially used to age fish by counting annual rings, and in more recent decades, young-of-the-year fish have been aged by counting daily rings (Stevenson and Campana 1992). Determining ages of fish of known length can be used to estimate population growth trajectories and may be used in fishery growth models (Stevenson and Campana 1992). Age determination has facilitated study of the timing of critical life history events, such as hatching and migration (Jones 1992). Otolith morphology and microchemistry have also been used to identifying stock structure and migration rates (Jones 1992).

Size at previous ages may be estimated through otolith back-calculation. After validating that the rings form in a predictable manner, and that fish length and otolith radii retain a functional relationship, it is possible to count back and estimate the size of a fish at some point in its history. By comparison with direct observations of sizes through time, back-calculated sizes provide a valuable time series that allows us to track the size of survivors.

Typically size is assumed to be the most important trait impacting an individual's survival (Rice at al 1997). In the early life of fishes, mortality is exceedingly strong and as much as 99.9% of fish that hatch do not survive to year 1 (Houde 1997). This mortality can be size-biased and otolith back-calculation has been used to study the size-dependence of mortality (e.g. Meekan and Fortier 1996). Similarly, otoliths can be used to measure natural selection for size. This is obtained by calculating a selection differential which is equal to the mean length of survivors minus the mean length of original population (Gagliano et al. 2007, Vigliola et al.

2007, Perez and Munch 2010). Evaluating selection on size is important for understanding the evolution of growth in the early life history of fish.

Many methods for back-calculation have been proposed; Vigliola and Meekan (2009) list twenty-two that use various approaches to address statistical artifacts, nonlinearity, and temporal variation. Here, I restrict my attention to four models that are commonly used. The earliest is the Fraser-Lee method, which assumes a linear relationship between otolith radius and length (Lee 1920). However, slower growing individuals tend to have larger otoliths than predicted by the Fraser-Lee method, leading Campana (1990) to develop the 'biological intercept model' to decrease the bias generated by this growth rate effect. Francis (1990) recommended the 'body proportional hypothesis' which assumes a constant proportional difference between mean length and otolith radius (Francis 1990). Further increasing flexibility, the 'modified Fry model' does not depend on a linear relationship between fish length and otolith radius but instead allows the relationship to be allometric (Vigliola et al. 2000). Sirois et al. (1998) developed the time-varying growth rate model, which takes into account the fact that growth varies with age. Although all of these models are based on the idea that increment width is a function of the change in length, Secor and Dean (1992) showed that predictions of length at age can vary widely between different back-calculation models. Further, in some applications, the back-calculated sizes are used to estimate the size frequency of survivors; in others only the mean or variance are of interest. It is therefore critical to evaluate which model performs best for any given species and the optimal model may depend on the characteristics of the size distribution that is being estimated.

Some back-calculation models, including the modified Fry model and the body proportional hypothesis model, include a more flexible relationship between fish length and

otolith radius. Vigliola and Meekan (2009) have noted the importance of determining whether the relationship between length and otolith radius is linear or allometric. Since the primary use of otoliths in many studies is to predict unobserved sizes, the best model should be the one that has the most accurate 'out-of-sample' performance, meaning that predictive ability is tested on samples not incorporated when estimating model parameters (Stone 1974). My first objective in this study is to determine whether fish length and otolith radii have a linear or an allometric relationship. Here, I use leave-one-out-cross validation (Stone 1974) to determine the 'out-of-sample' performance of either the linear or allometric model. Using the best model form, my next objective is to determine which back-calculation model is best for recovering the initial size distribution and its first few moments using Atlantic silversides (*Menidia menidia*) as a model system.

Methods

Lab-rearing and otolith techniques

The Atlantic silverside, *Menidia menidia,* is an abundant, annual marine fish that ranges from Northeastern Florida to New Brunswick, Canada (Johnson 1975). Adults spawn in the spring in inshore waters and the young-of-the-year migrate offshore in autumn (Conover and Murawski 1982). This species is an important prey item for many recreationally and commercially valuable fishes, such as bluefish (*Pomatomus saltatrix),* striped bass (*Morone saxatilis),* and summer flounder *(Paralichthys dentatus)* (Buckel et al. 1999; Manderson et al. 2000; Rudershausen et al. 2005). Silversides are easily cultured in the laboratory making it possible to obtain precise estimates of their size distributions through time and daily increment formation has previously been validated (Barkman and Bengtson 1987).

In order to test the assumption that fish length and otolith radii are proportional in Atlantic silversides, I collected a wide size range of *Menidia menidia* in May 2007-July 2007 from wild populations in Great South Bay, NY (40° 38'17.58"N 73° 17'16.39"W). For each fish, total length was measured using digital calipers (nearest 0.5 mm). Fish were then frozen until the otoliths could be removed and measured. Saggital otoliths were removed following the dissection techniques of Stevenson and Campana (1992) or by dissolving the surrounding tissue in hypoclorite bleach. After otoliths were thoroughly dry they were mounted on slides with Krazy glue, sulcus side up. The otoliths were polished with 3 micron lapping film (US Supply Corporation, North Carolina) until the daily rings were visible. Digital microscopic images of the otolith were analyzed using Image pro plus (V. 6 Media Cybernetics). Rings were counted and increment widths measured along the same axis for all otoliths. Each otolith was read a single time by the same reader (KOP). A total of 167 wild fish were used to determine the relationship between otolith size and fish size. To test whether there were any differences between field and laboratory fish, I also determined the otolith size-fish length relationship for 60 lab-reared fish collected at 4, 6 and 8 weeks old.

To test the accuracy of 4 different back-calculation models under ideal, predator-free conditions, I reared juvenile Atlantic silversides in the laboratory for two months. Adult silversides were collected from Great South Bay, NY (40° 38'17.58"N 73° 17'16.39"W) in May 2006 using 100ft beach seines and transported to the Flax Pond Marine Laboratory, Old Field, NY. Eggs were stripped from adults and allowed to hatch into two replicate 1600L aquaria kept in a greenhouse under ambient photoperiod and temperature. After hatch, fish were fed *Artemia* nauplii *ad libitum* to ensure growth commensurate with that observed in the field (Conover and Present 1990). Over the duration of the experiment, the mean temperature in both replicates was

21°C. All fish in both replicates were live-lengthed at weeks 4 and 6 post-hatch to obtain population length distributions. At 8 weeks post-hatch a random sample of 40 fish from each replicate was removed for otolith sampling. Sizes at weeks 4 and 6 were back-calculated using the four back-calculation models (described below). Back-calculating over this short timespan was chosen because back-calculation over longer intervals to the date of fish hatch has poor accuracy (Pers. Obs.).

Determining the functional form between length at capture and otolith radius at capture

First, I tested whether an allometric model ($L_{cpt}=L_0-bR_0^c+bR_{cpt}^c$) or a linear model ($L_{cpt}=L_0-bR_0+bR_{cpt}$) was better for predicting the fish length-otolith radius relationship using a leave-one-out cross validation approach (LOOCV) (Stone 1974). In the above equations, L_{cpt} and R_{cpt} is the observed length and otolith radius at fish capture. L_0 and R_0 are the length and otolith radius at the biological intercept (measured at hatch). The parameters b and c are statistically determined. The LOOCV method removes each observation from the total sample and estimates model parameters from the remaining observations. The LOOCV sum of squares is then calculated as the squared deviation between the model prediction and actual value for each left-out observation summed over the whole data set. The model form, either linear or allometric, that produced the smallest LOOCV residual sum of squares is the best model for predicting the relationship between fish length and otolith radii and is the most appropriate model for deriving back-calculation estimates. To facilitate comparison of the model performance, I calculated an approximate coefficient of determination (r^2) (Sokal and Rolf 2001). I used the following equation:

$$r^2 = 1 - \left({\text{residual ss}}/{\text{total ss}}\right) \quad (1)$$

where total ss is the overall sum of squares error $\left(\sum(\text{observed length}_i - \overline{\text{observed length}})^2\right)$ and residual ss = $\sum(\text{observed length}_i - \text{predicted length}_i)^2$. Note that this differs from standard practice in that residual ss is based on out-of-sample prediction.

To evaluate whether the otolith radius-fish length relationship differed between the lab and field data, I used likelihood ratio tests to compare residual sum of squares calculated using the allometric model on the lab and field data pooled versus fitting separate models for each. The negative log likelihood (NLL) was given by

$$\text{NLL} = \frac{n}{2}\ln[\frac{ss}{n}] + \frac{n}{2} \quad (2)$$

where n is the sample size of fish length- otolith radius observations for field or lab fish and ss is the residual sum of squares.

Back-calculation models

I used four models of otolith back-calculation that have been commonly used for the early life stages (Sirois et al. 1998; Vigliola et al. 2000; Wilson et al. 2009). The biological intercept model (BI) is given by:

$$L_i = L_{cpt} + (R_i - R_{cpt})(L_{cpt} - L_o)(R_{cpt} - R_o)^{-1} \quad (3)$$

where L_i is the length at age, L_{cpt} is the length at capture, R_i is the otolith radius at age, R_{cpt} is the otolith radius at capture, and L_o and R_o are the length and radius at the biological intercept. The second model for back-calculation is the body proportionate hypothesis (BPH), given by:

$$L_i = (a + bR_i{}^c)(a + bR_{cpt}{}^c)^{-1}L_{cpt} \quad (4)$$

where the constants a, b, and c are equal to the y-intercept, slope, and exponent obtained by fitting an nonlinear regression with radius at capture as the independent variable and length at capture as the dependent value. I also used the time varying growth model (TVG), given by:

$$L_i = L_o + \sum_{t=1}^{i}[W_t + M(W_t - \bar{W})]\left(L_{cpt} - L_o\right)\left(R_{cpt} - R_o\right)^{-1} \quad (5)$$

where W_i is increment width at age i and $\bar{W}$ is mean increment width for a single individual. For this validation study I took the mean increment width across all daily growth rings. M is a growth constant that was calculated as in Sirois et al. (1998). Noting that the sum of increment widths over any period of time is given by the difference between final and initial radii, this equation simplifies to

$$L_i = L_o + (R_i - R_o)\frac{L_{cpt} - L_o}{R_{cpt} - R_o} + M\left(L_{cpt} - L_o\right)\left[\frac{R_i - R_o}{R_{cpt} - R_o} - \frac{i}{T}\right] \quad (5a)$$

where T is the age at capture. The main advantage of (5a) is that it is clear that when otolith growth is constant the term on the right cancels and the TVG model essentially reduces to a BI model. The modified Fry model (FRY) is given by:

$$L_i = a + \exp\left\{\ln(L_0 - a) + \left[\ln\left(L_{cpt} - a\right) - \ln(L_o - a)\right][\ln(R_i) - \ln(R_o)]\left[\ln\left(R_{cpt}\right) - \ln(R_o)\right]^{-1}\right\} \quad (6)$$

where a is equal to the y-intercept, i.e. fish size at time of otolith formation (Vigliola et al. 2000). I calculated this parameter using the following methods described by Vigliola et al. (2000). First, I calculated the parameter b_1 (slope) and c_1 (exponent) from a regression where length is the dependent variable and otoliths radius is the independent variable ($L=L_0-b_1R_0^{c1}+b_1R^{c1}$). I then calculated the parameter b_2 and c_2 from a regression where otoliths radius is the dependent

variable and length is the independent variable ($R=[(L-L_0+b_2R_0^{c2})/b_2]^{(1/c2)}$). The parameter a is the mean of the two y-intercepts described below, where $a_1=L_0-b_1R_0^{c1}$ and $a_2=L_0-b_2R_0^{c2}$:

$$a = (2L_0 - b_1R_0^{c1} - b_2R_0^{c2})/2 \quad (7)$$

Because a_1 and a_2 tend to be very similar, Vigliola and Meekan (2009) have suggested setting a equal to a_1 however in this study I followed the original technique described in Vigliola et al. (2000).

Statistical Analysis

For many back-calculation studies, re-capturing the moments (mean, variance, skewness, and kurtosis) of the population is of primary importance. I calculated the moments of the distributions produced by each of the back-calculation models and compared them to the observed moments of the original population. To evaluate the back-calculation model match with the observed population I calculated square-root mean square errors (*RMSE*) for the mean, skewness, and kurtosis. I summed errors over both replicate aquaria to make these comparisons. The model with the smallest *RMSE* between back-calculated and observed moments is likely to be the most accurate method. To evaluate variance I calculated the ratio of variances in the initial observed population and in the back-calculated estimated population, i.e. $V_{back\text{-}calculated}/V_{observed}$, and determined whether this ratio differed significantly from 1 using an F-test.

I also used the LOOCV method to compare model accuracy in predicting the mean. As individual fish were not tracked from measurement to measurement, I am unable to calculate the sum of square errors between the predicted and observed on an individual level. Instead, I remove each individual from the total sample and calculate the mean from the remaining individuals. The LOOCV sum of squares is then calculated as the squared deviation between the

model prediction and actual value for each left-out observation summed over the whole data set. Using this LOOCV approach at a population level distinguishes outliers and their potential impact on the original calculation of the mean.

I compared observed and back-calculated size distributions using two methods: the Kullback-Leibler test (Kullback and Leibler 1951), and the Kolmogorov-Smirnov test (Sokal and Rohlf 2001). The statistic calculated for the Kullback-Leibler test is the divergence between the distributions (D), where the smaller the value, the better the model is at predicting the expected population. For the Kullback-Leibler divergence analysis, I used frequency bins based on quintiles of the original size distribution. I used these same bins to group the back-calculated populations and then compared the original and back-calculated frequencies in each bin. I also used a 2 sample Kolmogorov-Smirnov test carried out in Matlab version R2009B.

Because no model performed best at all back-calculation metrics, I constructed a performance index to evaluate overall model performance. I used the following 7 measures of accuracy to determine overall model performance: ratio of variances, mean *RMSE*, Skew *RMSE*, Kurtosis *RMSE*, Kolmogorov-Smirnov K statistic, Kullback-Leibler D statistic, and absolute % bias. I re-scaled the 7 measures of accuracy so they ranged from 0, indicating the smallest bias, to 1, which indicated the largest bias. To do so, I used the following equation:

$$\text{Index} = \frac{AM_i - \min(AM_i)}{\max(AM_i) - \min(AM_i)} \quad (8)$$

where AM_i is one of the performance metrics. In this performance index I included the seven measures of accuracy calculated for both replicates over both back-calculation intervals. I then summed the re-scaled metrics for each model over replicates and time intervals and compared the total for each model to identify the overall best model. Importantly this method of re-scaling

allows information on the model value to be taking into account instead of the more traditional ranking technique which would separate all model values by a consistent amount regardless of how similar the values are to each other. All statistics were calculated in Minitab version 16 unless otherwise noted.

Results

In the laboratory rearing study, mean otolith radius at hatch was 33.4μm (SD=2.9, N=34), and mean length at hatch was 4.5mm (SD=0.45, N=490). These values were used as the biological intercept in the back-calculation models. Mean length was slightly larger (~6%) in replicate A than in replicate B at all the time points when fish were measured (Table 1), though this was not significant ($p>0.05$). Skewness tended to be negative and ranged from 0 to -0.7. Kurtosis tended to be positive and ranged from -0.7 to 0.9.

The relationship between fish length (mm) and otolith radius (μm) was positive (Figure 2) for both the lab-reared fish as well as for the field-collected fish. The allometric model generated lower LOOCV residual sum of squares than the linear model for both the lab data (1427.3 v. 1448.3, N=126 lab data, N=167 field data) and the field data (3716.7 v. 4325.9). Additionally, the allometric model for both the field data and lab data had higher coefficients of determinations, though these are quite close (field data $r^2 = 0.94$ for linear v. $r^2 = 0.95$ for allometric, lab data $r^2 = 0.75$ for linear v. $r^2 = 0.76$ for allometric, Figure 1). I also found that the field and lab data were significantly different ($P=0.002$ in the likelihood ratio test using the allometric model).

In light of the superiority (although slight) of the allometric model, I incorporated the allometric form into each of the back-calculation models and calculated model parameters using

the lab fish. For some of the back-calculation models, the results would have changed had I used the field fish or a linear model to generate the model predictions of back-calculated size. Below, I note those differences.

Validation of Back-Calculation Models for Estimation of Population Mean, Skew, Kurtosis, and Variance

The TVG model had the lowest *RMSE* between observed and back-calculated means when back-calculating to week 6 (Table 2A., Figure 3A. and 2B.). When back-calculating to week 4 the BPH model had the lowest mean *RMSE*. On average, the *RMSE* in means was lowest for the BPH model (1.0 mm). Similarly, the BPH model had the lowest overall LOOCV sum of squares (ss=184.3). The FRY model was the worst, with a *RMSE* between the observed and back-calculated means of 1.7 mm.

When back-calculating to week 6 the skew *RMSE* was equally low for the FRY, BI, and BPH models and when back-calculating to week 4 the BPH had the lowest skew *RMSE* (Table 2A.). The kurtosis *RMSE* was lowest for the FRY and BI models when back-calculating to week 6 and was lowest for the FRY model when back-calculating to week 4. Overall, on average the skew and kurtosis *RMSE* was smallest for the BPH and the FRY models respectively indicating that these models were most accurate in estimation of the third and fourth moments.

I had 2 replicates and 2 back-calculated dates for each of the 4 back-calculation models resulting in 16 comparisons of original and back-calculated populations. The ratio of variances was larger than 1 in 100% of cases, indicating that back-calculated variance is greater than observed variance regardless of the model used (16 of 16, Table 2). This increase in variance due

to back-calculation was statistically significant in 81% of cases (13 of 16) (F-test, N=16, $P \leq 0.05$).

Temporal trends in model accuracy

Because many studies seek to back-calculate sizes over extended periods of time, it is worthwhile to ask how the prediction accuracy changes with time. All 4 back-calculation models were included in the following analysis of the temporal trend in back-calculation accuracy. The average *RMSE* for the population mean was 1.9 when back-calculating to week 6 and was 1.0 when back-calculating to week 4. In order to account for differences in fish population length at these different intervals I also calculated percent bias. This was calculated as:

$$\% \text{ Bias} = \frac{(\text{Estimated mean} - \text{observed mean})}{\text{observed mean}} \text{ x } 100 \quad (9)$$

Averaging over all models, the mean percent bias between the observed and back-calculated mean size was -6.7% when back-calculating to week 6 and was 0.5% when back-calculating to week 4. All models underestimated the mean when back-calculating to week 6, whereas when back-calculating to week 4, both underestimation and overestimation occurred. To account for the sign of the bias not always being the same, I also calculated the absolute bias. The mean absolute percent bias was similar when back-calculating to week 6 or week 4 (6.7 and 5.7 respectively). Although all were greater than 1, the average ratio of variances was lower when back-calculating to week 6 (1.4) then when back-calculating to week 4 (2.6), indicating that as the back-calculation interval increases, the overestimation of observed population variance also increases. Both skew and kurtosis showed a similar trend where *RMSE* increased with increasing back-calculation interval. The *RMSE* for these moments were lower when back-calculating to week 6 than to week 4 (0.2 vs 0.5 respectively for kurtosis, 0.1 vs 0.5 respectively for skew).

The Kullback-Leibler divergence ranged from 5.2 to 15.8 (Table 3). Samples back-calculated to week 6 had the lowest divergence with the TVG model whereas samples back-calculated to week 4 had the lowest divergence with the BI model. The Kolmogorov- Smirnov test showed that the smallest difference between the two distributions (K) when back-calculating to week 6 was obtained with the BI model and with the TVG and FRY models when back-calculating to week 4. When averaging across both back-calculation intervals, the Kolmogorov-Sminov test and the Kullback-Leibler divergence test both showed that the BPH model produced distributions that were most similar to the observed distributions.

Given that no single model stood out as the best for all back-calculation criteria, I evaluated overall performance based on their re-scaled metric across criteria (Table 4.). The re-scaled BPH was slightly better than the rescaled FRY model and BI model summed overall (8.7 vs. 9.3, 9.4). Additionally, when the rescaled values are ranked, the BPH model has the lowest rank sum (14 vs 16.5 for BI model, Table 4). The BPH model had a rank of 1 (most accurate model) 4 of 7 times, whereas the TVG model never had a rank of 1. Thus, assuming that all back-calculation metric are equally important, it appears that the BPH model is the best overall model.

If I had used the otolith-length relationship estimated from the field instead of lab fish the expected means for the BI model and the TVG model would not have changed, however model predictions from the FRY and BPH would have become less accurate. The *RMSE* for the mean would have been higher for the FRY model (2.5 vs 1.7) and higher for the BPH model (2.6 vs. 1.0). When using a linear model for the lab data, the models produce similar predictions as when using an allometric model with lab data. The BPH model had the lowest *RMSE* for the mean if a linear model form or an allometric model form was used with lab data (1.2 v. 1.0). However the

mean *RMSE* for the FRY model was lower when using the linear model (1.5 v. 1.7) than when using the allometric model. Furthermore, although the prediction of the population mean when using the linear FRY model was less accurate than when using the linear BPH model, the overall re-scaled metric sum for the FRY model was only slightly lower than the BPH model, but considerably lower than the TVG. This indicates that the overall best model depended on the model form as well whether I used the lab or field data.

Discussion

Many studies have used back-calculation models to measure size-selective mortality in the field (Meekan and Fortier 1996; Sirois and Dodson 2000; Cotano and Alvarez 2003). However, for these results to be interpreted, it is important to validate the main assumptions of the back-calculation approach. While the proportionality between fish length and otolith radius and the frequency of ring deposition are commonly validated (Stevenson and Campana 1992), relatively few studies have validated the back-calculation method (Sirois et al. 1998; Vigliola et al. 2000; Wilson et al. 2009; for a review Vigliola and Meekan 2009).

A recent review of back-calculation studies recommended using within-sample statistics to determine whether the relationship between otolith radius at capture and fish length at capture was allometric or linear, an important first step to determining the back-calculation model functional form (Vigliola and Meekan 2009). When I tested the length and otolith radius relationship using a 'within-sample' method the linear and allometric model were not significantly different (t-test, $p=0.06$). Instead I used the LOOCV technique, a method to determine the out-of- sample performance and found that the allometric model has a higher (albeit slightly) r^2 value and thus fits the data better than the linear model. I suggest that 'out-of-

sample' performance is the appropriate method because the back-calculation model will ultimately be used to predict new values.

I used 4 common back-calculation models to predict length at previous ages and found that overall the BPH model was preferred. In contrast, Wilson et al. (2009) validated five back-calculation models at an individual level in tropical cleaning gobies and found that the FRY model produced the smallest bias between back-calculated length and observed length (5%). The bias between observed and back-calculated mean length in this study ranged from 0.8 to 18.0%. The BPH model had an average bias in mean length of 4.2%. Furthermore, the BPH model also produced the lowest rank sum, indicating that overall it is the most accurate back-calculation model for Atlantic silversides. A previous review of back-calculation models by Vigliola and Meekan (2009) suggested that the most conservative and therefore best back-calculation model to use was the FRY. When comparing the FRY model to the BPH model, the BPH tends to be poorer at predicting small fish sizes than the FRY model (Vigliola and Meekan 2009). This is because the FRY is constrained to a biological intercept whereas the BPH model has a statistically derived intercept. In the current study, however, I found that the FRY model had the lowest accuracy in predicting the population mean. I suspect that the BPH model does well in the present study even at small sizes because I used radius-length data over the entire range of sizes to which I was back-calculating.

All of the models overestimated back-calculated variance. The models produced greater overestimates of variance when back-calculating over a larger interval. Previous work has found a similar trend where variance increases with increasing back-calculation interval (Secor and Dean 1992). In contrast, *RMSE* for population mean did not increase when increasing the back-calculation interval from 2 weeks to 4 weeks. These results support the argument that back-

calculation errors result primarily from random variation in growth or measurement errors rather than a persistent bias inherent in back-calculation.

An important assumption in this validation study is that mortality is not size-biased. I took several measures in setting up the experiment to limit the nature of this bias. Firstly, the fish were reared in a predator-free environment with unlimited food. However, the live measurement technique may have increased mortality, particularly for the smallest fish. I was not able to record lengths of the individuals that died until the final week of the study when they were large enough to be visible at the bottom of the tank. The lengths of these individuals that died in the final week of the experiment were measured and were likely to have come from the same distribution as the original population (KS test, N=33, P=>0.05).

However, I can estimate what the population mean would have been had the mortality been selective. From week 4 to week 6 I had 12% mortality (44 of 347) in replicate A and 27% mortality (76 of 279) in replicate B. From week 6 to week 8 mortality slightly increased; 35% died in replicate A (78 of 223) and 29% died in replicate B (59 of 203). The decreasing population sample size over time was partly due to mortality, but also due to removal of individuals for otolith extraction. The individuals I removed for otolith extraction were not included in the mortality estimation. To estimate how the best fit back-calculation models would change had this mortality been strongly selective, I truncated the left hand tail of the distribution and re-estimated the observed mean at week 4 and week 6 after. Adjusted in this manner, the mean increased by no more than 12% across all trials. This would have resulted in the TVG model being the most accurate in predicting the population mean although the BPH model was second best (*RMSE* of TVG= 2.7, *RMSE* of BPH= 3.6).

One important use of otolith back-calculation is to determine the size-dependence of mortality or calculate selection differentials for size. This validation study has demonstrated that differences in mean size of 4.2% (% bias for BPH) or less cannot unambiguously be attributed to selection. However, differences greater than 4% likely indicate size selective mortality.

Table 1. Observed moments for the two populations (Replicate A and B). The date that the fish were measured is given in the first column. In the second column the replicate is listed. The moments of the distribution (mean length in mm, variance in length, skewness and kurtosis as well as sample size from which these were calculated) are in the remaining columns.

Collected	Replicate	Mean	Variance	Skew	Kurtosis	Sample Size
Week 4	A	18.2	6.9	-0.7	0.8	347
Week 6	A	28.6	8.4	0	0.9	220
Week 4	B	17.2	6.8	-0.5	0.2	279
Week 6	B	26.8	9.4	-0.1	-0.7	200

Table 2. ---Mean *RMSE* (mm), Skew *RMSE*, and Kurtosis *RMSE*, for the 4 back-calculation models when back-calculating to week 4 and week 6. Replicate A and B are grouped together. The ratio of variance (V_{bc}/V_{orig}) is listed in the final column. The values in bold are associated with the model most accurate in predicting the observed population.

Back-calculation Estimate	Model	Mean	Skew	Kurtosis	Variance
Week 6	BI	2.4	**0.2**	**0.2**	**1.4**
	BPH	1.5	**0.2**	0.3	1.6
	FRY	2.6	**0.2**	**0.2**	**1.4**
	TVG	**1.0**	0.4	0.7	1.5
Week 4	BI	0.7	0.6	0.6	**2.2**
	BPH	**0.6**	**0.4**	0.6	3.5
	FRY	0.8	0.5	**0.5**	2.3
	TVG	2.0	0.7	1.0	2.4

Table 3. ---Results for Kullback-Leibler divergence tests and Kolmogorov-Smirnov tests for the 4 back-calculation models when back-calculating to week 6 and week 4. Replicate A and B are grouped together. The Kullback-Leibler statistic is the divergence between the two distributions (D), and the Kolmogorov-Smirnov statistic is the maximum difference between the two distributions (K). The values in bold are associated with the model most accurate in predicting the observed population.

Back-calculation Estimate	Model	KL div.	KS test
Week 6	BI	13.9	**0.2**
	BPH	7.3	0.3
	FRY	15.2	0.4
	TVG	**5.2**	0.4
Week 4	BI	**6.4**	0.4
	BPH	8.1	0.3
	FRY	9.9	**0.2**
	TVG	15.8	**0.2**

Table 4. ---Below are sums of re-scaled metrics for the 7 statistics used to determine model accuracy (Ratio of Variances, Mean *RMSE*, Skew *RMSE*, Kurtosis *RMSE*, Kolmogorov-Smirnov K statistic, Kullback-Leibler D statistic, and % bias of the mean). The bottom half of the table shows the ranks of the re-scaled statistics. The re-scaled or rank sum is listed in the final column.

Model	Ratio of Var	Mean	Skew	Kurtosis	KS test	KL div.	% bias	Re-scaled sum
BI	1.9	1.4	1.2	0.9	1.3	2	0.7	9.4
BPH	1.2	0.8	1.3	2.1	0.9	1.5	0.9	8.7
FRY	2.1	1.1	1.1	1	1.8	1.8	0.5	9.3
TVG	1.9	1.7	3.2	1.1	1.4	1.7	2	13

Model	Ratio of Var rank	Mean rank	Skew rank	Kurtosis rank	KS test rank	KL div. rank	% bias rank	Rank sum
BI	2.5	3	2	1	2	4	2	16.5
BPH	1	1	3	4	1	1	3	14
FRY	4	2	1	2	4	3	1	17
TVG	2.5	4	4	3	3	2	4	22.5

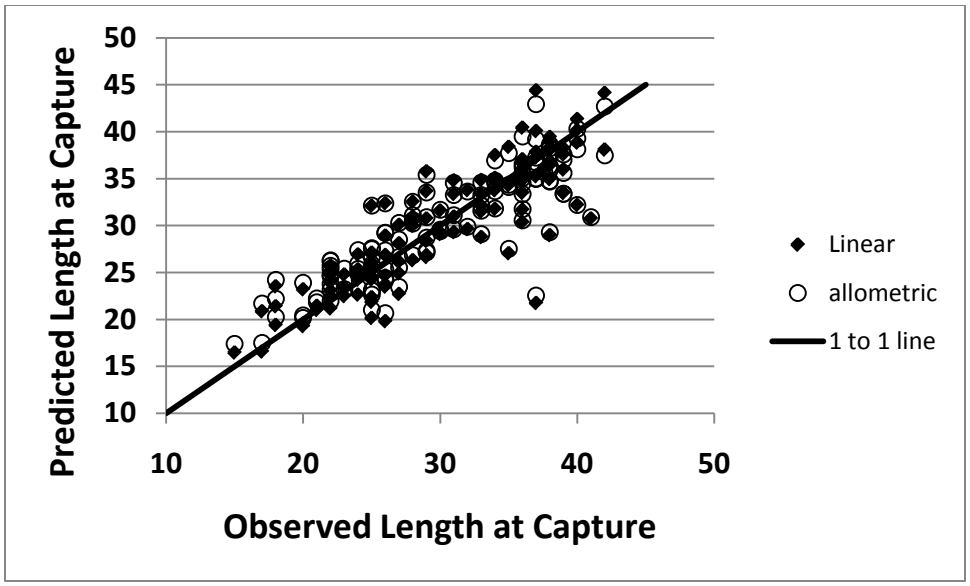

Figure 1. The predicted length at capture for lab reared fish (Y axis) is plotted versus the observed length at capture (X axis) for both the allometric and linear models. Data for the linear model is shown by the black markers and data for the allometric model is shown by the open white markers.

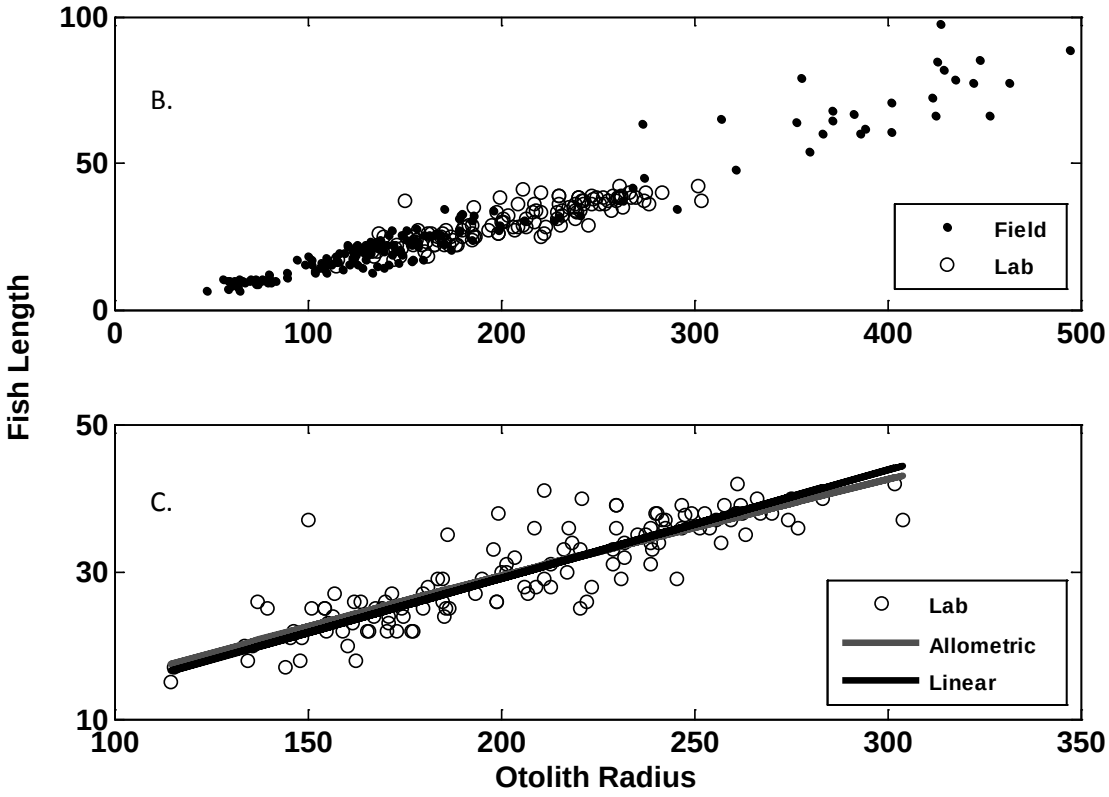

Figure 2.In panel B, black dots represent measurements of observed otolith radii(μm) and fish length (mm) from field collected fish, open circles are from lab-reared individuals. In panel C, the grey line is the allometric model fit to the lab data (a=-2.5, b= 0.4, c=0.9). The black line is the linear model fitted to lab data where (a=-0.4, b=0.2).

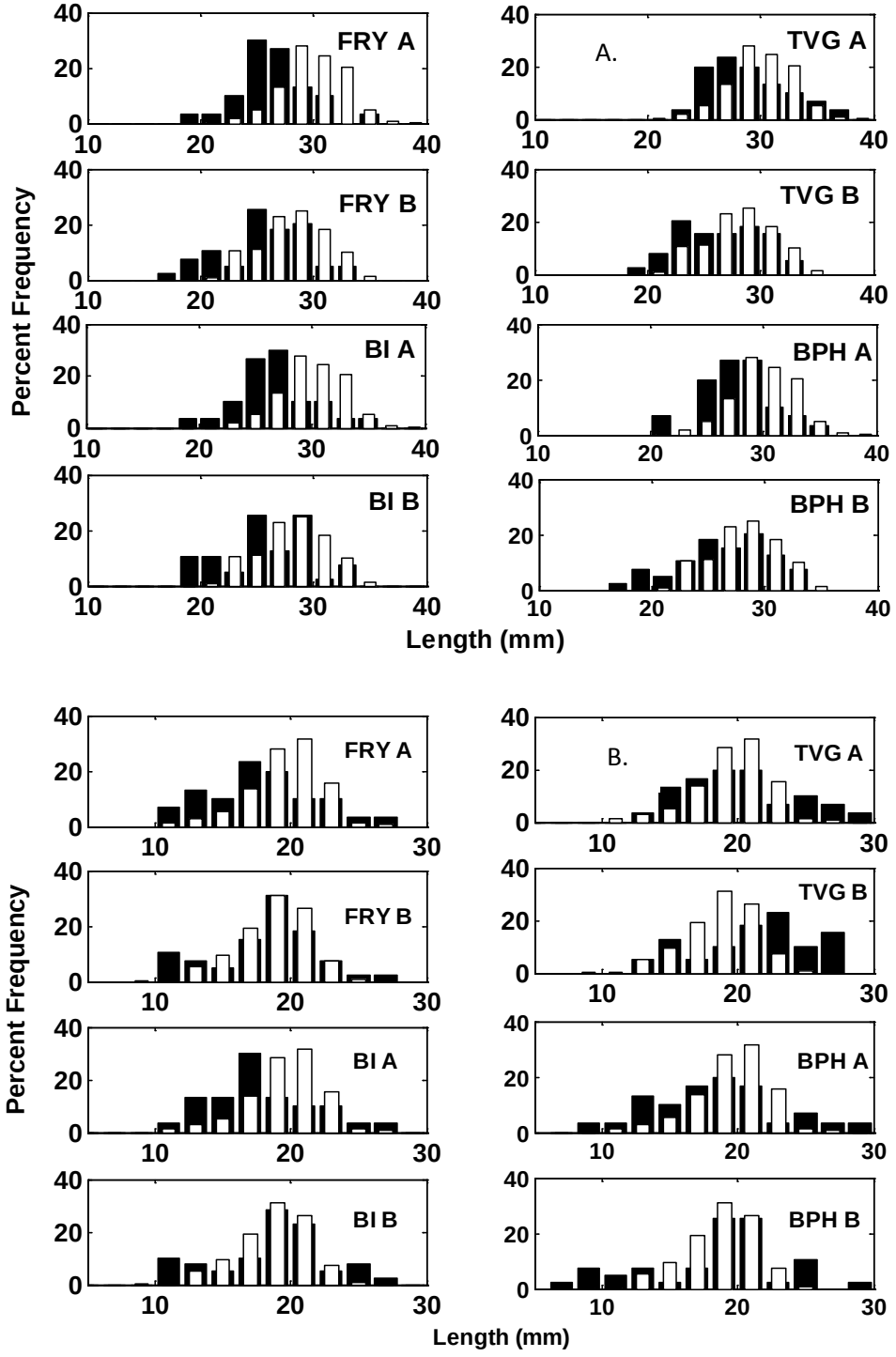

Figure 3. A. The white bars indicate the observed population in late July. The black bars indicate the expected population that was back-calculated to week 6 to late July. The back-calculation model (defined in the text) and replicate (A or B) is given in the upper right of the figures. Subplot B. The white bars indicate the observed population in early July. The black bars indicate the expected population that was back-calculated to week 4 to early July. The back-calculation model (defined in the text) and the replicate (A or B) is indicated in the upper right of the figures.

Chapter 3: Variation in the intensity of size-selective mortality over the growing season of the Atlantic silverside (*Mendia mendia*)

Abstract

Although large body size is typically found to be beneficial for fitness, most studies of selection on size focus on narrow intervals of the life history. Whether size selection fluctuates over larger portions of the life history is a largely an open question. To better understand overall selection pressures for size, I made a series of field collections in Long Island south shore bays following Atlantic silversides (*Mendia mendia)* over their growing season for two years. I used otoliths to back-calculate the length of survivors from which I estimated standardized selection differentials. In 2006 standardized selection differentials were positive in the early life and then switched to negative later in the growing season. In 2007, the standardized selection differentials switched back and forth from being negative to positive multiple times. Averaging over the season, natural selection for size was quite weak.

Introduction

Large body size is often tied to increased rates of survival and fecundity and considerable support has been established for the 'bigger is better' paradigm (Miller et al. 1988, Bailey and Houde 1989). Estimates of selection for body size from a wide range of terrestrial taxa, including vertebrates, plants, and insects, primarily favor larger size at age (Kingsolver and Pfennig 2004). More recently, Perez and Munch (2010) compiled estimates of selection on size in the early life of fishes, a group not included in previous reviews (Chapter 1). Selection in the early life history of fishes was 5 times stronger than selection for size in terrestrial taxa and 77% of the standardized selection differentials were positive showing strong support for the 'bigger is better' paradigm (Perez and Munch 2010). Similarly in juvenile fishes, 76% of cases show large individuals have a survival advantage, whereas only 8% of cases suggest the opposite pattern (Sogard 1997).

Mortality in the early life history, which can be due to predation, starvation, and oceanographic processes, is exceedingly high and it has been estimated that only 1 in 1000 to 1 in 10,000 fish make it to 1 year (Houde 1997, Pepin 1991). Since this mortality is often size-dependent, variation in growth rates in the early life stages may drive fluctuations in recruitment (Houde 1997) and Anderson (1988) proposed the growth-mortality hypothesis where higher growth rates result in lower net mortality. Because faster growers are able to reach a larger size and undergo metamorphosis sooner than slow growing counterparts, they are able to decrease the time spent in the high mortality early stages ('stage duration hypothesis' Houde 1987, but see Leggett and Deblois 1994). Fast growth is also associated with earlier settlement or migration to safer habitats (Bergenius et al. 2002, Theriault and Dodson 2003). Despite the evidence that

selection generally favors larger size, local adaptation in growth appears to be ubiquitous (Conover et al. 2009) suggesting that trade-offs with growth must be commensurately common.

Previous reviews of selection in fishes focused on brief periods of the life history, leaving open the possibility that selection on size fluctuates through time. Fluctuations in selection can occur both within and across generations. Fluctuations within a generation can have an overall stabilizing effect resulting in cumulative selection that is quite weak (Stearns 1992, Merila et al. 2001, Gingerich 1983, Price and Grant 1984). Such fluctuations in selection within a generation can lead to maintenance of genetic variance for that trait (Merila et al. 2001). It is important to note that selection on size at different ages can lead to indirect selection on size or other traits at all ages through phenotypic correlations which could maintain or erode genetic variation depending on its intensity and form. Additionally, fluctuations in selection across generations have also been suggested to help maintain genetic variance (Merila et al. 2001, but see Sasaki and Ellner 1997 and refs within), however theoretical approaches testing this often assume selection within a generation is constant, which is unlikely to be true. Fluctuation across generations, however, can demonstrate how the trait optimum moves, possibly due to environmental changes (Gibbs and Grant 1987, Grant and Grant 1995).

After reviewing the literature on a variety of traits, Schulter et al. (1991) suggested that selection switching direction was more common than selection staying the same direction in a single generation. Hoekstra et al. (2001) found that selection for morphological traits, including body size, in terrestrial taxa was less intense as the duration over which selection was measured increased. Selection on body size commonly fluctuates from year to year across generations (Carlson and Quinn 2007, Carlson et al. 2008, see review by Siepielski et al. 2009). Importantly,

the reviews of selection by Hoekstra et al. (2001) and Siepielski el al. (2009) focused on terrestrial taxa and did not distinguish fluctuations within and between generations.

Fluctuating selection in fish is expected because the factors that influence selection on size are not constant. For example, most habitats have multiple predators which can have their own unique prey selectivity functions (Baber and Babbitt 2003) resulting in strong size-selection where they are present and relaxed selection when absent (Holmes and McCormick 2006) as well as changes in the direction of selection (McCormick and Meekan 2007). Abiotic conditions including temperature and tidal currents can also modify the intensity of selection (McCormick and Holmes 2006, Gagliano et al. 2007b). Both the intensity and direction of size selection in bicolor damselfish was found to be influenced by temperature (Rankin and Sponaugle 2011). Selection has been found to reverse direction in a cohort of damselfish tracked over several months (Gagliano et al. 2007). Additionally, while selection favored larger size in the larval stage of a short lived clupeid, selection was not size-biased in the juvenile or adult stages (Meekan et al. 2006).

Objectives

My two main objectives for this study were measuring selection variation both within a generation and across generations. I took three approaches to looking at selection within a generation. First, I measured how selection varied week to week over the first half of the growing season. Second, I evaluated selection on a specific size class across several cohorts within a single year class. Third, I measured how selection in early otolith growth rates carried over into adult fish the following year. To evaluate variation in selection across generations, I compared selection estimates obtained in 2006 to selection measured in 2007 at one site. For all

of these comparisons, I removed otoliths from a subsample of fish on a weekly basis, measured growth rates, back-calculated sizes, and then calculated standardized selection differentials to evaluate how selection varied over years and over the season. To evaluate cumulative selection on early growth, I compared the early otolith growth rates of adults with early otolith growth of the same year class as larvae.

Methods

Study Species

The Atlantic silverside, *Menidia menidia,* is an abundant marine fish that ranges from Northeastern Florida to New Brunswick, Canada (Johnson 1975). This species exhibits counter-gradient variation in growth, in which individuals from low latitudes grow much slower than individuals from high latitudes when reared in a common environment (Conover and Present 1990). Adults reach sexual maturity at age 1, and few adult fish survive to age 2 (0.2-6.0% total population) (Conover and Ross 1982, Jessop 1983). Juvenile fish spend the spring and summer in warm inshore waters and then migrate offshore in the fall as water temperature decreases (Conover and Murawski 1982). In NY, the spawning season for Atlantic silversides begins in late April and ends in early July. Larvae and juvenile fish inhabit shallow bays until they migrate offshore at the end of the growing season in November, when water reaches 12°C (Conover and Present 1990).

Silversides experience substantial mortality during the juvenile stage as they are an important prey item for bluefish (*Pomatomus saltatrix),* striped bass (*Morone saxatilis),* and summer flounder *(Paralichthys dentatus)* (Buckel et al. 1999, Manderson et al. 2000,

Rudershausen et al. 2005). Both predation mortality (Scharf et al. 2003, and Lankford et al. 2001) and winter mortality (Munch et al. 2003) appear to be more severe for small individuals.

Evaluating Selection over the Growing Season within a Generation

Field Collections and Fish Measurement

I collected Atlantic silversides throughout their growing season in Great South Bay and Moriches Bay, New York in 2006- 2007. I collected fish from 4 sites from west to east (Pine neck (P)= N 40°44' 54" W 72°59'38", Smith point (S)= N 40°44' 14" W 72°51'22", Rowley (R)= N 40°47' 03" W 72°48'29", Atlantic (A)= N 40°47' 26" W 72°45'16"); however not all sites were sampled over all years (for sampling schedule see Appendix 1, Figure 1). Larval and juvenile fish were collected in 2006 and 2007. These fish were collected in weekly intervals during the spring and summer, and then at a bi-weekly interval during the late summer into fall.

At each site the collection effort was standardized in the usual way (Hayes et al. 1996): I stretched a fixed length of rope perpendicularly from shore, spread the seine parallel to shore, and then pulled it directly back to shore keeping it open to the maximum extent possible. Each site was sampled at least two times in locations at least 30m apart. If fish were scarce, sampling was conducted until either I collected 200 individuals or reached a maximum of 5 seine pulls for each gear type (5 pulls needed on 6/5/06, 7/6/06, 7/12/06 7/18/06, 7/25/06 at P, 5/22/2007 at R, 7/17/2007 at P). Silversides in each pull were either counted directly or their numbers estimated from the total volume of fish and the average number of fish per liter (volumetric enumeration occurred at site S on 3 of 12 dates, at site P on 2 of 10 dates, at site A on 5 of 9 dates) in 2007.

I used a 9m seine to collect larvae and small juveniles and a 30m seine to collect larger juveniles and adult fish in 2006 – 2007. The 9m seine, hereafter referred to as the larval seine,

was 9m long by 1.2m deep and had a 1.2m^3 centered bag. The mesh size on the wings was 1.6mm and in the bag was 0.8mm. The 30m seine, hereafter referred to as the adult seine was 30m long by 1.8m deep and had a centered bag that was 1.8m^3. In the wings of this net, mesh size was 6.4mm, and in the bag, mesh size was 3.2mm. I collected 200 fish from each gear at each site, and transported them on ice back to the lab where individuals were measured for total length. The observed length frequency distributions were determined by measuring standard lengths of 400 fish in 2006-2007 (200 from each gear type).

Measuring Selection within a Generation

To evaluate selection on size, I calculated standardized selection differentials, also known as the intensity of selection (Falconer and MacKay 1996):

$$i = (\bar{z}_t^{\,*} - \bar{z}_t)/SD_t \qquad 1.$$

where $\bar{z}_t^{\,*}$ is the mean length of the surviving population back-calculated to age *t* and $\bar{z}_t$ is the mean length of the initial population observed at age *t*. In order to facilitate comparisons with earlier selection reviews, I standardized the selection differentials by dividing by the initial population standard deviation (SD_t) (Gagliano et al. 2007, Vigliola et al. 2007, Perez and Munch 2010). Because data for other traits was not collected, I was unable to control for selection acting on other characters. Consequently these standardized selection differentials include both direct selection on size and indirect selection through phenotypic correlations with other selected characters. To approximate the standard deviation for the selection intensity, I used the following equation:

$$SD(i) = \left. sqrt\left(\left(\frac{v_t}{N_t}\right) + \left(\frac{v_t^*}{N_{bc}}\right)\right) \right/ SD_t \qquad 2.$$

where v_t and N_t are the variance and sample size in the original observed population. v_t^* and N_{bc} are the variance and total sample size in the back-calculated population. This equation is only approximate because it ignores sampling variation in the initial population SD and assumes independence of sampled means. Although other methods of measuring selection are available (Lande and Arnold 1983), these generally require knowledge of the fates of individuals which are logistically impossible to obtain for Atlantic silversides.

To measure the intensity of stabilizing selection, I calculated *j* where

$$j = \left[v_t^* - v_t \ + \left(\bar{z}_t^* - \bar{z}_t\right)^2\right] / v_t$$

$v_t^*$is the variance in the back-calculated population and v_t is the variance in the observed population (Lande and Arnold 1983, Chapter 1). The difference in trait means, here length, is added to the difference in variances to account for the bias towards decreased variance in the survivor population whenever selection occurs (Lande and Arnold 1983).

Atlantic silversides spawn in the field every two weeks (Conover 1985). Therefore, some means of accounting for recruitment is necessary when measuring selection. To exclude new recruits I truncated each size distribution (week $_t$), eliminating all fish smaller than the projected minimum size from the preceding week (week $_{t-1}$). I projected this minimum size, on a week to week basis, throughout the growing season based on the otolith-back calculated growth rates. Specifically, I modeled growth over a week as

$$L_t = a_t + b_t L_{t-1} + \varepsilon \quad 3.$$

where, L_t is length in week t and L_{t-1} is size in the previous week. I used linear regression to estimate parameters a_t and b_t and the residual variance, V_ε, from individuals whose back-calculated sizes (L_{t-1}) were obtained from otoliths beginning at t=2. Next, I projected the minimum size, M_{t-1} in the first week into the next week using the following equation:

$$M_t = a_t + b_t * M_{t-1} - 2\sqrt{V\varepsilon} \quad 4.$$

where the $-2\sqrt{V\varepsilon}$ term accounts for the spread of the distribution due to variation in growth. Any fish smaller than M_t, were excluded from of the calculation of selection over the interval t-1 to t. I repeated these methods to calculate minimum size bounds for all dates where I had extracted otoliths.

Otolith Back-calculation

The measured fish from each gear and 13 collection dates were placed into size class bins with 2 mm increments. I then selected a sub-sample of 40 fish for otolith removal based on their abundance in each bin to ensure that the subsample was representative of the overall size distribution. Based on the maximum coefficient of variation in silverside lengths, this otolith sample size permitted me to detect 5% differences in means with 90% probability (Sokal and Rohlf 1997).

More detailed methods of the techniques I used for otolith extraction, polishing, and reading are in Chapter 2. I used the body proportional hypothesis to back-calculate size at age. Briefly, in fish larger than 30mm, saggital otoliths were removed following the dissection techniques of Stevenson and Campana (1992). When fish were less than 30mm, I used Clorox

bleach to dissolve the fish head, leaving the bones behind. Following removal, the otoliths were air dried overnight, glued on a slide, and then polished with 3 micron US Supply Corporation lapping film until the daily rings were visible. I used Image Pro Plus to count the rings and measure distances between each of the rings. The same axis was measured on each otolith. Re-reading Atlantic silverside otoliths is highly accurate (N=168, measurement error variance=192.7 μm^2 for reading rings 1 week before capture, 63.8 μm^2 for reading ring 15). Previous evaluation of these methods indicate that it is possible to back-calculate size at age with < 4.2% uncertainty (Validation study chapter).

As is common with otolith microscopy, rings were not clear enough to read over the entire axis selected for reading on every otolith (Campana 1992). I determined an otolith to be unreadable due to the following: otolith cracking or greater than 20% of the otolith axis not clear. It is very common in otolith analysis to estimate some rings along an axis (Campana 1992), however, it is not recommended to estimate more than 20% of the axis (Campana pers.comm). I estimated rings for a region of the axis that was unclear using the SOLVER function in EXCEL. I used a second order polynomial to relate radius to ring number:

$$Oto\ radius = a * ring\#^2 + b * ring\# \quad + c \qquad 5.$$

where the otolith radius and ring number data were from 5 rings before and 5 rings after the unclear portion. Because I did not know the rings numbers for the 5 rings after the unclear portion they were defined as a function of the last ring before the unclear portion plus N (number missing rings). Restricting N to integers, I solved for the parameters from the second order polynomial function (a, b, c) and N that minimized the sum of squared error between the predicted and actual otolith radius (For example see Appendix 1). I then used the function to

determine the predicted otolith radius for each missing ring. For otoliths that were not clear at their edge, I estimated the rings in the unclear portion using the 10 rings immediately before this region.

I evaluated whether the back-calculated population and the original population came from the same distribution using Kolmogorov-Smirnov tests. To account for multiple testing (5 tests in 2006, 10 tests in 2007), I used a Bonferoni correction factor, which was equal to B=α/#tests. The Bonferoni correction factor defines a new significance level and makes no assumption about test independence. I calculated the observed original population length frequency distribution by weighting each gear type by the area that they sampled. The total weighted length frequency distribution, hereafter referred to as *LFD*, was calculated as:

$$LFD_{tot} = \left(LFD_{adult} * \left(\frac{area_{adult}}{Total\ area}\right)\right) + \left(LFD_{larval} * \left(\frac{area_{larval}}{Total\ area}\right)\right) \qquad 6.$$

Here, the *LFD* were vectors of total counts of fish that were in a 3 mm size class bin. The *LFD* ranged from 4mm to 100mm which spanned the entire size range of Atlantic silversides present in the field over the time sampled. The total area was equal to the combined larval seine area and adult seine area.

I used the same bin intervals when calculating the *LFD* for the back-calculated population as I had used for the observed population. Although I selected fish for otolith removal from the observed population in a manner that would allow the back-calculated population to be representative of the observed distribution, not all fish selected for otolith removal could be used. Some otoliths were not read because 20% or more of the otolith radius was not clear (335 unclear of 1200 otoliths, 28%). To ensure that the distribution of individuals used for back-calculation was indeed representative of the frequencies in the observed

population, I re-scaled the back-calculation frequencies when calculating the mean size. For each size bin in the *LFD (i)*, I multiplied the back-calculated length of each fish (L_{bci}) by the observed frequency in that size bin (*Tot obs*) divided by the total frequency used for otolith back-calculation (*Tot bc*). Here, the mean back-calculated size is the sum of the back-calculated lengths weighted by the number of observed fish in each size bin ($\sum RS_{bc_i}$) divided by the sum of the weights ($\sum\left(\frac{Tot\ obs_i}{Tot\ bc_i}\right)$).

$$avg\ bc\ size = \sum L_{bc_i} * \left(\frac{Tot\ obs_i}{Tot\ bc_i}\right) \Big/ \sum\left(\frac{Tot\ obs_i}{Tot\ bc_i}\right) \quad 7.$$

Selection for Size at Age across Cohorts in a Year Class

Field Collections and Fish Measurement across Cohorts

In 2008, I tracked fluctuations in selection on recently hatched larval fish throughout the spawning season (May-end June). I collected recently hatched larval fish at site A and site S (Figure 1). I sampled every couple of days at full and new moons. To collect recently hatched larvae, I used a 0.6m dip net. This dip net was $0.6m^3$ and had the smallest mesh size compared to the seines, 0.4mm. As Atlantic silversides are known to spawn on vegetation in the intertidal region (Conover and Kynard 1984), I dragged this net through the water parallel to the shore. Periodically, I checked the net for larvae. To minimize mortality, larvae were scooped out with a 2in. cup and then were placed in a 2 liter Pyrex glass with seawater of ambient temperature. Shrinkage occurs in larvae very quickly after death so I made every effort to keep the fish alive and measured 200 fish from each site on each date within 2 hours of collection (Radtke 1989).

Measuring Selection and Otolith Back-calculation methods across Cohorts

As the recently hatched larvae were very small and did not withstand handling well, I did not place these fish in size-class bins to obtain a representative otolith sub-sample. Instead I separated out every second or third measured fish for otolith analysis. I back-calculated otoliths and measured the intensity of selection (*i)* defined above. I also ensured that all individuals used for back-calculation were greater than the minimum predicted size M_i *(i. e.* from the initial cohort sampled). To make certain that the individuals used for back-calculation were indeed representative of the frequencies in the observed population, I re-scaled the back-calculation fish when calculating the back-calculated mean size.

Comparing Early Growth of Juveniles with Early Growth of Returning Adults from the Same Year Class

Field Collections and Fish Measurement of Juveniles and Returning Adults

To evaluate the cumulative selection on early growth over the life history, I examined the record of early growth in the otoliths of reproductive adults. I collected returning adult fish on one date in 2008 and 2009 from the S site using the adult seine. I measured 200 fish on each date, and placed fish into 2mm size class bins. I then selected a subsample of 40 fish based on their overall abundance in each size-class bin for otolith removal. I followed the otolith processing techniques described above. As rings near the edge of adult otoliths are not readable, I was limited to reading rings near the core, specifically, the first several weeks of fish life.

Measuring Selection in Juveniles and Returning Adults

I compared the larval growth rates of spawning adults in 2008 and 2009 with the early growth of larvae and juveniles from the corresponding year classes in 2007 and 2008. For all comparisons, I calculated the average otolith growth rate over the first two weeks of life. In 2007, I calculated the average early otolith growth of individuals collected in the first cohort

(May 22, 2007), as well as when all cohorts were in the system (June 26, 2007). I determined that spawning was largely over by this time due to the absence of adults and a marked decrease in my catch per unit area (Figure 9). The sampling method in 2007 was such that I collected a mix of cohorts. By predicting the minimum size on a week to week basis, I statistically removed individuals from younger cohorts in 2007. I also compared the average early growth of these individuals from the younger cohort with the returning adult fish.

In 2008, I also calculated the average early growth of the first cohort collected (May 22, 2008), the final cohort (June 12, 2008), and the average early growth when all cohorts were in the system (June 12, 2008). The sampling method in 2008 allowed me to specifically target various cohorts so I calculated the average growth of all cohorts as the mean early otolith growth of fish collected on 5/22/2008, 6/3/2008, and 6/12/2008. In this calculation of the mean growth, I did not have a way to estimate relative abundance of the older cohort, and thus am assuming that they are at equal frequencies in the juvenile population. I compared the average growth of larvae and juveniles collected in 2007 and 2008 with the average early otolith growth of the adult fish in the same year classes when they returned the following spring.

To compare the average early otolith growth in the larvae and juvenile collections with the average growth in the adults I used an ANOVA where age group (i.e. adult or larvae) was the predictor. To satisfy the assumption of normality the data were log transformed.

Evaluating Selection Variation across Generations

To determine whether selection for size fluctuates across generations, I compared selection estimates for juveniles collected through the growing season in 2006 and 2007 from the P site. The field collections, otolith back-calculation methods, and methods for measuring

standardized selection differentials, including the exclusion of new recruits, were identical to those described in above methods sections.

Results

I collected a total of 7,631 fish from 2006-2009 from 4 different sites (Figure 1). Atlantic silversides did not appear to hatch at all the sites at the same time. I collected larvae (Mean=8.0mm±0.9 SD) from site S a week before the other sites in both 2007 and 2008. In 2006, I only collected fish from the P site, so was unable to make spatial comparisons of hatch timing for that year.

Evaluating Selection over the Growing Season within a Generation

In both years, the strength and direction of selection on size varied considerably. In 2006, in the beginning of the season the standardized selection differentials were positive (6/5, 6/12) indicating selection favored larger size at age at site P (Figure 2A). Later in the season, at this same site, selection favored smaller size at age (7/12, 7/18). Three out of 5 of these selection differentials exceeded the 4.2% bias determined by the validation study (Chapter 2). The back-calculated distributions were significantly different than the observed distributions in all cases except when back-calculating to 6/16/2006 and 7/18/2006 (KS test: $p=<0.008$ for samples back-calculated to 6/5, 6/12, 7/12, Figure 2A).

In 2007 the pattern of selection showed considerable fluctuation between favoring larger and smaller size at age (Figure 2B, Figure 3). The fish back-calculated to the first observed collection on 5/15/2007 were smaller on average than the observed population. In the following week, selection favored larger individuals (5/22). Overall, selection changed direction 4 times between May and July 2007, ending with selection favoring small fish. KS tests showed that all

the back-calculated distributions except for 5/22/2007, 5/29/2007, and 7/24/2007 were significantly different than the observed distributions (all p=<0.005, Figure 2B).

Both in 2006 and 2007 on the dates where multiple gears had been used, the standardized selection differentials that were calculated by re-scaling to either the larval seine or the adult seine showed the same pattern (Figure 4). The intensity of stabilizing selection was greater than 0 in all cases, indicating that the variance after selection was greater than the variance before selection. All of the measures of j except for the first 3 dates in 2007 were outside the limit expected with 99.9% mortality (Perez and Munch 2010, Chapter 1) indicating that the back-calculated variance is likely to be inflated due to back-calculation error and thus are unlikely to biologically feasible (Chapter 2).

Selection for Size at Age across Cohorts in a Year Class

In 2008, the first cohort (May 19) of larvae experienced selection against large size at both sites (Figure 5, Figure 6). In contrast, selection favored larger size in the second and third cohorts (June 2nd and June 6th). All differences between observed and back-calculated mean size exceeded the 4.2% bias except for the A site when back-calculating to 5/19. Observed and back-calculated size distributions were all significantly different at the S site (N=565, P=<0.009), whereas at the A site, the back-calculated distribution was only significantly different from the observed distribution on the last date (N=547, P=9.9E-6).

Comparing Early Growth of Juveniles with Early Growth of Returning Adults from the Same Year Class

I measured the early growth rates from 33 adults in 2008 and 33 adults in 2009, and a total of 118 larvae/juveniles in 2007 and 167 larvae in 2008. To evaluate the cumulative effects of selection on early growth, I compared the first two weeks of otolith growth in spawning adults

in 2008 and 2009 with the otolith growth of larvae collected in 2007 and 2008. There appeared to be some temporal variability in indirect selection for early growth over winter (Figure 7). Adults that survived over the winter in 2007 had growth rates that were no different than the first cohort of juveniles in 2007 but were slower than the average growth when all cohorts were in the system (ANCOVA, P=<0.001, N=129). When predicting the minimum size of the first cohort forward through time, the average early otolith growth of individuals collected from the younger cohort on 6/26/2007 were also slightly higher than the returning adults, although this difference was marginally non-significant (ANCOVA, p=0.06, N=10). Adults that survived over winter in 2008-2009, however, had growth rates that were faster than both the first cohort, final cohort, and the average of all cohorts, although adult growth rates were only significantly different from the early cohort (ANOVA, P=0.001, N=60). Differences between adults and growth in all the cohorts was marginally nonsignificant (ANOVA, P=0.06,N=159)

Variation in selection across Generations

I compared standardized selection differentials at the P site in 2007 to what was measured in 2006 (Figure 8). Of the 4 dates that were sampled within 3 days of each other over both years, on average the standardized selection differentials had an absolute difference of 1.2. This difference would have a % bias of 17.9%, which is considerably larger than the 4.2% cut-off found in the validation study (Chapter 2). This suggests that selection is not consistent from year to year, however if I remove the most extreme value in 2007 (6/5/2007), the % bias is 12.2%, and both years appear to follow the same general pattern of selection favoring larger size early in life and then switching to favor smaller size later in life.

Discussion

My results indicate that size selection in Atlantic silversides varies both within and across generations. Selection for size fluctuated between favoring smaller and larger size when back-calculating size on a weekly basis both in 2006 and in 2007. Additionally, in 2008, when collecting recently hatched fish from 3 cohorts, I found that at both sites early in the season (5/22) selection favored smaller size whereas selection favored larger size in cohorts hatched in mid season (6/3) and late season (6/12).

As with any study measuring natural selection by back-calculating size at age, a critical bias may be size-based movement out of the sampling region. Fortunately much work has been conducted evaluating the natural history of this species. Young of the year Atlantic silversides are known to remain in shallow inshore bays during the spring and summer months until they migrate offshore in late October to November at mid latitudes (Conover and Ross 1982). In both 2006 and 2007, my last otolith subsample was removed from fish in late July, several months before their seasonal migration. Additionally, I evaluated whether larger Atlantic silversides were in deeper water in the Long Island south shore bays, outside of the area that is possible to sample with the adult beach seines. The maximum size of fish collected with an otter trawl in Fire Island inlet or a 200ft seine pulled by a boat, were no different than the maximum size I was collecting with the adult seine in July and August 2007(Appendix 2). Thus, the selection differentials I calculate are unlikely to be the result of movement.

In 2007, cumulative selection favored adults that had been slow growing as larvae whereas cumulative selection in 2008 favored adults that had been faster growing as larvae (Figure 4). Following offshore migration in the fall, much mixing occurs over winter and returning adult Atlantic silversides collected from Long Island south shore bays had a ~ 10% probability in 2004 and a >50% probability in 2005 of originating from Long Island south shore

bays (Clarke et al. 2010). Atlantic silverside adults that were found unlikely to be from Long Island South Shore bays in both years were likely to have originated from more northern sites, including Long Island sound and Waquoit, Massachusetts (Clarke et al. 2010). Evidence from Clarke et al. 2010 of high overwinter mixing of populations in one year and lower mixing in the following year suggests that all the returning adults I collected in 2008 and 2009 are unlikely to have originated from Long Island south shore bays. If a large proportion of the adults I collected in 2008 and 2009 had originated from northern waters, they may have been exposed to a very different selection regime as juveniles than adults originating in Long Island south shore bays. Unfortunately, with the current data for 2007-2008 and 2008-2009 it is impossible for me to determine the % of adults that originated in Long Island south shore bays. Despite this uncertainty in whether I am comparing individuals that are from the same cohort, it is still valuable to compare early otolith growth rates to obtain a cautious estimate of cumulative selection.

Overwinter temperature averaged at 0.6 m depth in 2007 was milder than in 2008 (6.6°C vs 5.9°C t-test, p=<0.0001, N=3478, NOAA Buoy DATA, Station 44025, 33 nautical mi south of Long Island). These differences in selectivity may be due to differences in overwinter condition severity in the two years. Environmental conditions are known to influence the size-selectiveness of mortality (Munch et al. 2003, Rankin and Sponaugle 2011, Gagliano et al. 2007). When combining the standardized selection differentials from 2006-2008, however, temperature did not appear to have an influence on selection (linear regression, p=0.3, N=21, Figure 9, Temperature Data from Charles Flagg, DOS Great South Bay Project).

It is important to note that I have not tracked the agent of selection, which would be valuable for explaining rapid shifts in the direction of selection. However, it is possible to

suggest potential causal agents of selection consistent with prior knowledge of silverside natural history. It is likely that the main agents of selection for size in Atlantic silversides are predation and fecundity benefits gained with larger size. Although large size is associated with increased fecundity in Atlantic silversides (Conover 1985) selection due to this component of fitness would not be apparent until maturity. On the other hand, silversides are an important prey item of numerous predatory fish species (Buckel et al. 1999, Manderson et al. 2000, Rudershausen et al. 2005). Furthermore, abundance of these predatory species varies over the season and from site to site (Nyman and Conover 1988, McBride and Conover 1991, Buckel and Conover 1997). Gagliano et al. 2007 suggested that slow growers are more resistant to starvation, and thus would be favored when food availability is limited. I do not expect starvation to be as important as predation for influencing size selective mortality as growth rates of fish in the field are comparable to fish reared in the laboratory under unlimited food. Furthermore, intrinsically fast growing Nova Scotia and intrinsically slow growing South Carolina fish are equally resistant to starvation (Conover 1992).

In Atlantic silversides, fast growth is known to be associated with costs, including increased predation risk (Lankford et al. 2001, Munch and Conover 2003) and poorer swimming ability (Billerbeck et al. 2001, Munch and Conover 2004). Interestingly, individuals that had grown slowly over the past two weeks of their life had positive standardized selection differentials, indicating that the largest of the slow growers had a survival advantage (growth =0.5 mm/day or less, Figure 10). Individuals that had grown fast over the past two weeks of their life tended to have negative selection differentials (or zero), indicating that the smallest of these fast growers had the survival advantage (growth =0.74mm/day or greater). The overall pattern relating selection to recent growth is significantly quadratic (R^2=0.65, SS=2.5, N=11)

with a minimum at around 1.1 mm/d. In my prolonged cost of growth experiment, I found that individuals that had grown 1.4mm/day had poorer swimming ability than individuals growing 0.6mm/day, compared at a common size. Munch and Conover (2004) found that growth rates less than 0.75 mm/d had very little effect on swimming, but that faster growth caused a sharp reduction in swimming performance. In light of this and the general decrease in mortality with size, I had expected to see selection favor increased size whenever fish were small or growth was slow and that periods of rapid growth would reduce or reverse selection on size. These results are loosely consistent with that hypothesis.

Although standardized selection differentials are positive in the early life of many fish species (Perez and Munch 2010), evolution towards larger size at age does not appear to be occurring (Arendt 1997). Fluctuating selection for size may maintain genetic variance and thus explain the lack of evolution (Stearns 1992, Roff 1992, Merila et al. 2001). Evolutionary stasis is promoted when fluctuations in selection, which accumulate over the life history, balance out, resulting in no overall selection pressure. To evaluate this, I calculated an average standardized selection differential in 2006 and 2007, where I averaged the initial and final sizes over all sampled dates and then calculated *i*. In 2006 the average standardized selection differential over the entire season was 0.1±0.4, whereas in 2007, the average standardized selection differential was -0.1 ±0.2 (%bias in 2006=1.7%, %bias in 2007=2.8%). To account for mortality, I re-calculated the overall mean selection by weighting each weekly standardized selection differential by the average catch per unit area on each sampling date (Figure 11). The weighted average standardized selection differential in 2006 was 0.1±0.4, and the weighted average selection differential in 2007 was -0.3 ±0.3.

Another possible explanation for the lack of evidence of evolution towards larger size at age is low heritability for this trait. Although additive genetic variance for life history traits that are closely associated with fitness tends to be lower than for other traits, adequate heritability is generally found (Stearns 1992, Roff 1992, Mousseau and Roff 1987). Additionally, heritability for adult size in the Atlantic silverside was 0.2, large enough to expect evolution (Conover and Munch 2002). As significant heritability for growth and size is common in fishes (Gjerde et al. 2004, Shimada et al. 2007), it is unlikely that low heritability impedes evolutionary response.

Although selection for size in the early life history appears to strongly favor larger fish with a mean intensity of 1.12 (Perez and Munch 2010), selection for size in silversides fluctuates through the growing season (from -1.3 to 0.8, but is quite weak on average). These results have important implications for fisheries induced evolution of size and demonstrate the importance of evaluating the natural selective landscape. Fishing typically selects for a specific size range, which can be due to targeting the largest fish for economic benefit, size limits imposed by managers, and selectivity intrinsic to the gear itself (Myers and Hoenig 1997, Thompson and Stokes 1996). Size may evolve due to the non-random removal of fish from fisheries (Conover and Munch 2002, Swain et al. 2007).

However, we need to better understand how natural selection and harvest selection oppose one another to predict the evolutionary outcome (Conover et al. 2009). Edeline et al. 2007 found that natural selection, which favored larger size, was opposite to harvest selection, which favored smaller size in pike. If the fluctuating natural selection that I found in this study is common across fish taxa, then natural selection is unlikely to counteract fisheries selection assuming that the pattern of selection is unchanged in harvested adults. Furthermore, the rebound rate for fish size may be slower than expected and this highlights the need to better understand

natural fitness landscapes. Thus, in light of our poor understanding of the long-term effects of fishing and the reversibility of harvest selection a precautionary approach to managing the selection imposed by fisheries seems warranted (Garcia 1994, but see Hilborn et al. 2001).

Figure 1. Fish were collected from 4 sites in Long Island south shore bays. The legend in the upper left hand corner of the map shows the site labels and the years where I collected fish.

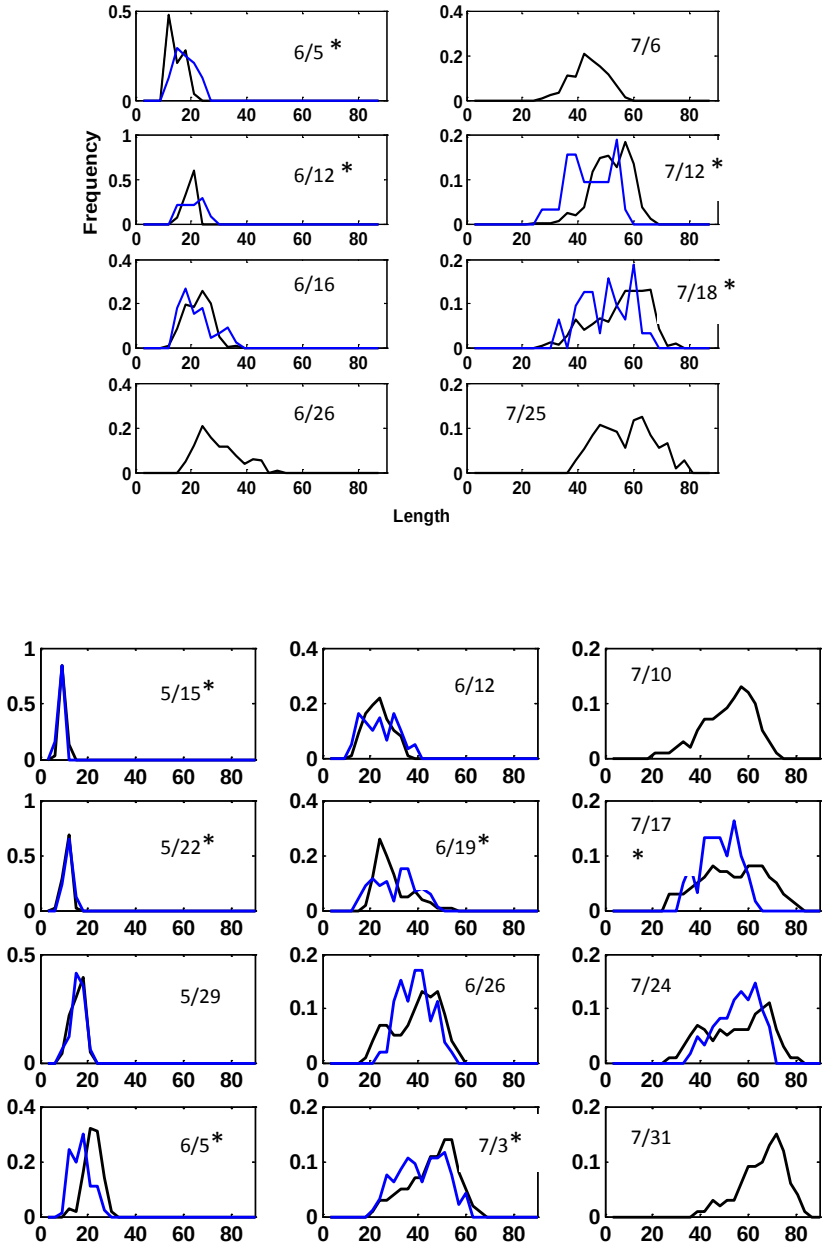

Figure 2. Panel A shows the observed length frequency distributions, weighted by area sampled, of fish collected in the field in 2006 (shown in black). The back-calculated length frequency distributions are shown in blue. All fish were back-calculated to the week before except fish collected on 7/25/2006 and 6/16/2006 were back-calculated to 1 and 2 weeks before. Panel B shows the observed length frequency distributions in black and the back-calculated length frequency distributions in blue for 2007. These data are for all 4 sites combined. The otolith samples were back-calculated to the week before except for samples collected on 7/31/2007 and 6/26/2007 that were also back-calculated to 2 weeks before. Both in Panel A and B asterisks show the weeks where the observed and back-calculated distributions were significantly different (KS Test $p<0.01$ in 2006, KS Test $p<0.005$ in 2007)

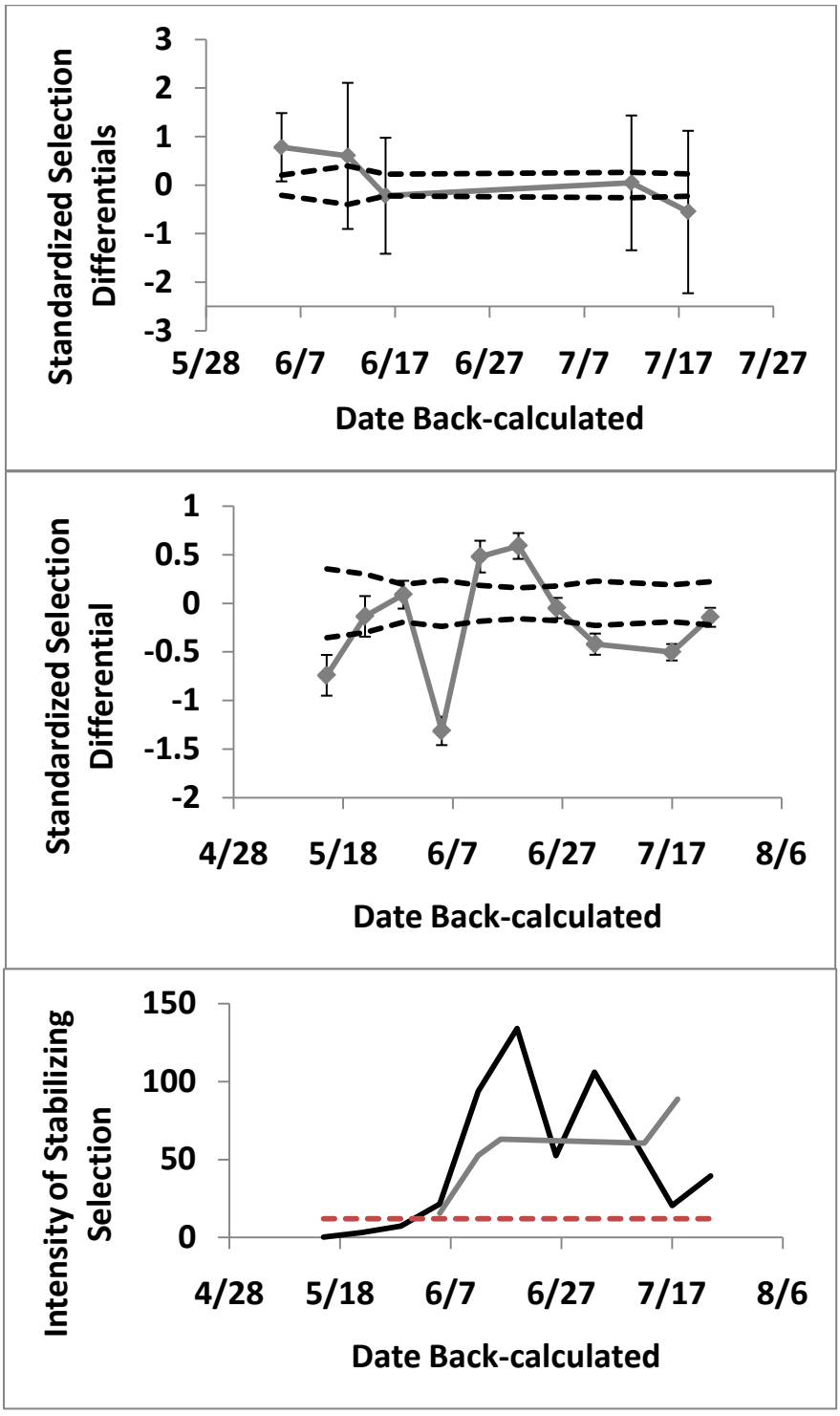

Figure 3. Subplot A shows the standardized selection differentials calculated in 2006 at site P. The black dashed lines are the standardized selection differential for a % bias between the

observed population and back-calculated population equal to 4.2%. Subplot B: Data shows the standardized selection differentials for all sites combined in 2007. The date that fish were back-calculated to is shown on the x-axis. Subplot C: All measures of j in 2006 and all measures of j in 2007 except for fish back-calculated to May were outside the limit predicted with a 99.9% mortality rate.

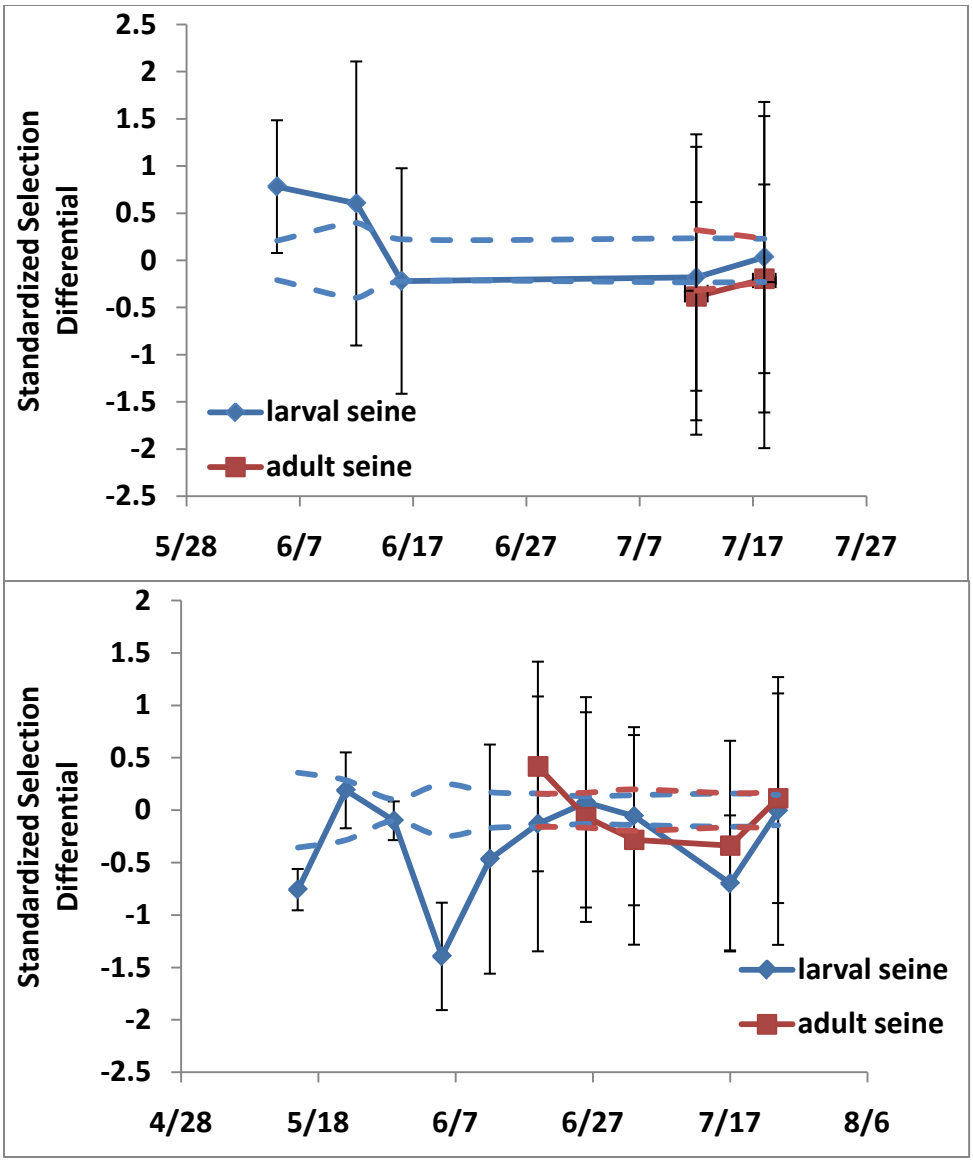

Figure 4. Subplot A shows standardized selection differentials in 2006 rescaled to the larval seine (in blue) or to the adult seine (red). Data from 2007 is shown in subplot B.

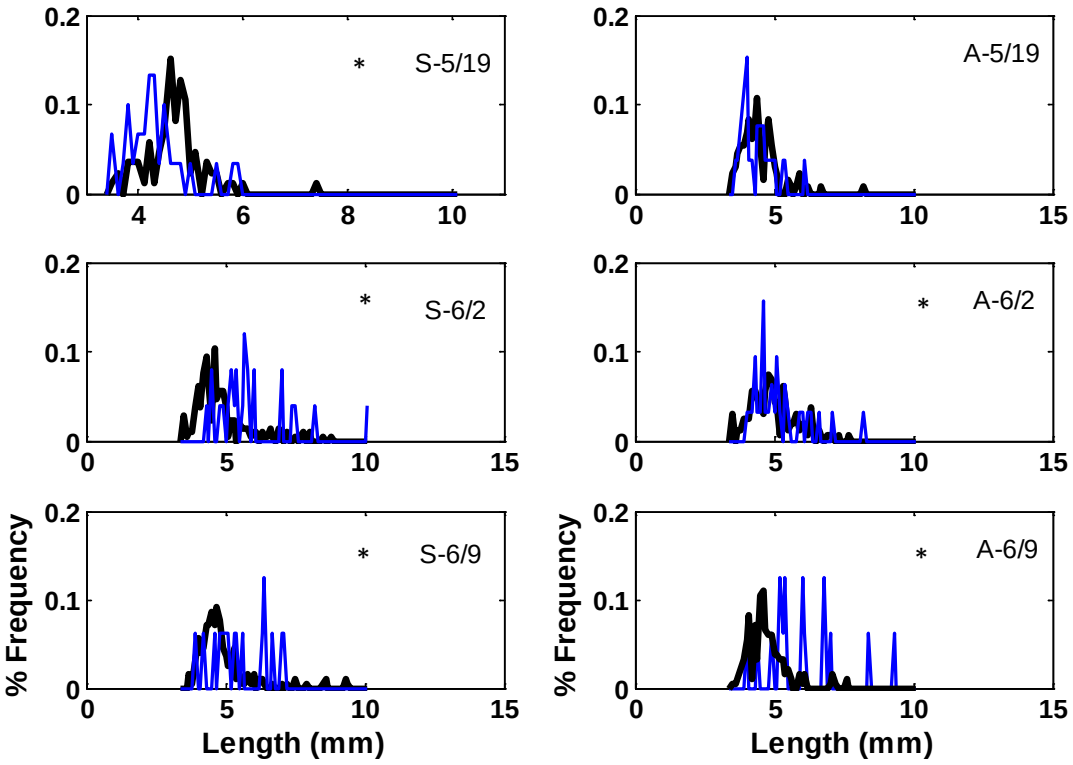

Figure 5. Back-calculation of fish collected in 2008 from two sites. The first column shows fish collected from the S sites whereas the second column show fish collected from the A site. Asterisks indicate differences between the observed and back-calculated population are greater than 4.2%.

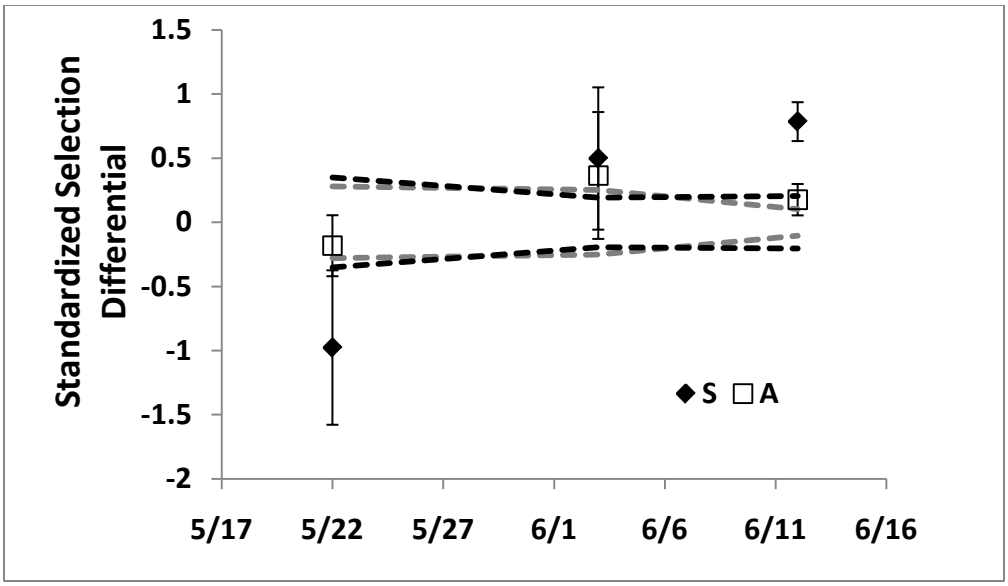

Figure 6. Selection for size in recently hatched fish collected from 3 cohorts spawned over the season. The date fish were back-calculated to is shown on the x- axis and the standardized selection differential is shown the y-axis. Cohorts collected from the S sites are plotted with black markers whereas cohorts collected from the A site is plotted with white open boxes. Error bars are ± 1 SE. The black dashed and grey dashed lines are the standardized selection differentials at a 4.2% bias of the observed population mean for the S and A sites respectively.

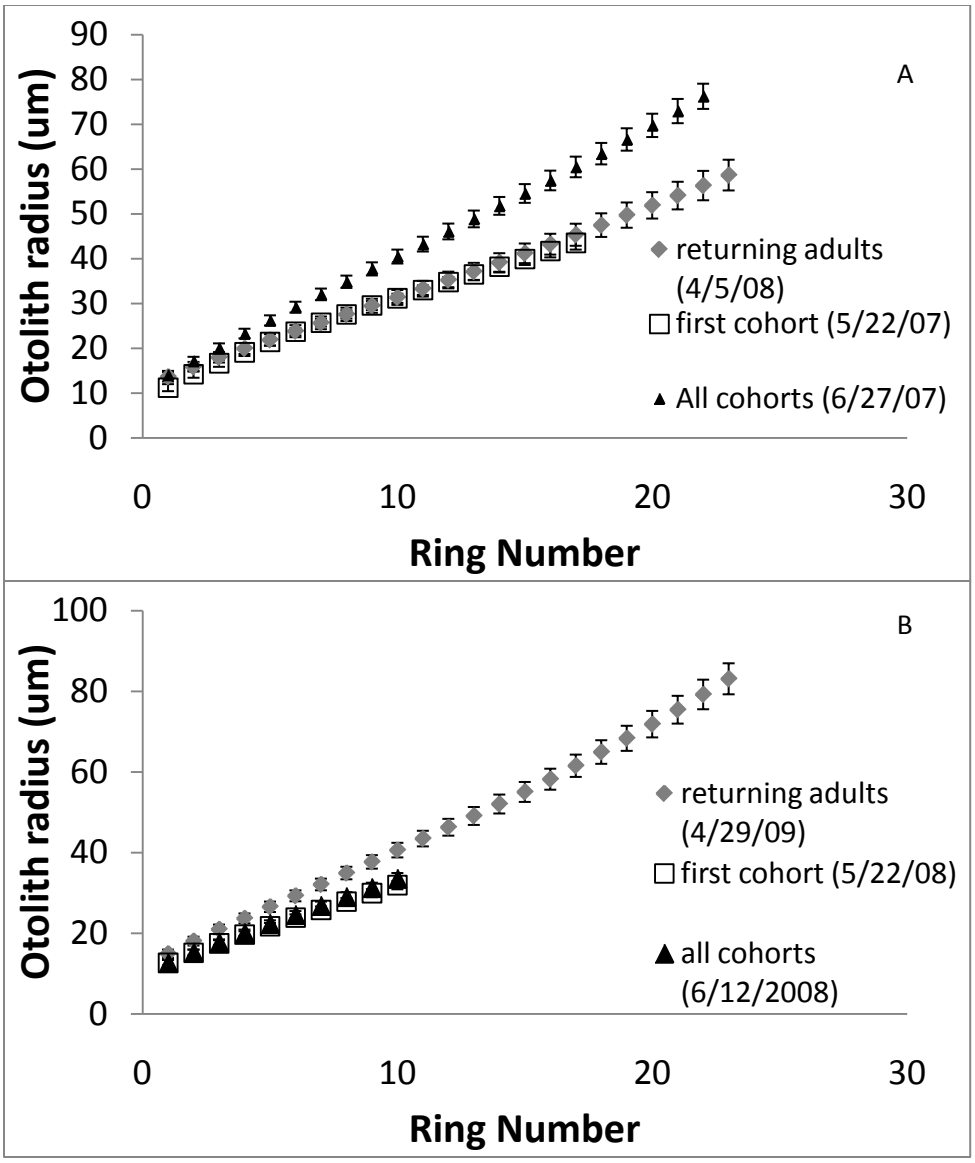

Figure 7. Panel A shows the mean otolith radius growth trajectory ±1SE for juveniles collected in 2007 and returning adults in 2008. Panel B shows juveniles collected in 2008 and returning adults collected in 2009. In both panels the returning adults are labeled in grey. The first cohort I collected is shown by the white squares. In 2007, my sampling protocol allowed for the collection of all cohorts at the end of the spawning season (Panel A black triangle), whereas in 2008 I averaged the early growth rates of the three cohorts I had previously collected to obtain values for all cohorts (Panel B black triangle).

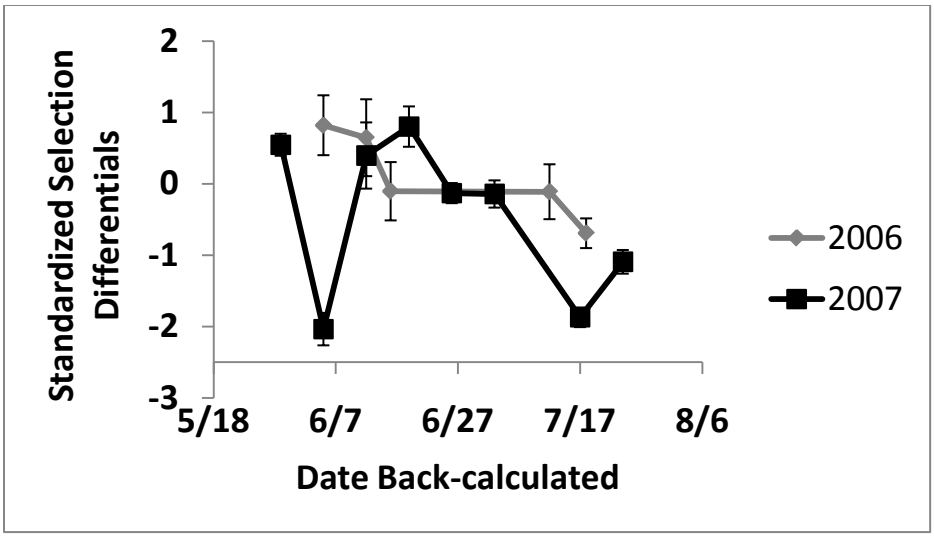

Figure 8. Comparison of standardized selection differentials calculated over the season in 2006 and 2007. Data is shown for site P from 2006 (grey) and from 2007 (black). The date that fish were back-calculated to is shown on the x-axis.

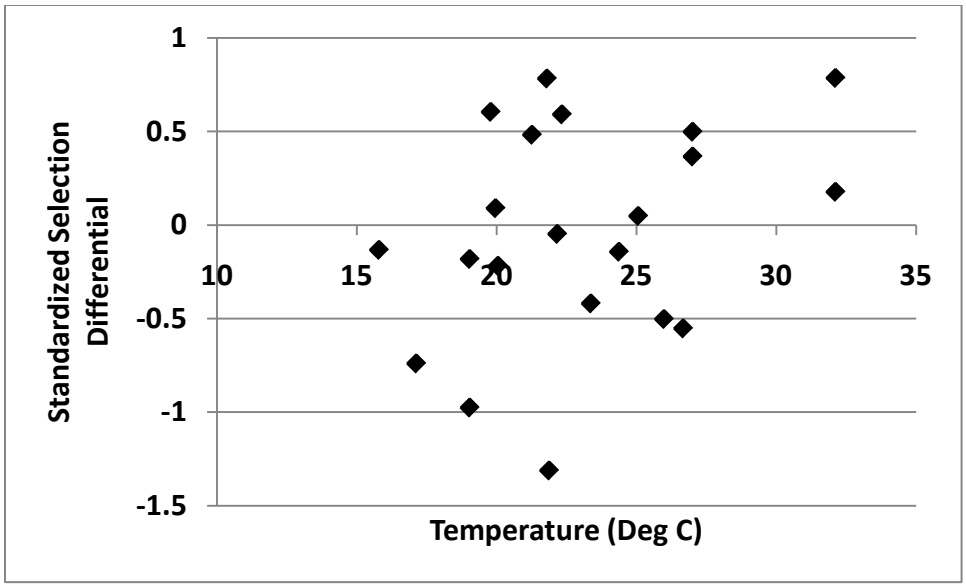

Figure 9. The pattern of standardized selection differentials measured in 2006- 2008 over a range of water temperatures (°C).

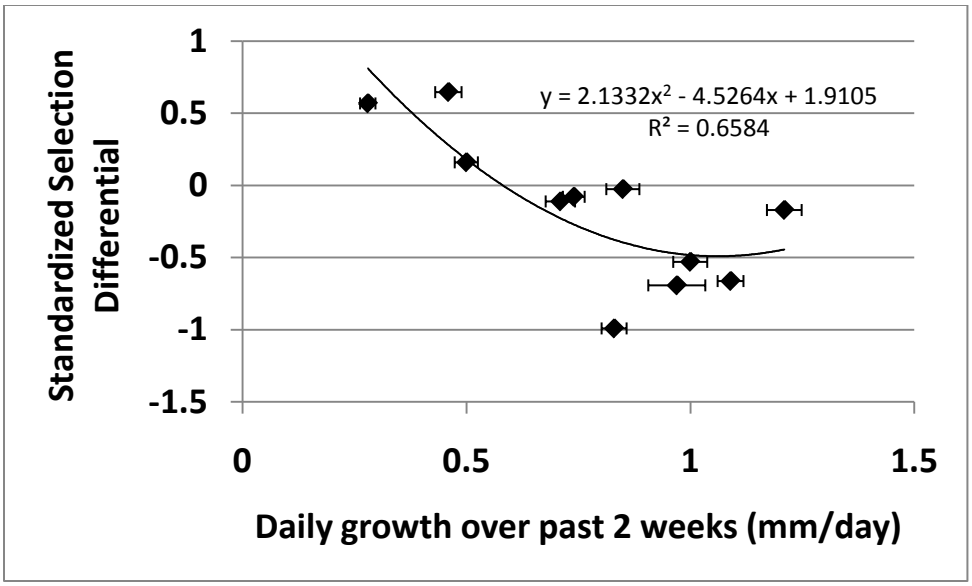

Figure 10. Standardized selection differentials measured in fish collected from 2006-2007. Plotted on the x-axis is the average daily growth of the sample of fish collected on weekly intervals over their growing season. Error bars are ±1 SE.

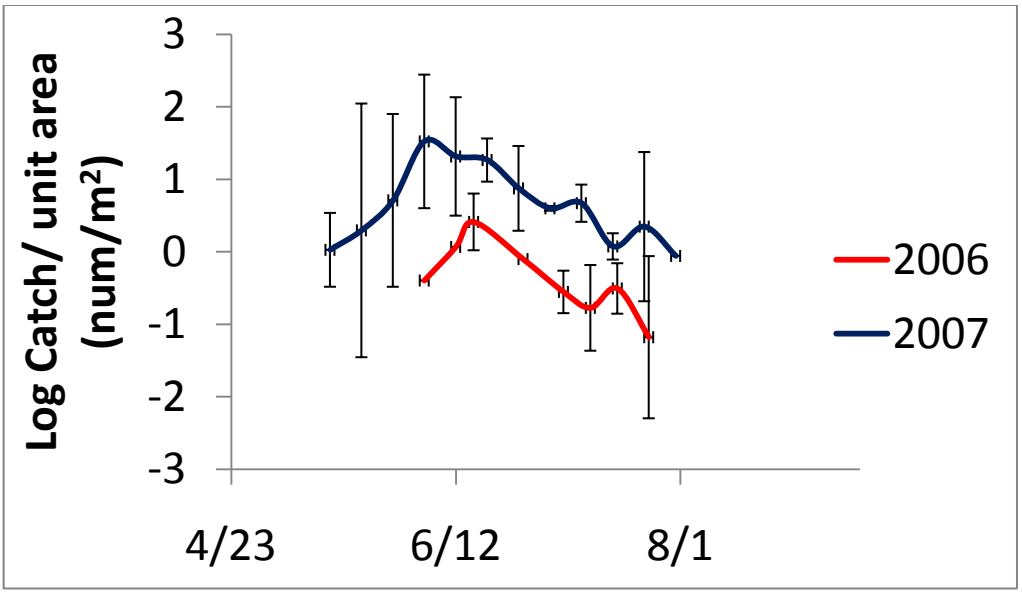

Figure 11. Catch per unit area (Logged) for the site sampled in 2006 (P) was compared to catch per unit area in 2007 (Panel A). Error bars are ±1 SD.

Appendix 1.

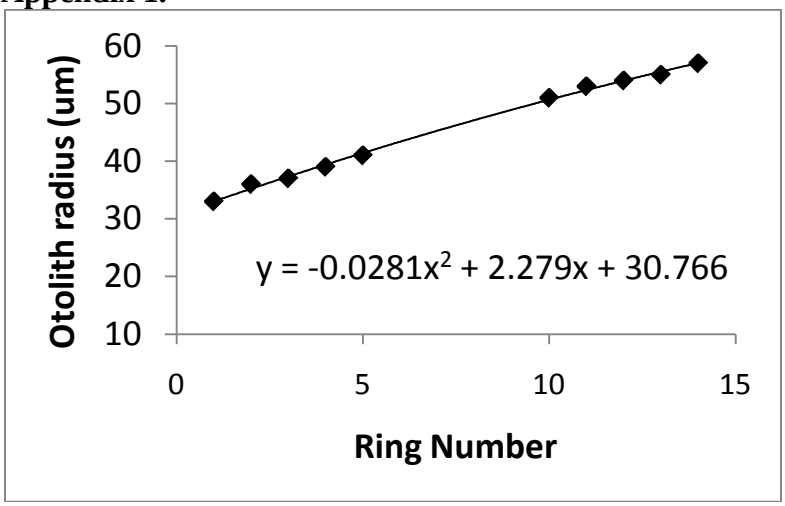

Figure A shows the otolith radius and ring estimation example for an individual where rings were missing in the middle of the otolith (between ring 5 and 10).

Table A shows the calculations used to determine the number of missing rings (N) and the otolith radius for each missing ring. The first two columns show the visible otolith radii's and ring number before and after the unclear portion. The next 5 columns show the calculations using solver in EXCEL to solve for the 4 parameters that minimize the sum of squares between the predicted and observed otolith radii's. Using the parameters that SOLVER estimated I then calculated the otolith radius for each missing ring.

before estimation		after estimation						predicted otolith radius for missing rings	
ring #	observed otolith radius	ring #	observed otolith radius	Y pred	(y-ypred)^2	parameters used for estimation		ring #	predicted otolith radius
1.0	33	1.0	33.0	31.8	1.4	n	5.0	6.0	43.1
2.0	36	2.0	36.0	34.5	2.4	a	-0.1	7.0	45.2
3.0	37	3.0	37.0	37.0	0.0	b	2.8	8.0	47.1
4.0	39	4.0	39.0	39.4	0.2	c	28.3	9.0	48.8
5.0	41	5.0	41.0	41.7	0.5				
N+5	51	10.0	51.0	51.2	0.0				
N+6	53	11.0	53.0	52.7	0.1				
N+7	54	12.0	54.0	54.1	0.0				
N+8	55	13.0	55.0	55.4	0.2				
N+9	57	14.0	57.0	56.6	0.2				

Table B Otolith Estimation Statistics and Sampling Schedule. The appendix below shows the summery of otolith ring estimation and the sampling schedule. The first column displays the site where the fish were collected. The second column show the date I sampled. The third, fourth and fifth columns show the gear used, the number of fish measured, and the number of otoliths measured. The sixth and seventh columns show the number of otoliths where ring estimation was needed and the average amount of the otolith that I estimated. The final three columns show qualitative descriptions of where the estimation took place.

Site	***Date collected***	***Gear*** *Larval=L Adult=A L Dip=D*	***Fish Measured***	***Otoliths***	***Number Otoliths Estimated***	***Average % estimated***	***# core***	***# mid***	***# edge***
A	6/5/2007	L	200	-	-	-	-	-	-
A	6/12/2007	L	199	36	10	7.4	4	5	1
A	6/19/2007	L, A	400	-	-	-	-	-	-
A	6/27/2007	L, A	400	37	3	11.9	3	0	0
A	7/3/2007	L, A	400	-	-		-	-	-
A	7/10/2007	L, A	400	34	9	11.8	6	3	0
A	7/17/2007	L, A	399	-	-	-	-	-	-
A	7/24/2007	L, A	400	-	-	-	-	-	-
A	5/22/2008	D	185	27	2	10.7	0	1	1
A	6/3/2008	D	191	33	0	0	0	0	0
A	6/12/2008	D	195	26	5	10.8	0	5	0
P	6/5/2006	L	83	-	-	-	-	-	-
P	6/12/2006	L	203	-	-	-	-	-	-
P	6/16/2006	L	228	26	8	10.9	4	4	0
P	6/27/2006	L	164	45	10	12.2	6	3	1
P	7/25/2006	L, A	128	32	17	11.3	2	5	10
P	5/29/2007	L	201	-	-	-	-	-	-
P	6/5/2007	L	200	28	3	6.1	2	1	0
P	6/12/2007	L	201	24	11	10.1	4	6	1
P	6/19/2007	L, A	384	-	-	-	-	-	-
P	6/26/2007	L, A	401	29	0	0	0	0	0
P	7/3/2007	L, A	400	32	9	11.3	8	1	0
P	7/10/2007	L, A	400	31	9	12.1	6	3	0
P	7/17/2007	L, A	174	-	-	-	-	-	-
P	7/24/2007	L, A	200	-	-	-	-	-	-
P	7/31/2007	A	198	32	12	8.4	0	7	5
R	5/22/2007	L	164	-	-	-	-	-	-

R	5/29/2007	L	192	30	3	8.2	2	1	0
R	6/5/2007	L	200	34	9	11.3	3	3	3
R	6/12/2007	L	200	24	8	9.5	4	1	3
S	5/15/2007	L	177	-	-	-	-	-	-
S	5/22/2007	L	200	25	0	0	0	0	0
S	5/29/2007	L	199	36	3	7.4	1	2	0
S	6/5/2007	L	200	31	4	13.5	1	3	0
S	6/12/2007	L	200	30	0	0	0	0	0
S	6/19/2007	L, A	400	-	-	-	-	-	-
S	6/26/2007	L, A	400	27	14	9.1	3	11	0
S	7/3/2007	L, A	400	31	12	10.7	7	5	0
S	7/10/2007	L, A	400	33	10	8.2	0	7	5
S	7/17/2007	L, A	367	-	-	-	-	-	-
S	7/24/2007	L, A	400	-		-	-	-	-
S	7/31/2007	L, A	400	29	7	12.1	0	4	3
S	4/5/2008	A	201	33	10	13.7	1	9	0
S	5/22/2008	D	172	30	0	0	0	0	0
S	6/3/2008	D	242	25	0	0	0	0	0
S	6/12/2008	D	218	26	3	14.2	0	3	0
S	4/29/2009	A	117	33	0	0	0	0	0

Appendix 2.

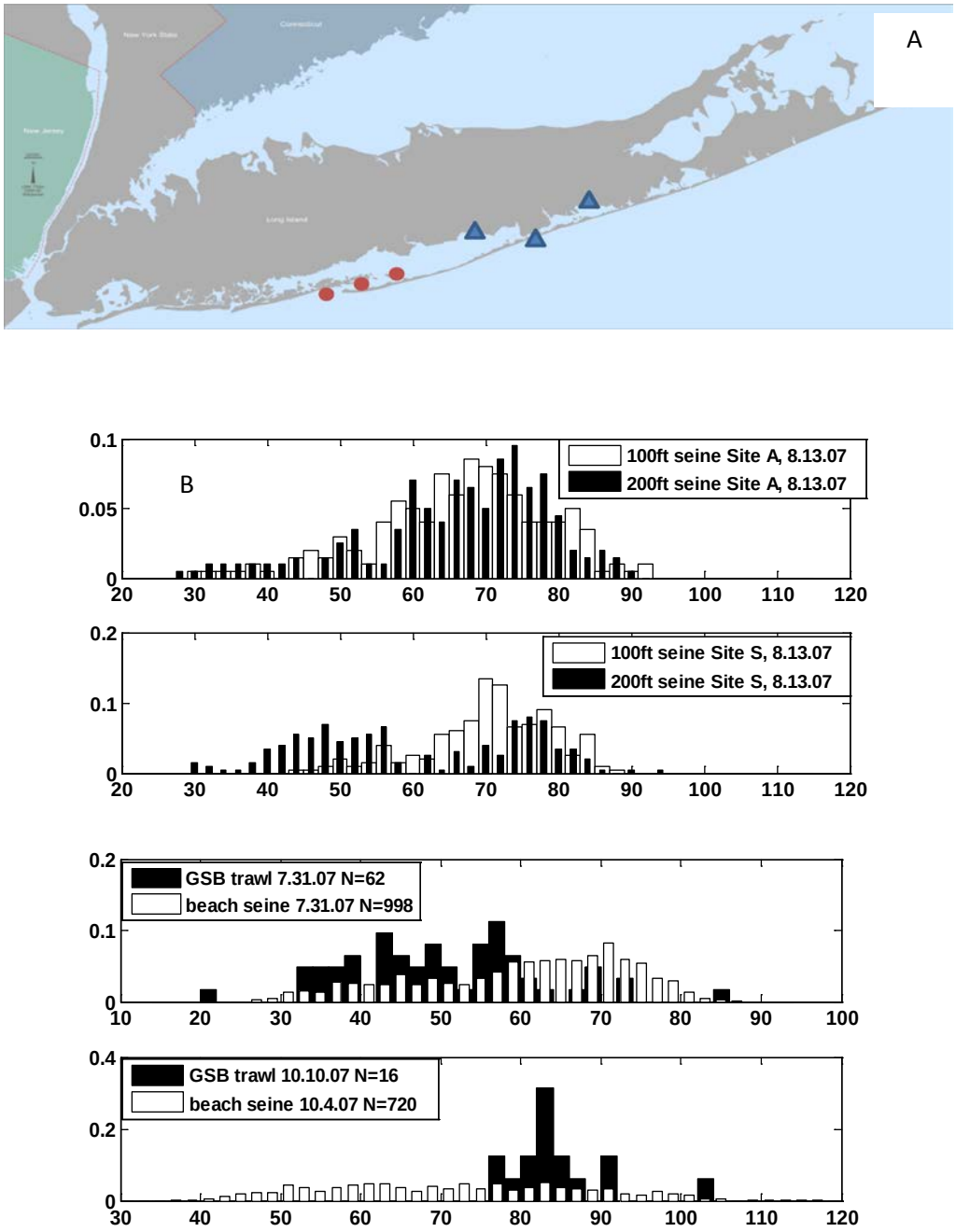

Figure A. Panel A shows the sites plotted by red markers that were sampled with an otter trawl in a survey conducted by M. Frisk and S. Munch in Great South Bay (GSB) in 2007. The sites marked by the blue markers were sampled by K. Perez and S. Munch using a 200ft seine pulled by a boat in order to sample water inaccessible to the adult seine used throughout this experiment. Panel B shows the length frequency distributions collected with the different gears.

Chapter 4: Recovery from early life fitness costs:

Identification of prolonged costs of rapid growth and evaluation of a potential mechanism

Abstract:

Although larger body size is often associated with increased fitness, many species do not grow at their physiological maximum, suggesting that rapid growth results in costs. Despite evidence for growth costs on some timescales (i.e. immediate and end of life), prolonged growth costs, or a cost that continues to manifest somewhere in-between these two time scale extremes, have not been well studied. To evaluate prolonged growth costs, and how long they continue to manifest, I measured swimming ability, lipid mass, and muscle morphology in growth manipulated Atlantic silversides (*Menidia mendia*). Fish were either grown fast (1.4mm/day) or slow (0.6-1mm/day) for 2 weeks. Following this, all fish were grown slowly (~0.6mm/day) for up to 37 days. Fast-grown fish had both significantly poorer swimming ability and less lipid mass than fish that had grown slowly early in life. Additionally, fish were able to recover their swimming ability after 36-37 days following their switch to slow growth. These results indicate that the costs of rapid growth are prolonged and a new life history theory that accounts for such costs is needed.

Introduction:

Larger body size increases survival in the early life, increases mate acquisition and leads to better competitive ability (Lindstedt and Boyce 1985, Janzen 1993, Honek 1993, Fleming and Gross 1994, Kissner and Weatherhead 2005, Sokolovska et al. 2008). A review of the literature in a wide range of terrestrial taxa showed that selection favors larger size at age (Kingsolver and Pfennig 2004). Selection also favors larger size in fish, and the intensity of selection is much stronger than selection for size in terrestrial taxa (Perez and Munch 2010). Many hypotheses have been developed to explain the benefits of size in the early life of fish, including the 'bigger is better' paradigm (Miller et al. 1988, but see Leggett and Deblois 1994). The prevalence of support in the literature for the benefits of larger size has led to the suggestion that growth rate be used as a surrogate of fitness (Schluter 1995).Thus, in the absence of physiological or phylogenetic constraints, we should expect to see evolution towards larger size at age.

Despite all the benefits of faster growth and larger size, many species display growth rates slower than the maximum possible and growth commonly varies among populations (Calow 1982). Many species of fish display sub-maximal growth including the Atlantic silverside (*Menidia menidia*), mummichog (*Fundulus heteroclitus*), turbot (*Psetta maxima*), spotted seatrout (*Cynoscion nebulosus*), Atlantic cod (*Gadus morhua),* Atlantic halibut (*Hippoglossus hippoglossus*), pumpkinseed (*Lepomis gibbosus*), and striped bass (*Morone saxatilis)* (Conover and Present 1990, Imsland and Jonassen 2001, Schultz et al. 1996, reviewed in Conover et al. 2009). Compensatory growth, which is accelerated above normal growth,

allows an individual to balance out an earlier period of slow growth and is further support for the routine occurrence of sub-maximal growth (Metcalfe and Monaghan 2001).

The ubiquity of sub-maximal growth, despite the obvious benefits of larger size, suggests that fast growth must come with costs (Conover and Schultz 1997, Arendt 1997). Growth costs, or trade-offs, occur when a change in one trait, growth, which positively impacts fitness, results in a change in another trait that has a negative effect on fitness (Stearns 1989). Traditional theory assumes that an organism has a finite amount of energy that can be expended, so increased energy allocation towards one trait results in energy being diverted from the other trait (Cody 1966, Gadgil and Bossert 1970, Ford and Seigel 1994). In keeping with this, many immediate costs of fast growth have been reported in both aquatic and terrestrial species. Plants that have grown fast have fewer defense compounds in their leaves and are more vulnerable to herbivorous predators (Cronin and Hay 1996, Coley 1988). Fast-growing butterflies were less resistant to starvation (Gotthard et al. 1994). Some fish species with a high capacity for growth have lower survival because they spend more time exposed to predators in highly productive habitats (Biro et al. 2006) and they also are more aggressive than slow growers (Nicieza and Metcalfe 1999). Higher mortality can also result from the trade-off between rapid growth and disease resistance or shell strength (Boulding and Hay 1993, Lochmiller and Deerenberg 2000). In addition to survival costs, rapid growth may decrease reproductive capacity, e.g. in guppies (Reznick 1983).

Another cost of rapid growth is poorer muscle fiber recruitment (McCormick and Molony 1992). The distribution of muscle fiber area in tropical goatfish that had been growing fast was shifted towards larger fibers indicating poor recruitment of new fibers (McCormick and Molony 1992). Differences in muscle fibers in fish that have different growth trajectories are likely to be

important for fitness because they are required for locomotion. White muscle fibers are associated with anaerobic pathways because they tend to be poorly vascularized and electromyographical studies have shown these muscles are used by fish to produce fast starts and burst speeds (Greer-Walker and Pull 1975, Altringham and Ellerby 1999). Red muscle fibers, also known as slow fibers, have much higher hemoglobin content and have a high aerobic capacity and thus are associated more with sustained swimming. In keeping with these observations, fast growing Atlantic silversides and rainbow trout had poorer swimming ability than fish that had been growing slowly (Billerbeck et al. 2001, Munch and Conover 2004, Gregory and Wood 1998).

Trade-offs may also manifest over longer times scales. Long-term costs of reproduction are also commonplace, where high reproduction in early years will result in a trade-off (Ackerman and Montalvo 1990). The long-term tradeoff for reproductive investment could occur on a variety of time scales (Reznick 1984). For example, increased resources into current reproduction could result in poorer reproduction later in life, and could also result in decreased longevity (Reznick 1984, Bell 1980). As both reproduction and fast growth require a considerable allocation of energy, this suggests that long-term costs of growth are also likely to be fairly common. In both lizards (Olsson and Shine 2002) and butterflies (Gotthard et al. 1994) fast growing individuals had decreased life spans relative to those that had grown slower. Similarly, pinon pines that had been fast growing as juveniles were more susceptible to herbivorous insects later in life (Ruel and Whitham 2002). Studies on compensatory growth also support this. Months after experiencing compensatory growth, salmon had fewer lipid reserves and slower sexual maturation (Morgan and Metcalfe 2001). Asian ladybird beetles exhibit long-term costs of compensatory growth as well, but only under stress (Dmitriew and Rowe 2007).

Existing studies on long-term growth costs primarily focus on effects that manifest long after the individual experienced the fast growth. Prolonged growth costs, where the cost is manifested for an extended period of time following an interval of rapid growth are largely unstudied. To the best of my knowledge, no one has explicitly attempted to estimate the duration of such a cost of growth and whether they decrease or increase over time following the resource allocation.

Objective of the study

I set out to determine whether prolonged costs of growth exist and how long they continue to manifest. To do so, I manipulated growth rates in Atlantic silversides (*Menidia menidia)* and measured two traits that are closely related to fitness, lipid content and swimming ability. To provide a mechanistic foundation for understanding differences in swimming performance, I also evaluated muscle morphology.

Study Species

The Atlantic silverside is an abundant, annual marine fish that ranges from Northeastern Florida to New Brunswick, Canada (Johnson 1975). This species exhibits counter-gradient variation in growth where individuals at high latitudes grow nearly twice as fast as individuals at low latitudes (Conover and Present 1990). It is thought that fast growth rates in the northern populations compensate for a short growing season and a long winter (Conover and Present 1990). This species experiences high mortality during the juvenile stage as they are an important prey item for many recreationally and commercially valuable fishes such as bluefish (*Pomatomus saltatrix),* striped bass (*Morone saxatilis),* and summer flounder *(Paralichthys dentatus)* (Buckel et al. 1999, Manderson et al. 2000, Rudershausen et al. 2005). Winter

mortality is also strongly size-dependent, as larger individuals survive better likely due to greater lipid storage (Munch et al. 2003, Schultz and Conover 1999).

Because the Atlantic silverside is a species that naturally displays a wide range of growth rates, much work has been conducted on the costs and benefits of fast growth in this species. Fast growing individuals from northern populations are more fecund, have higher rates of food consumption, and are more efficient at converting food into body mass (Klahre 1997, Present and Conover 1992). There is, however, a trade-off between growth and swimming ability (Billerbeck et al. 2001) that is nonlinear (Munch and Conover 2004) and results in increased risk of predation (Lankford et al. 2001). Fast growing Atlantic silversides had a lower metabolic scope than slow growing Atlantic silversides (Arnott et al. 2006) and this was suggested as a potential physiological basis underlying the differences in swimming ability. Munch and Conover (2003) found that fast growing northern silversides continued to suffer higher mortality even after the attainment of significantly larger sizes than slow growing conspecifics, suggesting that prolonged costs of growth exist. However, their design mixed northern and southern genotypes leaving open the possibility that some other difference between populations was responsible for the sustained difference in mortality.

Lipid storage is also relevant for Atlantic silverside fitness, as it is known to be an important factor determining over-winter survival (Schultz and Conover 1999). Fish from northern populations, which are exposed to severe winters, rapidly build up lipid stores before winter, whereas fish from southern populations add lipid slowly (Schultz and Conover 1997). However, when reared in the lab, slow-growing southern Atlantic silversides have more lipid mass than fast-growing northern individuals as juveniles (Schultz and Conover 1997). When fish are exposed to temperatures (4°C and 8°C) that are similar to over-winter temperatures,

small fish deplete their lipid reserves faster than large fish, which may explain why over-winter selection favors larger size (Schultz and Conover 1999).

Fish Collection and Rearing

I collected eggs from intertidal root masses in the Annapolis Royal Basin in 2009 (N 44 48.714', W 65 21.582'). Individuals from this location have higher intrinsic growth rates than fish collected from mid or low latitudes (Conover and Present 1990). To account for possible maternal effects, I repeated the experiment two times, once in 2009 with juveniles reared from field-collected embryos and again in 2010 with the F1 offspring of these fish that had spent their entire lives in the lab. Approximately 200 fish collected in 2009 were reared to adulthood and used as brood stock to produce the F1 generation. They were induced to spawn following the procedures described by Billerbeck et al. (2000).

The rearing set-up and growth manipulation methods, which are described below, were the same for both replicate experiments. Fish were hatched at 21°C. After hatch, I raised the temperature in the rearing chambers 1 degree daily until they reached 27°C, a temperature at which they grow rapidly (Conover and Present 1990). Temperature in the rearing chambers was checked daily to ensure the temperature was maintained at 27°C. Buckets were cleaned several times weekly (primarily in the first two weeks of the experiment), or as needed. To limit unhealthy levels of ammonia building up in the water, I exchanged 1/3 of the total water mass on a weekly basis. Prior to the new water being exchanged into the experimental baths, it was allowed to age for at least 24 hours and was heated to 27°C.

Ten days post hatch, density was standardized to 45 individuals per rearing bucket and then buckets were separated at random into two growth-rate manipulation treatments, 'FAST'

and ‘SLOW’. I manipulated growth by restricting the amount of brine shrimp (*Artemia)* nauplii that were available for consumption. Billerbeck et al. (2000) previously produced slow growth by feeding Atlantic silversides between 20% and 75% of wet body mass. Following this study, Billerbeck et al. (2001) manipulated fish to grow slowly by feeding them 50% wet body mass rations to generate a growth rate of 0.6mm/day. Swimming ability is greatest when fish are maintained at this growth range (Munch and Conover 2004, Billerbeck et al. 2001). Unlimited rations at 27°C produce Nova Scotia fish that have a growth rate from 1.3-1.6mm/day (Conover and Present 1990). Individuals in the SLOW growing treatment were fed 60% of their wet body weight daily. Individuals in the FAST treatment were fed unlimited rations. Present and Conover (1992) showed that 9000+ brine shrimp nauplii per liter of water were in excess of what a Nova Scotia juvenile could eat in 24 hours. Similarly, I fed the FAST treatment twice daily, and checked the buckets several times during the day in-between feedings, and in the mornings before the first feeding to ensure that food always remained in the FAST treatment buckets. Variation in growth is typically high, especially in limited ration treatments (Billerbeck et al. 2000). To ensure that fish density in the buckets remained constant, individuals that grew faster or slower than the target ranges were kept in the experiment although they were not used to measure swimming ability.

This period of growth manipulation continued for two weeks after which all individuals were placed on restricted rations (60% per day). Two to three weeks of fast growth is sufficient to result in a significant decrease in swimming ability (Munch and Conover 2003) without generating excessive differences in size among treatments. I used the SLOW growth treatment as the control for studying the effects of the 2-week period of rapid growth. Space constraints and limited sample sizes prevented me from also having a FAST growing control.

To evaluate the growth rate in each treatment, and allow for the adjustment of the SLOW growth feeding ration with increasing size, I measured a subsample of fish on a weekly basis. Subsamples of 5 individuals per bucket were weighed after 1 week of growth manipulation and were sacrificed using MS-222. Two weeks into the experiment fish were large enough to be live-measured without causing mortality. At this time, all fish were measured to the nearest millimeter and sorted into size-matched groups (± 1 mm). Density was standardized to 30 size-matched individuals per bucket. A subsample of 5 individuals per size class had both their mass and length measured to obtain a length-weight relationship every two weeks. This relationship was used for updating the feeding ration. During weeks 3 to 8, a subsample of 5-10 fish was measured from each bucket weekly to ensure that fish were growing at an appropriate rate and to adjust the quantity of food as necessary.

One week prior to swimming trials, fish were acclimated to swimming in low velocities (5-6cm/sec) in their rearing bucket. To ensure that all individuals were kept swimming, velocity was maintained throughout the entire depth of water.

Measuring Critical Swimming Ability

Three measures of swimming performance are commonly used, burst swimming, sustained swimming, and critical swimming speed (U_{crit}, Plaut 2001). Although the ecological relevance of U_{crit} is less obvious than burst or sustained speeds, it is easier to measure precisely and is highly repeatable (Plaut 2001). Billerbeck et al. (2001) tested the effect of growth on swimming performance using all 3 performance measures and found that all three exhibit an immediate growth cost. Therefore, in keeping with Billerbeck et al. (2001) and Munch and

Conover (2004), I measured critical swimming speed (U_{crit}) to test whether prolonged reductions in swimming performance exist and how long they continue to manifest.

The swim tunnel used to measure U_{crit} (Loligo Systems 100-240v) was calibrated prior to swimming trails by timing dye movement over a set distance. This was also used to ensure the current was non-turbulent. I calibrated the swim flume before starting the swim trails each year and had to re-calibrate several times (twice in 2009, once in 2010) due to equipment failure, where the screen restricting fish from swimming into the motor broke off. Each time I calibrated the swim chamber, I measured how long it took dye to travel 23.5cm, which was the length from the single opening in the top of the flume to the end of the flume. I timed 10 dye runs for each quarter of an increment on the speed control knob, from 2.0 to 4.5. I then estimated the velocity of higher increments on the knob using a second order polynomial.

Fish were size-matched to ±2mm and fasted for 24 hours before the swimming trials. I swam Atlantic silversides in groups of 3, because they are a schooling species and swim poorly when alone (Munch and Conover 2004). During the U_{crit} trials fish were allowed to acclimate in the swim tunnel for 20minutes at 5cm/sec. A pilot study showed that longer acclimation times (40 min) did not affect the swimming ability of the fish (ANOVA $p=0.93$ $N=20$). Following the 20 minute acclimation period velocity was increased by 5cm/sec every 10 minutes (Munch and Conover 2004). The swimming speed and failure time (nearest second) was recorded for each fish. After all three fish failed, the fish were re-measured for total length to get a more precise measure of size for each swim trial and preserved (frozen in 2009, 5% formalin in 2010) for later study. I used 2 different preservation methods because lipid extraction requires frozen tissue whereas muscle histology requires tissue to be fixed in formalin. The extrapolated failure speed, U_{crit} (cm/sec), was determined using the following equation:

$$U_{crit} = V + vt/T$$

where V is the last speed maintained for the entire interval, v is the velocity increment, and t/T is the fraction of the 10 minute interval the fish completed before failing.

I measured U_{crit} in the FAST growers at the end of their fast growth period (after 2 weeks) in both 2009 and 2010. Following the switch to SLOW growth, critical swimming speed was also measured at 18, 22, and 50 days in 2009 and at 28, 37, and 52 days in 2010. These intervals can also be defined in the number of days since the fish were switched to SLOW growth (4, 8, 36 in 2009, 13, 22, 37 in 2010). To control for the relationship of U_{crit} with body size I measured swimming performance in the SLOW growers when they reached the same size as the FAST growers and FAST/SLOW growers. In order to evaluate the long term cost of growth, I compared U_{crit} of individuals that had only experienced SLOW growth to those in the other growth treatment.

Analysis of Fish Condition

I evaluated whether fish condition, measured as total lipid mass, was affected by the growth treatment. Following methods of measuring somatic energy stores developed previously (Shultz and Conover 1999), I extracted natural storage lipids using a custom built Soxhlet extractor that allowed for processing multiple samples at once. I haphazardly chose 1 fish out of each swim trial from the first phase of the experiment. I dried cellulose extraction thimbles (Whatman single thickness 10mmx50mm) at 50°C for 24hrs and then measured their weight. Fish were diced and placed into the dried cellulose thimble, freeze-dried for 24hrs, and then weighed. Pilot experiments showed that 24 hours was sufficient to achieve constant weight.

Using a Soxhlet extractor and petroleum ether as the solvent, I then extracted the lipids from the samples. The Soxhlet extractor ran for 6 hours and cycled every 20 minutes. Following this extraction, I re-dried the thimbles with samples at 50°C for 24 hrs and obtained a final weight. The total mass (mg) of storage lipids was calculated as the difference in weights (sample +thimble) before and after extraction:

$$\text{Lipid} = \text{total weight}_{\text{after}} - \text{total weight}_{\text{before}}$$

Analysis of Muscle Morphology

The fish that were tested for swimming performance in 2010 were preserved in 5% neutral buffered formalin. From each trial one fish was haphazardly selected for histology. Fish bodies were decalcified for 24 hours in a 10% EDTA solution.The histological sectioning and staining was performed by AML Labs, Baltimore MD. The caudal peduncle of the fish was sectioned in 5 µm increments in a stepwise fashion. Four caudle peduncle sections were collected from each individual and stained with a standard haematoxylin and eosin stain following the methods from Greer-Walker and Pull (1975). Using this method of histological sectioning and staining, it is not possible to unambiguously separate pink muscle fibers from red or white muscle tissue. As a consequence, I was restricted to classifying muscle fibers as either red or white.

For both red and white muscle tissue I made three measurements. The following equations are defined for red muscle; however the same equations were also used for white muscle calculations. First, I measured the proportion of the sectioned body area that was comprised of either red muscle or white muscle. This was calculated as:

$$\text{Proportion Red} = \text{area red}(\mu m^2) \Big/ \text{total area}(\mu m^2)$$

I also selected groups of fibers (roughly 15-25 fibers) both from the red and white area and measured individual cross sectional fiber area using Image Pro Plus software. This was repeated 4 times on different sets of fibers for each fish. I measured the same 4 regions of fibers on each fish, to standardize across individuals. Finally I calculated fiber density for sections of both red and white muscle.

$$\text{Red fiber density} = {}^{\text{Number red fibers in area measured}}/_{\text{red area measured}(\mu m^2)}$$

I used the proportion and density measures to estimate the total numbers of fibers. This was calculated as:

$$\text{Total red fiber number} = \text{fiber density x area red}(\mu m^2)$$

White muscle fibers tend to be much larger and more numerous than red muscle (Stickland 1983). To compare how the fiber area and number of these different muscle types varies with fish size I standardized them so they both had units of standard deviations. To standardize, I used the mean fiber area of either red muscle or white muscle over the entire experiment. Similarly, to calculate the standard deviation of the fiber area I calculated the standard deviation of either red fiber area or white fiber area over the entire experiment. I repeated these calculations for total fiber number, and proportion of muscle for both muscle types. The equation below is an example of the standardization technique for a given muscle measure.

$$\text{Standardized muscle measure} = \frac{\text{individual muscle measure} - \text{mean muscle measure}}{\text{standard deviation muscle measure}}$$

To ensure that the measurements were repeatable I randomly selected 10 individuals to be re-measured one month following their initial analysis. Because these fish that were re-measured had a high degree of correlation between the first and second read, all remaining fish were read once (Pearson correlation coefficient range from 0.61-0.99).The highest degree of correlation between the first and second reads was seen for total area of red muscle, total area of white, and fiber density. The lowest correlation coefficients were seen when measuring the average fiber area of either red or white muscle fibers indicating that these measures are less repeatable than density or total areas.

Statistical Analysis

For statistical analysis, I calculated growth rate as:

$$\text{Avg growth} = \frac{\bar{L}(t + \Delta t) - \bar{L}(t)}{\Delta t}$$

where $\bar{L}$ is the mean size over all individuals in a treatment at time t. I used the mean size in a treatment because I was unable to track individuals over time steps. The difference between t+1 and t is the number of days between measurements. When t=0, $\bar{L}$ is equal to the initial average length. To evaluate differences between growth rate in the FAST/SLOW (FS) and SLOW (S) growth treatments I used a repeated measures ANOVA to test for differences where both year and treatment were factors and growth rate was the response.

Past work has found linear relationships between fish length and swimming ability when fish grow at a fairly constant rate (Billerbeck et al. 2001). However, in the present study one treatment of fish is switching from fast growth to the slow growth trajectory. Thus, to control for the potential non-linear relationship of length in this growth treatment with swimming ability,I

separated the growth treatments into size class groups with a range in size from 5-8mm (Figure 2). I analyzed the data in four groups based on fish length. I used the groupings for the FAST and FAST/SLOW fish described below and compared swimming ability of the SLOW treatment fish when they had reached the same size. The first group, initials, were the FAST group swam after 14 days in 2009 and 15 days in 2010 (Table 1). The second group, small, were the FAST/SLOW treatment swum on days 18 and 22 in 2009, and day 28 in 2010. The third group, medium, were FAST/SLOW treatment swum on day 37 in 2010 only, and the final group, large, were the FAST/SLOW treatment who were swum after 50 days in 2009 and 52 days in 2010. It is important to note that in 2009, I was limited by sample size and did not have a medium size group to examine for this year. The SLOW treatment fish were compared to the FAST or FAST/SLOW treatment when they were of comparable sizes (Figure 1).

To evaluate the effect of mean fish length on critical swimming ability and lipid mass I used a least squares linear regression. I tested the null hypothesis that treatments (year, growth manipulations) had no effect on immediate or long-term swimming performance using a 2-way ANCOVA. For all statistical analysis I used the median critical swimming ability, and the average length (mm) from each trial with three fish. I tested for differences in median U_{crit} values and lipid mass, where fish length was treated as the covariate. Both Ucrit and lipid mass residual values were tested for normality using a Shapiro-Wilk *w*-test. The covariate, length, was not significantly different between the growth treatments. To determine if there were significant differences between the poorest, or the best swimmers from the FAST/SLOW or SLOW growth treatments, I repeated the ANCOVA, where length was the covariate using either the maximum or minimum value for Ucrit from each trial as the dependent variable. Comparing

the ANOVA using the maximum or minimum values from the U_{crit} trials also allowed me to compare how variable swimming ability is within and between the different growth treatments

To evaluate whether the FAST/SLOW growth treatment showed signs of rebound in swimming ability I used a normal likelihood to predict swimming ability using two linear equations (swimming ability=a+b*length) with parameters determined separately for the FAST and SLOW fish. The mean and variance of the normal likelihood were from the linear regression U_{crit} on length. I then calculated the probability of being SLOW which was:

$$P_{SLOW}=L_{SLOW}/(L_{SLOW} + L_{FAST})$$

When the likelihood of being SLOW is near zero, the fish have not shown signs of swimming ability rebound. If L_{SLOW} approaches 1, the FAST/SLOW fish have recovered from their initial growth cost. To account for the effect of fish age on recovery, I repeated these calculations with age as the independent variable instead of fish length.

Muscle fiber analysis

I used a MANOVA to determine how if the different growth treatment groups had differences in their muscle characteristics (red fiber area, white fiber area, red fiber number, white fiber number, upper (75^{th}) and lower quantile (25^{th}) red, upper (75^{th}) and lower (25^{th}) quantile white). I grouped the data into their respective growth treatment (either FAST, FAST/SLOW, or SLOW). All data were log transformed to satisfy the assumption of normality, except the total counts, which were square root transformed. To control for the size range of fish used in this experiment and the increasing fish size over the experiment, I used the residuals from a regression of the predictor on length as the data for analysis.

I compared the muscle fiber area frequency distributions of the different growth treatments to test whether the FAST/SLOW growth treatment has poorer muscle fiber recruitment than the SLOW treatment. Importantly, I would expect that the growth treatment may have impacted the recruitment and growth of small muscle fibers that had recruited during the experiment, whereas large muscle fibers that likely were present before the experiment began may not show major differences. To evaluate whether the shape of the distribution of fiber areas changes with fish length or growth treatment I separated the data into 3 groups based on fish size described above and then calculated an average frequency distribution for each group. I calculated cumulative distribution functions of fibers that were in each bin increment. For the three size groups I then tested for differences between growth treatments using a G- test. I also evaluated any differences within size groups of FAST/SLOW and SLOW growth fish.

I also used a least square linear regression to determine if red fiber area, white fiber area, total fibers red, or total white fibers significantly influenced the swimming ability of the FAST/SLOW treatment or SLOW treatment. If the linear regressions were significant, I also tested if the FAST/SLOW or SLOW treatments had significantly different regression coefficients (slope, y-intercept).

Results:

Growth Trajectories

Fish in the SLOW treatment (labeled as S), grew on average 0.6 mm/day (+/- 0.4 SD) in 2009 and 0.7 mm/day (+/- 0.2 SD) in 2010 (Figure 1 and Appendix 1) . Fish in the FAST/SLOW treatment (labeled as FS) grew on average 1.4mm/day (+/- 0.2SD) in 2009 and1.3mm/day (+/- 0.10 SD) in 2010. After 2 weeks of fast growth, the FS treatment was

switched to limited rations and thereafter grew similarly to the SLOW treatment: the average growth rate of the FS fish in 2009 after they were switched to SLOW growth was 0.6 mm/day (+/- 0.3 SD), and in 2010 the average growth was 0.6 mm/day (+/- 0.2SD). Average growth over the roughly 2 week intervals did vary by year and growth interval (Figure 1). In both treatments, growth from week 2-4 and week 4-6 were not significantly different from each other, but did differ significantly from growth in weeks 6-end (repeated measures ANOVA, p=<0.001, Figure 1). From week 6 to end, growth did not significantly differ between the treatments, but growth was significantly higher in 2009 than in 2010 (p=<0.0001 for year, P>0.05 for growth). This slightly higher growth at the end of the experiment in 2009 is likely because of a change in the concentration of food in the buckets. Although I fed the fish the same percentage of their body weight throughout the experiment, the screens on the buckets that allow water exchange likely became slightly clogged towards the end of the experiment in 2009 allowing less nauplii escape. In 2010, bucket screens were individually cleaned weekly to prevent clogging. What is most relevant, however, is that within years, the S and FS treatments grew at the same rate.

Effect of size and year on median swimming performance

I ran a total of 164 swimming trials over 2 years, which took 331 hours of swim tunnel use (Table 1). In keeping with previous research on U_{crit}, I found that critical swimming ability, and thus the time that it took for the fish to tire, increased with mean size (Billerbeck et al. 2001, Munch and Conover 2004, Table 2, Figure 2 least squares regression p=<0.001).

There was no effect of year on swimming performance in the initial or small treatment groups of FAST and SLOW growing fish (2- way ANCOVA p=0.72, F=0.13, N=62 initial

group, p=0.78, F=0.08, N=70 small group, Figure 2). Since only fish in the medium size group were used in 2010, I was unable to account for a year effect in that size group. There was a significant effect of year in the large treatment group where fish swum in 2009 had higher swimming ability than fish swum in 2010 (ANCOVA p=0.02, F=6.12, N=38). This difference is likely because the fish were bigger in 2009 than in 2010, and when truncating the 2010 fish so that the exact same size range was compared, the differences in swimming were not significant (compared fish 46-49mm 2-way ANCOVA, p=0.08, F=3.32, Figure 2).

Effect of growth treatment on median swimming performance

A quadratic model (U_{crit}=a*length^2 + b*length + c) fit the SLOW treatment data and the FAST/SLOW treatment data significantly better than a linear model (U_{crit}=a*length + b) (Likelihood ratio test, SLOW treatment, p<0.001, N=66, FS treatment, p<0.0012.8E-5, N=82, Figure 3B). For the SLOW growing fish however, the significance is solely driven by the first 10 swim trials (initial SLOWs) in 2010. Over the initial swim period in 2010, the calibration of the swim flume was slightly slower than in 2009. I swam the initial FAST and initial SLOW at this calibration before realizing it was different. If the first 10 swim trials are removed from regression of swimming ability on length, the quadratic model is not significantly better than the linear model for the SLOW fish (p=0.81). For the FAST growing fish before they were switched to slow growth, the quadratic model is not significantly better than the linear model (Likelihood ratio test, p=0.56, N=29, Figure 3B).

Recent growth significantly impacted swimming performance (Figure 3). In the initial group, the FAST growing fish had significantly poorer swimming performance than SLOW growing fish (2-way ANCOVA, p<0.001, F=31.1, N=62). Even after FAST growing fish had

switched to slow growth for up to 22 days, they still maintained a cost of the initial FAST growth (small group= 4,8, and 13 days post switch $p=0.01$, $F=6.4$, $N=70$, medium group=22 days post switch $p<0.001$, $F=86.7$, $N=22$). However, the FAST/SLOW treatment recovered swimming ability by 36-37 days after the switch to slow growth. By this time, the FAST/SLOW growers and the SLOW growers had equal swimming performance (large group= 36-37 days post switch $p=0.08$, $F=3.25$, $N=38$).

It was also of interest to determine if swimming performance differed between growth treatments if the minimum or maximal value for U_{crit} was used instead of the median. Comparing both the minimum and maximum values for U_{crit} allows me to compare the variability of swimming ability in the different growth treatments. For the initial treatment of FAST growers compared to SLOW growers and the medium treatment of FAST/SLOW fish compared to SLOW fish, analysis of both the maximum and minimum U_{crit} values showed significant differences ($p=<0.0001$, $p=0.001$). The minimum value of U_{crit} for the small group of FAST/SLOW fish was not significantly different than the minimum value for the SLOW treatment ($p=0.16$), however the maximal values were significantly different ($p=0.004$). For this size group, the range in swimming ability of SLOW growers is greater than the range of FAST/SLOW treatment swimming ability, which is indicated by both the median and maximum swimming ability being better for SLOW growers than FAST/SLOW fish, but no differences between the minimum U_{crit} values. For large fish the result was similar: minimum swimming performance values did not differ across treatments, while maximum swimming performance values of the SLOW fish were significantly greater ($p=0.04$).

Rebound rate of median swimming performance

There was considerable variation in the likelihood of being a SLOW grower in all size groups until the fish were between 45-50mm (Figure 4 A, B). The mean likelihood of being a SLOW grower decreases until the fish reach 40mm, which may indicate a lag in when the cost completely manifests. It may also simply reflect the considerable degree of overlap between the swimming speeds of FAST and SLOW growers early in the experiment. After 40mm, the fish in the FAST/ SLOW treatment show signs of rebound. The mean likelihood in the 45-50mm size group was 0.98 (+/-0.08 SD) indicating a high probability of being equal to the swimming ability of the SLOW growers.

When calculating the probability of being SLOW given the age of the fish, the pattern of rebound was clearer than when taking into account size. Young FAST/SLOW fish had a low probability of being equal to the SLOW treatment (Figure 4 C, D). As the FAST/SLOW fish reached 25 days since the start of the experiment, some trials show signs of rebound, however full rebound for all trials is not seen until fish were 43 days since the start of the experiment.

Lipid analysis: trends with fish size

I extracted total lipid mass from 61 fish in 2009 (Table 3). Lipid mass was positively related to fish size (Log mm) (figure 5).

Effect of growth treatment on lipid mass

Lipid mass differed significantly among the growth treatments (Figure 6). FAST growing fish had significantly less lipid mass than SLOW growing individuals after correcting for length (ANCOVA $p<0.001$, $F=16.9$, $N=38$). FAST/SLOW growing fish that had been slow growing for only 4-8 days since fast growth had similar lipid mass as fish that were always SLOW growing (ANCOVA $p=0.53$, $F=0.34$, $N=32$). Fish from the FAST/SLOW treatment had

been slow growing for 36 days also had no differences in lipids compared with fish that were always growing slowly (ANCOVA p=0.98, F=0.00, N=11). I also tested for differences in lipids between the growth treatments using fish dry weight as the covariate instead of fish length. The results remain the same except the initial comparison between FAST and SLOW treatments became marginally non-significant (P=0.09, F=2.89). The dry fish weights were quite noisy, which might be due to measuring such a small fish mass per weight of the thimble, as fish averaged 15% of thimble mass (Figure 6 B).

Effect of growth treatment on muscle composition

All fish used in our study were in the second phase of muscle development, known as mosaic growth (Stoiber et al. 1999). Once fish reach this growth phase they have distinct regions of red and white muscle and may continually add new fibers to each region into adulthood (Stoiber et al. 1999). The differences between red and white muscle fibers were clearly distinguishable in the fish sections. Red muscle fibers were on average 20% of the size of white muscle fibers (Table 4). The size range of red muscle fiber area (min=4.6μm^2, max=15.4μm^2) was also much smaller than the size range of white muscle fibers (min=18.6μm^2, max=117.3μm^2).

The white fiber area, standardized by the mean and standard deviation from the entire experiment, changed more over the range of fish sizes evaluated than standardized red fiber area (Figure 7 A). This was indicated by a significantly greater slope for white muscle than red muscle (t-test p=0.007, N=68). Although both total number of white fibers and total number of red fibers increased with fish size, the regression slope for red fibers was greater than that for white fibers (t-test p=0.02, N=66, Figure 7 B). There was no difference in the regression slopes

for the proportion of red muscle or proportion of white muscle over the range of fish sizes (T-test P=0.3, N=62, Figure 7C).

Although the upper limit of white muscle fiber area increased with increasing fish size, the lower limit did not show the same trend (Figure 8 A). All fish sizes had small fibers (<10μm^2) indicating new recruitment. The small and medium fish from the SLOW growth treatment had slightly more small white fibers than the FAST or FAST/SLOW growth treatment. In large fish, the SLOW growth treatment had a greater proportion of large white muscle fibers than FAST/SLOW growth treatment. FAST/SLOW and SLOW growth treatments muscle fiber distributions were significantly different from each other in all size groups (G-test p=<0.002, df=1). For small fish the SLOW growers had more small white fibers than the FAST growers, however for the large fish that had regained their swimming ability, the FAST/SLOW growers had more small white fibers than the SLOW growers. The results differed for red muscle; for both small and medium fish, SLOW growers had significantly more small red muscle fibers than the FAST or FAST/SLOW treatment (G-test p=<0.0005, df=1). There were no differences between the FAST/SLOW and SLOW treatments in the distribution of red muscle fiber area for large fish (Figure 8 B, G-test P=0.2, df=1). Additionally, the 8 different muscle characteristics were not significantly different between the different growth treatments (MANOVA p=0.3, F=1.1, N=68, Figure 9). Comparing average fiber area and number of fibers below 5μm for red muscle and 25μm for white muscle also yielded no significant patterns between the growth treatments (MANOVA, p=0.58).

Linear regressions showed that white muscle fiber number, red fiber area, and white fiber area significantly affected critical swimming ability (subplot A, C, D Figure 10) (Least-square linear regression, p=0.01, N=68). I also tested if the regression coefficients (slope, y-intercept)

significantly differed due to the growth treatment. The slope and y-intercept for both white fiber number and white fiber area from SLOW growing fish were not significantly different than from FAST or FAST/SLOW growing fish. However, the slope of the linear regression of red fiber area was significantly larger for SLOW growers than the slope from the FAST or FAST/SLOW growth treatment (slope FAST, FAST/SLOW=28.6, slope SLOW=96.4, t-test, p=0.03, N=68).

Discussion

Swimming ability of the FAST growing fish showed signs of both an immediate cost and a prolonged cost. This immediate cost of fast growth had been demonstrated previously in Atlantic silversides (Billerbeck et al. 2001, Munch and Conover 2004) and my results agree with these, despite the fact that the fish I used were considerably larger (> 5mm) than fish in either of these prior studies. Furthermore, fish were able to recover their swimming ability after 36 days and lipid stores before 4 days following their return to slow growth. The pattern of recovery (Fig. 4) indicated that FAST/SLOW fish at 40mm have swimming ability that is considerably more similar to the FAST treatment swimming ability than the SLOW treatment, despite them having been on limited rations for several weeks. After fish reached 40mm they showed signs of rebound as the likelihood of being a SLOW grower increased. When fish were 45- 50mm, the likelihood of being a SLOW grower was very close to 1 indicating a full recovery in swimming ability. This result suggests that fish are able to begin recovery from a cost of rapid growth shortly after the cost manifests. This study is the first to my knowledge to determine the number of days it will take for individuals to recover from a cost of growth. The potential for recovery of a swimming ability cost is valuable for increasing our understanding of growth rate evolution.

Median swimming ability, lipid content, and muscle fiber characteristics were found to be positively related to fish size (Figures 2, 5, 7). A quadratic model fit the swimming ability vs.

fish size data significantly better than a linear model for the SLOW treatment and the FAST/SLOW treatment fish. This was expected because the relationship between fish size and swimming ability is changing over time in the FAST/SLOW treatment. However, the fit for the SLOW treatment fish depended on the initial data points from 2010. When these points were not included in the likelihood ratio test, the linear model was not significantly different from the quadratic model. Furthermore, the quadratic model was no different than the linear model for the FAST/SLOW fish before they were switched to slow growth. Previous swimming performance work on Atlantic silversides has found a linear relationship between fish size and swimming ability (Billerbeck et al. 2001). However, Stobutzki and Bellwood (1994) found an allometric relationship between fish size and swimming ability in coral reef fish. It seems likely that the relationship between fish size and swimming ability is linear when growth remains constant, as is demonstrated by the FAST growers and the SLOW growers. The relationship may be something other than linear when a switch occurs in fish growth rate, as it appears that the trajectory of FAST/SLOW swimming ability shifted from the FAST trajectory towards the SLOW trajectory. It may be that the relationship between U_{crit} and length measured by Stobutzki and Bellwood (1994) is being influenced by changing growth rates or development. Pre-settlement juveniles had higher swimming ability than the post-settlement juveniles immediately after they settled, which may indicate that there is a cost associated with preparing for settlement (Stobutzki and Bellwood 1994).

Maternal effects have commonly been found to influence larval condition, survival, and critical swimming ability (Marshal et al. 2010, Venturelli et al. 2010, Green and McCormick 2005). Tropical clownfish larvae from a small mother were found to be better swimmers than larvae from a medium size mother (Green and McCormick 2005). The fish collected in 2009

were wild collected embryos, whereas the fish from 2010 were the F1 generation. No clear indication of maternal effects is present in our data as the wild population responded to growth manipulations similarly to the lab-reared population. In general, maternal effects tend to be most pronounced early in life (Bernardo 1996) which indicates the difference in swimming ability between years in the largest fish in the present study is unlikely to be due to maternal effects.

In order to compare the swimming ability of individuals from the FAST/SLOW and SLOW growth treatments at the same size, the SLOW growers were always swim tested at an older age, leaving open the possibility that observed differences were due to age effects. However, since trials were carried out over several weeks, there is some variation in age that can be used to address this issue. Adding age as a covariate into the ANCOVA model did not qualitatively alter the results except that the swimming performance differences among FAST/SLOW and SLOW growing fish in the small size group became marginally non-significant (ANCOVA, $p=0.06$, $N=68$). This result, that recent growth rather than age is the primary determinant of swimming performance at a given size is consistent with prior work; Munch and Conover (2004) controlled for both size and age and found that the difference in swimming performance between FAST and SLOW growing fish was the same as that found by Billerbeck et al. (2001) who only controlled for size.

Lipid mass was significantly less in FAST growing fish than in their slower growing counterparts (Figure 7). However, no significant differences were seen between the SLOW growing treatment and the FAST/SLOW growing treatment. This might indicate that fish recover lipids faster than fish recover their swimming ability. The non-significant differences in lipid mass may also be due to a low statistical power (Power=0.43).

Energy allocation to lipid mass is known to result in poorer allocation to other traits, including reproduction and movement (Lima 1986, Maes et al. 2006). In field-collected Atlantic silversides, energy allocation to lipids varies seasonally as well as with latitude (Shultz and Conover 1999). Fast growing northern individuals, which are exposed to intense over-winter mortality, show dramatic fattening in the fall, whereas slow growing southern individuals, which are not exposed to harsh winters, do not display fall fattening (Shultz and Conover 1999). However, when individuals from both latitudes are reared in a common environment in the laboratory, in the summer juveniles from southern populations tend to have more lipid mass per body mass than juveniles from northern populations and also grow slower. In trout, Biro et al. (2005) suggested that early in life, strong pressure exists to reach a specific size, so allocation favors building muscle tissue over lipid mass. Also, salmon injected with growth hormone grow faster, but have fewer lipid reserves than normal growers (Johnsson et al. 2000). Thus, past research and the present results suggest that for northern populations of Atlantic silversides, FAST growers put on lean tissue early in life to increase their size quickly at the expense of lipid reserves and swimming ability. SLOW growers, however, may invest more in lipid reserves and have higher swimming performance at the expense of getting big as quickly. My results indicate that lipid mass increases rapidly after growth is slowed while swimming performance takes more than a month to recover. Theoretical arguments (Perrin and Sibly 1993) suggest that the timing of allocation to a suite of traits is directly tied to their expected fitness payoffs. In light of this, it may be more important to build lipid reserves than improve swimming ability. On the other hand, it may be that the physiology of muscle growth constrains the rate at which swimming performance can be restored.

Muscle fiber size is commonly associated with fish growth, though the magnitude and direction of this association is quite variable (Rasmussen and Ostenfeld 2000, Johnston et al. 2000, Ayala et al. 2001, Galloway et al. 1998, Vieira and Johnston 1992,Valente et al. 1999). In juvenile fish, the general pattern is that faster growth from increased rations results in equally sized fiber diameters but higher fiber number (Arendt 2007). However, faster growth due to increased temperature may increase fiber size, fiber number, or both (Arendt 2007). Farmed salmon that did not swim as well as wild salmon have smaller muscle mitochondria, less dense triads which underlie muscle contraction, and lower activity in muscle enzymes (Anttila and Manttari 2009).

I found that SLOW growing Atlantic silversides had more newly recruited fibers than FAST growers. Similarly, McCormick and Molony (1992) found that tropical goatfish that were fed limited rations had a higher proportion of small muscle fibers than fish that had been fed unlimited rations. Following the pulse of rapid growth, I found that red muscle fiber distributions remained different among medium- sized fish, but were no different at the end of the study. In contrast, FAST/SLOW growers had significantly more newly recruited white fibers (Figure 9) by the end of the study, suggesting the continued recruitment of new fibers. These results suggest that in Atlantic silversides recruitment of red and white fibers trades-off against rapid growth and that the recruitment of muscle fibers may be valuable in determining the duration of prolonged growth costs.

Despite the potential importance of prolonged growth costs for better understanding the evolution of size, apparently only two studies have modeled evolution of growth trajectories under delayed or long-term costs (Yearsley et al. 2004, Mangel and Munch 2005). The model developed by Yearsley et al. (2004) incorporated immediate costs and costs that had a delay in

when they manifested. They found that fast growth was most adaptive when the organism did not have to pay the cost until much later in life, after reproduction (Yearsley et al. 2004). Mangel and Munch (2005) model the accumulation and repair of metabolic damage which results in prolonged growth costs, which are conceptually more similar to my results than the delayed costs of Yearsley et al (2004). In keeping with my results, Mangel and Munch (2005) predict that most energy would go towards repair of damage when the individual is kept on a limited ration. However, in their model, the pattern of swimming recovery over time would be asymptotic, meaning that most recovery would occur shortly following the return to slow growth. The pattern actually observed in the present study is the opposite; minimal recovery of swimming performance occurred until the fish reached 40mm after which they recovered rapidly, reaching full recovery at 45-50mm.

At the moment it is not clear why swimming ability, the primary determinant of survival in juvenile silversides, should exhibit a prolonged, initially slow recovery. Several reasons are plausible. First, it may be that physiological constraints on muscle development prevent more rapid recovery of swimming performance. That is, recovery of swimming performance begins rapidly following reduced growth but takes a long time to complete. However, rather than viewing the delay in recovery as the result of a constraint, it may be optimal for the fish to delay until more information on its new ration level is available. Fish on unlimited rations are physiologically primed for high consumption rates and thus maintain high feeding metabolic rates. The increase in metabolism that occurs after food consumption, called specific dynamic action (SDA) is due to many physiological factors including enzyme secretion and stomach peristalsis (McCue 2006). SDA uses a considerable amount of energy, and a recent review of fish species found that on average 15% of consumed energy goes towards feeding metabolism

(McCue 2006). In Atlantic silversides SDA can use up 7.2% of energy ingested from food (Billerbeck et al 2000). In environments where food availability is variable, individuals facing a period of privation may initially maintain high feeding metabolism in anticipation of higher food availability in the near future in an effort to reduce start-up costs. In this light, the reduced swimming performance of FAST growers isn't a 'cost' of growth per se, but an allocation decision implying that attaining larger size is more important than motility, at least initially.

In light of much empirical evidence for immediate and long-term growth costs (Munch and Conover 2004, Billerbeck et al. 2001, Olsson and Shine 2002), and the ubiquity of physiological delays (Akçakaya et al. 1988), prolonged growth costs are likely to be commensurately common. My observations on the prolonged recovery from a period of rapid growth indicates a need for a more mechanistic theory of growth rate evolution that would include prolonged costs via physiological delays or alternate allocation strategies and the role of environmental fluctuations.

Table 1. Summary of swimming ability comparisons between the FAST/SLOW treatment and the SLOW treatment. The 1st column shows the group names for the swimming comparisons. The 2nd column shows the year that the swimming was tested, the 3rd column shows the FAST, FAST/SLOW treatment, and the 4th column shows the size range over which fish were measured. The 5th column shows the day that the FAST or FAST/SLOW treatment was swim tested on, and the final column shows the SLOW group that the FAST or FAST/SLOW treatment was compared with. In 2009, limited sample size prevented tests on a medium size group.

Name	Year	Treatment	Size Range	Swam on	Compared to
Initial	2009	FAST	25-33mm	d 14	SLOW 1
Small		FAST/SLOW	28-35mm	d 18, d 22	SLOW 2
Large		FAST/SLOW	44-49mm	d 50	SLOW 4
Initial	2010	FAST	25-27mm	d 15	SLOW 1
Small		FAST/SLOW	28-35mm	d 28	SLOW 2
Medium		FAST/SLOW	36-43mm	d 37	SLOW 3
Large		FAST/SLOW	44-49mm	d 52	SLOW 4

Table 2. The following table lists the number of different swimming trials for the different growth treatments in 2009 and in 2010. Swimming ability in the FAST/SLOW treatment was measured 4, 8, 13, 22, 36, or 37 days after fish were switched to slow growth. Individuals from the SLOW treatment (in bold) were measured when they had reached the same size as the FAST/SLOW treatment. The average critical swimming speed (cm/sec) is listed in the 4th column.

Treatment	Year	N	Avg U crit (mm/s)
FAST	2009	18	52.66
SLOW 1	2009	19	58.74
FAST/SLOW 4 d	2009	19	55.59
SLOW 2	2009	11	58.86
FAST/SLOW 8 d	2009	7	59.90
SLOW 3	2009	3	75.52
FAST/SLOW 36 d	2009	10	106.95
SLOW 4	2009	9	109.81
FAST	2010	10	51.38
SLOW 1	2010	9	62.13
FAST/SLOW 13 d	2010	9	59.93
SLOW 2	2010	9	82.95
FAST/SLOW 22 d	2010	13	70.24
SLOW 3	2010	10	83.02
FAST/SLOW 37 d	2010	8	90.91
SLOW 4	2010	9	98.59

Table 3. Lipids of growth manipulated fish for which swimming performance had been measured in 2009. The number of fish analyzed for each treatment group is listed along with the Ln average lipid mass (mg) and the Ln average size of the specimen.

Treatment	N	Ln Avg Lipid mass (mg)	Ln Avg Size (mm)
FAST	19	1.67	3.36
FAST/SLOW 4,8 d	12	2.62	3.53
FAST/SLOW 36 d	8	3.09	3.84
SLOW 1	15	2.47	3.33
SLOW 2	4	2.93	3.52
SLOW 3	3	4.04	3.87

Table 4. Below are summary statistics for muscle histology sections. The first column displays the growth treatment of fish. The fish in SLOW 1, SLOW 2, SLOW 3, were grouped so the range of fish size overlap with the fish sizes in the FAST and FAST/SLOW treatments. The number of fish sections that were scored are in the second column. The third through the final column represent averages. The average size (mm)is displayed in the third column. The proportion of the total section that is either red (R) or white (W) muscle, the average R or W fiber area (μm^2), the average number of R or W fibers that were measured and the total fiber number for R and W muscle are presented respectively.

Treatment	# Fish	Size (mm)	Prop R	Prop W	R fiber area (μm^2)	W fiber area (μm^2)	# R fiber meas	# W fiber meas	R total fiber #	W total fiber #
Fast	8	26.6	0.07	0.72	8.9	41.8	44	41	562	1229
F/S 15,24 d	15	38.9	0.08	0.77	8.86	44.7	46	55	939	2026
F/S 32 d	11	44.5	0.08	0.79	10.2	61.0	48	53	1199	1998
S 1	9	26.7	0.08	0.73	10.2	33.9	39	52	613	1517
S 2	17	37.3	0.09	0.77	7.8	43.5	46	44	996	1899
S 3	8	44.6	0.08	0.78	10.7	82.2	45	45	1151	1872

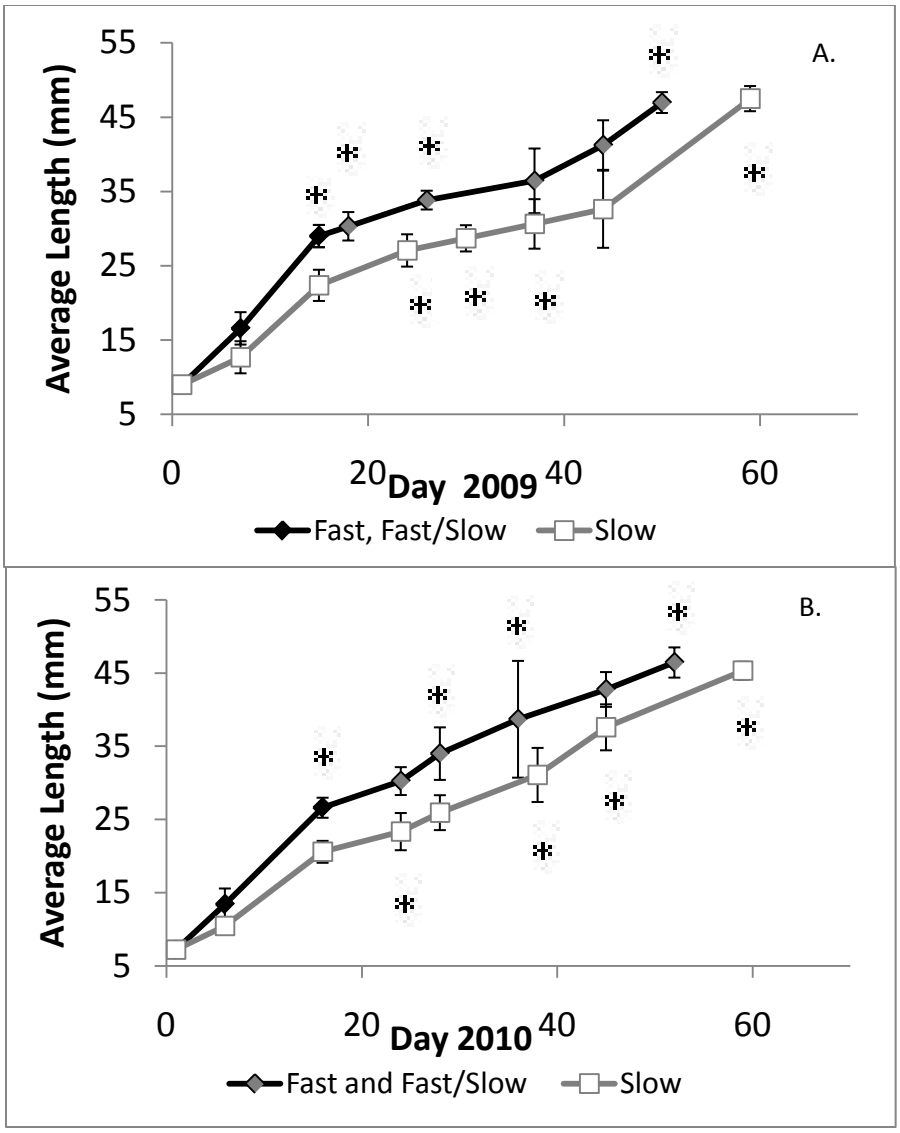

Figure 1 A, B. Panel A shows the grow trajectories for treatments in 2009; data from 2010 is shown in panel B. The FAST/SLOW treatment is shown by the black, for the FAST period, and then grey diamonds, for the SLOW periods, and the SLOW treatment is showed by the white squares. The error bars are ± 1 standard deviation. The asterisk markers indicate trait measurements. Swimming ability was tested in both 2009 and 2010. Lipid content was only tested in 2009 and histology was only measured in 2010. Length data from the final measurement were collected from fish size-matched for swimming trials.

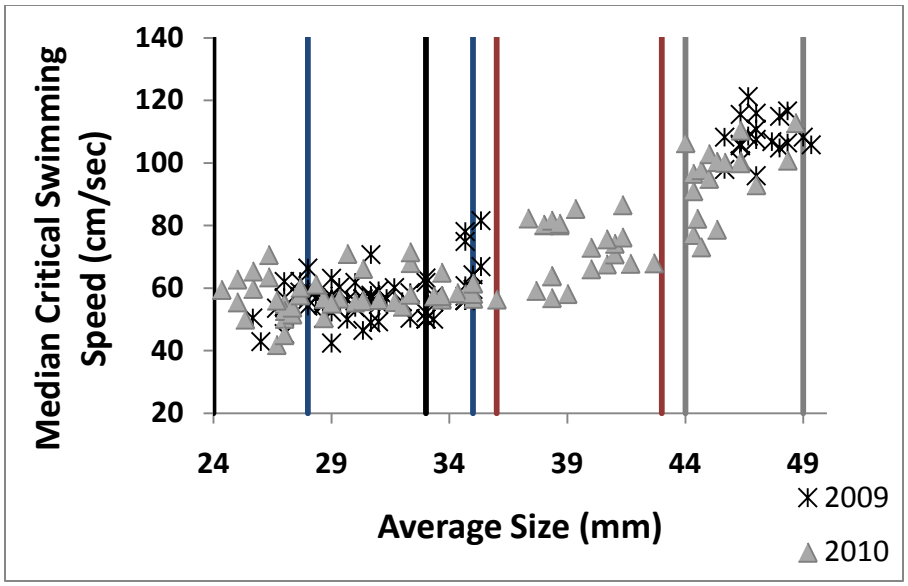

Figure 2. Median critical swimming speed (cm/sec) varied positively with average length (mm) (least squares regression of the mean- $P<0.001$). Data from both 2009 and 2010 are shown. Data from 24mm up to 33mm (black horizontal line) are the initial group, data from 28-35mm are the small group (in-between blue horizontal lines), data from medium size group are from 36-43mm (in-between red lines), and data from the large size group (44-49mm) are in-between the grey lines.

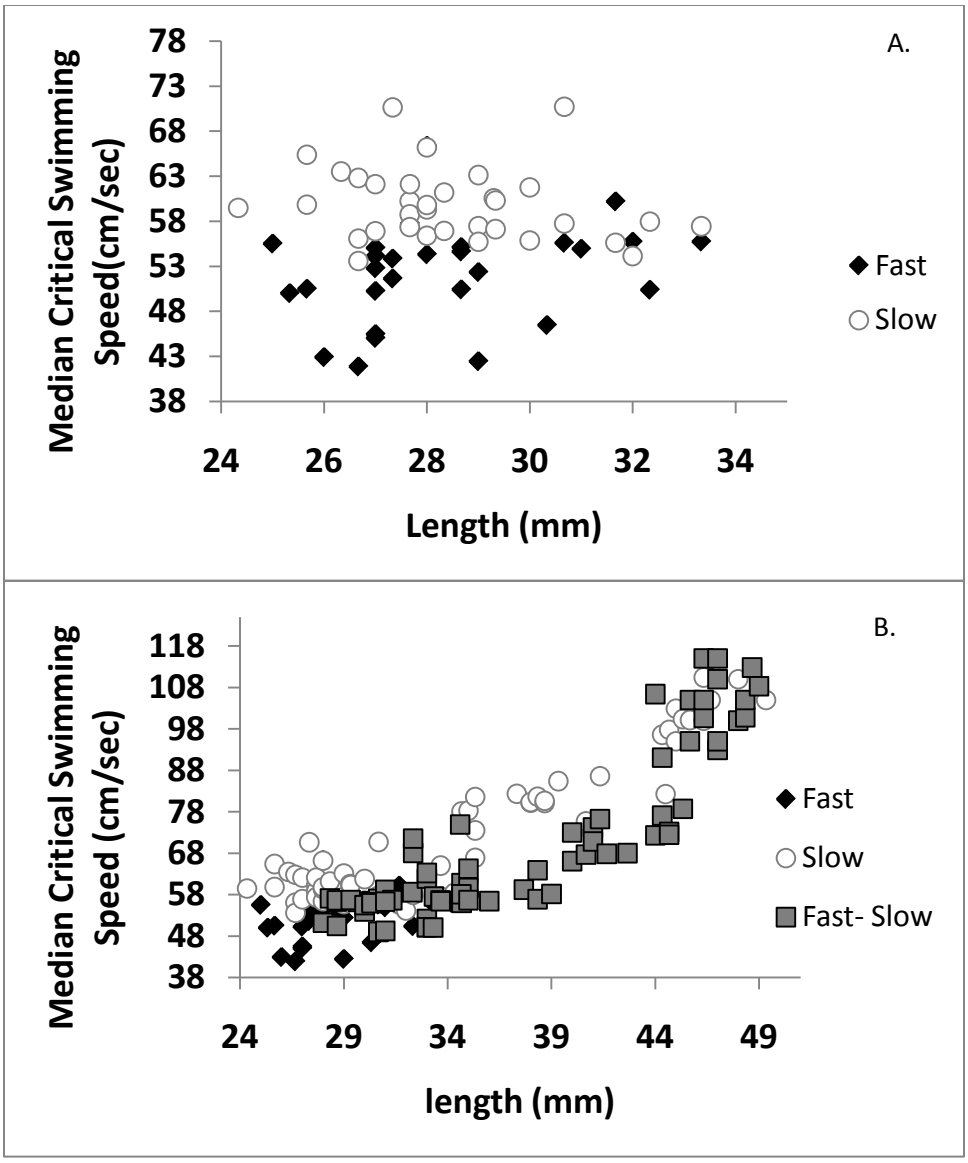

Figure 3. Data from 2009 and 2010 were pooled together. FAST growing fish are labeled with the black diamonds, SLOW growing fish are labeled with white circles, and FAST/SLOW growers are labeled with the grey squares. The swimming ability of the initial growth treatment of FAST and SLOW growth fish are compared in panel A. The SLOW treatment is compared to the FAST/SLOW treatment in panel B over the entire size range tested.

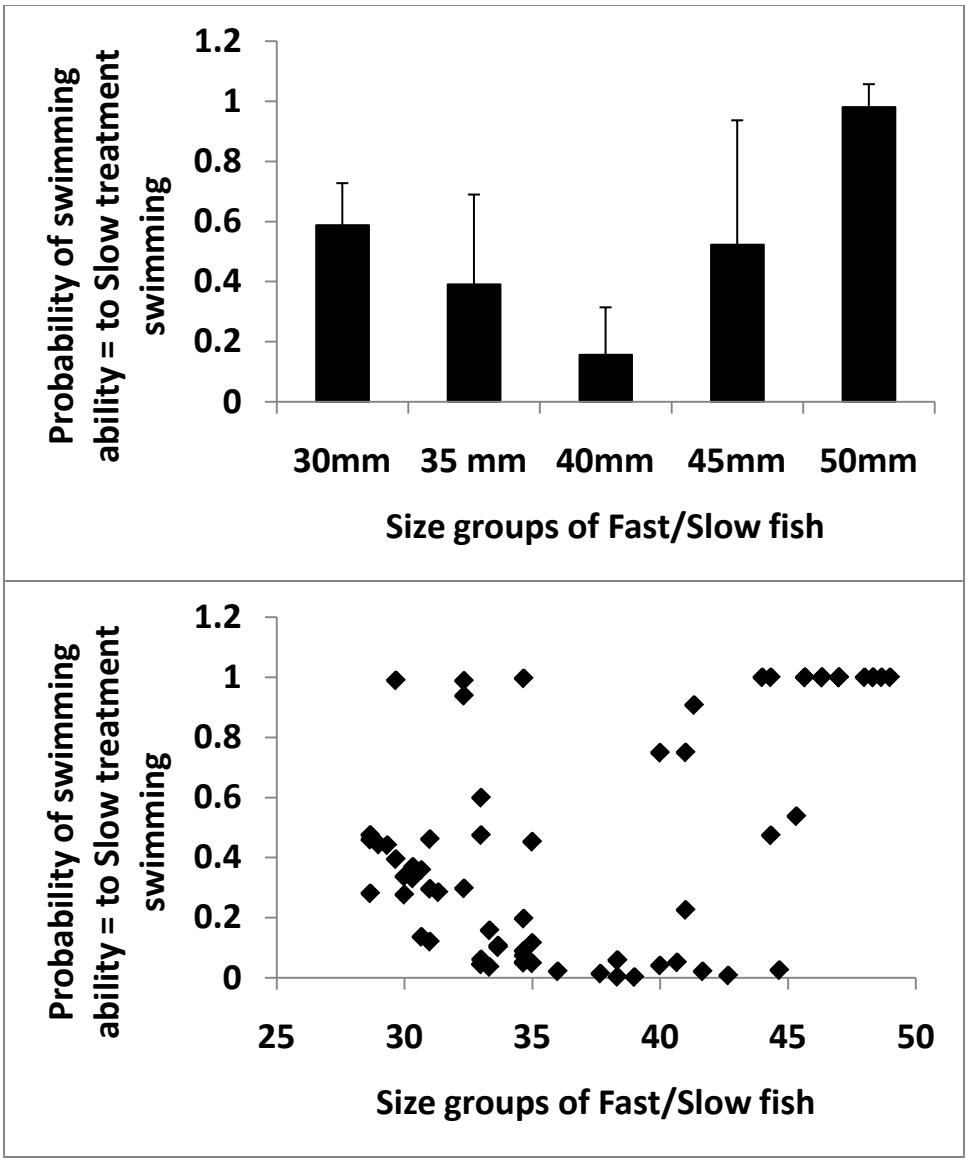

Figure 4A, B .The black bars show the likelihood of swimming ability in the FAST/SLOW treatment being equal to the SLOW treatment. The x-axis shows the 5mm size bins where fish were grouped. The first group shows all FAST/SLOW fish smaller than 30mm, and the next bin show fish from 30-35mm. The following bins show the mean over a range of 5mm. The errors bars are ±1 SD. Panel B shows the probability of being SLOW for each individual trial.

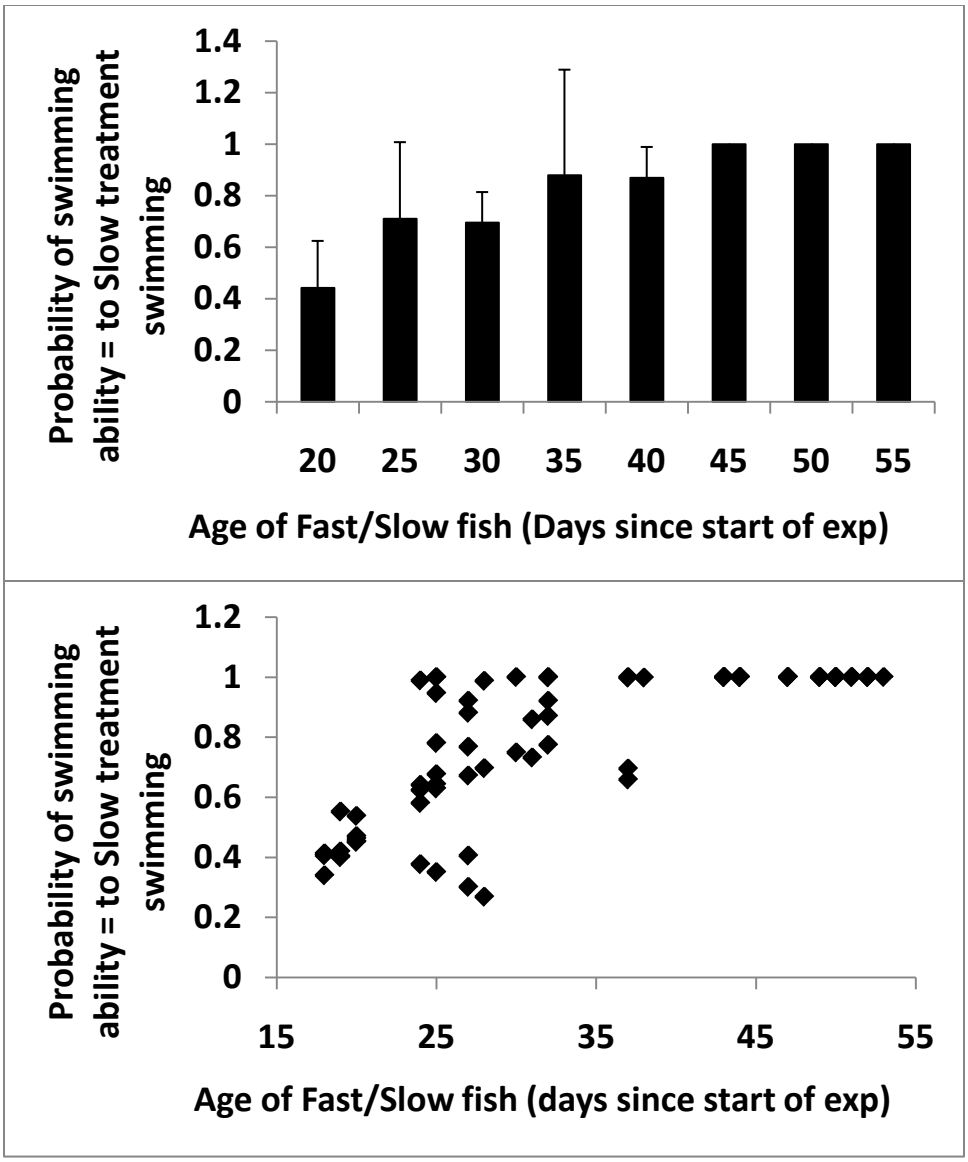

Figure 4 C, D . The black bars show the likelihood of swimming ability in the FAST/SLOW treatment being equal to the SLOW treatment over a range of fish ages on the x-axis. The first group shows all FAST/SLOW fish smaller than 20 days since the start of the experiment, and the next bin show fish from 20-25 days. The following bins show the mean over a range of 5 days. The errors bars are ±1 SD. Panel D shows the probability of being SLOW for each individual trial.

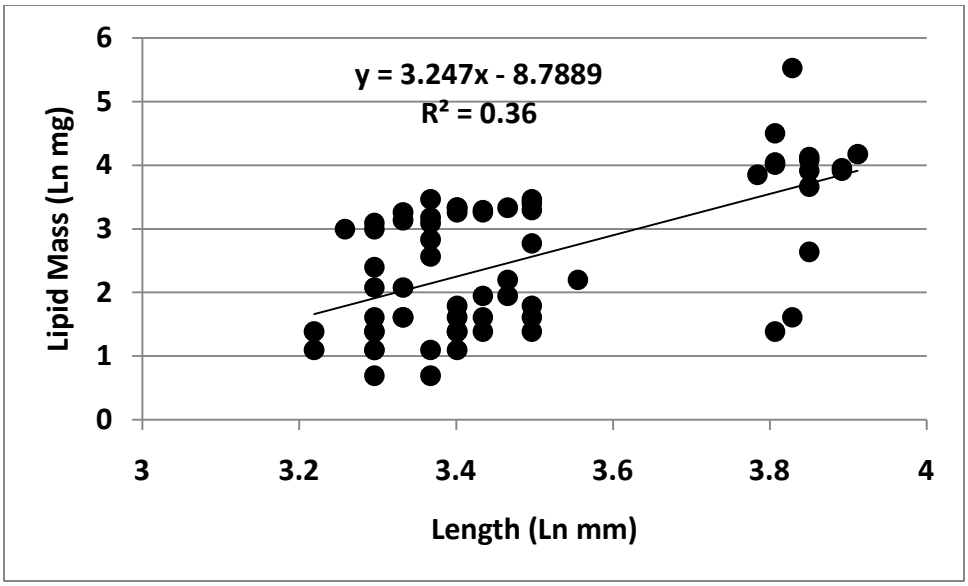

Figure 5. Lipid mass was positively related to fish size (Least squares regression $p<0.001$). Both fish length (mm) and lipid mass (g) were natural logged to report values.

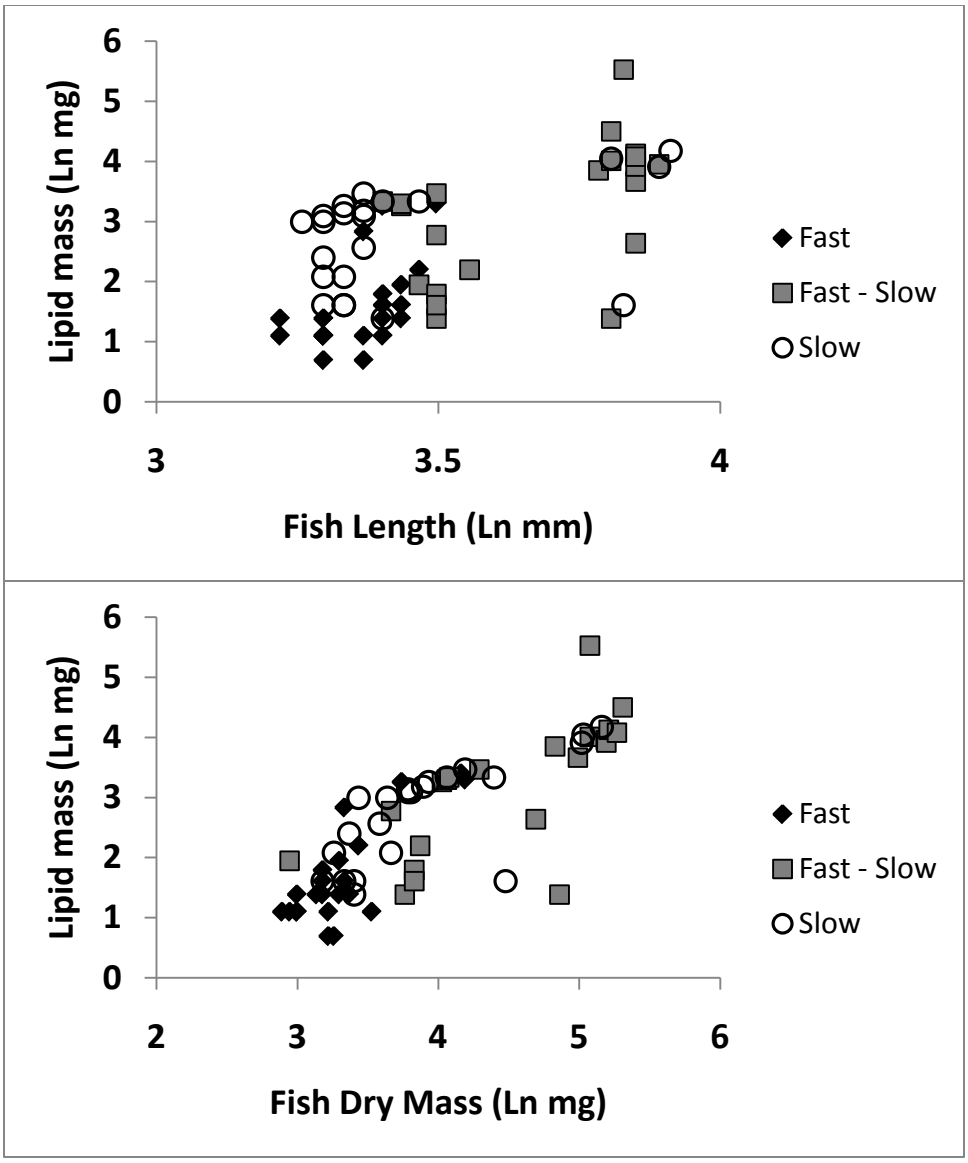

Figure 6. Subplot A. Mass of extracted lipids from growth manipulated fish. The values for both length (mm) and lipid mass (g) were logged. Subplot B. The values for both fish dry mass and lipid mass were logged. The SLOW growing fish are shown by the grey circles, the FAST growers are shown by the black diamonds, and the FAST/SLOW growers are shown by the white squares.

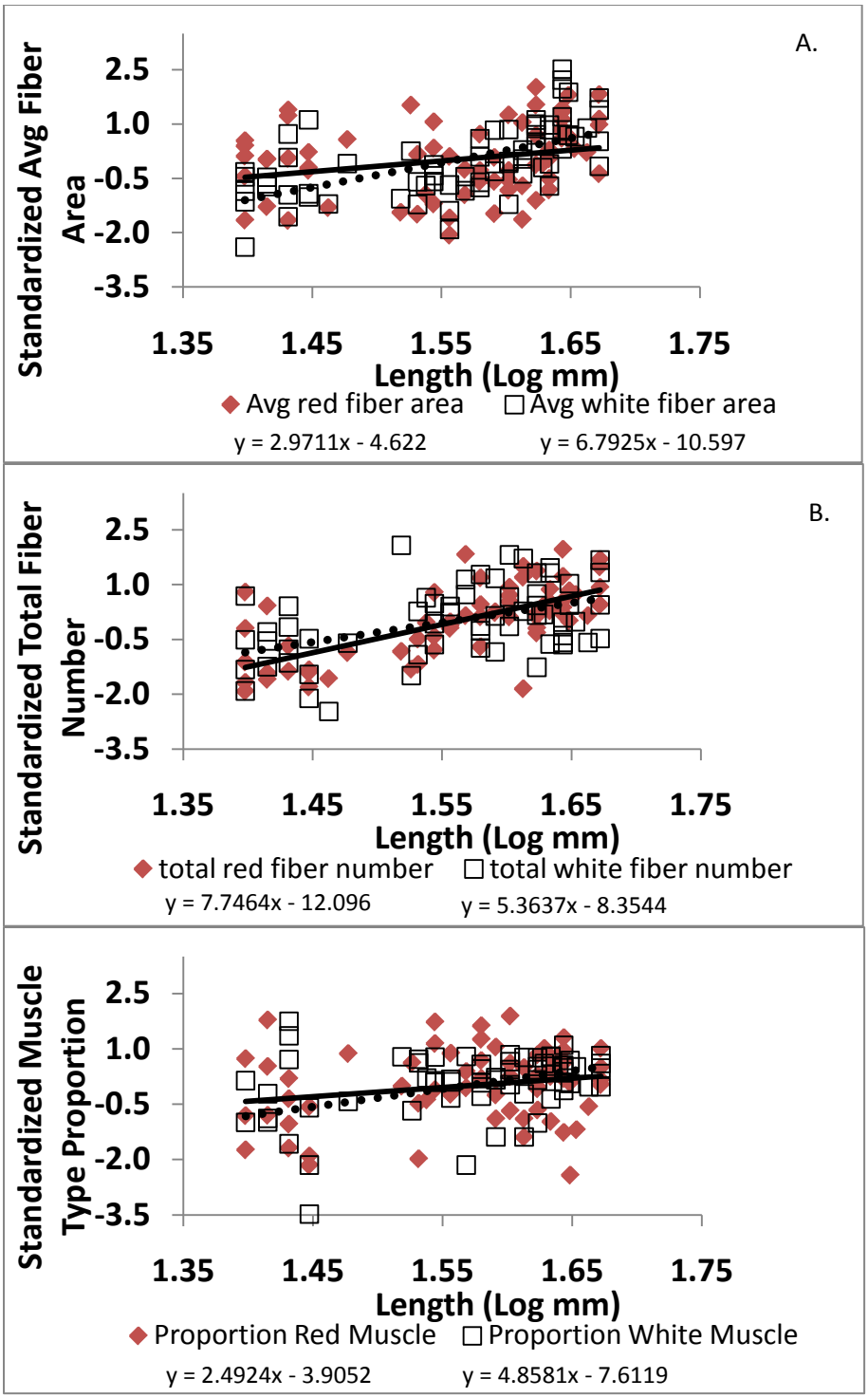

Figure 7.The standardized fiber area, total fiber number, and proportion muscle type of both red and white muscle is plotted over a range of sizes. Red fiber data is shown by the red diamonds and white fiber data is shown by the white squares. The linear regression equations (Standardized fiber area or number=m*length+b) for both fiber types are listed below the legend. The white muscle regression line is shown by the dotted line whereas the red muscle regression line is shown by the solid line.

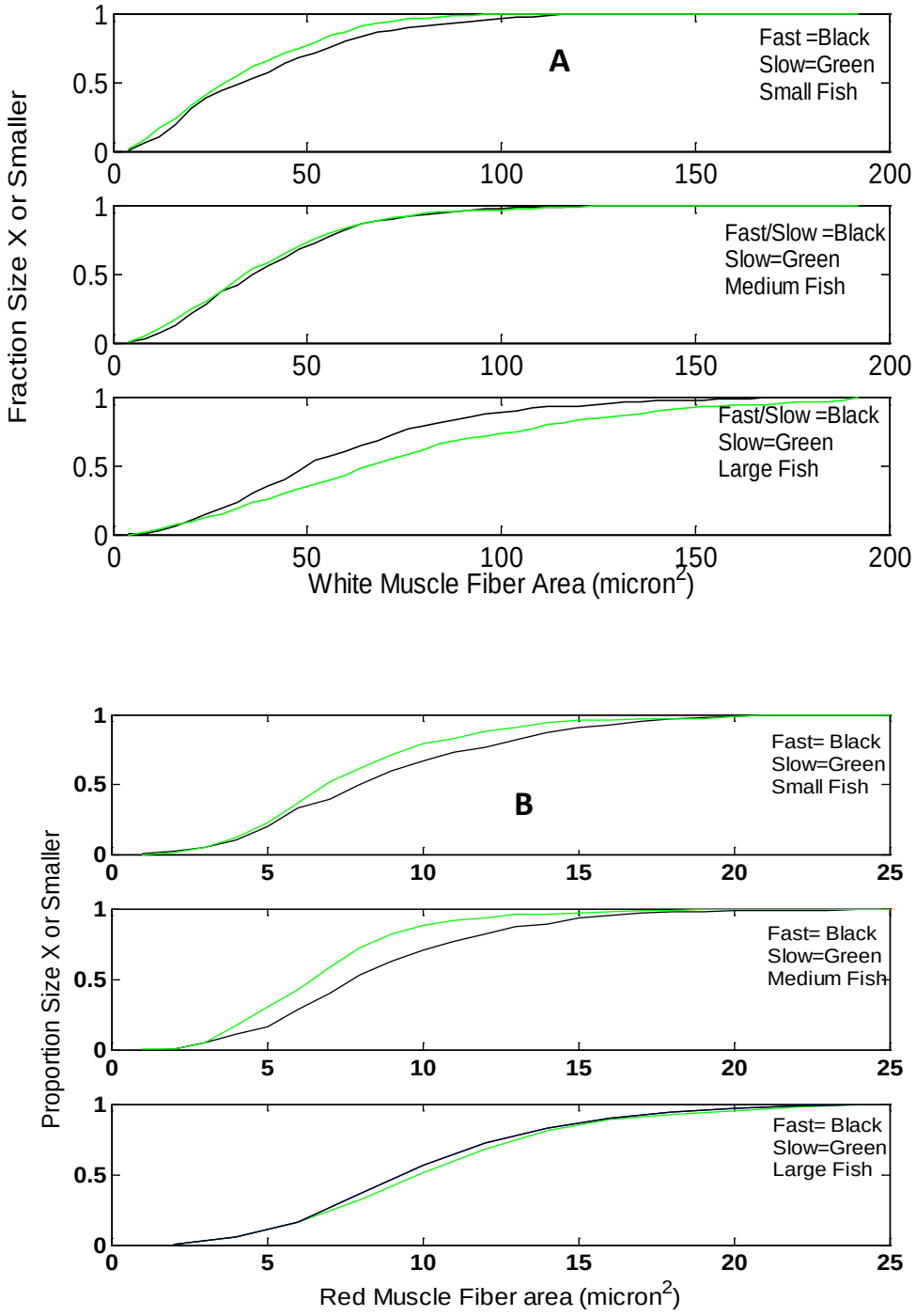

Figure 8. Plotted in Panel A are the frequency distributions of white muscle fiber area for small, medium, and large fish. The FAST growth treatment and then FAST to SLOW growth treatment are shown by the black line and the SLOW growth treatments are plotted with the green line. For large fish, the fibers greater than 190 μm^2 were summed and placed in the 190 bin. Panel B shows the cumulative distributions for red fiber area for small, medium, and large fish.

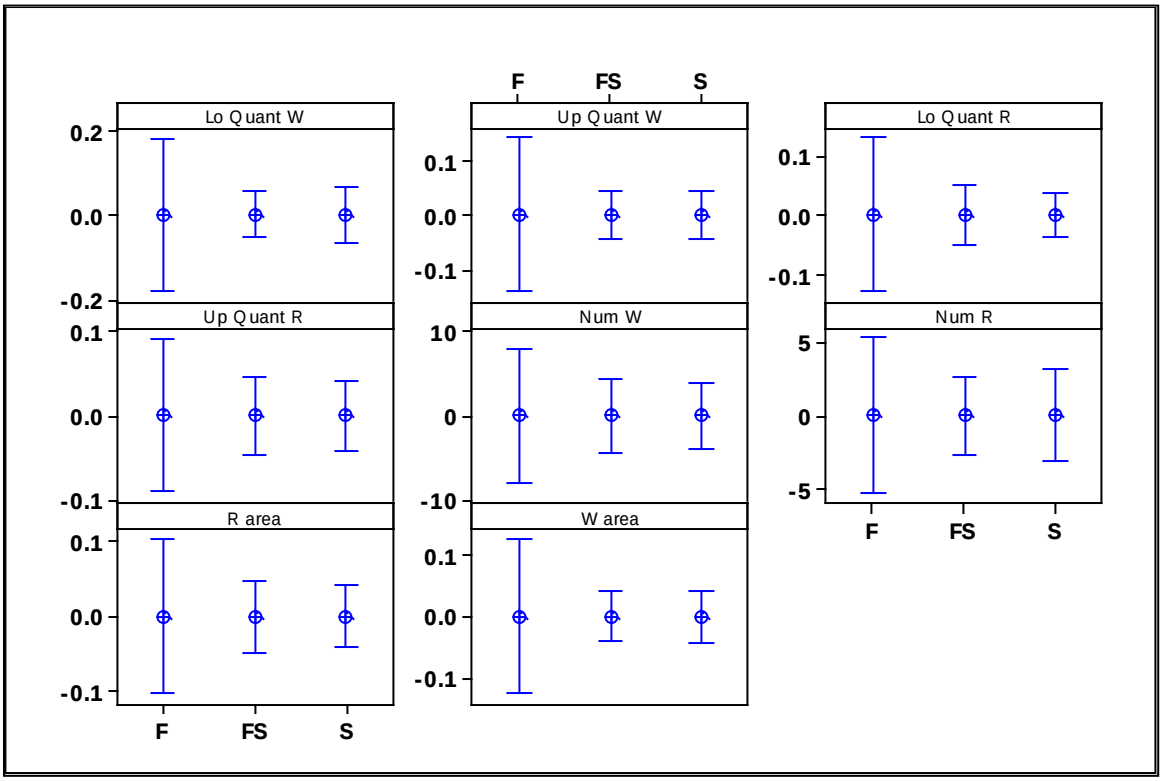

Figure 9. Plotted are the 8 predictors used to separate individuals from different growth treatments into groups. For each predictor I calculated the residual value from a regression of the trait on size. The error bars are 95% confidence intervals. The traits are listed from top left to bottom right as follows: Lower quantile white muscle, upper quantile white muscle, lower quantile red muscle, upper quantile red muscle, total number white fibers, total number red fibers, average red fiber area, average white fiber area.

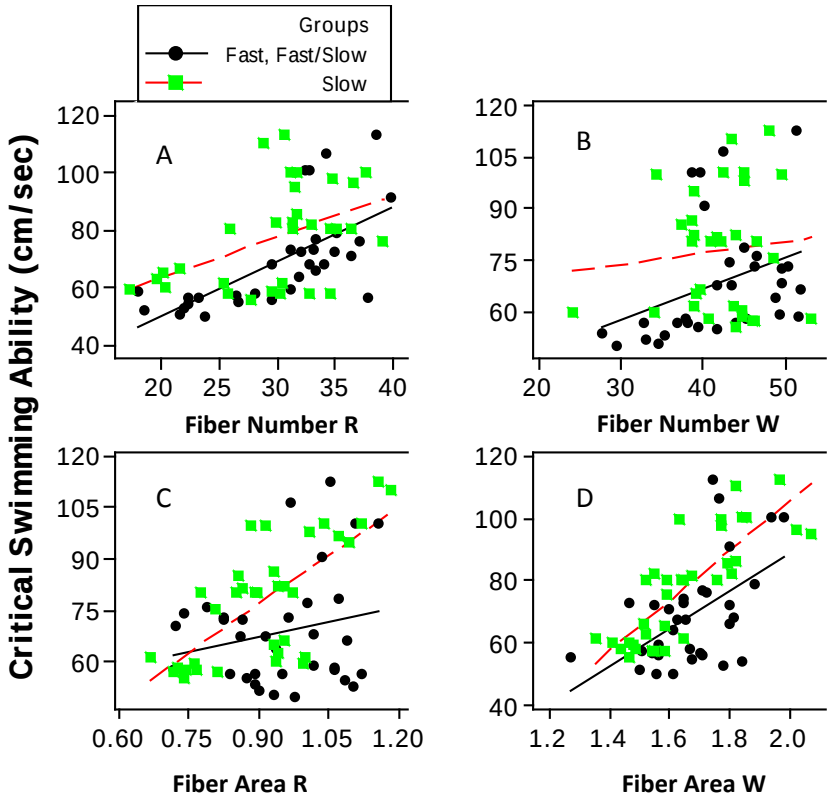

Figure 10. Subplot A shows the relationship between critical swimming ability and total fiber number red, subplot B shows relationship between critical swimming ability and total fiber number white, subplot C shows the relationship between critical swimming ability and red muscle fiber area, and subplot D shows the relationship between critical swimming ability and white muscle fiber area. For all subplots, the SLOW growth treatment is shown by the green markers and the FAST or FAST/SLOW growth treatment is shown by the black markers.

Appendix 1. Table A. The following appendix displays summary data for the FAST/SLOW growth treatment. The top half of the table are data from 2009 and the bottom half are data from 2010. The first column displays the number of days since the start of the experiment. The date that subsamples of fish were measured, as well as their average ration, length and standard deviation are in the next 4 columns. The growth treatment and total number of fish that were measured to obtain the growth rates are listed in the next 2 columns. Table B. These columns are then repeated with data for the SLOW treatment.

A.	**FAST/SLOW**						
Year	# D since start	date	Ration level for 30 fish (g)	Length (mm)	Stdev	Growth	N
2009	1	7/6/2009	Unlimited	9.0	0.8	F	22
	7	7/13/2009	Unlimited	16.6	2.2	F	155
	15	7/20/2009	Unlimited	29.0	1.5	F	450
	18	7/23/2009	2.24	30.3	1.9	FS	44
	26	7/31/2009	2.74	33.8	1.3	FS	38
	37	8/11/2009	3.11	36.4	4.4	FS	16
	44	8/18/2009	3.81	41.3	3.3	FS	16
	50	8/24/2009	4.62	47.0	1.4	FS	33
2010	1	5/23/2010	Unlimited	7.2	0.8	F	10
	6	5/28/2010	Unlimited	13.4	2.2	F	22
	16	6/7/2010	Unlimited	26.6	1.4	F	270
	24	6/15/2010	2.24	30.3	1.9	FS	40
	28	6/19/2010	2.77	34.0	3.6	FS	17
	36	6/27/2010	3.44	38.7	8.0	FS	26
	45	7/6/2010	4.02	42.8	2.4	FS	38
	52	7/13/2010	4.53	46.4	2.1	FS	18
B.	**SLOW**						
Year	**# D since start**	**Date**	**Ration level for 30 fish (g)**	**Length (mm)**	**Stdev**	**Growth**	**N**
2009	1	7/6/2009	0.1	9.0	0.8	S	21
	7	7/13/2009	0.22	12.7	2.2	S	65
	15	7/20/2009	1.03	22.4	2.1	S	270
	24	7/29/2009	1.79	27.1	2.2	S	37
	30	8/4/2009	2.01	28.7	1.8	S	43
	37	8/11/2009	2.28	30.6	3.3	S	22
	44	8/18/2009	2.57	32.6	5.2	S	26

	59	9/2/2009	4.69	47.5	1.7	S	24
	1	5/23/2010	0.06	7.2	0.8	S	10
	6	5/28/2010	0.12	10.5	1.2	S	28
2010	16	6/7/2010	0.75	20.6	1.5	S	330
	24	6/15/2010	1.26	23.4	2.5	S	20
	28	6/19/2010	1.62	25.9	2.4	S	32
	38	6/29/2010	2.36	31.1	3.7	S	34
	45	7/6/2010	3.28	37.6	3.1	S	32
	59	7/20/2010	4.38	45.3	1.2	S	28

Summary:

The Strength of Selection for Size in Fishes

Major Findings

The first issue I addressed in this dissertation was evaluating the strength of natural selection for size in the early life history (Chapter 1). Selection in the early lives of fishes overwhelmingly favored larger size at age, where 77% of the standardized selection differentials were positive. The mean standardized selection differential in fish, was more than 5 times greater than the mean in terrestrial taxa (1.12 SD in fish, 0.2 SD in terrestrial taxa, Kingsolver and Pfennig 2004). Although I found remarkably high selection pressures in fish early life, the remainder of fish life history must be evaluated before making predictions of size evolution. Evaluating how selection varies within or across generations of fish has only recently been addressed (see Gagliano et al. 2007, Meekan et al. 2007, Siepielski et al. 2009) as most studies measuring size selection focus on the early life history. The next major research project that I undertook, was measuring variation in selection intensity for size within and across generations of Alantic silversides (*Menidia menidia)* (Chapter 3). A common technique of measuring traits of wild survivors, otolith back-calculation, is highly accurate in Atlantic silversides with a bias of 4.2%, indicating that differences in populations smaller than this bias cannot be attributed to natural selection (Chapter 2). Selection evaluated over three months of the growing season in the Atlantic silverside fluctuated considerably between favoring larger and smaller size. Size selection completely switched direction approximately every two weeks from being significantly less than zero to being significantly greater than zero. However, the resulting overall average

selection is much weaker when looking over a longer period of the life history (overall in 2006=0.1, overall in 2007=-0.3).

Implications

Obtaining field estimates of the overall intensity of natural selection has important implications for predicting the evolution of size. An area of tremendous interest is determining whether fish size can recover following cessation of fishing (Conover et al. 2009). Edeline et al. (2007) measured selection differentials in Windermere pike and found that natural selection for size acted in opposition to harvest selection for size. Thus, if both harvest and natural selection have a similar intensity, they can cancel each other out. Larger changes in traits, including body size, were found in populations exposed to artificial selection, than in populations solely exposed to natural pressure, indicating the former is more intense (Darimont et al. 2009). Similarly, I found that the overall strength of natural selection averaged over the growing season is fairly weak in Atlantic silversides. Assuming this finding is common for many fish species, it seems unlikely that natural selection will be able to completely counteract fishery selection. This suggests that the best approach for management is to be precautionary, where limits are set with the goal of preventing non-reversible damage in the long-term to fish populations (Garcia 1994). Incorporating the likely effects of fishery-induced evolution in precautionary management practice would be ideal, where the intensity of selection is carefully managed to avoid rapid evolution of size.

Prolonged Growth Costs in the Atlantic silverside

Major Findings

I also evaluated prolonged costs of rapid growth in Atlantic silversides (Chapter 4). Metcalfe and Monaghan (2001) separated different growth costs into groups based on the time scale in which they occur. These groups were: immediate, short-term, medium-term, and long-term (end of life) (Metcalfe and Monaghan 2001). Despite much evidence for growth costs on immediate and end of life time scales, prolonged growth costs, or a cost that continues to manifest somewhere in-between these two extreme time scales, have not been well studied. After measuring 3 traits (swimming ability, lipid mass, and muscle morphology), I found several revealing results: firstly, poorer critical swimming ability is a prolonged growth cost. Fast-growing fish have an immediate cost of rapid growth as they swim poorer than their slow-growing counterparts. Decreased swimming ability continued to manifest for weeks after the fish were switched from fast growth to slow growth. Furthermore, Atlantic silversides recovered normal swimming ability 36-37 days after they had been growing slowly. This is the first study to my knowledge that has empirically determined the number of days that is takes for fish to recover from an early life growth cost. I also found differences in the muscle fiber area distribution of fast and slowing growing fish, which may explain some of the differences in swimming ability. Interestingly, although the fast growing fish also had less lipid mass than slow growing individuals, this cost appeared to recover faster than swimming ability.

Implications

The empirical demonstration of a prolonged growth cost and evaluation of swimming ability recovery following payment for the growth cost has improved our understanding of evolution of sub-maximal growth. The presence of sub-maximal growth, a common occurrence where normal growth in fish is less than the maximum that is physiologically possible, is often suggested to be due to growth costs (Arendt 1997). Although having faster growth and thus

reaching a larger size earlier is beneficial for fish survival (Chapter 1), prolonged growth costs can counteract positive selection for size. As both immediate trade-offs and end of life trade-offs are well documented (see chapter 4), and physiological delays are ubiquitous, prolonged growth costs may be equally as common. However, the time to recover may vary with the rate of growth, as well as the time period over which fast growth was experienced.

These results also have interesting implications for the evolution of allocation strategies. As the cost of poorer swimming ability was not re-paid immediately, this demonstrated that individuals make energy allocation decisions. The existing allocation decision theory should take into account physiological delays and prolonged growth costs.

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