DIVERGENCE AND SELECTION IN TROPHICALLY POLYMORPHIC

PUMPKINSEED SUNFISH (*Lepomis gibbosus*)

A Thesis

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ABSTRACT

DIVERGENCE AND SELECTION IN TROPHICALLY POLYMORPHIC

PUMPKINSEED SUNFISH (*Lepomis gibbosus*)

Christopher James Jastrebski   Advisor:
University of Guelph, 2001   Dr. Beren W. Robinson

I found evidence that selection acts on polymorphic pumpkinseed sunfish (*Lepomis gibbosus*) that inhabit littoral and pelagic habitats within single lakes. Strong parallel patterns of morphological divergence in traits related to habitat and resource use were found among 3 eastern Ontario populations, and among 26 populations spanning a wider geographic area, suggesting that selection repeatedly favours habitat-specific forms. To test for selection in one population, I estimated phenotype-related fitness in lake habitats using two measures of fitness that are expected to reflect long-term performance: seasonal reproductive onset and life history performance. Both suggested that more pelagic-like phenotypes had higher fitness than more littoral-like forms regardless of habitat, indicating that selection currently favours planktivorous phenotypes over all others. These findings suggest that the pattern of selection and the phenotypic distribution present in polymorphic populations may be related to the relative abundance of local pelagic and littoral resources, and that trophically related phenotypic polymorphism is itself insufficient evidence to infer disruptive selection is acting in a population.

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General Introduction

Recent research has come to recognize that anomalous morphological variation at many taxonomic levels is often associated with an increase in ecological opportunity that results from under-utilized resources or reduced interspecific competition (Skulason and Smith 1995, Smith and Skulason 1996). This phenomenon is particularly common in fishes, where cases of greater intraspecific morphological variation has been shown to increase with increasing latitude where species diversity is often reduced (Robinson and Schluter 2000).

One important characteristic of this variation in the fishes inhabiting northern postglacial lakes is a repeated divergence into forms either more specialized for benthic (or littoral) substrate feeding, or for planktivory in open water (pelagic) habitats. The presence of two specialized forms rather than a single generalist form has been explained on functional grounds as being the result of fitness tradeoffs that are expected to be present between using littoral and pelagic habitats, in response to different challenges associated with locomotion, feeding, and risk of predation (Robinson and Schluter 2000, Schluter 1995). The parallel repetition of morphological divergence into littoral and pelagic forms across a broad hierarchy of geographic and taxonomic levels suggests that natural selection often favours the adaptation of habitat-specific forms in lake fishes (Schluter 1996a). The evolution of specialized body forms may sometimes also result in the formation of new species, if ecological differences are large enough to allow the evolution of reproductive isolation between two forms (Robinson and Schluter 2000, Schluter 1996b, Smith and Skulason 1996). While speciation via this process may be rare, its implications have sparked a growing interest in how selection operates on

different forms across different lake habitats. The role of selection in the adaptive

divergence of closely related species in the wild is being increasingly studied and

understood (Robinson and Schluter 2000, Schluter 1996b), but the factors that influence

the evolution of reproductive isolation at or near the species level may hide the role

played by selection in originally creating the divergence (Nagel and Schluter 1998,

Rundle and Schluter 1998). Thus, studying the role of selection in divergence below the

species level may help us to better understand how selection creates and maintains

phenotypic variation in the wild.

The repeated occurrence of divergent intraspecific forms that use littoral and

pelagic habitats provides one system in which to study selection. However, the often

subtle phenotypic variation among divergent forms below the species threshold makes it

more difficult to detect selection, because relatively small phenotypic differences make it

difficult to test the relationship between phenotype and fitness within a specific habitat

(Robinson et al. 1996, Schluter 1995).

For this thesis, I investigated morphological variation and the selective forces that

act on pumpkinseed sunfish (*Lepomis gibbosus*) forms that occupy littoral and pelagic

habitats within single lakes. I tested the hypothesis that natural selection currently acts on

phenotypic variation in polymorphic sunfish populations. This was approached in

Chapter 1 by testing whether specialization of body form was consistent with eco-

morphological predictions for littoral and pelagic habitats (Schluter 1996a, Webb 1984).

Further, I tested for parallel patterns of divergence between littoral and pelagic forms by

comparing trends in body form across 26 populations studied by different researchers in

three systems over a large geographic area. Parallel patterns of morphological divergence

between similar littoral or pelagic lake habitats can provide strong evidence that selection

favours specific body forms in these different lake habitats (Schluter 1996b).

My next goal was to attempt to measure the pattern of selection acting on pumpkinseed phenotypes within one of my study lakes. As noted above, the challenge here was to detect variation in the relative fitness of only subtley different phenotypes. Direct tests of fitness, such as habitat specific feeding performance in artificial lab experiments or reciprocal transplant experiments in the field, can demonstrate such fitness variation at or near the species level (Schluter 1994, Schluter 1995). However, they may not be suitable in populations where phenotypic differences are small. Therefore, I focussed on indirect measures of fitness that are expected to integrate habitat-specific performance over longer periods of time, thereby providing a stronger signal of fitness. I used this approach to test the null hypothesis that fitness was unrelated to phenotype in littoral and pelagic lake habitats. By testing for phenotype-related fitness in both littoral and pelagic habitats, the rejection of my null hypothesis also allowed the comparison of the pattern of selection across both habitats, in order to distinguish between stabilizing, directional, and disruptive selection on body form. I implemented this approach using two measures of fitness that integrated performance over two temporal scales. In Chapter 2, I measured the association between body form and seasonal onset of reproduction in the littoral and pelagic habitats. Earlier reproduction during the breeding season was predicted in those phenotypes that could most rapidly recover from overwinter energy deficits (Danylchuk and Fox 1994a, Ridgway et al. 1991). Earlier onset of seasonal reproduction is expected to increase reproductive fitness by providing a longer growing season for offspring, thereby improving the survival of progeny (Cargnelli and Gross 1996, Danylchuk and Fox 1994a). In Chapter 3, I further attempted to measure fitness integrated over an entire lifetime by measuring life history

characteristics associated with fitness (growth rate and mean age at maturity). Higher life history performance is expected to reflect energy gains that result from more efficient habitat and resource use. Phenotypes yielding higher life history performance should be favoured by selection, as they should achieve greater lifetime reproductive output. I compared the life history performance of specialized and intermediate forms in littoral and pelagic habitats to test for a relationship between habitat-specific phenotype and this important component of fitness.

Chapter 1

Parallel Divergence in Habitat Use and Body Form in

Pumpkinseed Sunfish (*Lepomis gibbosus*) at Local and Regional Scales

Introduction

The expression of unusually high phenotypic variation associated with species

poor environments or a relaxation of interspecific competition is being increasingly

recognized in vertebrates (Schluter 1996b, Skulason and Smith 1995, Smith and Skulason

1996). This variation, believed to result from ecological opportunity, is particularly

common in the fishes that inhabit northern postglacial lakes. There, divergence is

commonly found between littoral (or benthic) and open water habitats, and can occur at a

variety of taxonomic levels: from variation among distinct species to variation among

forms of a single species within a single lake (Robinson and Schluter 2000, Robinson and

Wilson 1994, Schluter 1996a). Repeated and similar patterns of phenotypic divergence

among or within species associated with the same pairs of lake habitats among isolated

geographic sites may be a key to identifying the processes involved in the origin and

maintenance of diversity. Current evidence increasingly supports the hypothesis that the

open and shallow water environments of lakes present distinct feeding, survival, and

reproductive challenges to the fish that inhabit them (Schluter 1995). For this reason, we

may expect that natural selection favours the evolution of phenotypes best suited for each

habitat (Robinson and Schluter 2000, Schluter 1996a).

Many examples of parallel divergence across littoral (often similar to benthic) and

open water (pelagic) habitats are recognized within northern freshwater fishes. McPhail

(1984) identified the presence of a distinct but closely-related species pair of threespine

stickleback (*Gasterosteus aculeatus*) on the west coast of Canada. Distinct habitat-

specific forms are also known to coexist in populations of smelt (*Osmerus*

mordax)(Taylor and Bentzen 1993), lake whitefish (*Coregonus clupeaformis*)(Bernatchez et al. 1996), and Arctic charr (*Salvelinus alpinus*)(Malmquist 1992). In certain cases, variation in Arctic charr can rival species level differences, as in the polymorphic Arctic charr of Iceland (Sandlund et al. 1987). In yet other species, variation among forms in a single population is small, as in the case of brook charr (*Salvelinus fontinalis*)(Bourke et al. 1997, Dynes et al. 1999), pumpkinseed sunfish (*Lepomis gibbosus*)(Robinson et al. 1993), and bluegill sunfish (*Lepomis macrochirus*)(Ehlinger and Wilson 1988);(further examples are reviewed in Robinson and Wilson 1994). The distinctive feature in each of these examples is a general trend in body form towards specialized littoral (or benthic) and pelagic forms. Pelagic forms tend to possess more slender, fusiform bodies, smaller mouths and smaller paired fins, as well as more numerous and longer gill rakers, that are comb-like projections on the gill arches thought to play a role in the capture and manipulation of small prey items (Sanderson et al. 1991). Conversely, littoral forms generally possess a deeper, more robust body, larger paired fins, larger mouths, and shorter gill rakers (Robinson and Wilson 1994). This variation in body form conforms to our functional understanding of locomotion and prey search in littoral and pelagic habitats on prey that are respectively less cryptic and more evenly distributed versus more cryptic and patchily distributed (Webb 1984, Wainwright and Richard 1995).

Repeated and parallel patterns of phenotypic divergence across habitats have several important implications: 1) The repeated convergence of similar traits related to resource use in similar habitats (among fish species) suggests that natural selection favours those forms best suited to particular habitats (Schluter 1996b). This is because random processes such as genetic drift and founder effects are not expected to repeatedly yield the same patterns of divergence in different populations and species that occupy

different environments. 2) Parallel patterns of divergence within a single species repeated across wider geographic areas provides similar evidence that selection can favour particular trait combinations in a specific habitat. Molecular genetic analyses have indicated that in some cases such phenotypic divergence has occurred in sympatry (eg. Taylor and Bentzen 1993). 3) The range of taxonomic levels at which we observe parallel divergence presents an ideal system in which we can test the role of selection in the creation and maintenance of phenotypic variation (eg. providing the opportunity to investigate the strength of selection acting on phenotypes in each habitat at different stages of divergence).

The objective of this study was to explore morphological and dietary variation in pumpkinseed sunfish (*Lepomis gibbosus*) in a set of three lakes where individuals appear to use resources in either littoral or pelagic habitats to varying degrees. Sunfish are becoming increasingly well-known and valuable in the study of adaptive divergence, because of a large body of knowledge about their feeding habits (Keast 1978, Werner and Hall 1979), competitive interactions with closely related species (Keast 1977, Werner 1977, Werner and Hall 1976), and morphological adaptations (Lauder 1983, Wainwright 1996, Wainwright and Shaw 1999). Additionally, trophic or resource polymorphisms have been recognized in two species of sunfish (Ehlinger and Wilson 1988, Robinson et al. 1993). In pumpkinseeds, polymorphic populations have been found in the Kawartha Lakes area of eastern Ontario (Gillespie 2000), and in the Adirondack region of upstate New York (Robinson et al. 1993, 2000). In the Adirondack system, we additionally know that both additive genetic variation and phenotypic plasticity play a proximate role in the development of morphological traits related to resource use (Robinson and Wilson 1996), and that body condition factor can be related to phenotype in the pelagic habitat

(Robinson et al. 1996). Gillespie (2000) has also demonstrated that pumpkinseeds in one polymorphic population in the Kawartha area of eastern Ontario demonstrate a moderate level of habitat and site fidelity. Further study of this species may build further insights into the nature and strength of selection involved in phenotypic divergence between lake habitats.

Robinson et al. (2000) suggest that trophic polymorphism in pumpkinseeds is the result of character release, as populations respond to the availability of open water resources. The pumpkinseed is generally considered to be a littoral specialist that feeds on macroinvertebrates and gastropods (Keast 1978, Werner and Hall 1979). Lauder (1983) has shown that pumpkinseeds have a highly specialized neuro-muscular mechanism for crushing snails, which allows them to efficiently out-compete other sunfish species for mollusc resources in the littoral zone. Adult pumpkinseeds in turn are generally thought to be restricted to the littoral zone by competition with bluegill sunfish that are superior competitors for pelagic zooplankton resources (Keast 1977, Werner and Hall 1976). The post-glacial ranges of the two species do not completely overlap, however (Mandrak and Crossman 1992, Scott and Crossman 1973), providing portions of the pumpkinseed range where bluegill are absent. This presents an ecological opportunity to pumpkinseeds in the form of open water resources, provided that other zooplanktivorous taxa are not present or abundant. Morphological divergence between forms of trophically polymorphic pumpkinseeds appears to parallel that observed in many fish species between littoral and pelagic habitats. Pumpkinseeds using pelagic resources tend to have longer or more closely spaced gill rakers, a more fusiform or rear-weighted phenotype, and a reduction in the development of the pharyngeal jaws used in crushing snails. The littoral form more closely resembles the ancestral form that co-exists

with bluegill sunfish, and possesses an enlarged head region, well developed pharyngeal jaws, as well as shorter and more widely spaced gill rakers (Gillespie 2000, Robinson et al. 1993, 2000).

I quantified morphological variation of pumpkinseed forms in relation to habitat use in three eastern Ontario lakes to test for parallel patterns of morphological divergence. A replicated pattern of morphological divergence across littoral and pelagic habitats among the three lakes can be interpreted as evidence that natural selection favours the specialization of body forms. Morphological divergence is expected to be related to differences in feeding and habitat use in the following predicted ways: 1) The phenotypes of pumpkinseeds collected from littoral and open water habitats will differ in traits known to be important in habitat and resource use. 2) Trends in habitat-specific morphology will be similar across all three populations. 3) Divergence in morphology between habitats will be related to differences in feeding habits.

I further tested for parallel patterns of divergence in traits related to resource use among a larger set of populations from three geographically separated systems, as additional test that natural selection favours the specialization of body forms. I predicted that selection in similar habitats should favour similar divergences in traits related to habitat and resource use. This analysis used the morphological data collected from the three lakes in eastern Ontario, and compared them to published accounts of pumpkinseed body form in a nearby polymorphic system in eastern Ontario (Gillespie 2000), and to a geographically distinct pumpkinseed system in the Adirondack region of upstate New York (Robinson et al. 2000).

Methods

Study Area and Sample Collection

       Pumpkinseeds were collected from three lakes where they were observed in both

the littoral zone (that portion of the lake between the land and the open water) and in

close proximity to the pelagic zone (the free open water region of the lake)(Wetzel 1983).

All three lakes possess shoals (submerged or slightly emerged outcroppings of the bottom

substrate, usually composed of rock or rubble) that can be used as a refuge by

pumpkinseeds in or near pelagic areas. The lakes are located roughly within the Mazinaw

area of eastern Ontario, north and southeast of Bancroft, Ontario (Figure 1.1). The

physical characteristics, sampling information, and species composition for each lake are

provided in Table 1.1. Ashby Lake has a relatively large pelagic zone that is interrupted

by islands and rocky shoals. The littoral zone in the lake is small, as many rocky shores

drop off quickly into deeper waters, and shallow areas do not appear to support a high

abundance of macrophytes. Mayo Lake consists of twin basins that include numerous

islands and shoals rising from deep water areas. The littoral zone is also underdeveloped

(appears to have a relatively low abundance of macrophyte beds), and is restricted to

several shallow bays. Salmon Trout Lake is smaller than the above lakes, and has an

elongated shape with numerous points extending into deeper water in the form of shoals.

Littoral habitat is more common in this lake compared to the others, but is largely limited

to bays at each end of the lake.

       Littoral and pelagic habitat use by pumpkinseeds was based on extensive

snorkeling surveys in each lake at varying times of day during the spring and summers of

1999 and 2000. Samples of fish from each lake were collected by trapping with wire

funnel traps (35cm diameter x 90cm long; 8cm diameter aperture; constructed of 1.17 x 2.54cm wire mesh), and by angling. Collected fish were euthanized using clove oil, preserved in 10% formalin, then rinsed in water before being stored in 70% ethanol until analysis. In all populations, the site (habitat) where pumpkinseeds were captured was assumed to represent their long-term feeding habitat (henceforward referred to as native habitat, or habitat of origin). This assumption was based on evidence that pumpkinseeds in one nearby polymorphic population demonstrated moderate levels of habitat and site fidelity (Gillespie 2000).

Morphometric Analysis

Variation in body form was assessed using a morphological index that incorporated both external body shape and gill raker length. External morphometric analysis relied on a landmark-based geometric technique involving thin-plate splines to quantify variation in body form (Bookstein 1991). This analysis was based on digital images of the left side of each fish taken with fins extended, and calibrated against a known size standard. Each specimen was then digitized to 15 homologous coordinate landmarks using the TPSdig program to capture the x,y coordinates of each landmark (Rohlf 2001a)(Figure 1.2). I used the TPSRW program to estimate partial warp values (referred to as thin-plate splines) that represent changes in multivariate shape space in comparison to an average form estimated for the entire sample (both uniform and non-uniform components of shape variation were included)(Rohlf 2001b). Gill raker length was measured on the first left hand gill arch, which was excised from each fish and stained with alizarin red (Bell 1984). The average length of the second gill raker down from the apical raker (on the part of the arch connecting to the bottom of the oral

chamber) was calculated by measuring the length from the tip to both the upper and lower insertion points using a microscope equipped with an ocular micrometer at 4x magnification. Average values were then natural logarithm transformed, and regressed against centroid size to estimate residual size-free values.

Analysis of Morphological and Diet Variation

To test predictions 1 and 2, differences in body shape and gill raker morphology between littoral and pelagic habitat samples were quantified using multivariate discriminant function analysis (DFA). DFAs were first used on each population to test for evidence of significant morphological divergence between sunfish sampled from littoral and pelagic habitats. Separate DFAs of each population were expected to yield somewhat different linear combinations of traits because populations vary due to differences in local conditions, and because sample size varied among the three lakes. In order to address the similarity of divergence between littoral and pelagic samples among the three populations, the morphological data of all individuals were combined into a single DFA used to distinguish littoral and pelagic samples. The morphological scores of all individuals from each lake were then extracted, so that the pattern of morphological variation in each lake could be visualized by regressing external morphological form against DFA score using the TPS regression program by Rohlf (2000). Mean size-corrected gill raker length and standard length were also compared between habitat samples in each lake using t-tests. Analysis of Covariance (ANCOVA) was used to test the overall relationship between body shape (DFA score) and habitat, using centroid body size as a covariate.

Sunfish collected during 1999 and 2000 from Ashby Lake were pooled in the

analyses above because morphological variation in each habitat was not significantly different between years (Welch's modified t-test of body form (DFA scores): littoral: t=-1.10, ν=122.5, p=0.27; pelagic: t=0.49, ν=97.6, p=0.63). Tests for sexual dimorphism performed on the Ashby samples from 2000 found that females were significantly more littoral-like in body form in both habitat samples (t-test of body form (DFA scores): littoral: t=-2.63, ν=175, p=0.01; pelagic: t=-2.81, ν=142, p=0.01). The effect of sex on body form was not incorporated into further analyses because ineffective preservation prevented sex identification in all 1999 samples from the three lakes (gonadal tissue had deteriorated). Furthermore, the sex ratio was similar in both habitats, and so the effect of sexual dimorphism was not expected to bias variation in body form between habitats (sex ratio of Ashby Lake 2000 samples; littoral: 41% female, $n_{males+females}$=177; pelagic: 40% female, $n_{males+females}$=144).

To test the third prediction, that habitat specific morphological divergence should be related to diet, stomach contents were analyzed from a random sub-sample of littoral (n=21) and pelagic (n=18) fish from the Ashby 2000 sample. The ineffective preservation of all 1999 samples resulted in the almost complete digestion of stomach contents, preventing analysis in all three population samples. For every stomach sub-sampled from Ashby 2000, each prey item was identified to Order, then grouped into the following six categories: benthic prey (larval Trichoptera, Plecoptera, Ephemeroptera, Odonata, Chironomidae; Amphipoda, Decapoda), molluscs (Gastropoda, Bivalvia), cladocerans (*Daphnia, Bosmina*), Hydracarina (occurs in the water column in both habitats), non-aquatic invertebrates (adult insects and adult Odonata), and other (vegetation, seeds). The proportion of each prey category (number of prey divided by the total number of prey in

that stomach) was arcsin square-root transformed according to Zar (1999), and the transformed mean proportion of each category for all samples was compared between littoral and pelagic samples using Welch's t-tests. Individual p-values for each diet category were corrected using the sequential Bonferroni technique, since the prey abundances in each category were not independent (Rice 1989).

A Test for Parallel Patterns of Divergence Between Study Systems

I compared the variation in 17 traits (linear truss measurements between pairs of landmarks) between littoral and pelagic samples among 26 trophically polymorphic populations of pumpkinseeds from three separate study areas, in order to test the hypothesis that selection in littoral and pelagic habitats favours the parallel divergence of traits related to resource use over a wider geographic area. In the Mazinaw study area I used measurements from pumpkinseeds in Ashby and Mayo Lakes, as these were the only populations where the separate DFA results indicated significant body form differences between pelagic and littoral samples. These two 'Mazinaw' populations were compared with two populations in the Kawartha area of eastern Ontario (Looncall and Shadow Lakes) where Gillespie (2000) recently found significant morphological divergence between littoral and pelagic samples, and with the results of a meta-analysis of 22 populations in the Adirondack region of New York performed by Robinson et al. (2000). The 17 truss measurements were selected to facilitate the direct comparison of the different studies. In the Mayo and Ashby populations, trusses were estimated from measurements based on the x,y co-ordinate landmarks used in the geo-morphometric analysis described earlier. Comparisons were then made between littoral and pelagic samples using analysis of covariance (ANCOVA) for each body measurement, with

multivariate centroid size as a covariate to account for variation in body size (both measurements were natural logarithm transformed). Significance values of these individual ANCOVA's were adjusted using the sequential Bonferroni technique (Rice 1989), because the 17 measurements were measured on each individual, and thus were not independent. If the homogeneity of slopes assumption was met in each ANCOVA, the adjusted least-squares mean values for each habitat were compared to determine whether the littoral or pelagic group was larger for that trait. Similar data were extracted from the results of Gillespie (2000), who used the same ANCOVA approach. Comparable least-squares mean values were also extracted for the same set of traits measured in Robinson et al. (2000), although the effect of habitat on each trait was analyzed simultaneously in 22 populations using univariate analysis of variance (ANOVA) on size-free residuals of each trait (calculated from individual regressions against centroid size)(Robinson, unpublished data). The least-squares means of littoral and pelagic samples in that analysis qualitatively reflected the differences between habitats for each truss as in the ANCOVA analyses above. The trends in size differences between littoral and pelagic samples for each trait were then compared among study groups, to qualitatively determine if littoral or pelagic forms were consistently larger for each truss (trait). These data were tabulated, and the percent correspondence among studies was calculated by determining the number of cases out of five that gave the same qualitative result. For example, if for a given trait, pelagic forms were always larger than littoral forms, then the percent correspondence was 100% (note that the lowest value of percent correspondence possible is 50%).

Results

Morphological Variation in the Mazinaw Study Lakes

Separate DFA analyses of external body form and gill raker length indicated significant differences in morphology between littoral and pelagic samples in Ashby and Mayo Lakes (Ashby Lake: Wilk's λ=0.439, $F_{26,416}$=20.4, p<0.0001, 86% of 242 littoral and 89% of 201 pelagic correctly classified; Mayo Lake: Wilk's λ=0.418, $F_{26,69}$=3.70, p<0.0001, 88% of 41 littoral and 87% of 55 pelagic correctly classified). There appeared to be less morphological divergence between habitats in Salmon Trout pumpkinseeds, where DFA analysis correctly classified 100% of 17 littoral and 12 pelagic individuals (Salmon Trout Lake: Wilk's λ=0.007, $F_{26,2}$=11.7, p=0.08). Statistical results in Salmon Trout Lake were likely in part due to the low sample sizes from this population.

The combined DFA analysis of all three Mazinaw populations indicated significant overall divergence between habitats, correctly classifying 76% of 300 littoral and 79% of 268 pelagic origin fish (Wilk's λ=0.580, $F_{26,541}$=15.1, p<0.0001). The distribution of DFA scores from this analysis indicated similar trends in morphological divergence between littoral and pelagic forms in all three lakes (Figure 1.3). This trend was also evident in the visualization of the average forms from each habitat among lakes, despite large differences in the relative degree of variation among populations (Figure 1.4). Littoral pumpkinseeds tended to have body forms with enlarged head regions and eyes, and reduced depths of mid-body and tail regions. This resulted in an overall trend towards a forward-weighted phenotype (Figure 1.4). Pelagic pumpkinseeds tended to have a more rear-weighted phenotype, characterized by a smaller head region, and a

deeper mid- and rear-body (particularly in the region of the anterior caudal peduncle)(Figure 1.4). Pelagic pumpkinseeds from Ashby Lake had significantly longer gill raker lengths than littorals, while there was no such difference in Salmon Trout Lake and a weakly significant but opposite trend in Mayo Lake (t-test on size free residual gill raker length: Ashby: t=-10.6, v=441, p<0.0001; Mayo: t=1.89, v=94, p=0.06; Salmon Trout: t=1.46, v=27, p=0.16).

Based on Figures 1.3 and 1.4, it is apparent that Ashby Lake pumpkinseeds have the greatest degree of divergence between habitats. Although variation in Mayo Lake pumpkinseeds was significant (as indicated also in Figure 1.3), the specific morphological trends between habitats appeared to be very small, at least in comparison to Ashby or Salmon Trout Lake pumpkinseeds (Figure 1.4). This may represent high within-habitat morphological variability that resulted in a poor relationship between body form and habitat. While Salmon Trout Lake pumpkinseeds appeared to have a large amount of habitat related variation (Figure 1.4), the overall range of variation in each habitat was also large and sample sizes were small, creating an overlap in DFA scores between groups (Figure 1.3).

Pelagic pumpkinseeds had significantly larger mean body sizes than littorals in Ashby and Mayo Lakes, but no significant difference was detected in Salmon Trout Lake despite a parallel size trend (ANOVA on standard length: Ashby: $F_{1,441}$=11.8, p=0.0006, R^2=0.03; Mayo: $F_{1,94}$=11.2, p=0.001, R^2=0.11; Salmon Trout: $F_{1,27}$=3.17, p=0.086, R^2=0.11). While these results indicated significant differences in body size, actual differences appeared small (Table1.1). Statistical significance appears related to the large sample sizes, as indicated by the low R^2 values for each test. Additionally, the

relationship between standard length and body form was not equal between habitats, as indicated by a significant interaction between standard length and habitat in the ANCOVA of morphological score (ANCOVA (model R^2=0.10): Standard length R^2=0.06; Habitat R^2=0.05; Habitat by Standard length interaction R^2=0.01)(Table 1.2). While this suggests that the relationship between body form and size varies between habitats, the very low R^2 value suggests that these differences are not all that biologically significant and instead result from large sample sizes (eg. the interaction effect only explains 1% of the total morphological variation)(Figure 1.5). Using multivariate centroid size rather than standard length in the above analysis gave similar results, indicating that standard length does not reflect differences in body form related to habitat.

Analysis of pumpkinseed stomach contents from the Ashby 2000 sample demonstrated strong dietary differences between habitats. Significant differences in diet were found between littoral and pelagic samples in 5 of 6 prey categories (Table 1.3). Littoral pumpkinseeds were found to be generalist feeders, consuming a variety of mostly benthic prey types, while pelagic pumpkinseeds had a diet that was highly specialized on zooplankton, particularly *Daphnia* (Figure 1.6).

Parallel Patterns of Divergence Between Study Systems

Results summarized for 26 populations in the 3 study areas indicated an overall mean correspondence for the 17 traits of 77%. Correspondence across the 3 study areas was greater than 80% (eg. 4 out of 5 analyses confirmed the same qualitative trend in body form) in 9 of 17 traits (Table 1.4). Five traits displayed 100% correspondence among study areas, suggesting the following trends in body form: overall head size (greater in littoral fish), length of caudal peduncle (greater in pelagic fish), length of the

ventral mid-body (longer in pelagic fish), and location/size of the pectoral fin insertion (lower/larger in littoral fish)(Table 1.4). These same trends can be observed in the geomorphometric results of the Mazinaw area populations shown in Figure 1.4.

Discussion

I found repeated parallel trends in body form between littoral and pelagic habitats that were consistent with basic functional predictions for locomotion and prey searching in these two habitats. These trends were observed across a variety of geographic levels in the pumpkinseed system, and suggest that selection favours the development of similar combinations of traits in similar lake habitats. I discussed the trends in body form at each geographic level in further detail below.

Habitat-specific Variation in Body Form and Feeding Habits

The combined analysis of the three Mazinaw populations suggested similar trends in the morphological divergence of littoral and pelagic pumpkinseeds, with the magnitude of divergence being different among lakes (Figure 1.3). In the three lakes, littoral origin pumpkinseeds had enlarged heads, wider pectoral fin insertions, and reduced body depths in the caudal regions relative to pelagic origin pumpkinseeds. While reduced body depth was not predicted on functional grounds, a wider pectoral fin insertion may provide larger pectoral fin area that is better suited to search for and forage on littoral zone prey, a task which is expected to require considerable maneuverability (Webb 1984). Although not measured in this study, pectoral fin length was positively related to pectoral fin width in other studies of polymorphic pumpkinseeds by Gillespie (2000) and Robinson et al.

(2000), and further indicates littoral specialization by allowing increased maneuverability. Pelagic pumpkinseeds tended to have smaller heads, narrower pectoral fin insertions, and enlarged body depths (particularly in the caudal regions). Increased rear body depth is consistent with open water locomotion, where burst speed is required to either move quickly to prey that have been located, or to quickly escape predators (Webb 1984). Rapid, almost vertical bursts down 2-3 metres were commonly observed upon startling pelagic pumpkinseeds in Ashby Lake, and may be attributed to potentially heavy predation pressure from loons that were frequently observed foraging over pelagic shoals. Vertical responses, suggested by Barr (1973) to be a form of fish escape tactic from loons, were never observed in littoral pumpkinseeds. Pelagic pumpkinseeds from Salmon Trout Lake also exhibited greatly increased mid-body depth, a response not predicted in the open water habitat. Although I did not have sample sizes large enough to examine this trait in further detail, it is interesting to note that Salmon Trout Lake was the only study population to contain walleye, a warm water piscivore (Table 1.1). This increased body depth may reflect an adaptation to escape predation (eg. the response of the crucian carp to pike predation, Bronmark and Pettersson 1994).

The trend towards increased head size in littoral pumpkinseeds may increase their ability to feed on a wider range of large macroinvertebrates and molluscs in the littoral zone, as suggested by a positive relationship between mouth size (gape size) and ability to ingest larger prey noted by Wainwright and Richard (1995). Littoral pumpkinseeds (particularly molluscivores) are also known to have hypertrophied pharyngeal jaw apparatus (PJA) muscle and bone components (Gillespie 2000, Mittelbach et al. 1999). Although I did not measure this internal characteristic in the Mazinaw area pumpkinseeds, larger head size may also represent the space required to house larger PJA

structures. Finally, while significantly longer gill rakers are an adaptation in pelagic fish that is expected to increase feeding efficiency on zooplankton, this was only observed in the Ashby Lake population. However, Robinson et al. (1993) noted that there may be other morphological solutions to feeding on zooplankton, such as closer spacing between gill rakers. I did not test for such morphological variation in this study, but this trend has been observed in pumpkinseeds in the Kawartha area by Gillespie (2000).

Dietary differences between littoral and pelagic pumpkinseeds were consistent with the differences in habitat use, and both were related in predictable ways to variation in body form in Ashby Lake. The proportion of prey types in stomach contents were significantly different between pelagic and littoral pumpkinseeds with the exception of Hydracarina, which were present at low abundance in both groups. Littoral pumpkinseeds fed primarily on macroinvertebrates, molluscs, terrestrial insects, and to a small extent on cladocerans (Figure 1.6), exhibiting a generalist macroinvertebrate diet consistent with that found in Robinson et al. (1993), Gillespie (2000), and Keast (1977). Pelagic pumpkinseeds appeared to be specializing almost exclusively on *Daphnia* (over 99% of their diets), with only the rare inclusion of non-aquatic insects, molluscs, and Hydracarina (Fig. 1.6). This suggests that dietary specialization on zooplankton by pelagic pumpkinseeds in Ashby Lake is even greater than that observed in pelagic pumpkinseed forms by Robinson et al. (2000) in 22 Adirondack lakes and by Gillespie (2000) in five Kawartha area lakes. Although comparable diet data was not available for Mayo or Salmon Trout populations, haphazard observation of stomach contents in the field suggested that planktivory is common in pelagic pumpkinseeds from both populations.

Weak relationships between standard length and body form (DFA

score)($R^2=0.06$) indicated that the observed significant interaction effect of habitat and body size on body form was relatively weak, and required very large sample sizes to detect. This suggests that any differences in the allometry of littoral and pelagic pumpkinseeds are not particularly significant biologically (the interaction effect only explained 1% of the total morphological variation in the 3 Mazinaw area lakes (Figure 1.5). Littoral pumpkinseeds were on average smaller than pelagics in each lake, but such differences may reflect variation in growth rate due to differences in resource availability (Deacon and Keast 1987), predation risk (Belk and Hales 1993), size selective predation, or selective mortality between lake habitats (Bertschy and Fox 1999). Observed differences in body size do not suggest that it is a trait always predictably related to polymorphisms in pumpkinseeds, as appears to be the case in the smaller threespine stickleback (Schluter 1993), or between dwarf and normal Arctic charr morphs (Johnson 1980, Parker 1997). Among polymorphic Adirondack pumpkinseed populations, body size variation was not consistently related to habitat (Beren Robinson, personal communication).

Parallel Patterns of Morphological Divergence Among Geographically Separated Populations

I found evidence of parallel morphological divergence by pumpkinseeds using littoral and pelagic habitats among lakes from a wider geographic area, in addition to the evidence that ecological and morphological divergence was similar among the three Mazinaw populations. These parallel trends in divergent body form in relation to habitat strongly suggest that selection is favouring the development of the same pair of phenotypes in these two lake habitats. Although only qualitative comparisons were made

here, there were consistent differences between forms in several functionally important traits. Traits having the greatest correspondence among different populations and regions included head size, pectoral insertion width, and rear body depth, suggesting that pelagic phenotypes are better suited to open water movement and predator avoidance, while littoral phenotypes are better suited to maneuvering in three dimensions amongst macrophytes and other structure while foraging for larger cryptic macroinvertebrates and molluscs.

Patterns of Selection in the Polymorphic Pumpkinseed System

Specialization of body form according to habitat and diet in a manner consistent with functional predictions in the three lakes that I studied, as well as across a wider geographic area, suggests that selection favours similar divergence in body form in each population. That similar benthic and pelagic phenotypes are repeatedly observed suggests that either disruptive selection favours the evolution of alternative phenotypes in this system, or that selection has favoured the evolution of a plastic phenotype that develops a body form suited for the environment in which it lives (Nager et al. 2000, Stearns 1989). There is evidence that both additive genetic variation and phenotypic plasticity play a proximate role in the development of morphological variation in the Adirondack pumpkinseeds (Robinson and Wilson 1996). However, the evolution of adaptively plastic responses between habitats itself requires that divergent selection favours different phenotypes in alternate lake habitats, or that the patterns of selection vary over time. The difference is that the evolutionary response is not a genetic polymorphism in the latter case.

Not all habitat-specific trait differences had equally high levels of correspondence

among lakes, suggesting either heterogeneity in lake characteristics, or that some observed traits may be more functionally 'neutral' and thus not adaptive in a specific habitat. Despite this, the generally high level of parallel divergence between similar habitats over such a wide geographic area is consistent with the idea that divergence has occurred independently within lakes (eg. sympatric origin), as opposed to the evolution of separate forms in allopatry that subsequently invaded each lake. This hypothesis can be further tested using molecular genetic approaches to make such a distinction between origins.

The presence of disruptive selection has been invoked in the development of both littoral and pelagic forms within single lakes (Smith and Skulason 1996). Although the Mazinaw pumpkinseed system is consistent with the results of such a process, it cannot be assumed to be the cause of the observed morphological variation. The Mazinaw system does however provide an ideal opportunity to explore the ecological basis of selection in several populations. For example, selection may in fact be disruptive on the pumpkinseed population, favouring the evolution of specialized forms that reflect underlying genetic polymorphism. As noted above, disruptive selection can also favour the evolution of a single phenotypically plastic genotype capable of developing into specialized phenotypes in each habitat. Alternately, in some lakes, directional selection may favour the evolution of a more pelagic form than an ancestral littoral form, a process that may not have yet gone to fixation in these relatively young post-glacial lakes. Directional selection may also vary over time, favouring alternately pelagic and littoral phenotypes depending on the seasonal availability of resources between habitats, and maintaining both forms in a single population over the long-term. Even stabilizing selection could be operating to permit the coexistence of multiple forms in a single

population, if the different forms have allopatric origins and are now hybridizing in sympatry. Without additional information about selection across littoral and pelagic habitats in a particular lake, we cannot predict evolutionary changes in body form in any single population. Such as understanding of how selection operates during adaptive divergence may allow us to compare mechanisms that derive divergence below the species level to mechanisms that cause divergence among species, and so may increase our understanding of a complex processes by which new species evolve (Schluter 1996b).

In order to determine the pattern of selection acting on forms within a population, we need to determine the relationship between phenotype and fitness in each habitat. Strong evidence of habitat related specialization in this study suggests that the polymorphic pumpkinseed system may be ideal for such a study, especially since preliminary evidence suggests that fitness may be related to phenotypic specialization within habitats (Robinson and Wilson 1996). My results further suggest that the Ashby Lake pumpkinseeds may be the best population in which to test for relationships between phenotype and fitness because it exhibits the greatest divergence between forms, and the best evidence that morphological divergence is related to differences in diet and habitat use.

Table 1.1: Physical characteristics and sampling information for the three Mazinaw area lakes studied in eastern Ontario. Physical characteristics are from unpublished Ontario Ministry of Natural Resources (OMNR) data. Fish species composition represents OMNR data combined with personal observations for each lake. †Although bluegill sunfish are recorded by the OMNR as being present in Salmon Trout Lake, they have not been observed or captured there over 3 summers of research, and are assumed to not be in the lake.

	Ashby Lake	Mayo Lake	Salmon Trout Lake
PHYSICAL CHARACTERISTICS			
Location	$45^0$05'N, $77^0$21'W	$45^0$02'N, $77^0$35'W	$45^0$11'N, $77^0$49'W
Size (Ha)	259	182	100
Maximum depth (m)	36.6	38.1	14.0
SAMPLING INFORMATION			
Years sampled in this study	1999, 2000	1999	1999
Sample size (Littoral, Pelagic)	242, 201	41,55	17,12
Mean standard length (mm):			
Littoral ± SE	93.2 ± 1.2	96.0 ± 2.0	94.2 ± 5.4
Pelagic ± SE	99.4 ± 1.3	104.6 ± 1.6	111.3 ± 8.6
FISH SPECIES COMPOSITION Lake			
trout - (*Salvelinus namaycush*)	X	X	
Common white sucker- (*Catastomus commersoni*)	X	X	X
Walleye- (*Stizostedion vitreum*)			X
Yellow perch- (*Perca flavescens*)	X	X	
Smallmouth bass- (*Micropterus dolomieu*)	X	X	X
Largemouth bass- (*Micropterus salmoides*)		X	
Pumpkinseed sunfish- (*Lepomis gibbosus*)	X	X	X
Bluegill Sunfish- (*Lepomis mocrochirus*)			X†
Rock Bass- (*Ambloplites rupestris*)		X	
Fallfish- (*Semotilus corporalis*)	X		
Creek chub- (*Semotilus atromaculatus*)		X	
Bluntnose minnow- (*Pimephales notatus*)	X		
Iowa darter- (*Etheostoma exile*)		X	

Table 1.2: ANCOVA results testing the effect of habitat on body form (DFA score), with standard length (mm) as a covariate. The analysis combines pumpkinseeds from the three Mazinaw populations (see also Figure 1.5).

Factor	ν	SS	MS	F-ratio	P-value
Habitat	1	30.2	32.2	32.3	<0.0001
Standard Length	1	34.2	34.2	36.6	<0.0001
Habitat by Standard Length interaction	1	4.1	4.1	4.4	0.04
Error	564	527.2	0.9		
Total		595.7			

Table 1.3: Analysis comparing stomach contents between littoral and pelagic pumpkinseeds sampled from Ashby Lake in 2000. Mean proportions represent the proportion by item abundance (number) in the stomachs for each prey category averaged in each habitat. Habitat comparisons were performed using Welch's t-tests on arcsin square root transformed diet proportions. Bold-face values represent significant differences after sequential Bonferroni correction. See Figure 1.6 for the distribution of prey items in diets.

	Mean Proportion in Diet		Habitat comparison	
	Littoral zone	Pelagic zone	t-value; ν	p-value
BENTHIC PREY	0.46	--	10.3, 20.0	**<0.0001**
MOLLUSCS	0.14	1.3×10^{-4}	2.4, 20.0	**0.021**
CLADOCERA	0.07	0.99	-20.2, 20.4	**<0.0001**
HYDRACARINA	0.02	1.3×10^{-4}	0.9, 20.5	0.35
TERRESTRIAL INSECTS	0.18	6.6×10^{-4}	3.3, 20.2	**0.0017**
OTHER (eg. vegetation)	0.13	1.3×10^{-4}	2.7, 20.1	**0.0011**

Table 1.4: Summary of results on 17 individual linear truss measurements from the sides of pumpkinseeds sampled from 26 lakes used to assess parallelism in morphological divergence between polymorphic systems. Letters indicate the group with the greater average measure (least squares means) for each trait, littoral (L) or pelagic (P) for each sample. Results from the Mazinaw area (results of the current study) and the Kawartha area (Gillespie 2000) represent the results of ANCOVA tests of habitat origin difference (covariate centroid size). Adirondack area results represent univariate ANOVA tests of habitat origin across 22 lakes (using residuals from size correction with centroid size) taken from Robinson et al. (2000). Bold results indicate those significant differences between forms in individual studies.

BODY MEASUREMENTS	Mazinaw Area		Kawartha Area		Adirondack Area	% Correspondence
	Mayo Lake	Ashby Lake	Looncall Lake	Shadow Lake	22 lake analysis	
BODY LENGTHS						
Head region						
Pre-dorsal length	L	L	L	L	L	100
Pre-pectoral length	P	L	L	L	**L**	80
Pre-pelvic length	L	L	--	--	**L**	100
Mid-body						
Dorsal fin base length	P	P	--	--	L	67
Anal fin base length	L	L	P	L	L	80
Anterior anal fin - Anterior Pelvic fin	P	P	P†	P†	P	100
Tail						
Dorsal caudal peduncle length	L	--	P	L	P	50
Ventral caudal peduncle length	P	P	P	P	P	100
BODY DEPTHS						
Mid-Body						
Anterior dorsal fin to anterior pelvic fin	L	P	--	--	**L**	67
Anterior dorsal fin to anterior anal fin	L	**P**	P	L	**L**	60
Posterior dorsal fin to anterior anal fn	L	P	P	L	L	60
Posterior dorsal fin to anterior pelvic fin	P	P	P	L	---	75
Tail						
Anterior caudal peduncle depth	L	P	--	--	L	67
Posterior caudal peduncle depth	L	P	**P**	P	P	80
FIN SIZE / POSITION						
Pectoral fin altitude (from dorsal origin)	L	P	L	L	L	80
Pectoral fin insertion width	**L**	L	L	L	L	100
INTERNAL MEASUREMENTS						
Gill raker length	L	P	P	L	--	50

† Marked results indicate a truss measurement from the anterior anal fin to posterior pelvic fin. This was assumed similar for the purpose of this comparison.
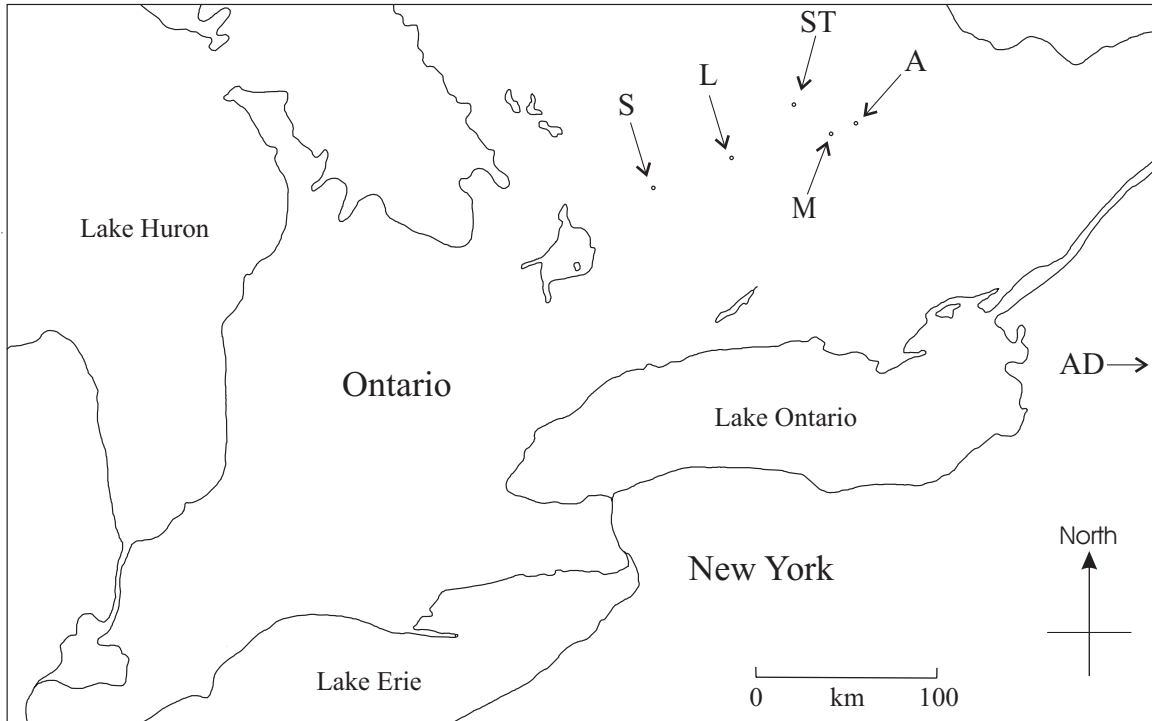
Figure 1.1: Location of the pumpkinseed populations used in this study. The three Mazinaw area study lakes in eastern Ontario are Ashby Lake (A), Mayo Lake (M), and Salmon Trout Lake (ST). Morphological data for pumpkinseeds from Looncall Lake (L) and Shadow Lake (S) (Kawartha area) studied by Gillespie (2000) were included in the analysis of parallel patterns of divergence. Similarly, morphological data for pumpkinseeds in 22 lakes in the Adirondack region of New York from Robinson et al. (2000) were included in the analysis of parallel patterns of divergence. The western boundary of the Adirondack region is shown (AD). Refer to Table 1.1 for exact locations, lake characteristics, sampling information, and fish species composition for the Mazinaw area lakes.
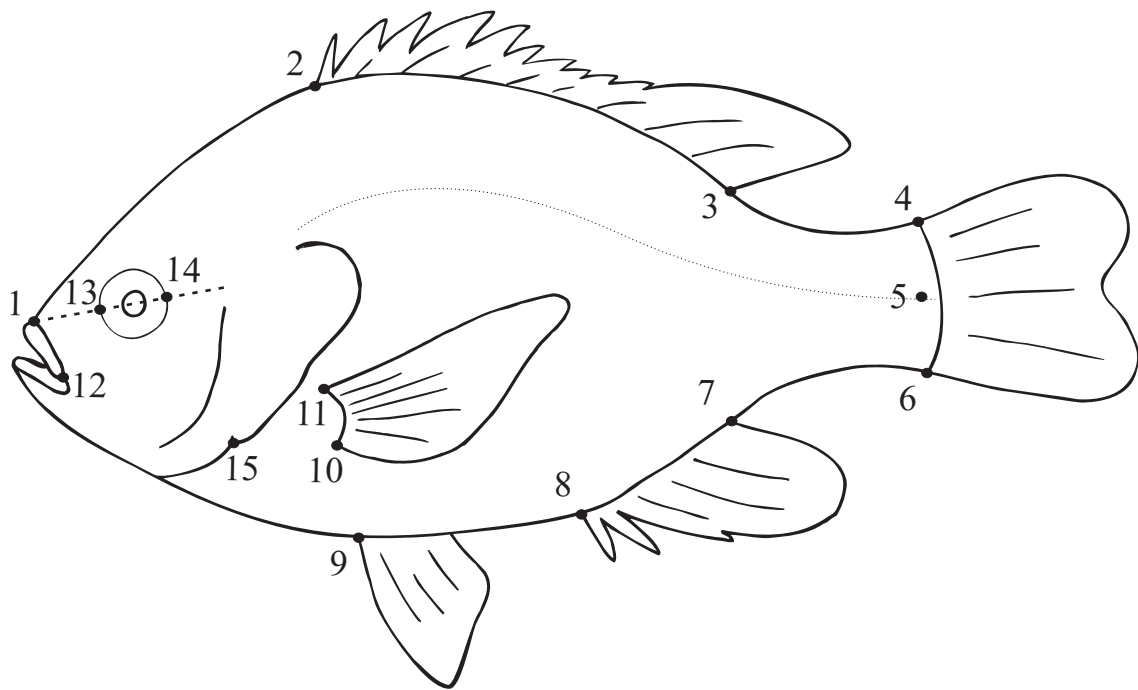
Figure 1.2: Location of the 15 landmarks used in all geomorphometric analyses and linear truss measurements. Landmarks for analysis were recorded from digital images of the left side of each pumpkinseed, and calculations were scaled to a size standard included in each photograph. Refer to this diagram for clarification of landmark location in all diagrams demonstrating changes in body form throughout the thesis.
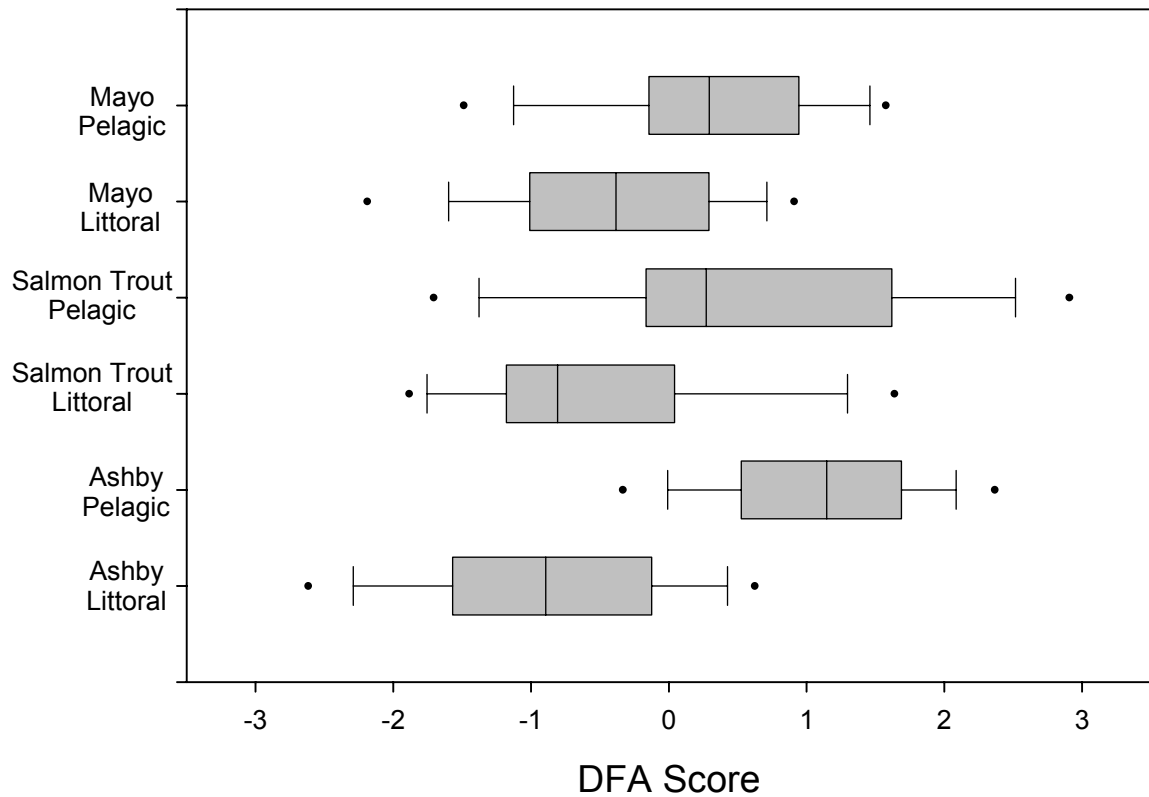
Figure 1.3: Distribution of body form (DFA scores) between littoral and pelagic habitats from a combined DFA analysis of pumpkinseed sunfish sampled from the 3 Mazinaw area lakes. Boxes represent the interquartile range, with median value inside. Outer whisker ends represent the 10th and 90th percentiles, while dots represent the 5th and 95th percentiles. See Figure 1.4 for the body form of average forms of littoral and pelagic groups from each population.

Mayo Littoral   (0.71)   Mayo Pelagic

Salmon Trout Littoral   (1.08)   Salmon Trout Pelagic

Ashby Littoral   (1.97)   Ashby Pelagic
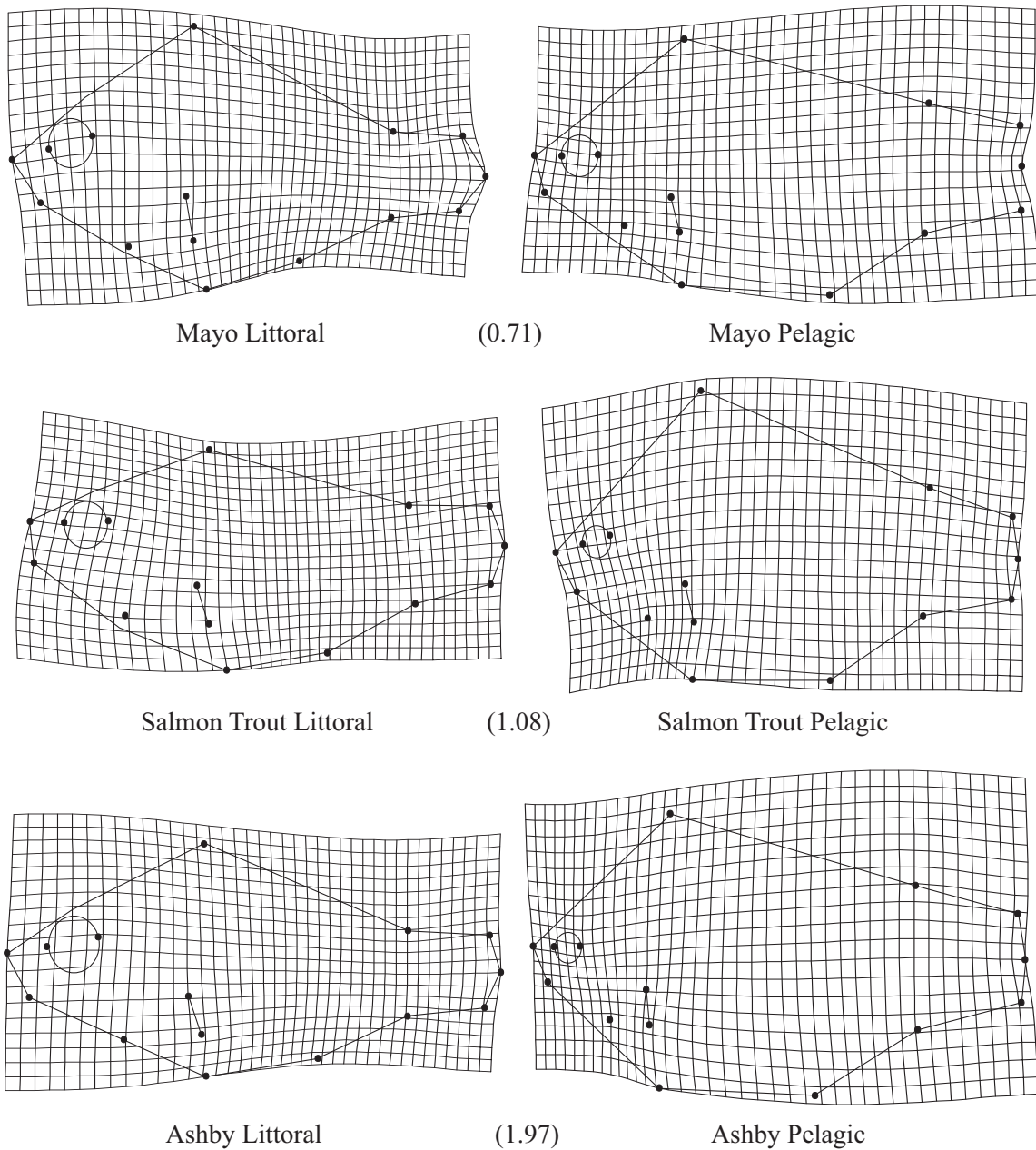
Figure 1.4: Differences in external pumpkinseed morphology between littoral and pelagic habitats in the 3 Mazinaw populations. Deformation of the gridlines represents the difference in body form between each group compared to the mean form for that lake (which would be represented by a figure composed of perfect squares). The outlined form is meant as a guide to locate landmarks, and does not represent the true location of the body outline. For clarity, shape change has been exaggerated 10X in Ashby and Salmon Trout Lakes, and 30X in Mayo Lake where body form was less variable. Numbers in parentheses represent the difference in mean body form (DFA scores) between habitat groups in each population (mean pelagic score - mean littoral score), and so indicate the relative amount of divergence between forms (from the combined analysis of all three populations, see Fig.1.3).
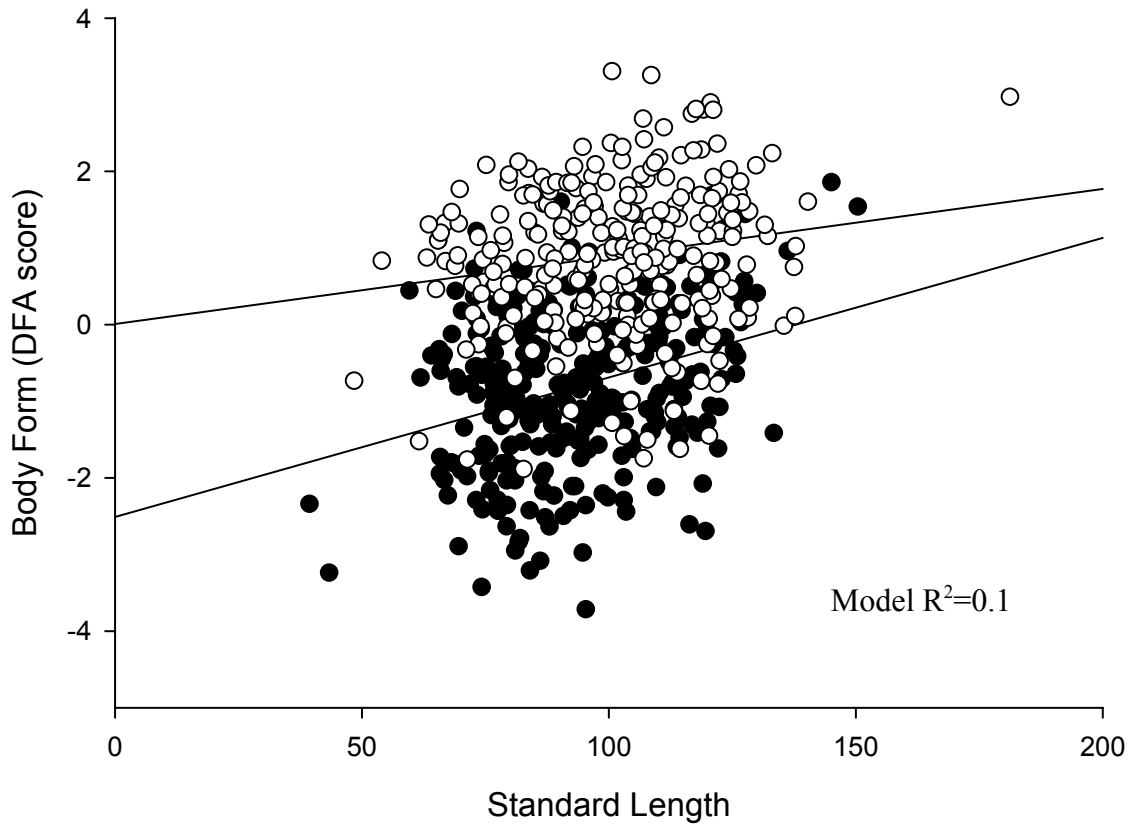
Figure 1.5: Overall relationship between body form (individual DFA scores shown in Figure 1.3) and standard length between littoral samples (closed circles) and pelagic samples (open circles) from all three study lakes. Different relationships between body size and body form are found in each habitat, as shown by the difference of slopes between the two groups (ANCOVA habitat by standard length interaction p=0.036). However, the effect appears to be relatively insignificant in biological terms because of the low R^2 associated this slope effect (R^2=0.007). See Table 1.2 for additional ANCOVA results.
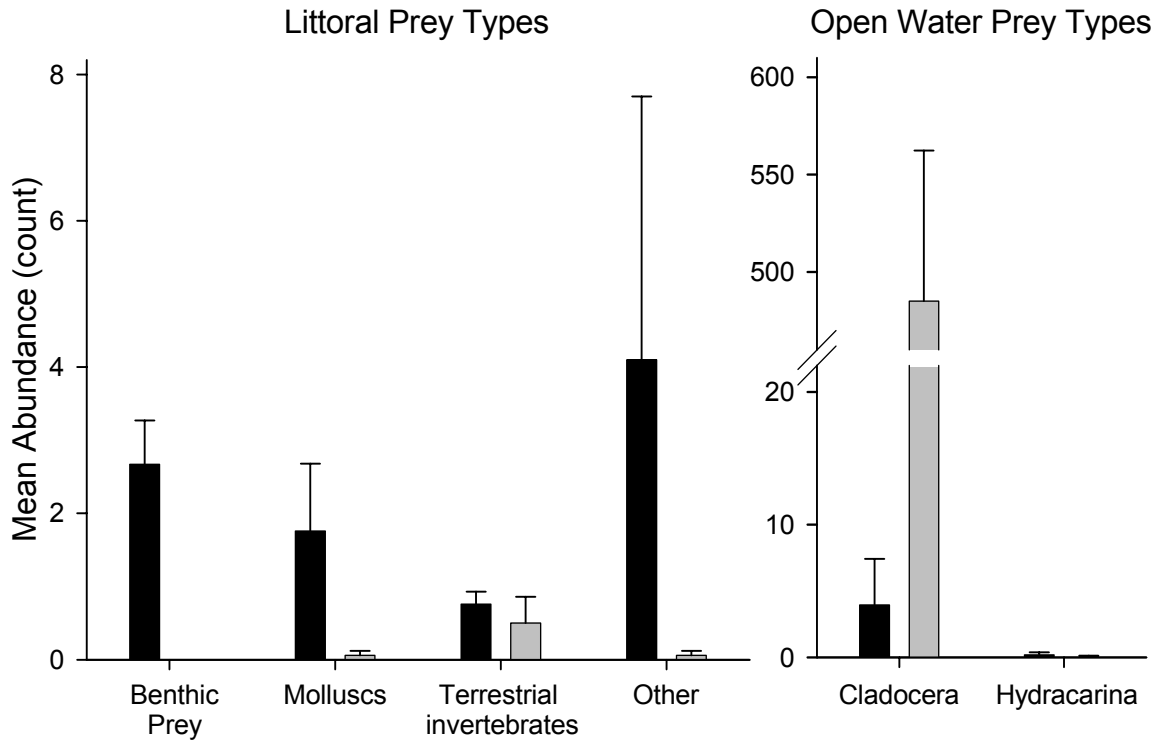
Figure 1.6: Distribution of stomach contents from a random sub-sample of Ashby 2000 pumpkinseeds. Bars represent the mean number of prey items per stomach (+SE) represented in 6 functional categories for littoral (black bars) and pelagic (grey bars) origin fish. Refer to Table 1.3 for diet analysis results.

Chapter 2

Patterns of Selection Inferred from Phenotype-related Onset of Seasonal

Reproduction in a Polymorphic Population of Pumpkinseed Sunfish

(*Lepomis gibbosus*)

Introduction

Recent studies suggest that natural selection imposed by the challenges of living and acquiring different resources in littoral versus open water lake environments favours the divergence of fishes into alternate body forms in northern post-glacial lakes. The evidence that selection favours multiple phenotypes has included: i) parallel patterns of morphological divergence in traits related to resource use between closely related species specialized for littoral and pelagic habitats (Schluter 2000), as well as between coexisting forms of a single polymorphic species (Chapter 1, Robinson et al. 2000, Robinson and Schluter 2000). ii) The measurement of habitat-specific fitness through experimental manipulations involving reciprocal transplants of morphological forms. Here, short-term performance has been measured as: a) growth rate in the field (Hatfield and Schluter 1999), or b) foraging efficiency in artificial laboratory habitats (Schluter 1993, Robinson 2000). iii) Longer-term growth experiments involving closely related species in semi-natural littoral ponds (Schluter 1994); and iv) significant relationships between habitat-related phenotype and fitness related traits such as condition factor and size at age (Robinson et al. 1996). Frequently, the method used to test the role of natural selection in phenotypic divergence appears biased by the degree of divergence present in the system.

Divergence between littoral and pelagic forms has been observed across a wide range of levels: from subtly different phenotypes forming a continuous morphological distribution within a single population, to discrete morphological forms, to closely related species that occupy alternate habitats (Robinson and Schluter 2000). While this variation in the degree of morphological divergence suggests that local conditions are important

(eg. characteristics of lake environments or the age of various systems), it also suggests that similar processes are at work in many such situations. This is advantageous because it permits us to compare patterns and mechanisms of divergence that operate between species with those below the species level, potentially filling the gap in our understanding of the origins of rapid divergence and species formation. While methods 1-3 above have been used to assess the possible role of natural selection in divergence between closely related species, only methods 1, 2b and 4 have been used to assess the role of selection in the divergence of forms below the species level. Phenotypic variation below the species level may be too subtle to allow the detection of selection measured as differences in performance during short-term experiments (eg. using growth or foraging efficiency as in 2a or 2b above). A potentially more effective way to measure performance in fitness-related traits when phenotypic differences between forms are small (such as in a continuously varying trophic polymorphism) may lie in measuring habitat-specific performance integrated over a longer timescale (eg. method 4 above). Here I attempt to use such an integrated measure of phenotypic performance by assessing whether reproductive fitness (measured as the onset of individual reproductive activity during the spawning season) is related to phenotypic variation in sunfish found in littoral and pelagic lake environments.

I assumed the following causal model that relates phenotypic variation to timing of reproductive activity. Phenotypic specialization between lake habitats (such as in many trophically polymorphic populations) is expected to improve foraging efficiency of more specialized forms 'native' to a habitat (Schluter 1996a, Webb 1984). Increased foraging efficiency of a phenotype is related to seasonal onset of reproduction because although the onset of reproductive activity in pumpkinseeds is regulated by environmental factors

such as temperature (Smith 1970, Scott and Crossman 1973), individual pumpkinseeds can only reproduce when sufficient energy is available to complete egg development in females, and to begin nest building and defense in males. These activities can be delayed by energy deficits that commonly accumulate over the winter period in centrarchid fishes (Danylchuk and Fox 1994b, Ridgway et al. 1991). In other words, morphological variation is expected to influence reproduction, an activity that is highly dependent on energy early in the growing season for gonadal development and for costs of reproductive behaviour (Danylchuk and Fox 1994a). Earlier onset of reproduction in the spring is thought to be selectively advantageous because it increases the length of the growing season for offspring to develop energy reserves for their first winter, thereby increasing their survival and recruitment (Cargnelli and Gross 1996, Danylchuk and Fox 1996, Shuter and Post 1990). We may then expect the earliest reproductive onset in those phenotypes with the greatest foraging efficiency in the previous or current year that has allowed them to limit or rapidly recover from overwinter energy deficits. Thus, variation in body form should be related to variation in the onset of reproductive activity in the population. This in turn may result in selection that favours further morphological and ecological specialization of forms in the population.

The pattern of selection imposed on the population will depend on the relationship between phenotype and onset of reproduction in each habitat as well as between habitats. There are three basic relationships to consider, each predicted to result in a different form of selection. 1) Onset of reproduction may be earliest in different specialized phenotypes that forage most efficiently in their respective 'native' habitats. Forms intermediate to these specialist forms (or even opposite to them) will be less fit by comparison, and selection is expected to be disruptive, favouring alternate specialized forms in each

habitat. 2) Highest foraging efficiency and earlier onset of reproduction will occur in intermediate phenotypes present in both habitats, and the reduced fitness of extreme specialist forms in both habitats will result in stabilizing selection in the population. 3) If higher foraging efficiency and earlier onset of reproduction occur in the direction of only one of the more specialized phenotypes in both habitats, with the alternate specialized form and intermediates being less fit, then directional selection would favour that more specialized phenotype in the population (either a littoral or pelagic form, but not both).

I tested the null hypothesis that there is no relationship between phenotype and the onset of reproductive activity. This was tested separately in males and females because the energy limitations with respect to reproduction can be different between sexes (Danylchuk and Fox 1994a). My tactic was to sample nesting males and gravid females very early in the reproductive season and to compare their body forms with a sample of males and females taken randomly from the population at the same time (referred to hereafter as the non-reproductive sample). Similar distributions of body forms in reproductive and non-reproductive males or females would indicate that the onset of reproduction is unrelated to phenotype. Differences in body form between reproductives and non-reproductives would allow rejection of the null hypothesis, indicating that some form of selection may be favouring habitat-specific body forms in the population. This result would allow an examination of the relationship between phenotype, habitat, and onset of reproduction to further reject some of the alternate selection hypotheses above (#1-3). For example, the disruptive selection hypothesis above (#1) predicts that the onset of reproduction will be earlier in the specialist forms in their respective habitats, as indicated by a significant interaction effect between reproductive status (reproductive or non-reproductive) and habitat (pelagic or littoral).

Stabilizing selection (#2) is predicted by a significant interaction between reproductive status and habitat, but that intermediate forms will have the earliest onset of reproduction in each habitat. The directional selection hypothesis (#3) predicts no interaction between reproductive status and habitat, and instead that only one of the more specialized forms will have the earliest onset of reproduction in both habitats.

I tested these predictions in a polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*) that appears to be in an early stage of ecological divergence. In this population pumpkinseeds occupy the shallow, littoral feeding habitats where they are known to be specialists on molluscs and benthic macroinvertebrates (Keast 1978). Additionally, a planktivorous form occurs at high density in the open water (pelagic) zone of the lake in areas surrounding shoals that rise from the deep water. The external body forms of pumpkinseeds in this population form a continuous but bimodal distribution ranging from a robust littoral form with a larger head and shallower tail region, to a pelagic form with a longer, taller caudal peduncle and smaller head region (see Chapter 1). There are also differences in gill raker morphology between habitats, with littoral forms possessing shorter, more rounded rakers and pelagic forms possessing longer, more pointed, and often highly curved rakers (Chapter 1, Unpublished data). Nesting occurs in both lake habitats and potentially allows habitat-specific phenotypes to spawn in their respective 'native' (feeding) habitat (Gillespie 2000). While an interaction between fitness (measured as size at age and condition factor) and phenotype has been shown in one population of pumpkinseed sunfish (Paradox Lake in the Adirondack region of New York) by Robinson et al. (1996), this current study uses a different component of fitness, seasonal reproductive timing.

Methods:

Study Location and Sample Collection

The study was conducted in the early spring of 2000 at Ashby Lake, in eastern Ontario (45^o05'N, 77^o21'W). Ashby Lake has a deep basin (36.6m maximum) and limited littoral areas along a convoluted shoreline (see Table 1.1 for details of further lake characteristics). Although open water areas are interrupted in many places by numerous islands and rocky shoals arising from the deep water, most shoreline areas drop off rapidly, apparently limiting the extent of the littoral zone.

Pumpkinseeds were collected during the first 10 days of the local reproductive season in both littoral and pelagic zones of the lake (during the first 15 days for nesting males in the pelagic zone). This time frame was within the first 20% of the 79 day reproductive season identified for this region by Danylchuk and Fox (1996), and was assumed to represent only the earliest of reproductive individuals for that year. Collection of nesting males began when nest construction was first observed in each habitat (June 11 for littorals, June 14 for pelagics), and was accomplished by angling males off of nests while snorkelling. The collections of gravid females were made using haphazardly placed funnel traps and by angling (as in Chapter 1), and began on June 10 when fully gravid individuals were first caught. Females were identified as being gravid and classed as reproductive by the presence of clearly distended abdomens and swelling surrounding the gonopore. This status was later confirmed by the presence of mature, yolked eggs when females were dissected (Michael Fox, personal communication). Littoral sample sizes were 30 nesting males and 75 gravid females, and for pelagic samples, 60 nesting males and 62 gravid females. Samples of both sexes of ages >2+ (determined in Chapter 3

analyses) totalling 178 fish in the littoral zone and 144 in the pelagic zone (the non-reproductive sample) were taken concurrently with reproductive individuals throughout the first 10 days. These samples were taken using haphazardly placed funnel traps and angling in the same locations as reproductive samples. All fish collected for analysis were euthanised using clove oil, fixed in 10% formalin, then rinsed in water and stored in 70% ethanol prior to analysis.

Analysis of Body Form

Morphological variation across samples was characterized separately for each sex using a morphological index that incorporated both body shape and gill raker length. Techniques used to quantify these characteristics are described in Chapter 1. Morphological variation of reproductive and non-reproductive fish was then analyzed for each sex separately because significant differences in morphology were observed between the sexes (Chapter 1), although littoral and pelagic groups were combined. The non-reproductive samples were additionally screened to include only individuals with body size (centroid) values within the range found for the reproductive sample. This prevented morphological differences between reproductive and non-reproductive groups due to possible ontogenetic effects, and reduced non-reproductive sample sizes to 103 littoral and 100 pelagic pumpkinseeds. Every individual was then assigned a morphological score by combining both external (partial warp scores) and internal (size-free residual gill raker length) morphological measurements into a single principal component analysis (PCA).

Statistical Analysis

I tested the prediction that members of the early reproductive group would have different habitat-related body forms than the sample of non-reproductive individuals using a two factor multivariate analysis of variance (MANOVA). The distribution of phenotype scores on the first 6 PCA axes (canonical loadings are shown Appendices 1 and 2) was compared between reproductive and non-reproductive groups (the reproductive status effect), and between littoral and pelagic sites (the habitat effect) separately for each sex. Including habitat as a factor allowed me to compare variation in body form between reproductive and non-reproductive groups to the variation in body form related to habitat.

Results:

Female Morphology

Female pumpkinseeds from the littoral zone had, on average, a larger head and buccal region, as well as a shortened and shallower posterior body region compared to pelagic females that had a more rear-weighted phenotype characterized by a reduced head region and taller, longer posterior regions and caudal peduncles (Figure 2.1a). Gill rakers were significantly longer in pelagic than littoral females (t-test of size-free residual gill raker length: $t=-8.72$, $v=264$, $p<0.0001$) (Figure 2.2a).

The variation in female body form was significantly related to habitat and reproductive status (2-Factor MANOVA based on PCA axes 1-6 comprising 51% of total morphological variation: habitat: $F_{6,262}=47.1$, $p<0.001$; reproductive status: $F_{6,262}=17.1$, $p<0.001$). More specialized phenotypes native to each habitat did not reproduce earlier or

later than their respective intermediate forms, as indicated by the non-significance of the interaction effect (habitat by reproductive status interaction: $F_{6,262}=1.1$, p=0.40). Gravid females had a more pelagic-like body form than non-reproductive females, regardless of the habitat effect (Fig. 2.3). This pelagic-like body form was characterized by shorter head regions, and lengthened and heightened body posteriors and caudal peduncles compared to non-gravid females (Figure 2.3). Non-reproductive females had more littoral-like body forms, with enlarged heads and compressed body posteriors. Although gravid females from both the littoral and pelagic habitats had more pelagic-like phenotypes, the effect of reproductive status on body form appeared to be stronger in littoral females (Figure 2.3). This effect is illustrated further with gill raker length, which was longer in reproductive females than non-reproductive females in the littoral zone, but not significantly different between reproductive and non-reproductive females in the pelagic zone (t-test of size-free residual gill raker length between reproductive and non-reproductive females in each habitat: littoral habitat: t=2.73, v=145, p=0.007; pelagic habitat: t=−0.80, v=117, p=0.42).

Male Morphology

Male pumpkinseeds demonstrated morphological trends similar to those of females with respect to habitat, although males differed from females in body form. Pelagic males had heightened and lengthened caudal peduncles, as well as smaller head regions (Figure 2.1b). Gill rakers were significantly longer in pelagic than littoral males (t-test on size-free residual gill raker length: t=-6.18, v=162, p<0.0001)(Figure 2.2b).

Both habitat and reproductive status explained significant amounts of the

morphological variation in males (2-factor MANOVA based on PCA axes 1-6,

comprising 54% of total morphological variation: habitat: $F_{6,160}=16.7$, p<0.001;

reproductive status: $F_{6,160}=10.4$, p<0.001). Forms most specialized to each habitat did not

both nest earlier or later than their respective intermediate forms. Instead, all nesting

males tended to have more pelagic-like phenotypes (habitat by reproductive status

interaction: $F_{6,160}=0.58$, p=0.75). Nesting males tended to have a taller caudal peduncle

and larger body posterior than non-reproductive males, although this effect was not as

pronounced as in females. Nesting males also tended to have larger heads and greatly

increased body height, resulting in a more hump-backed body form that was independent

of habitat effects (Figure 2.4). Although gill raker length in both habitats was not

significantly longer in nesting than in non-nesting males, those individuals with the

longest gill rakers nested early in the season, particularly in the pelagic zone (Fig. 2.2)(t-

test of size-free residual gill raker length between nesting and non-nesting males: littoral

habitat: t=0.82, ν=89, p=0.42; pelagic habitat: t=0.73, ν=71, p=0.47).

Discussion

Pattern of Selection

My findings indicated that reproductive status measured as early onset of

reproduction was related to body form in each sex, leading to rejection of the null

hypothesis of no relationship between morphology and reproductive status. This result

implied that selection can act on body form with respect to reproductive timing, enabling

further analysis of the relationship between body form and reproductive timing in the

context of alternate hypotheses 1-3 (designed to discriminate between the patterns of

selection). While the results indicated that both habitat and reproductive status explained significant portions of the total variation in body form in males and females, there was no significant interaction between these effects in either sex. Rather, individuals in each reproductive group were significantly more pelagic-like in body form than those in their respective non-reproductive groups. This allowed the rejection of alternative hypotheses #1 and #2, suggesting that directional selection for pelagic-like sunfish phenotypes may be present in Ashby Lake. Reproductive females demonstrated pelagic-like body traits such as a generalized shortening of the head region, increased caudal peduncle depth, heightening of the posterior body regions, and narrower pectoral fin insertions (Figure 2.3). These changes related to reproductive status were most distinct in littoral females. My results also indicated that nesting males had more pelagic phenotypes than non-nesting males, although the relative degree of morphological difference was not as extreme as in females. Nesting males had body forms characterized by a slight heightening of the posterior body and narrower pectoral fin insertions. Nesting males also had enlarged dorsal head regions that resulted in a 'humped' appearance that was unrelated to habitat (Figure 2.4).

Differences in Body Form in Relation to Reproductive Activity

These data suggest that the relationship between body form and reproductive timing could cause directional selection to favour more pelagic phenotypes in both habitats. However, there are two possible causes of such a relationship that need to be distinguished: 1) that reproductive status causes changes in body form, and 2) that body form influences reproductive status (eg. the onset of reproduction).

The first case suggests that the onset of reproductive activity causes changes in

body form and so results in the observed differences in body form between reproductive and non-reproductive groups. It is well documented that physiological and hormonal effects can cause significant morphological changes during reproduction, such as the kype often observed in male salmonids (Helfman et al. 1997). While I observed reproductive effects in both sexes, they appeared to be in addition to differences in body form associated with habitat. Nesting males (particularly those in the pelagic zone) displayed characteristics such as a dorsal enlargement (resulting in a larger, hump-backed appearance) that may play a role in nest guarding or inter-male competition, and could be plastic responses that are under hormonal control. In gravid females, distended abdomens were an obvious morphological change induced by reproduction (due to the presence of mature eggs). Both of these changes may cause an individual to have a deeper body, more like the pelagic form described earlier.

More importantly, however, reproductive sunfish were more pelagic-like in several key traits that have no predicted relationship with reproductive status: both reproductive females and males had smaller eyes and compressed snouts or head regions, narrower pectoral fin insertions, and wider caudal peduncle depths (this trait appeared to be less distinct in males than in females). In other words, changes in body form induced by reproduction may explain some differences in body form observed between reproductive classes of males and females, but they do not adequately explain all of the differences in body form between reproductive and non-reproductive groups in either sex.

My results suggest that more pelagic-like phenotypes are able to reproduce earlier in the season than other phenotypes in both lake habitats. This suggests first that pelagic forms generally have higher early season body condition, and second that directional selection should favour more pelagic phenotypes through their offspring having higher

survival in both habitats. The relationship between increased reproductive success and higher early season body condition has been well documented in the Centrarchidae (Cargnelli and Gross 1996, Danylchuk and Fox 1994b, Ridgway et al. 1991), and Danylchuk and Fox (1996) have suggested that this results from overwinter energy deficits that must be overcome before reproductive activity can begin. Energy deficits are known to be related to body size, such that larger individuals appear to have lower size-specific energy demands that allow them to reproduce earlier than smaller individuals (Danylchuk and Fox 1994a,b). In Ashby Lake, however, a similar relationship appears to exist between different body forms that have similar body size. In this case, I suggest that phenotype related variation in feeding efficiency can also effect body condition (as in Robinson et al. 1996).

The observation that more pelagic forms in both habitats reproduce earlier in the season than other forms suggests that open water plankton resources are very important for avoiding or recovering from overwinter energy deficits in Ashby Lake. This contradicts most conventional expectations about pumpkinseed sunfish, because they are widely believed to be littoral specialists that feed largely on macroinvertebrates and gastropods in the littoral zone (Keast 1978, Werner and Hall 1979). In Ashby Lake however, pumpkinseeds feed on different prey in littoral and pelagic habitats. One possible explanation for use of the pelagic habitat for feeding would be an opportunistic response to frequency- and density-dependent competition with conspecifics over littoral resources. Those individuals best able to compete in the littoral zone would maximize energy intake and fitness in that habitat, and thus could retain their conventional ancestral foraging niche. The observation of directional selection towards a pelagic phenotype suggests a different scenario in Ashby Lake, where the pelagic zone may be important to

all pumpkinseeds in the population.

Temporal Scale of Directional Selection

As with other centrarchid species, the ability to overcome overwinter energy deficits is crucial in allowing early reproduction, which in turn can improve reproductive fitness resulting from greater offspring survival (Danylchuk and Fox 1996). Dependence on resources in the pelagic zone may be necessary to overcome overwinter energy deficits in Ashby Lake. From ice-out in the spring of 2000 (approximately the third week in April) until just prior to reproduction (first week of June; approximately 5-6 weeks total), I observed no pumpkinseeds in the littoral zone of Ashby Lake. Instead, all observed pumpkinseeds (presumably both littoral and pelagic phenotypes) were in the open water (Smith 1970), where they may have been feeding on zooplankton resources. Significant zooplanktivory in the early spring may allow pumpkinseeds early and rapid energy gains compared to using only littoral resources. Many northern temperate lakes are known to undergo a turnover in early spring that frequently induces a rich plankton bloom that could provide a valuable energy boost long before littoral resources are available (Wetzel 1983). My results are consistent with the idea that an abundance of pelagic zooplankton resources may cause directional selection on those pumpkinseed forms that can efficiently forage on planktonic prey.

Unfortunately, while these results suggest a fitness advantage for sunfish with pelagic phenotypes, I cannot discriminate the temporal scale of this effect. Two possible temporal scales of selection seem plausible, depending on resource availability over the growing season. First, if zooplankton resources are the dominant resource available only in the early spring (when littoral resources are still relatively undeveloped), then I would

expect that directional selection will favour pelagic forms over the short-term only in the early spring. Increasing abundance of littoral resources later in the spring could allow resumption of divergent selection that favours both littoral and pelagic phenotypes in their respective habitats. Under this model, I would expect pelagic sunfish to be under directional selection for open water feeding ability throughout the year, while the ability to feed in both habitats would be beneficial to littoral sunfish (early season in the pelagic zone, summer in the littoral zone). The overall phenotypic distribution in Ashby Lake is consistent with this scenario because there is a wider range of intermediate forms present in the littoral than pelagic habitat (Figure 1.3).

The littoral zone habitat of lakes like Ashby is reduced due to steep rocky drop-offs near the shoreline (personal observation). A second hypothesis then is that the relatively small littoral habitat may limit the availability of littoral resources over the entire growing season, and so directional selection may favour phenotypes that use open water resources throughout the growing season. Although competition for pelagic zooplankton and functional constraints imposed by body form may reduce the efficiency of zooplanktivory in specialized littoral forms, those forms better able to feed on zooplankton would benefit throughout the year. Under this scenario, higher fitness throughout the growing season would result in long term directional selection that favours more pelagic phenotypes. My reproductive timing results are also consistent with this scale of selection, although this does not explain the presence of extreme littoral specialists in the Ashby Lake population.

My study suggests that selection favours more pelagic-like phenotypes in Ashby Lake. Using measures of fitness integrated over longer time intervals may be the key to better understanding selection pressures that may be acting on subtley divergent

phenotypes below the species level. The question is how to correctly choose traits related to fitness (ie. over what time scale should they incorporate feeding performance?). My results suggest that reproductive fitness advantages are associated with the ability to use pelagic resources, especially in females. However, they cannot distinguish whether directional selection acts over the long-term or the short-term in this lake (eg. complete growing seasons or only the early spring period. Short-term directional selection favouring the use of pelagic resources at one time may shift during other seasons, as the relative abundance of prey varies between habitats. Alternatively, if directional selection is long-term, then sunfish may possibly abandon ancestral littoral habitats and phenotypes in plankton-rich and littoral-poor lakes. Distinguishing between these possibilities will require measuring integrated fitness components and patterns of selection during other seasons or over even longer intervals, as in Chapter 3.

Chapter 3

Life History Performance as an Indicator of Relative Fitness Among

Coexisting Forms of Pumpkinseed Sunfish (*Lepomis gibbosus*)

Introduction

Adaptive life history characteristics are expected to represent the optimal allocation of available energy among growth, maintenance and reproduction (Roff 1984, Justus and Fox 1994). Under ideal conditions of unlimited resources, an organism may have enough energy to reach a large size that maximizes fecundity, while also maturing early in life, which increases reproductive lifespan. Unfortunately, when resources are limited, maximizing either of these activities may come at the expense of performing the other (Bertschy and Fox 1999). Thus, the optimum life history tactic is the particular solution to this tradeoff that an organism uses under its local conditions to achieve a balance between early maturation and fast growth that maximizes fitness. Both of these characteristics are related to fitness, because each influences lifetime reproductive output. Because increased energy gain should reduce the severity of the life history tradeoffs, any mutation that increased the efficiency of energy gain, such as feeding in a particular habitat, is expected to have increased reproductive output and should be favoured by selection. In this way, life history performance can perhaps be used to reflect feeding efficiency integrated over the lifespan of an individual. My goal was to test for an association between phenotype and fitness using these life history traits as a reflection of individual feeding performance, in order to determine the pattern of selection currently operating among forms of sunfish that are trophically polymorphic.

Optimal life history tactics are expected to vary between habitats that pose potentially different feeding and predation challenges, such as between the littoral and pelagic habitats of many postglacial lakes (Schluter 1995). However, if phenotype

influences foraging efficiency, then I additionally expect that the life history performance of an individual in a specific habitat will be related to phenotype. The results of Chapter 2 suggested a relationship between phenotype and fitness that directionally favoured pelagic-like pumpkinseeds in both pelagic and littoral habitats of Ashby Lake. However, I was unable to determine whether this fitness advantage persisted throughout the year and perhaps among years, or whether it was a temporary and perhaps reversible seasonal pattern of selection. For example, an early spring plankton bloom could be responsible for directional selection favouring pelagic body forms that was subsequently reversed by an abundance of prey in the littoral zone over the summer. If directional selection reverses on a seasonal timescale depending on the relative abundance of littoral and pelagic resources, then pelagic forms may not have any advantage over littoral forms when fitness is integrated over longer time periods. If life history performance reflects the conditions experienced by an individual pumpkinseed over its lifetime, then it should be possible to use it in order to test whether fitness is related to body form over the longer-term of an individual's lifespan.

The objective of this study was to measure the relationship between phenotype and life history performance within habitats, where predation risk and other environmental factors that influence life history responses (such as prey abundance) are likely to be more constant than between habitats. Patterns of selection within habitats can then be compared between the littoral and pelagic habitats to assess the shape of the fitness landscape experienced by the pumpkinseed population in Ashby Lake. I assumed a causal relationship between phenotypic variation and life history characteristics that was similar to that described in Chapter 2. In trophically polymorphic fish where multiple forms coexist in a single lake, I expect that phenotypic specialization will result in greater

feeding efficiency within a particular habitat, and in turn increased energy intake for specialized forms in their native habitat. Phenotypic and ecological specialization should be favoured by selection as it would reduce the energetic constraints, thereby reducing the severity of life history tradeoffs between growth and reproduction. This would result in increased reproductive output through earlier age at maturation and/or increased fecundity resulting from more rapid growth (Bertschy and Fox 1999, Stearns 1976). An additional positive effect of higher growth may be an earlier refuge from size-specific predation (Belk and Hales 1993, Hambright 1991, Werner et al. 1983). If phenotypic variation is related to variation in lifetime reproductive output, then selection should favour the most reproductively fit phenotypes. As outlined in Chapter 2, the pattern of selection acting within any population will depend on the relationship between body form and fitness in the littoral and pelagic habitats. Specifically, the fitness relationship between the two most specialized forms and their related intermediate forms within and between habitats will result in an overall fitness landscape consistent with stabilizing selection (if intermediate forms are found to be more fit than either native specialists), disruptive selection (if native specialists are more fit than intermediate forms in each habitat), or directional selection (if only one native form is generally favoured in both lake habitats).

I tested the null hypothesis of no relationship between phenotype and life history performance within habitats. This was accomplished by associating life history performance (mean size/age at maturity and growth rate) with phenotype in each habitat, and then comparing these relationships between littoral and pelagic habitats. No association between life history response and phenotype within habitats would result in the conclusion that there is little evidence that relative fitness varies in a consistent

fashion among pumpkinseed body forms, and hence little evidence of selection acting on body form in the system. A relationship between phenotype and life history performance within habitats would indicate that selection is acting in the population, enabling further examination of the relationship between phenotype, life history, and habitat, according to the alternative selection hypotheses outlined above and in Chapter 2. Rejection of some of these alternative hypotheses may permit a distinction between long term patterns of stabilizing, disruptive and directional selection that may be operating in Ashby Lake. More importantly, these results may indicate whether directional selection favouring pelagic phenotypes (Chapter 2) operates only over the short-term (eg. representing seasonally based selection) or over the long-term (eg. over many seasons and years).

Methods:

Study Location and Sample Collection

This analysis used the non-reproductive sample of pumpkinseeds that were also used in Chapter 2. Samples were collected during the first 10 days of the reproductive season in the spring of 2000 from 5 littoral and 4 pelagic sites, totalling 178 littoral fish (female n=72, male n=106) and 144 pelagic fish (female n=57, male n=87) between the ages of 2 and 9 years old.

Morphological Analysis

Morphological variation for each sex was quantified as in Chapter 2 using a combination of partial warp values from a thin-plate spline technique (Bookstein 1991) based on 15 homologous landmarks on the left side of every fish and gill raker length, in

order to collectively assess both external and internal traits thought to be related to habitat-specific performance. Partial warp values and residual average gill raker length were combined in a multivariate discriminant function analysis (DFA) separately for each sex, in order to quantify morphological differences between the two habitats. Individual gill raker length was measured from the tip to the upper and lower insertion points, then natural logarithm transformed and regressed against centroid body size. Individuals of each sex were classified into habitat-specific 'specialist' or 'intermediate' forms (to relate variation in body form to life history characteristics within each habitat group). This was accomplished by dividing the entire range of morphological scores (DFA values) into 4 equal quarters separately for each sex. In the littoral origin group, individuals falling within the lowest 1/4 of the total range of DFA scores were designated as littoral specialist (L), while individuals above this range (more pelagic-like) were designated as intermediate littoral (I_L). Conversely, pelagic origin individuals in the upper 1/4 of the total range were designated as pelagic specialist (P), while those with values lower than this range (more littoral-like) were designated as intermediate pelagic (I_P). These four morphological categories (hereafter referred to as forms) were made separately for each sex, and were used in all further analyses.

Calculation of Life History Characteristics

After digital images were taken for analysis of body form, all pumpkinseeds were dissected to determine sex and maturity status as in Deacon and Keast (1987). Mature males were identified as having thicker, opaque gonads compared to the translucent and threadlike testes of immature males. Mature females were identified by the presence of larger, yolked eggs in the ovary in addition to small developing eggs. Spent females

(having already spawned that spring) were easily identified by the presence of remnant

developed eggs, and so were classified as mature (M. Fox, personal communication,

Gillespie 2000). Maturity was assessed on two occasions separated by 90 days, with the

second scoring of maturity blind to the previous results. The two results were then

compared, and all individuals with discrepancies were viewed a third time (14 of 321

samples=4.4%). Age of each individual was determined from acetate impressions of 8-12

scales removed from the left-hand side of each individual approximately 5 rows below

the lateral line, in an area surrounding the caudal tip of the pectoral fin when laid parallel

to and below the lateral line. Annuli were identified and counted on one representative

scale from each fish, and the distance from the origin to each annulus and to the scale

margin were measured (Michael Fox, personal communication; Reiger 1962). Age was

scored independently by two individuals, providing results with over 99% agreement.

Standard length at age of each individual was calculated using the Fraser-Lee method

according to Carlander (1982):

$$L_i = a + \frac{(L_c - a)}{S_c} S_i$$

where L_i is the estimated standard length at age i, L_c is the standard length at capture, S_c is

the radius of the scale at capture and S_i is the distance to the annulus at age i. The

intercept of the rectilinear body-scale regression is a. For this analysis, L_c was estimated

from truss measurements based on landmarks used in the geometric morphometric

analysis. Length at age was only backcalculated for individuals age 4 or less to avoid

Lee's phenomenon (Bagenal and Tesch 1978), where length at age is underestimated due

to the selective earlier mortality of faster growing individuals in the population.

Life History Analysis

Mean age at maturity was calculated for each form and sex using the following formula adapted from DeMaster (1978):

$$\alpha = \sum_{x=0}^{n} (x)[k(x) - k(x - 1)]$$

where α is the average age at maturity within the population (age at which 50% of the population is mature), x is the age class in years (to the maximum age in the sample, n), and $k(x)$ is the proportion of the sample mature at age x. Mean length at age was also calculated using a modification of the above formula by Trippel and Harvey (1987) that uses 10mm size classes rather than age. Standard errors of mean age and length at maturity estimates were calculated as (G. Hines, personal communication):

$$SE = \frac{\sum_{x=0}^{n} (x)^2 \cdot \text{var}[k(x) - k(x - 1)] + [k(x) - k(x - 1)]^2 \cdot \text{var}(x)}{\sqrt{s}} \quad \text{Where } SE \text{ is the}$$

standard error of the mean maturity estimate for a particular group, s is the total sample size for that group, x is the age or size class, $k(x)$ is the proportion of mature individuals in that class, and n is the total number of age or size classes.

Growth Data

Backcalculated standard length at age was compared among body forms for each age class (1-4) using analysis of variance (ANOVA). Multiple comparisons among forms

were performed using a protected Fisher LSD test (eg. only after demonstrating a significant overall effect of phenotype).

Growth rate was compared among the four forms for each sex separately by testing the effects of body form category and age on standard length (natural logarithm transformed) using ANCOVA. Differences in growth rates among forms were indicated by a significant interaction between body form category and age in the model. This effect represents differences among the slopes of the length-age relationships of the four body forms, that were further examined using a multiple comparison of slopes test described in Zar (1999, p.372).

Results

Morphology

Significant differences in DFA scores (reflecting external morphology and gill raker length) were found between littoral and pelagic samples for both males and females. In females, 94% of 72 littoral individuals and 95% of 57 pelagic individuals were correctly classified to habitat ($F_{28,99}$=8.58; $p<0.0001$; Wilk's $\lambda = 0.285$). In males, 92% of 105 littoral individuals and 92% of 87 pelagic individuals were correctly classified to habitat ($F_{29,162}$=12.2; $p<0.0001$; Wilk's $\lambda= 0.314$). Canonical loadings of the morphological variables in male and female DFAs are shown in Appendix 3, while the characteristics of th pelagic and littoral groups are summarized in Table 3.1.

The classification of individuals from each habitat into either specialist or intermediate forms is shown for females in Figure 3.1 and for males in Figure 3.2.

Characteristics and sample sizes for each group are given in Table 3.2. Classification in

both sexes resulted in specialized forms consistent with the morphometric results in

Chapter 1. Female and male littoral specialists displayed an anteriorly exaggerated

phenotype with larger head regions, a shortening and reduction in height of the posterior

body region (Figures 3.3 and 3.4), and significantly shorter gill rakers than intermediate

phenotypes (t-test on size-free residual gill raker length: females: $t=-3.28$, $v=70$, $p=0.002$;

males: $t=-3.73$, $v=103$, $p=0.003$; Figure 3.5). Pelagic specialists of each sex displayed a

more rear-exaggerated phenotype with a smaller head, and enlarged body posterior

(Figures 3.3 and 3.4), as well as significantly longer gill rakers in pelagic males than in

corresponding intermediate phenotypes (t-test on size-free residual gill raker length:

females: $t=1.73$, $v=55$, $p=0.09$; males: $t=4.90$, $v=85$, $p<0.0001$; Figure 3.5). Average

body form of the two intermediate forms (I_L and I_P) was weakly biassed towards their

respective specialists. Otherwise, intermediate phenotypes were most similar to the

average form of the population (Figures 3.3 and 3.4). Comparison of mean body form

(DFA score) indicated that the two habitat-specific intermediate groups were different

from each other, however the associated R^2 values (particularly in males) suggested that

these groups were highly variable, and overlapped to a large extent (ANOVA on DFA

scores between habitat groups: females: $F_{1,73}=126.2$, $p<0.0001$, $R^2=0.63$; males:

$F_{1,108}=72.9$, $p<0.0001$, $R^2=0.40$).

Maturity

Littoral females were on average both younger and smaller at maturity than

pelagic females (Table 3.1). In the littoral sample, specialist forms (L) matured on

average at a later age but at approximately the same size as intermediate forms (I_L) (Table 3.2). In the pelagic zone, I found the opposite trend, where specialist forms (P) matured at a younger age and a slightly smaller size than intermediate forms (I_P)(Table 3.2). Males from the littoral zone were found to mature at an older age, but at approximately the same size as pelagic males (Table 3.1). Trends in maturity for males were similar to those in females, where specialist forms (L) matured on average later but at approximately the same size as intermediate forms (I_L) (Table 3.2). In the pelagic zone, specialist forms (P) and intermediate forms (I_P) matured at approximately the same age and size (Table 3.2).

Although the above estimates are commonly used for quantitative comparison of size or age at maturity, no formal statistical methods have been developed for their comparison. Calculations of standard errors associated with these maturity estimates have not previously been applied in published studies that compared age/size at maturity among groups. I use them here to better judge the relative precision of my estimates. Standard errors associated with estimates of size at maturity were very large (Table 3.2), possibly because this estimate used relatively more categories than did age at maturity (resulting in smaller sample sizes in each category). For this reason, mean age at maturity was assumed to be a more reliable estimator of maturation rate for the purposes of this study.

Growth

Backcalculated length at age was based on a significant linear body length-scale regression with an estimated body length intercept of 27.7mm (R^2=0.85, p<0.0001) (Figure 3.6), compared to the value of 25mm found in the literature for pumpkinseed sunfish (Carlander 1982). In both male and female samples, ANOVA indicated that backcalculated standard length at ages 1 through 4 was greater in the pelagic than littoral

samples (Table 3.3, Figure 3.7). These differences became significantly larger with increasing age, as indicated by significantly greater growth rates in the pelagic zone for both sexes (ANCOVA habitat by age interaction effect on standard length (natural logarithm transformed); females: $F_{1,259}=29.3$; $p<0.0001$, males: $F_{1,416}=36.9$; $p<0.0001$)(Table 3.1).

I found significant size at age effects between the 4 female forms in age classes 1 through 4 (Table 3.4a, Figure 3.8a). These differences increased with age, indicating significant growth rate effects (ANCOVA morphotype by age interaction effect on standard length (natural logarithm transformed): $F_{3,255}=14.0$; $p<0.0001$). In the littoral sample, there were no differences among forms in length at ages 1 and 2, after which intermediate forms (I_L) were larger than specialists (L) (Table 3.4a, Figure 3.8a). Pairwise growth rate comparisons indicated significantly higher growth in littoral intermediate forms (I_L)(Figure 3.9a). In the pelagic zone there were no significant differences in length at age between forms (Fig. 3.8a, Table 3.4a), but pelagic specialist forms (P) had significantly higher growth rates than pelagic intermediate forms (I_P) (Figure 3.9a).

In males, I found significant effects of body form on size at ages 2 through 4 (Table 3.4b, Figure 3.8b). These effects also corresponded to differences in growth rates among male morphs (ANCOVA morphotype by age interaction effect on standard length (natural logarithm transformed): $F_{3,412}=12.6$; $p<0.0001$). In the littoral zone, there were no significant differences in either length at age nor growth rate between specialist (L) and intermediate (I_L) forms (Tables 3.4b, Figures 3.8b, 3.9b). In the pelagic zone, specialists had significantly greater length at ages 2 - 4 (Table 3.4b), but not a significantly higher growth rate (Figure 3.9b).

Form of Selection Indicated by Life History Performance

Fitness measured as life history performance was associated with body form within habitats, suggesting that selection is operating on pumpkinseed phenotypes in Ashby Lake. My results also indicated that different life history tactics were being used between littoral and pelagic habitats. I estimated differences in overall fitness with respect to life history performance between habitats by assessing both age at maturity and growth rate simultaneously for each body form and sex. Results in both females and males indicated that the fitness of pelagic specialist forms was highest, suggesting that directional selection may favour this body form. In females, overall growth was highest in the pelagic zone, where specialist forms (P) grew faster and matured earlier than intermediate forms (I_P)(Table 3.3). In the littoral zone where overall growth rates were lower, intermediate forms (I_L) (the more pelagic-like forms within the littoral zone) had both significantly faster growth rates and earlier maturity than the native specialist form (L), suggesting an overall fitness advantage for littoral intermediate forms (I_L)(Figure 3.10a). A comparison of the two intermediate forms indicated that earlier maturity in the littoral zone was traded off against faster growth in the pelagic zone in females (Figure 3.10a). Mean male growth was greater in pelagic than in littoral forms, while age at maturity was nearly the same between habitats (Table 3.3). In the pelagic habitat, specialist forms (P) had significantly higher growth rates and similar maturity to intermediate forms (I_P), suggesting higher fitness for the specialist forms (P)(Figure 3.10b). In the littoral zone, where overall growth rates were lower, intermediate forms (I_L) may have higher fitness than specialists (L), because of earlier maturity without a significant reduction in growth rate (Figure 3.10b). I found significantly higher growth

rates and earlier maturity in the pelagic male intermediate forms (I_P) compared to male littoral intermediate forms (I_L), suggesting an overall fitness advantage for males using the pelagic habitat (Figure 3.10b), in contrast to the strong life history trade-off found between the two intermediate forms in females (Figure 3.10a).

Discussion

Patterns of Selection Within Ashby Lake

Life history performance was related to phenotype in both sexes, leading to rejection of the null hypothesis of no relationship between life history response and phenotype. This means that certain pumpkinseed forms achieve better life history performance than others. This may cause variation in lifetime reproductive output among forms within the population, thus allowing for selection to favour the most reproductively fit pumpkinseed forms. I then returned to the 3 alternative selection hypotheses outlined in Chapter 2, to determine which pattern of selection was most consistent with the data. I found no evidence of significant interactions between phenotype and life history response between habitats, prompting me to further reject that stabilizing or disruptive selection are acting in Ashby Lake (H_A #1, #2, Chapter 2). Rather, both males and females with more pelagic-like body forms had life history performances equal to or better than those with more littoral-like body forms in both habitats, suggesting that directional selection favours pelagic-like phenotypes in Ashby Lake.

In females, fitness measured as life history performance was highest for specialist forms in the pelagic zone (P) compared to specialists in the littoral zone (L)(Figure 3.10a). However, a tradeoff between growth and age at maturity was apparent between

the two female intermediate forms (I_L, I_P), making a comparison of their relative fitnesses difficult. This comparison was further complicated by evidence of significant morphological differences between these forms, although their morphological distributions overlap considerably (Figure 3.3, 3.4). This overlap in body form may allow the intermediate groups to have roughly equivalent feeding performance if placed in the same habitat. However, a fitness comparison between intermediate forms measured through life history performance is difficult because the two habitats may vary with respect to resource availability and predation risk, both of which are known to influence life history responses (Belk and Hales 1993, Deacon and Keast 1987). It is possible that female intermediate forms are using alternate life history tactics while more or less achieving similar overall fitness (Figure 3.10a). This trade-off between habitats was not simply a result of comparing age at maturity, because a similar but less distinct tradeoff was found between size at maturity and growth in females (Figure 3.11a). Although it was not possible to directly compare the fitnesses of intermediate forms, their implied habitat-related life history performances are consistent with predictions resulting from variation in predation risk between habitats. Belk and Hales (1993) demonstrated that increased predation risk imposed by largemouth bass favoured faster growth at the expense of delayed maturation in bluegill sunfish. This is consistent with the tactic adopted by the pelagic intermediate forms (I_P), where faster growth may provide a size refuge from predation. In contrast, sunfish in the littoral zone may face less predation risk because vegetative cover may provide a greater refuge from predation, and predators may target other fish prey species that are not observed in the pelagic zone. Hence, earlier maturity may be favoured over faster growth in the littoral zone because reduced predation risk provides an opportunity to mature earlier.

The life history performance of males also suggested that directional selection favours pelagic-like forms, although without the apparent complexity of possible life history trade-offs between habitats as observed in females. While less life history variation occurred within both the littoral and pelagic habitats, my results clearly show a fitness advantage in the form of faster growth and earlier maturity in the pelagic zone compared to the littoral zone (Figures 3.10b). Pelagic forms also had the highest fitness considering both growth rate and size at maturity (Figure 3.11b).

Fitness measured as life history performance here and as seasonal reproductive timing in Chapter 2 suggest that selection may act more strongly on phenotype in females than in males. One explanation for this is that energy acquisition may be more closely related to fitness in females because fecundity is positively related to body size (Stearns and Koella 1986). In males, alternate reproductive strategies (Ehlinger et al. 1997) and variation in parental care can make it difficult to predict life history responses under different levels of resource availability. Preliminary observations in Ashby Lake pumpkinseeds, however, show no evidence of cuckolding males (personal observation). Furthermore, all four male forms mature between 69 and 74mm standard length (unpublished results), which suggests a single minimum optimal size for male parental care (Table 3.2). These observations suggest that female fitnesses may vary more between littoral and pelagic habitats than do those of males, resulting in stronger selection on female phenotypes and perhaps different life history tactics in each habitat.

Long-term Selection in Ashby Lake

Pelagic-like pumpkinseed phenotypes in this study had greater life history performance than other phenotypes, and pumpkinseeds that used the pelagic habitat

(regardless of body form) appeared to have a fitness advantage over others that did not. This advantage was present despite several important differences in life history tactic predicted for pumpkinseeds in pelagic compared to littoral habitats. Male and female growth rates were both significantly higher in the pelagic than in littoral habitats (Table 3.1). Faster growth and larger size at maturity have been predicted and observed in response to predation risk in other sunfish systems (Belk and Hales 1993, Gillespie 2000). In Ashby Lake, smallmouth bass (*Micropterus dolomieui*) and the common loon (*Gavia immer*) have both been observed on and around the shoals where the pelagic samples were collected, and represent predation risks that target smaller individuals (Barr 1973, Hambright 1991, Werner et al. 1983). The reduced availability of cover on pelagic shoals in comparison to littoral habitats, and the almost complete absence of other fish prey species (personal observation) further intensifies the risk of predation and should result in stronger selection for fast growth in order to escape size-specific predation. The fact that this can be achieved in females without increasing age at maturity suggests greater nutrient and energy intake in pelagic compared to littoral habitats. Faster growth in pelagic pumpkinseeds is consistent with such a prediction made by Gillespie (2000), although the results of that study found an energy disadvantage for pelagic sunfish in Shadow Lake. While pelagic males did not mature at a larger size than littorals, they did mature earlier as a result of their faster growth rate (Figure 3.10b, Table 3.1). This relationship is similar to that observed by Fox (1994), where faster growth was correlated with earlier maturity in 27 southeastern Ontario pumpkinseed populations.

The results of this study parallel those of Chapter 2, where an analysis of seasonal reproductive timing also indicated that directional selection favours individuals with more pelagic body forms that use open water zooplankton resources. A fitness advantage

achieved through zooplanktivory by a nominally littoral species raises important questions regarding the long-term distribution of sunfish phenotypes in Ashby Lake and in similar polymorphic populations. One possible explanation is that the relative abundance of littoral and pelagic forms in a particular lake is proportional to the relative abundance of resources in each lake habitat (Robinson et al. 2000). Frequency dependent selection acting on phenotypes may only favour the stable coexistence of littoral and pelagic forms within a lake when both environmental niches are available over the long term (Wilson 1989). When either the littoral or pelagic niche is absent or severely limited (either by lack of resources or the presence of competing species), the overall pattern of selection may favour only a single phenotype best able to exploit the locally available niche. Under these conditions, an unstable polymorphism may coexist for a period of time as the distribution of phenotypes in a lake shifts from one form to another on a higher fitness peak. My results are consistent with this scenario because they indicate that directional selection in Ashby Lake is favouring a shift from a littoral-like ancestral form to a derived pelagic form. Such a process may not yet be completed in the relatively young populations that inhabit northern postglacial lakes (<15 000 years, Mandrak and Crossman 1992).

Life History Characteristics as Measures of Fitness

These results demonstrate that life history characteristics can be used as indirect measures of long-term performance perhaps related to foraging efficiency and morphology. Such measures can be used to study the evolution of phenotypic variation below the species level. This technique may be particularly useful in polymorphic populations characterized by only subtle variation in body form. Life history

characteristics, such as growth rate and age at maturity, should incorporate feeding performance over the entire lifetime of individuals, thus allowing the detection of fitness changes that are too weak to observe in short-term experiments that focus on growth rate over several weeks or months (Hatfield and Schluter 1999, Schluter 1993, Schluter 1994). Other advantages to using life history performance are that it reduces the risk of bias present when studying only a single cohort, and does not require the deployment of a massive amount of equipment to gain satisfactory sample sizes as required in field experiments. Nonetheless, large sample sizes are required to make precise estimates, particularly when samples are sub-divided by sex, habitat, and phenotype classes, as in this study.

My results suggest that life history performance varied in relation to resource availability and possibly predation risk between littoral and pelagic habitats in Ashby Lake. Such responses are more often associated with comparisons among isolated populations or related species that experience variation in resource availability or risk of predation (Belk 1998, Bertschy and Fox 1999, Deacon and Keast 1987, Reznick et al. 1996). This sensitive response to biotic factors among forms within a single population suggests that the patterns of selection observed in Ashby Lake may be different than that in other polymorphic sunfish populations, due to variation among lakes in the biotic factors listed above. It also suggests that evidence of trophic polymorphism is not necessarily evidence that disruptive selection currently maintains that polymorphism. From a different perspective, my results demonstrate that we can, in fact, measure selection on subtle phenotypic variation in the wild. This provides the opportunity to estimate the pattern of selection operating on similar phenotypes across many polymorphic populations where biotic characteristics such as predation risk and the

relative richness of littoral and pelagic habitat varies. Comparisons over multiple

populations will undoubtedly provide valuable insights into how selection creates and

maintains adaptive divergence below the species level.

General Discussion

I found strongly parallel patterns of morphological divergence among pumpkinseed populations known to use littoral and pelagic habitats in 3 lakes of the Mazinaw area of eastern Ontario, and among populations at a wider geographic scale. This indicates that selection favours habitat-specific divergence in body form. In a detailed study of one population, I also found strong evidence that selection favours pumpkinseeds with more pelagic-like body forms in both littoral and pelagic habitats.

In Chapter 1, my results demonstrated parallel trends in morphological divergence among 3 Mazinaw area pumpkinseed populations that was consistently related to differences in habitat use and diet. Additionally, trends in morphological specialization associated with littoral and pelagic habitat use demonstrated high correspondence across 26 trophically polymorphic populations at a wider geographic scale. This general pattern of morphological divergence is unlikely to be random and suggests that selection favours alternate morphological forms in littoral and pelagic lake habitats. While these repeated parallel patterns of divergence among many geographically isolated populations are consistent with the idea that littoral and pelagic forms have developed independently in each lake (eg. sympatric origin), I cannot confirm this without molecular genetic evidence that the populations are evolutionarily independent.

Regardless of origin, however, parallel patterns of divergence raise the question of how selection currently operates on the multiple forms of pumpkinseeds within single lakes. I used a novel approach to address how selection acts in a polymorphic sunfish population by measuring indices of the relative fitness of different phenotypes in each

habitat using traits expected to integrate the effects of subtle variation in feeding performance over longer periods of time.

Determining how selection operates in a polymorphic population using different lake habitats is key to understanding why the polymorphism exists. This subject has recently been of particular interest in the fishes inhabiting northern postglacial lakes, where mechanisms of divergent selection are thought to result in speciation in some cases. For this reason, there is interest in studying the mechanisms of morphological specialization in relation to habitat type between closely related forms of single species (where reproductive isolating mechanisms are not expected to be acting in addition to divergent ecological selection). However, the subtle differences between forms below the species level can prevent the measurement of the interaction between phenotype and habitat-specific performance if the latter cannot be measured with precision.

I approached this problem by attempting to measure habitat-specific performance integrated over the long-term, to compare indices of fitness among different pumpkinseed forms. In Chapter 2, I tested the relationship between phenotype and seasonal reproductive timing within habitats to assess whether specialized phenotypes could achieve higher reproductive fitness by being able to overcome overwinter energy deficits and reproduce earlier in the spring (presumably as a result of increased feeding efficiency). Earlier reproduction is expected to increase fitness by allowing more time for offspring to grow before their first winter (Cargnelli and Gross 1996, Danylchuk and Fox 1996). My results indicated that more pelagic-like phenotypes had an advantage over other forms in both habitats, suggesting that pelagic zooplankton resources may be a very important energy source early in the spring. However, these results could not distinguish if this process of directional selection acts only in the spring (short-term) or whether it

may continue throughout the year (long-term).

I attempted to distinguish between short- and longer-term patterns of selection in Ashby Lake by re-assessing the relative fitnesses of body forms in each habitat in a comparison of individual phenotype with life history performance. Life history performance represents the allocation of energy to growth and reproduction by an individual, and can be used to assess relative fitness if forms are compared within a common habitat so that predation risk and resource abundance are held constant. I predicted that higher feeding performance in more specialized forms would reduce the severity of tradeoffs between growth and reproduction, leading to an overall higher lifetime reproductive output. Again in Chapter 3, my evidence suggested a directional fitness advantage of more pelagic-like forms, regardless of habitat origin.

Both Chapters 2 and 3 suggest that natural selection is operating in Ashby Lake to favour body forms that are more efficient at using plankton resources and perhaps better at avoiding a presumably the higher risk of predation in the pelagic zone. Judging by the relatively greater abundance of pelagic habitat and many shoals that provide cover within it (in contrast to the scarcity of the littoral zone in Ashby Lake), my intuitive explanation is that the observed pattern of directional selection is related to differences in resource availability between habitats. This immediately raises the question: *what will happen to the distribution of pumpkinseeds in Ashby Lake over the long-term?* The results of this work suggest that the pumpkinseeds in Ashby Lake are taking advantage of plankton resources, possibly due to the relative abundance of these resources and limited competition from other planktivorous fish species. I expect that the fitness landscape in Ashby Lake currently favours a population-wide shift towards an open water form. That does not necessarily imply that the littoral form will become extinct in the future.

Currently, the relatively greater abundance of pelagic habitat and presumably pelagic resources favours individuals that can use it. However, phenotypic plasticity is likely an important characteristic of this population, which will allow the production of littoral forms that will use whatever littoral resources are most available.

Additionally, other fitness characteristics that I did not measure could be acting to favour the presence of some littoral phenotypes (ie. we may still need to study other indirect measures of fitness to realize the entire selective regime acting on forms within Ashby Lake). It is likely that in many northern postglacial lakes where species diversity is low , multiple forms of a single species will be favoured by the presence of multiple habitats. This model predicts that the relative abundance of littoral and pelagic forms that can coexist in a polymorphic population will be related to the relative abundance of resources available in each habitat. While the existence of multiple forms may be favoured, the selective pressures acting on each may change depending on the balance of resources between habitats. This effect may shift within lakes over time, or vary among lakes. This hypothesis needs to be tested, and the techniques used in Chapters 2 and 3 may make such a test feasible. A wide-scale comparison of the abundance of habitat-specific body forms and patterns of selection in lakes that vary with respect to the relative proportions of pelagic and littoral habitats and their resources may help us to better understand this relationship.

Without molecular genetic information, it is not yet possible to determine whether multiple forms of pumpkinseeds have sympatric or allopatric origins, and so my conclusions about selection relate only to the current maintenance of variation in Ashby Lake, and not to the origin of the variation. For example, if a single form had colonized Ashby Lake and multiple forms subsequently evolved in sympatry, then directional

selection may be favouring a shift in the population from the ancestral littoral form to a more derived pelagic form. Alternately, if littoral-like and pelagic-like forms evolved in allopatry and subsequently colonized Ashby Lake, then directional selection may be favouring the more pelagic form, even though both forms may be hybridizing to produce a continuous distribution of body forms. In other lakes, where both pelagic and littoral habitats are more equally abundant, either allopatric or sympatric origins could result in disruptive selection for the maintenance of alternate habitat-specific body forms. It is disruptive selection that has normally been of interest to studies focussing on divergence at higher taxonomic levels.

These possible scenarios, and the results of my studies, raise an important question about the general assumptions regarding morphological variation below the species level. There appears to be a tendency to equate intraspecific morphological divergence between littoral and pelagic lake habitats with disruptive selection, and the associated possibility of sympatric speciation (Skulason and Smith 1995, Smith and Skulason 1996, Schluter 1996b). The occurrence of morphological variation at different taxonomic levels has thus been viewed as representing different states along an evolutionary trajectory of divergence (Robinson and Schluter 2000). My results suggest that this may not always be the case, and that selection may be acting in different ways to yield similar patterns of morphological variation between littoral and pelagic habitats among different populations. For instance, my results indicate that directional selection favours pelagic-like phenotypes, leading to a transition in the Ashby Lake pumpkinseed population from an ancestral littoral form to a more derived pelagic form. Although we don't know to what extent this transition has occurred, or the rate of change in phenotype frequency in the population, the current distribution of phenotypes could be easily

assumed to be the result of disruptive selection.

Nonetheless, while this thesis suggests additional complexity in the mechanisms affecting intraspecific variation and that some assumptions concerning divergence below the species level may have to be re-evaluated with greater care, my work also suggests that selection is an important mechanism that influences patterns of phenotypic diversity below the species level. Lastly, this thesis demonstrates that we have the tools to better understand how ecological factors influence how diversity arises and changes in single populations composed of subtley different phenotypes able to use a heterogenous environment.

References

Bagenal, T.B. and F.W. Tesch. 1978. Age and growth. Pages 101-136 *in* T.B. Bagenal (ed.). Methods for assessment of fish production in fresh waters. Third edition. IBP Handbook Number 3. Blackwell Scientific, England.

Barr, J.F. 1973. Feeding biology of the common loon (*Gavia immer*) in oligotrophic lakes of the Canadian Shield. Ph.D. Thesis. University of Guelph, Guelph, Ontario. 204p.

Belk, M.C. 1998. Predator-induced delayed maturity in bluegill sunfish (*Lepomis macrochirus*): Variation among populations. Oecologia 113: 203-209.

Belk, M.C., and L.S. Hales, Jr. 1993. Predation-induced differences in growth and reproduction of bluegills (*Lepomis macrochirus*). Copeia 1993(4): 1034-1044.

Bell, M.A. 1984. Evolutionary phenetics and genetics: the threespine stickleback, *Gasterosteus aculeatus*, and related species. *In* Turner, B.J., ed. Evolutionary genetics of fishes. Plenum Press, NY, USA.

Bernatchez, L., J.A. Vuorinen, R.A. Bodaly, and J.J. Dodson. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). Evolution 50: 624-635.

Bertschy, K.A, and M.G. Fox. 1999. The influence of age-specific survivorship on pumpkinseed sunfish life histories. Ecology 80: 2299-2313.

Bookstein, F.L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge, UK.

Bourke, P., P. Magnan, and M.A. Rodriguez. 1997. Individual variations in habitat use and morphology in brook charr. Journal of Fish Biology 51: 783-794.

Bronmark, C. and L.B. Pettersson. 1994. Chemical cues from piscivores induce a change in morphology in crucian carp. Oikos 70: 396-402.

Cargnelli, L.M. and M.R. Gross. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). Canadian Journal of Fish and Aquatic Sciences 53: 360-367.

Carlander, K.D. 1982. Standard intercepts for calculating lengths from scale measurements for some centrarchid and percid fishes. Transactions of the

American Fisheries Society 111: 332-336.

Danylchuk, A.J. and M.G. Fox. 1994a. Seasonal reproductive patterns of pumpkinseed (*Lepomis gibbosus*) populations with varying body size characteristics. Canadian Journal of Fisheries and Aquatic Sciences 51: 490-500.

Danylchuk, A.J. and M.G. Fox. 1994b. Age and size-dependent variation in the seasonal timing and probability of reproduction among mature female pumpkinseed, *Lepomis gibbosus*. Environmental Biology of Fishes 39: 119-127.

Danylchuk, A.J. and M.G. Fox. 1996. Size- and age-related variation in the seasonal timing of nesting activity, nest characteristics, and female choice of parental male pumpkinseed sunfish (*Lepomis gibbosus*). Canadian Journal of Zoology 74: 1834-1840.

Deacon, L.I., and J.A. Keast. 1987. Patterns of reproduction in two population of pumpkinseed sunfish, *Lepomis gibbosus*, with differing food resources. Environmental Biology of Fishes 19: 281-296.

DeMaster, D.P. 1978. Calculation of the average age of sexual maturity in marine mammals. Journal of the Fisheries Research Board of Canada 35: 912-915.

Dynes, J., P. Magnan, L. Bernatchez, and M.A. Rodriguez. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. Journal of Fish Biology 54: 955-972.

Ehlinger, T.J. and D.S. Wilson. 1988. Complex foraging polymorphism in bluegill sunfish. Proceedings of the National Academy of Sciences, USA 85: 1878-1882.

Ehlinger, T.J., M.R. Gross, and D.P. Philipp. 1997. Morphological and growth rate differences between bluegill males of alternative reproductive life histories. North American Journal of Fisheries Management 17: 533-542.

Fox, M.G. 1994. Growth, density, and interspecific influences on pumpkinseed sunfish life histories. Ecology 75: 1157-1171.

Gillespie, G.J. 2000. Eco-morphological and reproductive isolation in populations of pumpkinseed (*Lepomis gibbosus*). M.Sc. Thesis, Trent University, Peterborough Ontario. 113 p.

Hambright, K.D. 1991. Experimental analysis of prey selection by largemouth bass: Role of predator mouth width and prey body depth. Transactions of the American Fisheries Society 120: 500-508.

Hatfield, T. and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution: 53: 866-873.

Helfman, G.S., B.B. Collette, and D.E. Facey, Eds. 1997. The diversity of fishes.

Blackwell Science, Inc. USA.

Johnson, L. 1980. The Arctic charr, *Salvelinus alpinus*. Pages 15-98 *in* E.K. Balon, (ed.)
　　　　Charrs: Salmonid fishes of the genus *Salvelinus*. Dr. W. Junk, The Hague,
　　　　Netherlands.

Justus, J.A. and M.G. Fox. 1994. The cost of early maturation on growth, body condition
　　　　and somatic lipid content in a lake pumpkinseed (*Lepomis gibbosus*) population.
　　　　Ecology of Freshwater Fish 3: 9-17.

Keast, A. 1977. Mechanisms expanding niche width and minimizing intraspecific
　　　　competition in two centrarchid fishes. Evolutionary Biology 10: 333-395.

Keast, A. 1978. Feeding interrelations between age-groups of pumpkinseed (*Lepomis
　　　　gibbosus*) and comparisons with bluegill (*L. macrochirus*). Journal of the
　　　　Fisheries Research Board of Canada 35: 12-27.

Lauder, G.V. 1983. Functional and morphological bases of trophic specialization in
　　　　sunfishes (Teleostei, Centrarchidae). Journal of Morphology 178: 1-21.

Malmquist, H.J. 1992. Phenotypic-specific feeding behaviour of two arctic charr
　　　　Salvelinus alpinus morphs. Oecologia 92: 354-361.

Mandrak, N.E. and E.J. Crossman. 1992. Postglacial dispersal of freshwater fishes into
　　　　Ontario. Canadian Journal of Zoology 70: 2247-2259.

McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (Gasterosteus):
　　　　Morphological and genetic evidence for a species pair in Enos Lake, British
　　　　Columbia. Canadian Journal of Zoology 62: 1402-1408.

Mittelbach, G.G, C.W. Osenberg, and P.C. Wainwright. 1999. Variation in feeding
　　　　morphology between pumpkinseed populations: Phenotypic plasticity or
　　　　evolution? Evolutionary Ecology Research 1: 111-128.

Nagel, L. and D. Schluter. 1998. Body size, natural selection and speciation in
　　　　sticklebacks. Evolution 52: 209-218.

Nager, R.G., L.F. Keller, and A.J. Van Noordwijk. 2000. Understanding natural selection
　　　　on traits that are influenced by environmental conditions. Pages 95-115 *in*
　　　　Mousseau, T.A. B. Sinervo, and J.A. Endler (Eds.) Adaptive genetic variation in
　　　　the wild. Oxford University Press. New York.

Parker, H.H. 1997. Growth or reproduction: The life history gamble of landlocked Arctic
　　　　char. American Fisheries Society Symposium 19: 214-223.

Reiger, H.A. 1962. Validation of the scale method for estimating age and growth of
　　　　bluegills. Transactions of the American Fisheries Society 91: 362-374.

Reznick, D.N., M.J. Butler IV, F.H. Rodd, and P. Ross. 1996. Life history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. Evolution 50: 1651-1660.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Ridgway, M.S., B.J. Shuter, and E.E. Post. 1991. The relative influence of body size and territorial behaviour on nesting asynchrony in male smallmouth bass, *Micropterus dolomieui* (Pisces: Centrarchidae). Journal of Animal Ecology 60: 665-681.

Robinson, B.W. 2000. Trade-offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. Behaviour 137: 865-888.

Robinson, B.W. and D. Schluter. 2000. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. Pages 65-94 *in* Mousseau, T.A. B. Sinervo, and J.A. Endler (Eds.) Adaptive genetic variation in the wild. Oxford University Press. New York.

Robinson, B.W. and D.S. Wilson. 1994. Character release and displacement in fishes: A neglected literature. The American Naturalist 144: 596-627.

Robinson, B.W. and D.S. Wilson. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evolutionary Ecology 10: 631-652.

Robinson, B.W., D.S. Wilson, A.S. Margosian, P.T. Lotito. 1993. Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. Evolutionary Ecology 7: 451-464.

Robinson, B.W., D.S. Wilson, and A.S. Margosian. 2000. A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). Ecology 81: 2799-2812.

Robinson, B.W., D.S. Wilson, and G.O. Shea. 1996. Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes. Ecology 77: 170-178.

Roff, D.A. 1984. The evolution of life history parameters in teleosts. Canadian Journal of Fisheries and Aquatic Sciences 41: 989-1000.

Rohlf, F.J. 2000. Tps regression v.1.2. Free computer software for exploring the relationship between shape and one or more independent variables. Ecology and Evolution, SUNY at Stoney Brook. Available online at: http://life.bio.sunysb.edu/morph/

Rohlf, F.J. 2001a. Tps Dig v.1.28. Free computer software for collecting landmark data from images. Ecology and Evolution, SUNY at Stoney Brook. Available online at: http://life.bio.sunysb.edu/morph/

Rohlf, F.J. 2001b. Tps relative warps v.1.22. Free computer software for calculating partial and relative warp scores from landmark data. Ecology and Evolution, SUNY at Stoney Brook. Available online at: http://life.bio.sunysb.edu/morph/

Rundle, H.W. and D. Schluter. 1998. Reinforcement of stickleback mate preferences: Sympatry breeds contempt. Evolution 52: 200-208.

Sanderson, S.L., J.J. Cech, and M.R. Patterson. 1991. Fluid dynamics in suspension-feeding blackfish. Science 251: 1346-1348.

Sandlund, O.T., B. Jonsson, H.J. Malmquist, R. Gydemo, T. Lindem, S. Skulason, S. Snorrason, and P.M. Jonasson. 1987. Habitat use of arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. Environmental Biology of Fishes 20: 263-274.

Schluter, D. 1993. Adaptive radiation in sticklebacks: Size, shape, and habitat use efficiency. Ecology 74: 699-709.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science 266: 798-800.

Schluter, D. 1995. Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. Ecology 76: 82-90.

Schluter, D. 1996a. Ecological speciation in postglacial fishes. Philosophical Transactions of the Royal Society of London, Series B 351: 807-814.

Schluter, D. 1996b. Ecological causes of adaptive radiation. The American Naturalist 148: S40-S64.

Schluter, D. 2000. Ecological character displacement in adaptive radiation. American Naturalist 156: S4-S10.

Scott, W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184. Ottawa.

Shuter, B.J. and J.R. Post. 1990. Climate, population variability, and the zoogeography of temperate fishes. Transactions of the American Fisheries Society 119: 314-336.

Skulason, S. and T.B. Smith. 1995. Resource polymorphisms in vertebrates. Trends in Ecology and Evolution 10: 366-370.

Smith, R.J.F. 1970. Control of prespawning behaviour of sunfish (*Lepomis gibbosus* and *Lepomis megalotis*) II. Environmental factors. Animal Behaviour 18: 575-587.

Smith, T.B. and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fish, amphibians and birds. Annual Review of Ecology and Systematics 27: 111-133.

Stearns, S.C. 1976. Life-history tactics: A review of the ideas. The Quarterly Review of Biology 51: 3-47.

Stearns, S.C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience 39: 436-445.

Stearns, S.C. and J.C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. Evolution 40: 893-913.

Taylor, E.B. and P. Bentzen. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in Northeastern North America. Evolution: 47: 813-832.

Trippel, E.A. and H.H. Harvey. 1987. Reproductive responses of five white sucker (*Catostomus commersoni*) populations in relation to lake acidity. Canadian Journal of Fisheries and Aquatic Sciences 43: 194-202.

Wainwright, P.C. 1996. Ecological explanation through functional morphology: The feeding biology of sunfishes. Ecology 77: 1336-1343.

Wainwright, P.C. and B.A. Richard. 1995. Predicting patterns of prey use from morphology of fishes. Environmental Biology of Fishes. 44: 97-113.

Wainwright, P.C. and S.S. Shaw. 1999. Morphological baisis of kinematic diversity in feeding sunfishes. Journal of Experimental Biology 202: 3101-3110.

Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. American Zoologist 24: 107-120.

Werner, E.E. 1977. Species packing and niche complementarity in three sunfishes. The American Naturalist 111: 553-578.

Werner, E.E. and D.J. Hall. 1976. Niche shifts in sunfishes: Experimental evidence and significance. Science 191: 404-406.

Werner, E.E. and D.J. Hall. 1979. Foraging efficiency and habitat switching in competing sunfishes. Ecology 60: 256-264.

Werner, E.E., J.F. Gilliam, D.J. Hall, and G.G. Mittelbach. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540-1548.

Wetzel, R.G. 1983. Limnology, second edition. Saunders College Publishing, USA.

Wilson, D.S. 1989. The diversification of single gene pools by density- and frequency-dependent selection. Pages 366-385 *in* Otte, D. and J.A. Endler (Eds). Speciation and its consequences. Sinauer Associates, Inc. USA.

Zar, J.H. 1999. Biostatistical Analysis. Prentice Hall. New York.

Appendix