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Microevolutionary processes in natural populations of the waterstrider *Aquarius remigis*

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A Thesis in
The Department of Biology

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Abstract

Microevolutionary processes in natural populations of the waterstrider *Aquarius remigis*

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Concordia University, 2000.

Traits are often assumed to be optimal, but evolution may be constrained by genetics, the adaptive landscape, development, and functional limitations. Optimal body size is determined by the net effect of selection acting throughout the life history of the organism. Body size is correlated between the sexes, and sexual size dimorphism (SSD) provides natural experiments in the evolution of traits constrained by genetic correlations. In this thesis I relate sex-specific fitness functions, opportunity for selection, and genetic correlations among body size components to the adaptive significance of SSD in the waterstrider *Aquarius remigis*. Laboratory studies have revealed significant genetic correlations among body size components but Lynch's (1999) method of "estimation in the absence of pedigree information" does not provide useful field estimates in this study, and the use of polymorphic genetic markers to improve these estimates remains untested. I use multiple regression to estimate fitness functions for total length and components of body size, and compare these with observed trait distributions. My analysis shows that female-biased SSD in total length is favoured in *A. remigis*, but evolution of total length is better understood through analysis of selection on body size components. Partitioning opportunity for selection, *I*, indicates that although the strongest selection reported in *A. remigis* occurs during the reproductive season, the greatest determinant of fitness through the adult stage is survival ability from eclosion to first reproduction. I suggest that while *I* is a useful adjunct to selection gradient analysis in studies of the adaptive significance of trait values, its most important contribution may lie in studies of the evolution and adaptive significance of life history components, which are themselves components of fitness. I find evidence of spatial and temporal heterogeneity in fitness functions, and conclude that variance in the pattern and intensity of selection on total length observed in this species may be due to interactions between phenotypic distributions of components of body size and locally variable fitness functions. This suggests that evolutionary equilibrium may be dynamic, and estimating selection in one population or generation may fail to detect long-term or broad-scale fitness functions actually driving trait evolution.

Acknowledgements

I am proud of the accomplishment that this thesis represents, but I am humbled by the assistance and support that I have received. Although it sounds like a cliché, I certainly would not have made it without that help. Dr. Daphne Fairbairn lured me out of a somewhat unfocused undergraduate degree and led me down the path that finally arrived here. I have the highest regard for her ability as a scientist, but it is her skill and dedication as a tutor that have made it possible to complete this project. I owe her a great deal as a supervisor and as a friend.

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Long before I came to Concordia University, my family helped to build in me many of the tools that I would rely on to succeed here. The memory of my parents, John and Thelma Ferguson, continues to inspire me, while my brothers Rob, Bruce, Neil, and Glen, and their families, have supported me at every turn. The future also inspires me, thanks to my fiancé, Barbara MacDonald. She has suffered through the long and uncertain road that I chose, and held my hand every step of the way. I cannot imagine those years without her, nor the future.

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Contributions of Authors

All chapters in this thesis were prepared for submission to peer-reviewed journals for publication. Dr. Daphne J. Fairbairn is the co-author of all three papers, being instrumental in planning and methodology as well as contributing to the material in the normal supervisory role. This thesis falls within Dr. Fairbairn's research program funded by the National Sciences and Engineering Research Council of Canada.

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General Introduction

Evolutionary biologists tend to think of traits as adaptive. That is, we tend to assume that a trait "helps its bearer to survive and reproduce" (Ridley, 1993, p. 330). More specifically, we often assume that traits are at an optimum (i.e. a change in the trait will tend to reduce fitness, Futuyma, 1998, p. 528) and are therefore at evolutionary equilibrium (Endler, 1986, p. 232; Price et al, 1987, p. 280; Charlesworth, 1990). Although evolution by natural selection is important in shaping organisms, it does not follow that all observed traits are necessarily optimal or even adaptive (Futuyma, 1998, pp. 355-356). Evolution by natural selection may be constrained, at least transiently, by genetics (e.g. genetic correlations, lack of genetic variance), the adaptive landscape (e.g. fitness 'valleys'), development (ontogeny), and functional limitations (e.g. due to the laws of physics) (Gould, 1977; Lande, 1980; Cheverud, 1984; Partridge and Endler, 1987; Price et al, 1987; Charlesworth, 1990; Arnold, 1992).

Body size is "one of the most important aspects of an animal's endowment" (Schmidt-Nielsen, 1984), and significant correlations have been found between body size and many life history traits (Peters, 1983; Schmidt-Nielsen, 1984). The optimum body size is therefore expected to be determined by the net effect of selection acting throughout the life history of the organism (Ralls, 1976; Price, 1984; Clutton-Brock, 1988b; Preziosi and Fairbairn, 1996). Sexual size dimorphism (SSD) is simply a difference in mean body size between males and females of a population at a given life history stage, usually sexual maturity. Such differences are found in many animal taxa (Reiss, 1989, pp. 91-128; Andersson, 1994, p. 252; Fairbairn, 1997). In endotherms, males are often larger than females, but in most ectotherms it is the females that are larger (Ralls, 1976; Arak, 1988; Reynolds and Harvey, 1994; Fairbairn, 1997). These differences have evolved despite the presence of genetic correlations between male and female body size (e.g. Price, 1984; Cowley and Atchley, 1988; Preziosi and Roff, 1998). Thus, SSD provides us with natural experiments on the evolution of traits constrained by genetic correlations.

To determine whether or not a SSD is at equilibrium, we may estimate the relationship between lifetime fitness and body size in males and females of a population of interest. These relationships (fitness functions) may then be compared to the distribution of body sizes in the sexes. If the SSD is at equilibrium, we expect the mean body size of each sex to be at the optimum for that sex (Arnold, 1992).

The waterstrider *Aquarius remigis* is well-suited to the study of the adaptive significance of SSD. It is a semiaquatic bug (Hemiptera: Gerridae) found in small rivers and streams across much of temperate and subtropical North America (Preziosi and Fairbairn, 1992; but see Gallant and Fairbairn, 1996, 1997). In this species females are about 10% larger than males in total length, but the pattern of SSD varies with different components of body size. In particular, females have longer abdomens, males have longer external genitalia, and the femora of the mid-legs are about equal in size (Fairbairn, 1992; Preziosi and Fairbairn, 1996, Appendix 4). Thus, we may examine different patterns of SSD within the same population.

Directional selection on total length and body size components has been found in certain life history stages in each sex (Fairbairn, 1988; Blanckenhorn, 1991a; Sih and Krupa, 1992; Kaitala and Dingle, 1993; Krupa and Sih, 1993; Fairbairn and Preziosi, 1994; Blanckenhorn et al., 1995; Sih and Krupa, 1995; Fairbairn and Preziosi, 1996; Preziosi and Fairbairn, 1996; Preziosi et al., 1996; Preziosi and Fairbairn, 1997). There is also evidence that total length was near an optimum, for net adult fitness, in both sexes in a study population (Preziosi and Fairbairn, 2000). However, patterns of selection on body size in this species appear to vary in both space and time (see Chapter 1).

This thesis examines microevolutionary processes in natural populations, using *A. remigis* as a model organism. Patterns of selection on specific traits in the wild are estimated and the adaptive significance of those traits is assessed. Organisms may experience changing environmental conditions from population to population and from generation to generation, and therefore spatial and temporal heterogeneity in fitness functions from natural populations is examined and related to evolutionary equilibrium in the traits under selection. The contributions of different life history stages to net adult fitness are estimated for these wild populations, and the consequences for microevolutionary processes are discussed. To put these processes in the context of possible genetic constraints, a new statistical method for estimating genetic correlations between traits in the field is employed.

In Chapter 1, we use multiple regression techniques to estimate sex-specific fitness functions for body size in two wild populations of *A. remigis* through two generations. These estimates are based on field data that include mark-recapture data, the results of fecundity experiments, and estimates of mating success that include the encounter rate between males and females. Separate fitness functions are estimated for each of three episodes of selection and for net adult selection. By comparing these fitness functions with the observed distributions of body size measurements, we assess the adaptive significance of SSD in total length and body size components in this species. We also assess spatial and temporal variance in patterns of selection, and discuss the role of selection on components of body size in determining the adaptive significance of SSD in total length.

The estimation of daily fecundity in the field necessitates confining females in small containers for at least 24 hours. Because the amount of food available to females in the wild is difficult to assess, the question arises as to how much food to provide to the confined females. In Appendix 2 we report the results of a laboratory experiment designed to determine whether or not daily fecundity estimated in such experiments depends on whether or not the females are fed. The results of this experiment suggest that the fecundities estimated for Chapter 1 were not influenced by the amount of food available during the experiment.

The analysis in Chapter 1 suggests that the relationship between fitness and total length is best understood as the net result of selection acting on different body size components. Because this selection on body size components is often specific to particular life-history stages, it is important to look at the relative contribution of each stage to net adult fitness. A useful statistic that may be partitioned among episodes of selection is the opportunity for selection, *I*, defined as the variance in relative fitness. Although most evolutionary biologists understand that *I* is an estimate of the potential for selection and not of actual selection on a trait, there remains some confusion as authors continue to use *I* as an estimate of the 'strength' of selection. In Chapter 2 we partition *I* among the three episodes of selection used in the selection analysis of Chapter 1, for each sex in each population and generation. The insights gained into selection on body size in

A. remigis are discussed, and the appropriate use of the opportunity for selection is clarified.

The adaptive significance of SSD is being examined in this thesis as an example of a trait evolving under constraints, specifically body size evolving in the presence of strong genetic correlations. It is generally assumed that male and female body size will be genetically correlated and this has been found in many species, including *A. remigis* (e.g. Price, 1984; Cowley and Atchley, 1988; Preziosi and Roff, 1998). However, given the importance of selection acting on specific components of body size, correlations between these components may constrain the evolution of body size in each sex. Genetic correlations among body components and between the sexes have been estimated in laboratory assays of *A. remigis* using standard half-sib (Preziosi and Roff, 1998), full-sib and offspring-parent designs (Fairbairn and Reeve, unpublished). Extending these results to the field can only be done with caution (Simons and Roff, 1994, 1996), but obtaining field estimates of genetic correlations has been extremely difficult. Lynch (1999) proposed two methods of estimating genetic correlations from field data. The second requires using polymorphic molecular markers to estimate of the relatedness of individuals in the sample ("Marker-assisted estimates"), but the first requires only measurements that may easily be made during a mark-recapture study ("Estimation in the absence of pedigree information"). The latter method is well-suited to studies of evolution such as this one, but has not been tested in the field. In Chapter 3 we estimate genetic correlations among body size components in the field using the "Estimation in the absence of pedigree information" method of Lynch (1999). The results are compared to the half-sib results of Preziosi and Roff (1998) and the full-sib results of Reeve and

Fairbairn (unpublished), and the potential utility of this new method is discussed.

Chapter 1. Sex-specific selection and sexual size dimorphism in the waterstrider *Aquarius remigis.*

Estimation of fitness functions for body size, comparison of these functions with current distributions of body size, and assessment of spatial and temporal variance in these functions comprise the core of this thesis. In this chapter, multiple regression is used to estimate patterns of selection on total length and components of body size for each sex. These patterns are then compared to the mean sizes in the population, and differences between populations and generations are examined. The adaptive significance of the sexual size dimorphism in *Aquarius remigis* is discussed.

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Abstract

We estimated selection on adult body size for two generations in two populations of *Aquarius remigis*, as part of a long term study of the adaptive significance of sexual size dimorphism (SSD). Net adult fitness was estimated from the following components: prereproductive survival, daily reproductive success (mating frequency or daily fecundity), and reproductive lifespan. Standardized selection gradients were estimated for total length and for thorax, abdomen, genital and mesofemur lengths. Although selection was generally weak and showed significant temporal and spatial heterogeneity, patterns were consistent with SSD. Prereproductive survival was strongly influenced by date of eclosion, but size (thorax and genital lengths in females; total and abdomen lengths in males) played a significant secondary role. Sexual selection favoured smaller males with longer external genitalia in one population. Net adult fitness was not significantly related to body size in females, but was negatively related to size (thorax and total length) in males.

Introduction

Sexual size dimorphism (SSD) is common (Reiss, 1989, pp. 91-128; Andersson, 1994, p. 252; Fairbairn, 1997) despite the generally high genetic correlation between male and female body size (e.g. Price, 1984; Cowley and Atchley, 1988; Preziosi and Roff, 1998), and therefore provides a useful system in which to study the evolution and maintenance of traits constrained by genetic correlations (Lande, 1980). Male-biased SSD is often attributed to sexual selection favouring large males (Darwin, 1890; Selander, 1972, p. 187; Andersson, 1994), while female-biased SSD is widely attributed to fecundity selection favouring large females (Darwin, 1890; Shine, 1988; Andersson, 1994). Such mechanisms, however, are insufficient to explain the existence of SSD since sexual selection for larger males has been detected in species with female-biased SSD (e.g. Howard, 1988; Shine, 1994; Fairbairn and Preziosi, 1994, 1996), while fecundity selection is a very general pattern (Darwin, 1890; Clutton-Brock, 1988a, p. 7; Roff, 1992,

p. 126; Honek, 1993) and is neither necessary nor sufficient to explain female-biased SSD (Arak, 1988; Hedrick and Temeles, 1989). Since SSD is just a difference in body size between the sexes, and body size is correlated with many life history traits (Peters, 1983; Schmidt-Nielsen, 1984), selection acting at different life history stages (i.e. different episodes of selection) may influence SSD. By extension, we expect differences between the sexes in lifetime selection on body size to result in SSD at equilibrium (Ralls, 1976; Price, 1984; Clutton-Brock, 1988b; Preziosi and Fairbairn, 1996), unless constrained by genetic correlations (Charlesworth, 1990).

We report estimates of selection on body size in the waterstrider *Aquarius remigis* (Hemiptera, Gerridae), and relate contemporary patterns of selection on males and females to the pattern of SSD. Three components of lifetime fitness were measured: survival from eclosion as adult to the reproductive season, daily reproductive success (mating frequency or daily fecundity), and reproductive lifespan. These were then combined in an estimate of fitness through the entire adult lifespan. This study extends and expands the longitudinal study of selection on body size in *A. remigis* reported in Preziosi and Fairbairn (1996, 1997, 2000). We present analyses of two more generations (1993-95), giving us estimates of selection through four consecutive generations (1991-95) in the same population. Analyses are also presented for two generations (1993-95) in a second population.

Study animal

Adult *A. remigis* are 10-17 mm long and live on the surfaces of streams and small rivers throughout most of temperate and subtropical North America (Preziosi and Fairbairn, 1992). As in most insects, females are larger than males, but components of body size display divergent patterns of SSD (Fairbairn, 1992; Preziosi and Fairbairn, 1996; Table 1.1, Appendix 4).

Table 1.1: Patterns of selection and sexual size dimorphism in *Aquarius remigis*. Dimorphism is reported as mean female size divided by mean male size (data from this study). Patterns of selection are taken from the literature.

Component of Size (length)	Dimorphism (F/M)	Pattern of Selection
total body	1.08	sexual selection for increased male size[1-8], fecundity selection for increased female size[1,9-12], selection through reproductive lifespan for smaller size in both sexes[8,10,12,13], stabilizing net adult selection in both sexes[13]
external genitalia	0.30	sexual selection for increased size in males[8]
abdomen	1.72	fecundity selection for increased size in females[11-12]
thorax	1.06	net adult selection for smaller size in females[13]
mesofemur	0.99	sexual selection for smaller size in males[7], net adult selection for larger size in females[13]

[1] Fairbairn, 1988; [2] Sih and Krupa, 1992; [3] Sih and Krupa, 1995; [4] Kaitala and Dingle, 1993; [5] Krupa and Sih, 1993; [6] Fairbairn and Preziosi, 1994; [7] Fairbairn and Preziosi, 1996; [8] Preziosi and Fairbairn, 1996; [9] Blanckenhorn, 1991a; [10] Blanckenhorn et al., 1995; [11] Preziosi et al., 1996; [12] Preziosi and Fairbairn, 1997; [13] Preziosi and Fairbairn, 2000

In southern Quebec, females lay eggs from late April through early July on rocks under water. Young climb to the surface and develop through five nymphal instars. Adults do not become reproductively mature until the following spring, emerging from overwinter diapause as soon as the waters become free of ice. Mortality over the winter is 70-90% (Fairbairn, 1985a; Blanckenhorn, 1994). Both sexes mate repeatedly with different partners (Krupa and Sih, 1993; Preziosi and Fairbairn, 1996). During mating, males ride on the back of females, often remaining in copula for many hours (Wilcox, 1984; Weigensberg and Fairbairn, 1994). The mean reproductive lifespan is about four weeks (Preziosi and Fairbairn, 1997), and all overwintered adults die before mid summer (Fairbairn, 1985a). *Aquarius remigis* move poorly overland, are rarely winged (≤3%), and when winged, seldom fly (Fairbairn, 1986; Fairbairn and Desranleau, 1987). Even on water, adult movements of greater than 100 m are rare in our study populations (Fairbairn, 1985b, 1986). This low mobility results in genetic isolation of populations on streams separated by as little as a few hundred metres (Preziosi and Fairbairn, 1992).

Previous evidence of selection on body size in Aquarius remigis

If SSD in *A. remigis* is adaptive, selection should differ between the sexes, favouring smaller males than females. Paradoxically, sexual selection favouring large body size in male *A. remigis* has been found in numerous studies (Table 1.1). However, such selection was lacking in some samples, and smaller males may occasionally be favoured through higher encounter rates with females (Krupa and Sih, 1993; Blanckenhorn et al., 1995). Multivariate analysis of selection on body components suggests that the general pattern of sexual selection favouring larger total length may actually be due to selection for greater genital length (which makes up about 20% of male total length), with selection on the rest of total length being either neutral or negative (Preziosi and Fairbairn, 1996). Such a pattern would be consistent with the pattern of SSD, with males having generally smaller bodies but much longer external genitalia than females.

Total length of females has been found to be positively associated with fecundity in a variety of experiments (Table 1.1). Multivariate analysis has revealed that fecundity selection actually targets abdomen length (Table 1.1), indicating that larger females lay more eggs because they have larger abdomens. Food availability also influences fecundity, and the influence of body size may be small or absent when food is limited (Blanckenhorn, 1991a; Blanckenhorn et al., 1995). Thus, fecundity selection favouring large female body size appears to be general in *A. remigis*, but is sensitive to food availability.

Body size may also influence SSD through differential prereproductive survival or reproductive longevity (Table 1.1). However, the lack of strong, general trends in either the pattern or intensity of selection revealed by these data suggests that selection through differential survival is variable in *A. remigis* populations.

Only Preziosi and Fairbairn (2000) have attempted to measure selection through the adult lifetime (hereafter net adult selection) in this species. They found significant stabilizing selection on total length in both sexes (Table 1.1), which they attribute to negative relationships between total length and reproductive lifespan combined with positive relationships between total length and daily reproductive success. However, absence of these relationships in the second generation of males suggested temporal variance in selection regimes. To assess the generality of the fitness trade-offs suggested in Preziosi and Fairbairn (2000), we have measured selection on body size for two additional generations and in a second population. We have also altered the assay of sexual selection to determine if the results are robust to a change in methodology. Whereas Preziosi and Fairbairn (1996) estimated sexual selection in artificial pools (as did Sih and Krupa, 1992; and Kaitala and Dingle, 1993), possibly reducing the influence of encounter rate on mate acquisition, we included encounter rate in our estimates of sexual selection by measuring mating frequency directly on the streams. Sexual selection may be divided into two stages: pre-encounter, when traits that maximize a male's chances of encountering a female are favoured, and post-encounter, when traits that maximize a male's chances of mating with the female encountered are favoured. Smaller males may be more efficient at finding females, and thus may be favoured in the first stage (Blanckenhorn et al, 1995). Studies in which mating success is measured in an artificial enclosure or even a small naturally occurring pond, where encounter rates are high, are mainly measuring selection in the second stage. Sexual selection for longer external genitalia in male *A. remigis* occurs in the second stage, when it has been hypothesized that males with longer external genitalia may be better able to subdue females (Preziosi and Fairbairn, 1996). If the first stage is important, then studying mating activity in a pool will provide an incomplete description of sexual selection.

Methods

We studied *A. remigis* on two streams at the McGill University Research Station on Mont St.-Hilaire, Quebec, Canada, about 35 km SE of Montreal (Figure 1.1), which support genetically distinct populations of *A. remigis* (Preziosi and Fairbairn, 1992). The study area on South Creek was identical to that used by Preziosi and Fairbairn (1996), including an upper recapture-only area (30 m long), a central mark-recapture area (100 m), and a lower recapture-only area (100 m). On West Creek, the areas are 50, 200, and 100 m long respectively. On both streams the study areas are bounded by barriers to dispersal such as waterfalls. Since *A. remigis* on these streams rarely move more than 100 m from where first captured (Fairbairn, 1985b), any individuals marked in the central mark-recapture areas are unlikely to move out of the study areas. Nevertheless, 100-200 m beyond the study areas were searched monthly for marked individuals. Fewer than 1% of the marked individuals were ever observed outside of the study area, and thus death and dispersal are unlikely to be confounded in this study.

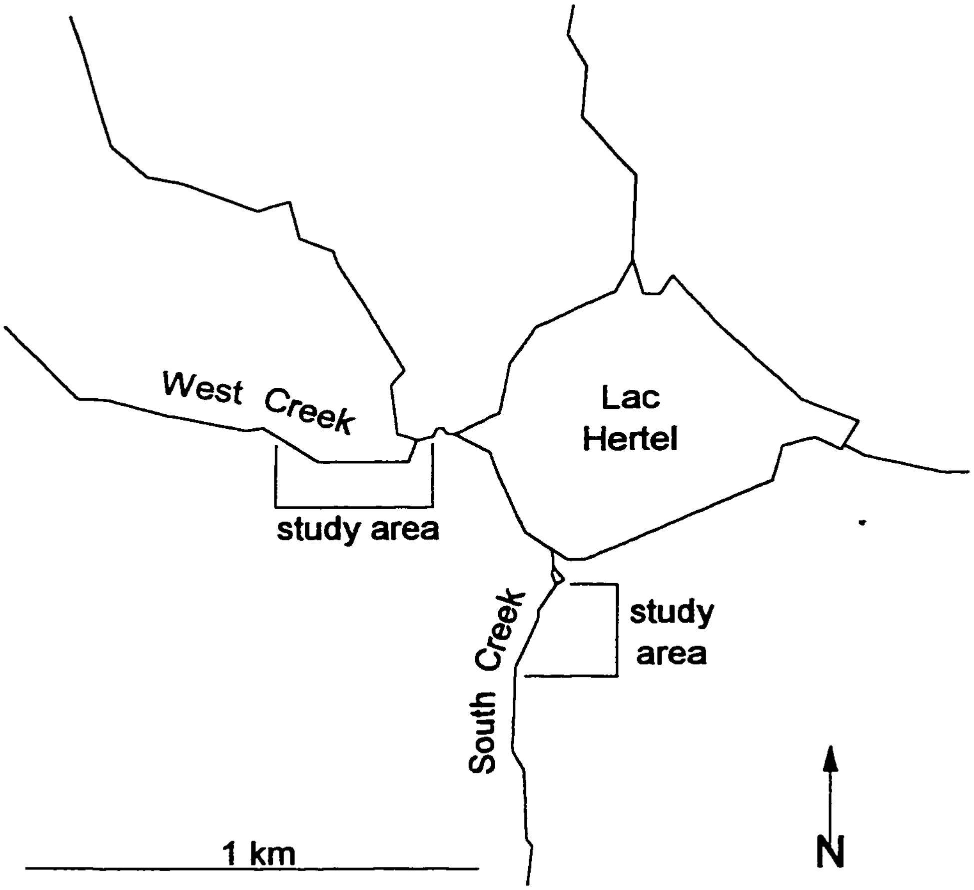

Figure 1.1. Study site on Mont St.-Hilaire, Quebec, Canada. Study areas included the extents of the creeks indicated, not including tributaries (which had few or no waterstriders).

The study areas were carefully searched weekly for *A. remigis*, and the number on each marked adult was recorded. All unmarked adults found in the mark-recapture areas were captured using hand nets, sexed, photographed in a ventral aspect, and marked with a unique number on the dorsal surface using enamel paint (see Preziosi and Fairbairn, 1996, 1997). These marks have no detectable effects on survival or mating success (Wheelwright and Wilkinson, 1985; Butler, 1987; Appendix 1). For each individual, total length, genital length, abdomen length, thorax length and (mean) mesofemur length were measured from the photographic negatives using a computerized digitizing system with MTV software (Updegraff, 1990). The definitions of these components are detailed in Preziosi and Fairbairn (1996, 1997). Repeatabilities for these measurements are all at least 0.97 (R. F. Preziosi, unpublished data).

Adults were followed through two complete generations. Sampling continued as long as adults were active on the water surface: from 15 July through 19 October in 1993, from 27 April through 12 October in 1994, and from 12 April through 18 July in 1995. Catchabilities (Krebs, 1989) in the reproductive season ranged from 67%-91% with a mean of 79%, indicating that the mean probability of not capturing an individual known to be alive was 21% for 1 week, 4.4% for 2 weeks and 0.92% for 3 weeks.

Assessing components of fitness

Sexual dimorphism in total length does not occur in this species until the last nymphal instar (V. Simoneau, unpublished data), and since development time does not differ between the sexes (Fairbairn, 1990), selection during the immature stages is unlikely to influence adult SSD. Thus, we have restricted our study to selection on adults.

Prereproductive survival (survival from eclosion to the spring reproductive season) was recorded as 0 (= did not survive) or 1 (= survived) for all individuals that were marked before the winter. Reproductive longevity of the surviving individuals was then calculated as:

$$\text{Reproductive longevity} = (D_L - D_F) + 1 \text{ day}$$

where D_L is the Julian date on which the individual was last captured and D_F is the Julian date on which it was first captured after diapause.

To measure daily fecundity, buckets with screened lids, holes in the bottoms, and a carefully cleaned rock for oviposition, were placed in the streams so that water partially covered the rocks. Marked females were collected twice from each stream each year (on 15 June and 6 July 1994, and on 24 May and 14 June 1995) and held individually in the buckets for two days. Daily fecundity was estimated as the mean number of eggs laid per day for each individual over the two-day trial.

The calculation of fecundity was straightforward in the first generation (1993-94), but in the second generation (1994-95) the mean fecundity differed between trials on both streams. To control for these differences, all fecundities in the second generation were converted to relative fecundity within each trial before the trials were combined for analysis.

To assess male mating success, we recorded the mating status (mating or single) of all males seen during the mark-recapture sampling. The study areas were also searched for mating males on one (1994) or two (1995) additional days each week. Mating frequency was then calculated for each marked male as the proportion of times that a male was found mating during the reproductive season.

Net adult fitness was calculated as prereproductive survival x reproductive lifespan x daily reproductive success (daily fecundity for females, mating frequency for males). While estimates of fitness for each episode were made for all individuals present for each episode, net adult fitness was only estimated for those individuals marked the previous fall. Although the net adult fitness of females is an estimate of lifetime fecundity, that of males can only be interpreted as an index of lifetime fitness. Mating status was assessed only once in any one field day, providing an "instantaneous" estimate, rather than a true count of number of matings per day. Multiplying this by reproductive lifespan therefore gives a good index of relative mating success, but does not translate into total number of matings in a lifetime.

Female fecundity was determined for only a proportion of those surviving to the reproductive season. To avoid exaggerating the effects of prereproductive mortality, net adult fitness for females was calculated using the same proportion of the females that did not survive the winter. For example, on West Creek in 1994 fecundity was measured for 17 females out of 57 surviving from those marked in 1993 (29.8%). We therefore randomly selected 29.8% of the 152 females that did not survive the winter (i.e., 45) to include in the analysis of net adult selection, giving us a sample of 62. The random selection of non-surviving females and subsequent analysis was repeated 10 times for each sample, and we report the mean coefficients and probabilities.

Statistical analysis

Date of eclosion (hereafter DOE: Julian date on which the individual ecloses from the last nymphal instar to the adult) is negatively correlated with total length in this species (Blanckenhorn, 1994; this study, Table 1.2): waterstriders eclosing later in the year are significantly smaller. If DOE directly influences any component of fitness, its correlation with body size might result in a spurious correlation between body size and fitness. Therefore DOE was included as an independent variable in the regression models to ensure that any selection on body size that was detected would be independent of DOE (see Mitchell-Olds and Shaw, 1987; Wade and Kalisz, 1990). For sexual and fecundity selection, DOE is not available for many of the individuals included in the data set. However, regression analysis using the subset of data for which DOE is available revealed no significant influence of DOE on the relationships between body size and male mating success or female daily fecundity. In the analysis of net adult fitness, DOE was available for all individuals and was included in the analyses.

Table 1.2: Correlations between Julian date of eclosion and total length in *Aquarius remigis* at Mont St.-Hilaire.

		South Creek		West Creek	
		1993-94	1994-95	1993-94	1994-95
Males	r	-0.69	-0.58	-0.30	-0.26
	P	<0.001	<0.001	<0.001	0.004
	n	466	427	151	121
Females	r	-0.67	-0.62	-0.31	-0.25
	P	<0.001	<0.001	<0.001	0.002
	n	476	484	209	146

We assessed the relationships between our estimates of components of fitness and body size using multivariate regression techniques (Lande and Arnold, 1983). Within each population and generation the estimates of fitness were converted to relative fitness ($w_i' = w_i / \bar{w}$ where w_i' is relative fitness, w_i is absolute fitness, and $\bar{w}$ is mean absolute fitness), and each trait (body size component or date of eclosion) was standardized to a mean of 0 and a standard deviation of 1 {$z_i = (x_i - \bar{x}) / s_x$ where z_i is the standardized trait value, x_i is the (unstandardized) trait value, $\bar{x}$ is the mean trait value, and s_x is the standard deviation of the trait values}. Relative fitness was regressed on standardized traits in four different models for each episode of selection. The linear model for total length included standardized total length and DOE, while the full model included the linear terms plus all possible quadratic terms: (standardized total length)^2, (standardized DOE)^2, and (standardized total length) x (standardized DOE). To identify possible selection on different components of body size, relative fitness was also regressed on the standardized lengths of the external genitalia ("genital length"), abdomen, thorax, and (mean) mesofemora; and DOE (both the linear and full models were estimated). The linear regression coefficients from the linear models (referred to as *linear selection gradients*, the "independent" β' of Koenig et al., 1991) and the quadratic coefficients from the full models (referred to as *quadratic selection gradients*, γ) estimate selection on each trait, independent of selection on any other traits included in the model, for each episode of selection (Lande and Arnold, 1983; Endler, 1986).

Both linear and quadratic selection gradients for DOE were estimated, but DOE was included only as a control variable, and this paper is concerned with sex-specific selection on body size. Therefore selection gradients for DOE are not reported. Within each population, generation, and sex we estimated 5 linear and 11 quadratic selection gradients on body size for each episode of selection and for net adult selection. The number of estimates leads to two problems: first, after Bonferroni correction for multiple tests there remains little power to detect significant selection; second, the large number of gradients generated makes interpretation difficult (5 + 11 = 16 gradients x 2 sexes x 2 populations x 2 generations x 4 episodes of selection [including net adult] equals 512 gradients). We therefore simplified the analysis by combining the standardized data from the different populations/generations before further analysis. This allowed us to test hypotheses about general patterns of selection, rather than estimating selection gradients within each population/generation. Only the linear selection gradients for net adult selection, for each sex in each population and generation, are reported in Table 1.3 (linear and non-linear selection gradients for total length in each episode are reported in Tables 2.2 and 2.3, linear, non-linear, and correlational selection gradients for components of body size in each episode are reported in Appendix 3, Tables A3.1 – A3.6).

We tested for differences in selection gradients between samples (spatial and temporal heterogeneity in the fitness functions), so that we could avoid combining samples with different patterns of selection. This was accomplished by regressing relative fitness on standardized total length and DOE using the combined data set, then using partial F tests (Neter et al., 1985, p. 281) to test whether the addition of the interactions between standardized total length and population or generation (included as indicator or "dummy" variables as per Neter et al., 1985, p. 328) improved the model (see Mitchell-Olds and Bergelson, 1990). The main effects of these dummy variables are always nil because traits were standardized within each population/generation. If an interaction was significant (indicating heterogeneity in the fitness function), the data were split as appropriate and the analysis repeated separately for each population/generation for all subsequent analyses in that episode of selection.

Patterns of selection on total length were estimated using the linear model (with standardized DOE and total length) and the full model (including all linear and quadratic terms) (Lande and Arnold, 1983; Phillips and Arnold, 1989). If one of these models was found to be significant (F test), then there must have been a significant relationship between fitness and at least one of the independent variables included in the model (Neter et al, 1985, p. 289). Stepwise regression (stepwise, forward selection, and backward selection; Neter et al, 1985, p. 430) was used to reduce that model to the significant variable(s) (see Mitchell-Olds and Bergelson, 1990). Similarly, the linear and full models with standardized genital, abdomen, thorax, and mean mesofemur lengths, and DOE, were estimated and reduced by stepwise regression where significant. Because Preziosi and Fairbairn (1996) found that sexual selection on male genital and total lengths may be antagonistic, we also regressed relative mating frequency on standardized genital length and "pregenital body length" (total length - genital length). In all cases, stepwise, forward selection, and backward selection techniques produced the same reduced models. The statistical software SPSS 8.0 (SPSS Inc., 1997) was used to calculate all regression models.

The residuals from these regression models were not normally distributed, therefore we confirmed significances using the program RT 1.02 (Manly, 1992) to randomize the dependent variable (fitness) 9,999 times. The randomization results were nearly identical to the parametric regression results ($r^2 = 0.99966$ for probabilities generated by the two methods).

Results

Through the entire study, a total of 1165 males and 1315 females were marked before the reproductive seasons. Prereproductive survival differed significantly between the sexes in the second generation on West Creek (males 10.7%, females 23.3%, $\chi^2 = 7.18$, df = 1, $P = 0.007$), but this did not represent a general trend: overall 17% of males and 19% of females survived to the reproductive season ($\chi^2 = 1.54$, $P = 0.12$). Similarly, reproductive longevities estimated for the 194 males and 244 females that survived were not significantly different (male $\bar{x} = 26.12$ days, female $\bar{x} = 26.40$ days, $U = 23522$, $P = 0.91$). Mean daily fecundity estimated for 179 females was 4.98 eggs/day, while mean mating frequency estimated for 588 males was 23.7%. Factorial comparisons including population and generation indicated that males and females did not differ significantly in either DOE (date of eclosion to adult, F = 3.05, df = 1, 2472, P = 0.081), or date of emergence from winter diapause (F = 0.002, df = 1, 430, P = 0.96).

Male net adult fitness

Selection generally favoured smaller males in this study. Three of the four linear selection gradients for total length were negative, though none were significant (Table 1.3). The interactions between standardized total length and population or generation were not significant (F = 1.15, df = 3, 1159, P = 0.33), indicating no significant temporal or spatial heterogeneity in selection through net adult fitness. The linear model for total length using the combined data set was significant (F = 4.83, df = 2, 1162, P = 0.008), and stepwise regression reduced the model to [fitness = 1 - 0.33(total)]. Therefore, there was a general pattern of selection favouring smaller males. Analysis of the components of body size also revealed significant selection for smaller size. The linear model was significant (F = 2.36, df = 5, 1159, P = 0.038), and reduced by stepwise regression to [fitness = 1 - 0.34(thorax)], suggesting that selection favouring smaller males may have been targeted at thorax length. The selection gradients in Table 1.3 are consistent with this result, the strongest pattern being negative gradients for thorax length in all four samples.

Male prereproductive survival

There was a significant interaction between standardized total length and generation (F = 4.48, df = 1, 1161, P = 0.034), indicating temporal heterogeneity in the relationship between male prereproductive survival and total length. The two generations were therefore analysed separately. In the second generation the linear model for total length was highly significant (F = 12.00, df = 2, 545, P << 0.001). The model reduced by stepwise regression was [fitness = 1 + 0.30(DOE) - 0.24(total)], indicating that males with greater DOE (eclosed to adult later) and males with shorter total length had significantly higher survival (Figure 1.2). The same trend was observed in the first generation, but it was not significant {F = 0.73, df = 2, 614, P = 0.48

Table 1.3. Standardized multivariate selection gradients for net adult selection on body size in *Aquarius remigis* at Mont St.-Hilaire, with DOE in the model. Standard errors are in parentheses. n = sample size.

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Males	n = 466	n = 427	n = 151	n = 121
Total length	-0.10 (0.29)	-0.34 (0.20)	0.26 (0.21)	-0.72 (0.44)
Genital length	0.22 (0.24)	-0.12 (0.18)	-0.02 (0.21)	0.13 (0.45)
Abdomen length	-0.05 (0.26)	-0.13 (0.20)	0.11 (0.24)	-0.69 (0.47)
Thorax length	-0.40 (0.36)	-0.32 (0.23)	-0.28 (0.27)	-0.03 (0.52)
Mesofemur length	0.20 (0.42)	0.23 (0.25)	0.53 (0.30)	-0.57 (0.55)
Female	n = 97		n = 62	
Total length	0.24 (0.40)		0.00 (0.35)	
Genital length	0.57 (0.42)		-0.12 (0.38)	
Abdomen length	0.23 (0.60)		-0.16 (0.43)	
Thorax length	-0.25 (0.67)		0.27 (0.43)	
Mesofemur length	0.10 (0.68)		-0.01 (0.49)	

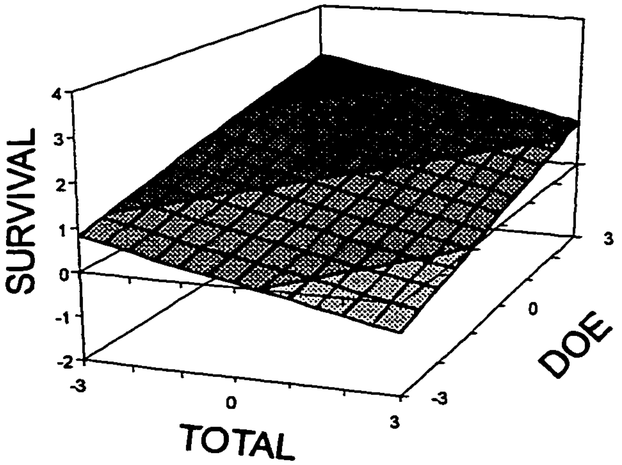

Figure 1.2. Contour plot of estimated selection on male standardized total length (TOTAL) and Julian date of eclosion to adult (DOE) through prereproductive survival in *Aquarius remigis* at Mont St.-Hilaire, in 1994-95. Fitness is estimated using the empirically-derived equation: [prereproductive survival = 1 + 0.30(DOE) - 0.24(TOTAL)]. Darker shades of grey indicate higher survival.

[fitness = 1 + 0.063(DOE) - 0.072(total)]}. Therefore, selection favoured later eclosion and reduced total length, these trends being much stronger in the second generation.

The full model with components of body size was also significant in the second generation (F = 1.73, df = 20, 527, P = 0.025), reducing to [fitness = 0.92 + 0.41(DOE) - 0.21(abdomen x DOE)] by stepwise regression. The interaction between abdomen length and DOE is difficult to interpret because of the strong direct effect of DOE. A contour plot based on the regression equation (Figure 1.3, right panel) indicates that fitness is maximized if males eclose late and have small abdomens. However, the effect of abdomen length depends on DOE such that long abdomens actually appear to be favoured among males eclosing early, resulting in minimal fitness for males eclosing early and having small abdomens. Perhaps the most striking aspect of the regression solution is the suggestion that the relationship between DOE and prereproductive survival is contingent upon abdomen size, being virtually absent among males with large abdomens.

Male reproductive lifespan and mating frequency

We found no evidence of any relationship between body size and reproductive lifespan (all models with P > 0.10). In the analysis of mating frequency, there was a significant interaction between standardized total length and population (F = 5.53, df = 1, 585, P = 0.019), and thus the two populations were analysed separately. On West Creek there was no significant relationship between body size and mating frequency (all P > 0.16). On South Creek, the models with standardized total length were not quite significant, but the linear model suggested a trend favouring shorter males [fitness = 1 - 0.092(total), F = 3.52, df = 1, 396, P = 0.061]. The components of body size (genital, abdomen, thorax, and mesofemoral lengths) significantly predicted mating frequency (F = 1.88, df = 14, 383, P = 0.027). Reduction by stepwise regression revealed that sexual selection favoured males with shorter thoraxes and longer external genitalia

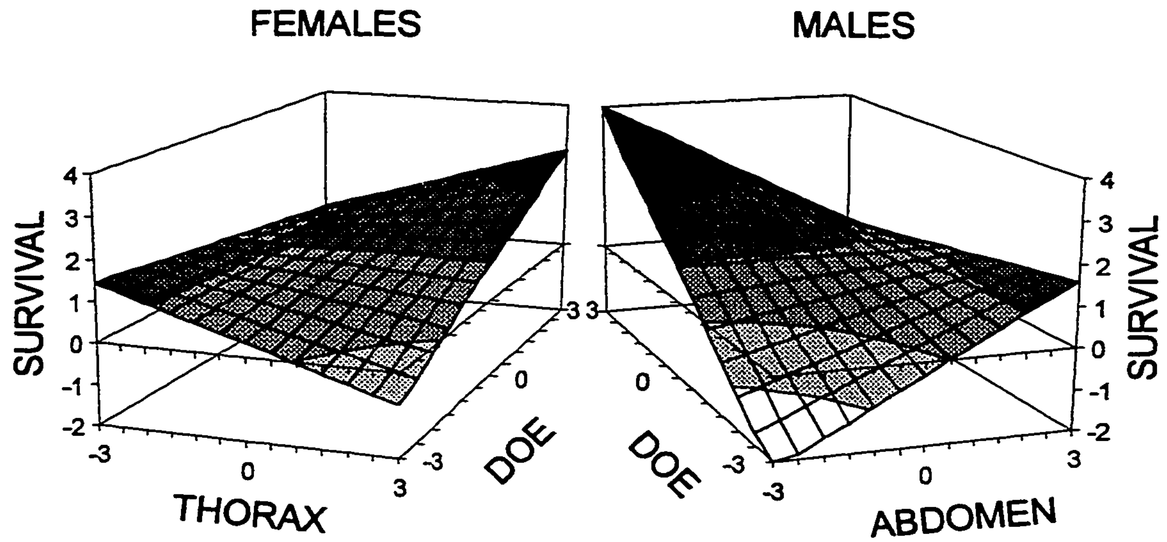

Figure 1.3. Contour plots of estimated correlational selection on components of body size and Julian date of eclosion to adult (DOE) through prereproductive survival in *Aquarius remigis* at Mont St.-Hilaire. Correlational selection for females (left panel) is estimated using the empirically-derived equation [prereproductive survival = 1 + 0.22(DOE) + 0.12(THORAX x DOE)]. Correlational selection for males in 1994-95 (right panel) is estimated from the empirically-derived equation [prereproductive survival = 0.92 + 0.41(DOE) -0.21(ABDOMEN x DOE)]. The traits have been standardized. Darker shades of grey indicate higher survival.

[fitness = 1 - 0.18(thorax) + 0.15(genital)]. The model with genital and pregenital lengths is a better predictor of fitness (F = 3.76, df = 5, 392, P = 0.0024): shorter males with longer external genitalia were favoured [fitness = 1 - 0.18(pregenital) + 0.15(genital)]. Thus, significant sexual selection on South Creek favoured smaller males with larger external genitalia.

Female net adult fitness

Because daily fecundity varied with time in the second generation, our estimates of daily fecundity could not be meaningfully multiplied by reproductive lifespan as an estimate of lifetime fecundity. Therefore, net adult fitness in females was only estimated for the first generation. One linear selection gradient for total length is zero and the other is positive but not significant (Table 1.3). Even after combining the data for the two populations we found no significant selection on total length or any component of body size (all $P > 0.32$).

Female prereproductive survival

The linear model with standardized total length and DOE was significant (F = 6.34, df = 2, 1312, P = 0.0018), but stepwise regression reduced the model to [fitness = 1 + 0.20(DOE)], suggesting that total length was unimportant. To confirm this, we regressed relative survival on standardized total length by itself: this model was not significant (F = 1.03, df = 1, 1313, P = 0.31), while the addition of standardized DOE significantly improved it (F = 11.63, df = 1, 1312, $P < 0.001$), therefore there was no significant selection on total length.

However, the linear model containing components of body size was significant (F = 3.82, df = 5, 1309, P = 0.0019), reducing to [fitness = 1 + 0.21(DOE) + 0.14(genital)] after stepwise regression. Therefore females with longer external genitalia were significantly more likely to survive. The full model was also significant (F = 1.67, df = 20, 1297, P = 0.032), and reduced to [fitness = 1 + 0.22(DOE) + 0.14(genital) + 0.12(thorax x DOE)]. The interaction between standardized thorax length and DOE indicates that the effect of thorax length depends upon DOE (Figure 1.3, left panel): thorax length is positively related to prereproductive survival for females that eclose late, but this relationship is negative among early-eclosing females. In spite of this interaction, the parametric solution suggests that fitness is maximized for late-eclosing females with long thoraces.

Female reproductive lifespan and daily fecundity

As in males, no significant selection through reproductive lifespan was found (all $P > 0.39$). Daily fecundity was also independent of total length or any component of body size (all $P > 0.23$), therefore female body size appears to have been selectively neutral during the reproductive season.

Discussion

Using fitness measured through the entire adult lifetime, we found that selection on total length differed between males and females in a manner consistent with the SSD: mean male total length is smaller than that of females, and significant selection favoured smaller male total length. In contrast, though selection was not significant on females, neither selection coefficient for female total length was negative (Table 1.3). This suggests that we are not merely missing selection for smaller females due to lack of statistical power. These results are consistent with optimum male size being smaller than optimum female size, a conclusion that Preziosi and Fairbairn (2000) arrived at for one of our populations through the two generations immediately preceding this study.

Surprisingly, the difference between the sexes in net adult fitness functions for total length did not result primarily from differences in the reproductive season. The only significant selection on female body size was found in the prereproductive survival episode, including selection favouring longer external genitalia. The same selection on female external genitalia was significant in one of two generations studied by Preziosi and Fairbairn (2000), however the external genitalia of females make up only about 5% of the total length (Fairbairn, 1992), therefore this selection did not significantly influence total length in either study. The only episode with significant selection on male total length was prereproductive survival: smaller males were favoured in the second generation, and trend was the same in the first generation, though not significant. Although a non-significant trend favouring smaller male total length through mating frequency on South Creek was found, the significant selection through prereproductive survival in one generation, coupled with the high mortality before the reproductive season (83%) suggests that the selection through net adult fitness favouring smaller males was due primarily to selection acting in the prereproductive episode.

Although prereproductive survival has generally been found to be independent of total length in *A. remigis* (Blanckenhorn, 1994; Preziosi and Fairbairn, 2000), some samples have shown significant selection for larger female total length (Blanckenhorn, 1994), or non-linear selection on male total length (Preziosi and Fairbairn, 2000). There are few life history differences between the sexes before the reproductive season that might be responsible for differences in the fitness functions. Males and females occupy the same habitat (Rubenstein, 1984; Fairbairn and Brassard, 1988; Krupa and Sih, 1993) and are similar in development time (Fairbairn, 1990), DOE (this study), patterns of movement (Fairbairn, 1985b; Fairbairn and Brassard, 1988), feeding behaviour (Blanckenhorn, 1991b), and adult prereproductive survival (this study). Galbraith and Fernando (1977) found that females emerged from diapause earlier than males in a small stream in southern Ontario, but no significant difference between the sexes was found in this study. Female bodies contain more lipids, both absolutely and as a proportion of body weight (Lee et al, 1975), suggesting physiological differences between the sexes. However, how this might contribute to the apparent difference in selection on size in males and females during the prereproductive season is unclear.

The only significant quadratic selection in this study was correlational selection found in the prereproductive episode: for lower (abdomen length x DOE) in the second generation of males and higher (thorax length x DOE) in females. Correlational selection on body size in *A. remigis* has generally been found to be weak and nonsignificant (Fairbairn and Preziosi, 1994, 1996), and for this reason correlational gradients are sometimes not reported (e.g. Preziosi and Fairbairn, 2000). However, DOE has not previously been included in these analyses, therefore the generality of the patterns found in this study is impossible to assess. Nevertheless, the difference between the sexes reported here is consistent with the hypothesis that males and females experienced different selective pressures through prereproductive survival.

We expected our estimate of mating success, which is sensitive to encounter rate, to yield an estimate of sexual selection that favoured smaller males, or at least favoured larger males less than in previous studies, and that is what we found on South Creek. The target of selection for larger males in previous studies, genital length, was also found to be under sexual selection for greater size in this population. At the same time, selection strongly favoured smaller pregenital body length, resulting in weak net selection for reduced total length. Such a result is consistent with the female-biased SSD in total length and pregenital length, as well as the male-biased SSD in genital length. The tendency for smaller males to have more opportunities to mate (Krupa and Sih, 1993; Blanckenhorn et al., 1995) could account for the advantage that smaller males appeared to have in acquiring mates on South Creek in this study. If small males are better at finding mates, small size is likely to be favoured when the density of the population is low and food is limiting (Ghiselin, 1974; Blanckenhorn et al., 1995). This sensitivity to population density and food level means that the balance between selection for longer genitals and for smaller overall size in *A. remigis* is expected to vary over space and time. This may account for some of the previously reported variation in direction and intensity of sexual selection in this species (Fairbairn, 1988; Kaitala and Dingle, 1993; Fairbairn and Preziosi, 1994, 1996; Sih and Krupa, 1995; Preziosi and Fairbairn, 1996).

Our results demonstrate spatial and temporal heterogeneity in fitness functions, for mating frequency and prereproductive survival. In males, patterns of sexual selection differed significantly between the two populations, and selection through prereproductive survival differed between generations. Our results also differ from those of Preziosi and Fairbairn (2000), who found net adult stabilizing selection on total length in both sexes on South Creek through one of two generations, attributed to opposing selection pressures through different episodes of selection. Preziosi and Fairbairn (2000) report significant net adult stabilizing selection only on female total length in their other generation, while the pattern of opposing selection pressures producing net adult stabilizing selection was lacking in males. We found no evidence of opposing selection pressures across episodes, or of stabilizing selection.

Although the differences between our study and that of Preziosi and Fairbairn (2000) suggest temporal heterogeneity of fitness functions on South Creek, these apparent differences could arise from either methodological differences between the studies or actual shifts in the body size distributions (rather than in the fitness functions) among generations. The methodological differences between the studies may explain the stronger selection for smaller pregenital body size detected in the present study, as our assay of sexual selection was designed to include selection through encounter rate. Differences in methodology might also account for some differences between the studies in estimates of the other components of reproductive fitness: we measured both daily fecundity and reproductive lifespan with less precision (i.e. fecundity over two rather than three days, and lifespan at weekly rather than twice-weekly intervals). This reduction in precision would be expected to increase the error variance of our estimates, and thus reduce the probability of detecting statistically significant selection. It may, for example, account for the lack of significant fecundity selection favouring longer abdomens on South Creek in our study. However, differences in methodology cannot explain our finding of significant directional selection through prereproductive survival, which Preziosi and Fairbairn (2000) did not find, because this component of fitness was estimated in exactly the same way in the two studies. Thus, methodological differences are insufficient to explain all the observed temporal variance in fitness functions.

The second alternative is that the underlying fitness functions remained relatively constant over the four generations, but the distributions of body sizes changed between generations. For example, if the mean total length was near the optimum in one generation, and much larger the next, selection could appear to be stabilizing in the first generation, but directional (favouring smaller size) in the second. If this was responsible for the differences between our results and those of Preziosi and Fairbairn (2000), we would expect the mean total lengths in our study to be higher in males and lower in females than in Preziosi and Fairbairn (2000). This was not the case (Table 1.4). Therefore, the most plausible explanation for the differences between studies remains temporal variance in fitness functions, particularly for prereproductive survival. Temporal and spatial variance in fitness functions has been reported for other taxa (Grant and Grant, 1989; Arnqvist, 1992; Endler and Houde, 1995; Blanckenhorn et al, 1999), and is doubtless typical for selection acting in local populations.

In conclusion, although spatial and temporal heterogeneity in fitness functions was observed, we found net adult selection generally favouring greater SSD, caused mainly by differences between the sexes in the fitness functions for prereproductive survival. However, the results of our studies of multivariate selection in this species indicate that selection acts antagonistically on different body size components, and therefore estimates of selection on total length can give misleading results. Patterns of selection on the main components of total length (genital, abdomen, and thorax length) are consistent with the dimorphisms in those components, while selection on total length is often weak and sometimes inconsistent with the SSD (Table 1.1). Thus, fitness in *A. remigis* may be relatively insensitive to overall body size (total length). Our results suggest, instead, that variation in the pattern and intensity of selection on total length observed in this and previous studies primarily reflects interactions between phenotypic distributions of components of body size and locally variable fitness functions.

Table 1.4: Comparison of patterns of net adult selection with means for total length. All data are from *Aquarius remigis* on South Creek at Mont St.-Hilaire.

Sex	Generation	Mean Total Length (mm)	Pattern of Selection
Male	1991-92	12.85[1]	stabilizing[2]
	1992-93	13.10[1]	ns (stabilizing)[2]
	1993-94	12.73	none
	1994-95	13.12	directional: smaller
Female	1991-92	13.75[1]	stabilizing[2]
	1992-93	14.14[1]	stabilizing[2]
	1993-94	13.77	ns (directional: larger)
	1994-95	14.14	not available

[1] from Preziosi (1997), [2] from Preziosi and Fairbairn (2000), ns = not significant

Chapter 2. Is selection ready when opportunity knocks?

Net adult fitness, as discussed in Chapter 1, resulted from components of fitness associated with the three episodes of selection considered. In Chapter 2, we look at the relative contribution of each component of fitness to net adult fitness by partitioning the opportunity for selection, I. The usefulness of I in the study of natural selection is discussed.

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Abstract

The opportunity for selection, *I*, defined as the variance in relative fitness, has been called an estimate of the "total amount of selection." However, while a non-zero *I* is a necessary condition for selection, it is not a sufficient one. We investigated the relationship between *I* and the magnitude of standardized linear and non-linear selection gradients for body size in the waterstrider *Aquarius remigis*, measured over three episodes of selection and for net adult fitness. Male *I* exceeded female *I* for daily reproductive success, but the difference was not statistically significant and had little impact on net adult *I*. Linear selection gradients were only weakly correlated with *I*, while non-linear gradients were uncorrelated with *I*. Partitioning *I* among the three episodes of selection revealed that variance in net adult fitness was largely generated by variance in prereproductive survival. The patterns of selection across the adult life stage suggested by analysis of the opportunity for selection differed qualitatively and quantitatively from those revealed by selection gradient analysis. In particular, the former identified prereproductive survival as the key component of net adult fitness, even though there is little selection on total length in this life stage. We conclude that *I* is a useful adjunct to selection gradient analyses, but is perhaps most useful in the analysis of life history evolution where the traits themselves are direct estimates of fitness.

Introduction

Crow (1958) suggested that the best measure of the "total amount of selection" is the variance in fitness divided by the square of the mean fitness, a statistic that he named the "Index of Total Selection," and designated *I*. Some authors have referred to *I* as the "Intensity of Selection" but "Opportunity for Selection" is preferable because it is nearer the intuitive meaning of *I*, and because it avoids confusion with the standardized selection differential *i*, also called the "Intensity of Selection" (Arnold and Wade, 1984).

The opportunity for selection can be calculated as the variance in relative fitness, which emphasizes its relationship to selection: the change in mean relative fitness within a generation is equal to *I*, and the square root of *I* is the maximum number of standard deviations that any trait can be moved by directional selection in one generation (Arnold and Wade, 1984; Walsh and Lynch, 1998, p. 306). Thus, opportunity for selection is a necessary but not a sufficient condition for natural selection, which requires an association between a trait and relative fitness (Sutherland, 1987; Wade, 1987; Wilkinson et al., 1987; McVey, 1988).

There is room for confusion when *I* is said to estimate the "amount of selection," independent of any character (Walsh and Lynch, 1998 p. 305-306). Futuyma (1998, p. 349) provides the following definition of natural selection: "*any consistent difference in fitness* (i.e., survival and reproduction) *among phenotypically different biological entities*." If fitness itself is considered a phenotype, then this definition becomes tautological. Therefore, fitness must be defined in terms of phenotypes other than fitness. Walsh and Lynch (1998, pp. 305-306) point out that *I* is independent of any particular character, which emphasizes that it is an estimate of the opportunity for selection, not of actual selection on any character.

Wilkinson et al (1987, p. 238) calls attention to two studies in which some estimates of *I* have either no correlation or a negative correlation with the estimated selection gradients on certain traits. However, the opportunity for selection is still sometimes presented as an estimate of the strength of selection. Nishida (1989) includes

I with selection gradients and differentials among "selection parameters," Vasi et al (1994, p. 441) discuss "opportunity for selection acting directly on each fitness component," Møller et al (1998, p. 606) uses an indicator of variance in male reproductive success as a measure of "the intensity of sexual selection," and Walsh and Lynch (1998, p. 308) state that partitioning *I* "allows the relative strength of selection to be compared across episodes." These statements suggest an expected correlation between *I* and selection acting on specific phenotypes.

In this paper, we explore the relationship between opportunity for selection and the strength of actual selection on body size in the waterstrider *Aquarius remigis*. First, *I* is compared between the sexes separately for each of three components of fitness, because differences between the sexes in selection on body size have been detected in this species (Ferguson and Fairbairn, 2000; Preziosi and Fairbairn, 2000). Second, the correlations between *I* and the standardized selection coefficients β and γ (Lande and Arnold, 1983) are estimated, to determine whether *I* provides information on the strength of observed selection. Third, *I* for net adult fitness is partitioned among three components to relate selection in each episode to total selection through the adult lifespan.

Study animal

The waterstrider *Aquarius remigis* is a large semiaquatic true bug found on the surfaces of streams and small rivers throughout most of temperate and subtropical North America (Preziosi and Fairbairn, 1992). In southern Quebec, females lay eggs from late April through early July on rocks under water. Young climb to the surface and develop through five nymphal instars to the adult, but the adults do not become reproductively active until the following spring. Mortality over the winter is 70-90% (Fairbairn, 1985a; Blanckenhorn, 1994; Ferguson and Fairbairn, 2000). Both sexes mate repeatedly with different partners through the reproductive lifespan (Krupa and Sih, 1993; Preziosi and Fairbairn, 1996), which lasts about four weeks (Preziosi and Fairbairn, 1997; Ferguson and Fairbairn, 2000).

Methods

Our mark-recapture study followed two populations of *A. remigis* through two generations (1993-94 and 1994-95). These populations are located on South Creek and West Creek respectively at the McGill University Research Station on Mont St.-Hilaire, Quebec, Canada, about 35 km SE of Montreal. The study area on South Creek included an upper recapture-only area (30 m long), a central mark-recapture area (100 m), and a lower recapture-only area (100 m). On West Creek, the areas are 50, 200, and 100 m long respectively. On both streams the study areas are bounded by barriers to dispersal such as waterfalls. Since *A. remigis* on these streams rarely move more than 100 m from where first captured (Fairbairn, 1985b), any individuals marked in the central mark-recapture areas are unlikely to move out of the study areas. Nevertheless, 100-200 m beyond the study areas were searched monthly for marked individuals. Fewer than 1% of the marked individuals were ever observed outside of the study area, and thus death and dispersal are unlikely to be confounded in this study.

From spring until fall, the study areas were carefully searched weekly for *A. remigis*, and the number on each marked adult was recorded. All unmarked adults found in the mark-recapture areas were captured using hand nets, sexed, photographed in a ventral aspect, and marked with a unique number on the dorsal surface using enamel paint (see Preziosi and Fairbairn, 1996, 1997). For each individual, total length was measured from the photographic negatives using a computerized digitizing system with MTV software (Updegraff, 1990). We estimated the following components of fitness: prereproductive survival (0 = did not survive to the reproductive season, 1 = did survive to the reproductive season), reproductive lifespan (in days), mating frequency for males, and daily fecundity for females. Mating frequency was estimated as the proportion of observation days that the individual was found mating. Observation days included all mark-recapture days plus one (1994) or two (1995) additional searches of the creeks each week (during the reproductive season). To assess daily fecundity, we confined solitary females in plastic containers in the creeks and counted the eggs laid over a 48 hour period. For a detailed description of these methods, see Ferguson and Fairbairn (2000).

Net adult fitness was estimated as prereproductive survival x reproductive lifespan x daily reproductive success (mating frequency for males, daily fecundity for females). Female fecundity varied through the reproductive season in the second generation, making meaningful estimates of lifetime fecundity impossible, therefore net adult fitness was not estimated for females in the second generation. Relative fitness was estimated within each population, generation, and sex, as absolute fitness divided by the mean absolute fitness. Standardized linear and non-linear selection gradients were estimated by regressing each relative fitness on standardized total length and standardized total length squared according to Lande and Arnold (1983). Statistical significances were determined by randomization (Manly, 1992), and are not corrected for experimentwise error. We use these significance levels only to indicate our degree of confidence in the estimates, rather than to test specific hypotheses about selection. For a complete analysis of selection in these samples, see Chapter 1 (Ferguson and Fairbairn, 2000).

The opportunity for selection, *I*, was estimated as the variance in relative fitness for each sex in each population and generation. The total opportunity for selection (through net adult fitness) was partitioned among prereproductive survival, daily reproductive success, and reproductive lifespan according to the methods of Arnold and Wade (1984) for each sex, population, and generation. We report both the relative and absolute contribution of each episodic *I*, and the contribution of covariance between components of fitness, to the total opportunity for selection. The contribution of covariance may be due to non-independence of selection (between episodes) or zero fitnesses at an early episode (Arnold and Wade, 1984). The latter is expected to be a factor in this study, because prereproductive survival includes zero fitness for many individuals. Partitioning was repeated with the second two components of fitness combined (reproductive lifespan x daily reproductive success) to isolate the contribution of covariance between prereproductive survival and overall reproductive success. Statistical analyses were done using RT 1.02 (Manly, 1992), Microsoft Excel 97 (Microsoft Corp., 1997) spreadsheet functions, and SPSS 8.0 (SPSS Inc., 1997).

Results

Variance in daily reproductive success is generally greater in male than in female animals (Futuyma, 1998, p. 587), and therefore we used one-tailed statistical tests to compare *I* in this episode and for net adult fitness which includes daily reproductive success. Mean opportunity for selection did not differ significantly between the sexes for prereproductive survival or reproductive lifespan. Males experienced slightly higher *I* for daily reproductive success, and this difference was borderline in statistical significance (Table 2.1). Although *I* for net adult fitness was higher for males than females, this difference was not significant.

When all 24 samples were used (2 populations x 2 generations x 2 sexes x 3 episodes of selection; Tables 2.2 and 2.3) the correlation between the absolute value of the linear selection gradients and the opportunities for selection is positive and just significant ($r_s = 0.34$, $n = 24$, one-tailed $P = 0.050$). However, this relationship is weak (Pearson $r^2 = 0.095$). The non-linear gradients were not significantly correlated with *I* ($r_s = 0.07$, $n = 24$, one-tailed $P = 0.37$).

Partitioning *I* revealed that the episode that contributed the most to *I* for net adult fitness in both sexes was prereproductive survival (Table 2.4 and Figure 2.1). Daily reproductive success never contributed more than 15% of net adult *I*, and in five of six samples reproductive lifespan contributed no more than 7% of net adult *I* (for West Creek 1993-94 males the contribution was 29%). In contrast, variance in prereproductive survival always contributed at least 36% of net adult *I*. However, the greatest single contributor to *I* was covariance between components of fitness (Table 2.4 and Figure 2.1). When reproductive lifespan and daily reproductive success were combined into one estimate of overall reproductive success, partitioning the opportunity for selection yielded similar contributions for covariance (43-55%), indicating that it was covariance between prereproductive survival and overall reproductive success (rather than between reproductive lifespan and daily reproductive success) that was responsible for the large contribution of covariance to the total opportunity for selection. No significant

Table 2.1. Between-sex comparison of opportunity for selection in *Aquarius remigis* at Mont St.-Hilaire. The Wilcoxon paired sample tests were conducted on square-transformed data (x^2) to correct for skew. Probabilities (P) were generated by a Monte Carlo procedure.

	mean *I* (SE)		Wilcoxon test	
	males	females	n[a]	P
Prereproductive Survival	5.61 (1.36)	4.22 (0.83)	4	0.62[b]
Reproductive Lifespan	0.81 (0.15)	0.82 (0.10)	4	0.97[b]
Daily Reproductive Success	1.51 (0.31)	0.92 (0.23)	4	0.061[c]
Net Adult Fitness	15.29 (3.88)	11.34 (4.15)	2	0.49[c]

[a] number of samples (= populations x generations), [b] two-tailed test, [c] one-tailed test

Table 2.2. Univariate linear (β) and non-linear (γ) selection gradients for total length, sample size (*n*), and opportunity for selection (*I*), in male *Aquarius remigis* at Mont St.-Hilaire. * indicates statistical significance at $\alpha = 0.05$.

		South Creek		West Creek	
		1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	β	-0.16	-0.38*	0.04	-0.42
	γ	0.14	0.13	-0.12	0.06
	n	466	427	151	121
	I	7.34	4.22	2.53	8.38
Reproductive Lifespan	β	-0.07	0.02	0.09	-0.41
	γ	0.19	-0.17	-0.13	0.29
	n	56	82	43	13
	I	1.25	0.71	0.59	0.70
Daily Reproductive Success	β	-0.14	-0.06	0.22	0.07
	γ	0.05	-0.17*	-0.02	0.18
	n	152	246	89	101
	I	1.50	0.63	1.96	1.95

Table 2.3. Univariate linear (β) and non-linear (γ) selection gradients for total length, sample size (n), and opportunity for selection (I), in female *Aquarius remigis* at Mont St.-Hilaire. * indicates statistical significance at $\alpha = 0.05$.

		South Creek		West Creek	
		1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	β	0.01	-0.30*	0.10	0.28
	γ	-0.05	-0.21	0.16	0.23
	n	476	484	209	146
	I	6.45	4.45	2.68	3.32
Reproductive Lifespan	β	0.07	0.04	-0.03	0.12
	γ	-0.03	-0.06	-0.10	0.09
	n	64	89	57	34
	I	1.03	0.56	0.79	0.91
Daily Reproductive Success	β	0.01	0.12	0.08	-0.10
	γ	0.12	0.18	-0.13	-0.14
	n	35	60	36	48
	I	0.86	0.45	0.81	1.54

Table 2.4. Opportunity for selection in *Aquarius remigis* at Mont St.-Hilaire, partitioned according to Arnold and Wade (1984).

	MALES				FEMALES	
	South Creek		West Creek		South	West
	1993-94	1994-95	1993-94	1994-95	1993-94	1993-94
Prereproductive Survival	7.21	4.01	2.57	6.32	6.46	2.47
Reproductive Lifespan	1.01	0.70	1.87	1.11	0.42	0.21
Daily Reproductive Success	0.70	0.39	1.00	0.67	0.46	0.90
Covariance	10.56	6.11	0.92	7.19	7.80	3.10
Net Adult Fitness	19.48	11.21	6.34	15.28	15.33	6.69

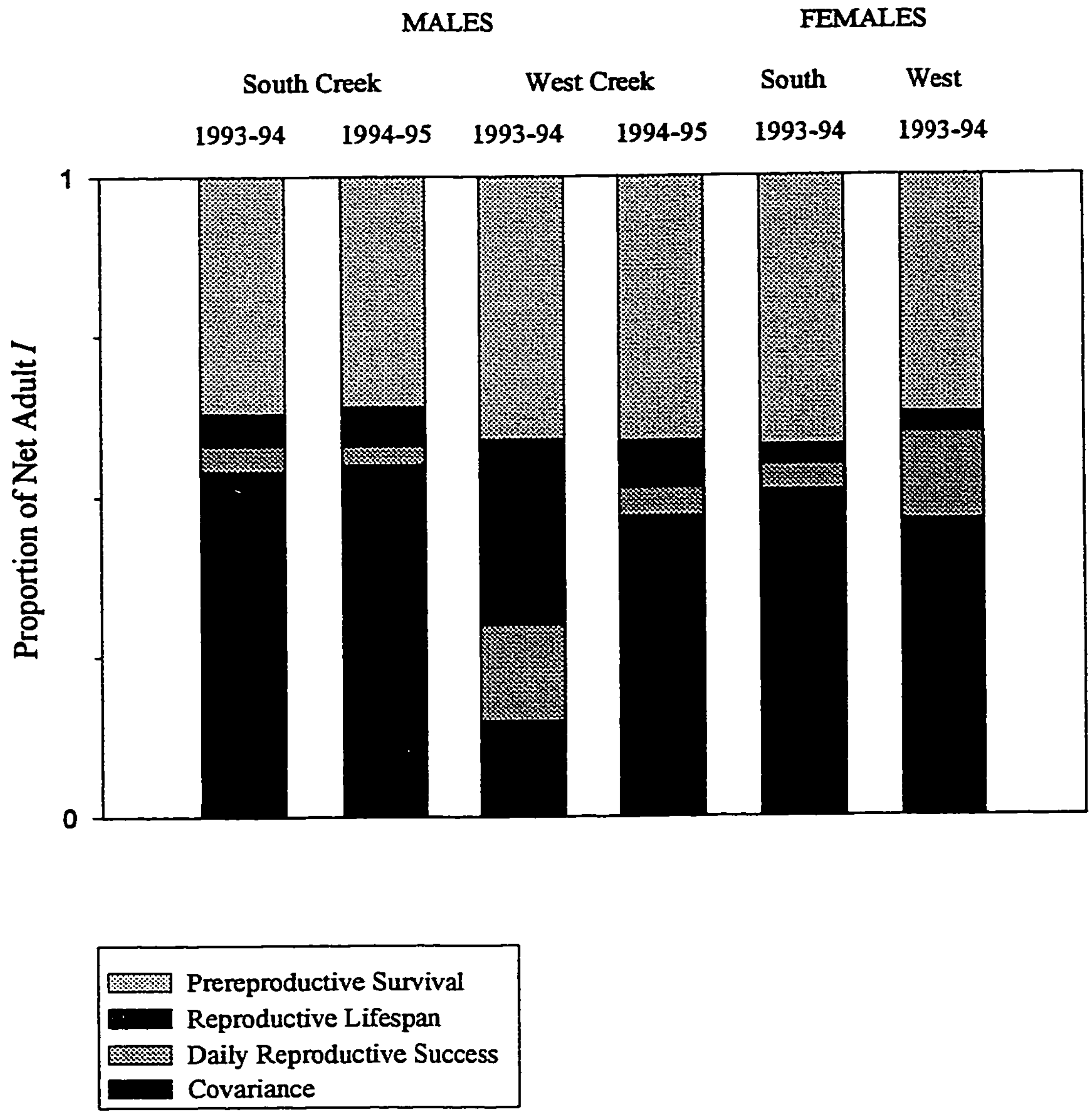

Figure 2.1. Partitioning the opportunity for selection, *I*, for *Aquarius remigis* at Mont St.-Hilaire. The proportion of net adult *I* is presented for each episode of selection in each sample.

correlation between daily reproductive success and reproductive lifespan was found in any sample (all $P > 0.10$).

Discussion

The estimated *I* for daily reproductive success tended to be higher for males than for females, though this pattern was not statistically significant. Despite this, no significant selection on total length was detected on males in this episode. This does not rule out selection on other male traits. In fact certain components of male body size were found to be under directional sexual selection in the same South Creek samples (Chapter 1; Ferguson and Fairbairn, 2000). The key point is that the presence of opportunity for selection does not indicate selection on any particular trait. We did find a weak correlation between the opportunity for and the strength of directional selection but, with r^2 less than 10%, *I* provides little information about the magnitude of the selection gradient. Further, the strongest selection on body size reported in other studies of *A. remigis* has acted through daily reproductive success and reproductive lifespan (Fairbairn, 1988; Sih and Krupa, 1992; Sih and Krupa, 1995; Kaitala and Dingle, 1993; Krupa and Sih, 1993; Fairbairn and Preziosi, 1994; Fairbairn and Preziosi, 1996; Preziosi and Fairbairn, 1996; Blanckenhorn, 1991a; Blanckenhorn et al., 1995; Preziosi et al., 1996; Preziosi and Fairbairn, 1997; Ferguson and Fairbairn, 2000; Preziosi and Fairbairn, 2000), even though our results indicate that the opportunity for selection in these episodes is low (Tables 2.2 and 2.3). We also found no evidence of a correlation between non-linear selection gradients and *I*. Thus, *I* is a poor predictor of estimates of selection on body size.

The opportunity for selection, *I*, has been reported in a number of studies (Table 2.5), and some of these studies also present estimates of the strength of selection (such as the selection differential, *s* or *C*, e.g. Anholt, 1991; or the selection gradient, β or γ, e.g. Howard, 1988) but none that we are aware of has estimated the relationship between *I* and the strength of selection. The values for *I* in this study fall into the range of previously published values (Table 2.5), suggesting that the lack of a strong relationship between the opportunity for selection and the strength of selection is not due to *I* being exceptionally high or low. The standardized linear selection gradient, β, is expected to be correlated with *I* only because *I* sets the upper limit for β, so it should not be surprising that the correlation is weak. The relationship with the non-linear gradient, γ, is expected to be even weaker as Arnold (1986) showed that non-linear (e.g. stabilizing) selection is not informatively limited by the opportunity for selection unless *I* is less than 0.5. The failure of the opportunity for selection to adequately predict the strength of selection is consistent with Sutherland's (1987) theoretical conclusion that "it is not useful to measure variance in reproductive success as this is neither evidence for sexual selection nor a good measure of its intensity."

Partitioning net adult opportunity for selection among the episodes of selection on these waterstriders revealed that variance in net adult fitness was largely generated by variance in prereproductive survival (the covariance component is due to zero fitnesses in prereproductive survival, see Arnold and Wade, 1984). This may explain why previously reported significant selection through prereproductive survival favouring smaller males resulted in smaller males enjoying a significant advantage in net adult fitness (Ferguson and Fairbairn, 2000). This pattern is not unique to waterstriders; Howard (1988) found

Table 2.5. The ranges of estimated opportunity for selection, *I*, in selected studies.

	opportunity for selection		
	minimum	maximum	taxon
Anholt, 1991	0.8	114.8	damselflies
Cabana and Kramer, 1991	0.01	1.49	birds
Houck et al., 1985	0.01	0.074	salamanders
Howard, 1988	0	3.18	frogs
LeBoeuf and Reiter, 1988	7.08	27.00	elephant seals
McVey, 1988	0.440	6.22	dragonflies
Pruett-Jones and Pruett-Jones, 1990	2.27	3.94	birds
this study	0.45	19.48	waterstriders

that survival contributed more to opportunity for selection than fecundity in two species of frogs. It may also explain why selection in the reproductive season may be swamped by random variance in prereproductive fitness, as described by Preziosi and Fairbairn (2000).

Thus, as we expected, I is not a good predictor of the strength of selection on total length (as estimated by linear and non-linear selection gradients). However, I is useful precisely because it is independent of any particular phenotype (Walsh and Lynch, 1998, p. 306). In a sense, I may be characterized as a measure of the relative impact of the various life history components on lifetime fitness. For example, we have identified prereproductive survival as a key life history component in *A. remigis*. Individual fitness through the adult stage is most strongly determined by ability to survive from eclosion to first reproduction, and hence we would expect selection to act most strongly in this phase of the adult life cycle. Because prereproductive survival is not strongly correlated with total length, our selection gradient analyses did not reveal the importance of prereproductive survival in determining net adult fitness (Ferguson and Fairbairn, 2000; Preziosi and Fairbairn, 2000). Our comparisons of the two methods of analyzing selection suggest that while I is a useful adjunct to selection gradient analysis in studies of the adaptive significance of trait values, its most important contribution may lie in studies of the adaptive significance of life history components, which are themselves components of fitness.

Chapter 3. Estimating genetic correlations from measurements of field-caught waterstriders.

The link between selection and evolution is the inheritance of traits. Offspring tend to resemble their parents largely because the offspring receive their genes from their parents (but see Falconer, 1989, pp. 158-160), but environmental factors often contribute to the offspring's (and the parent's) phenotype. The phenotypic variance in a trait may be divided into the *additive genetic variance* (responsible for the resemblance between parents and their offspring) and variance from other sources. The proportion of phenotypic variance in a trait that is additive genetic variance is called the ***narrow sense heritability***, or just ***heritability***, h^2 (Falconer, 1989, p. 126). If h^2 is zero, then there is no additive genetic variance for the trait and it will not evolve in response to selection. If h^2 is one, then all phenotypic variance in the trait is due to additive genetic variance and the trait may evolve quickly in response to selection. The relationship between the response to selection and heritability is expressed in the equation (Falconer, 1989, p. 192):

$$\Delta\bar{z} = ih\sigma_A$$

where $\Delta\bar{z}$ is the change in the trait mean from one generation to the next (the response to selection), i is the standardized difference between the trait mean before selection and the trait mean after selection (the selection intensity), h is the square root of the heritability of the trait, and σ_A is the additive genetic variance of the trait.

If a trait is genetically correlated with another trait that is also under selection, the response to selection in the first trait depends on the effect of direct selection on that trait plus the effect of indirect selection through the correlated trait. This may be expressed by the equation (Falconer, 1989, p. 318):

$$\Delta\bar{z}_X = i_X h_X \sigma_{AX} + i_Y h_Y r_A \sigma_{AX}$$

where r_A is the genetic correlation between traits X and Y, and the subscripts *X* and *Y* refer to those traits. The right side of this equation contains the effects of both direct ($i_X h_X \sigma_{AX}$) and indirect ($i_Y h_Y r_A \sigma_{AX}$) selection on the response in trait X. Note that the effect of indirect selection depends on the genetic correlation r_A between the two traits.

This equation can be generalized to selection on any number of traits with the matrix equation (Roff, 1992, p. 24):

$$\mathbf{Z} = \mathbf{G}\boldsymbol{\beta}$$

where **Z** is a column vector of the responses in the traits, **G** is the genetic variance-covariance matrix, and β is a column vector of the standardized multivariate selection gradients. The standardized multivariate selection gradients are the slopes from a multiple regression of relative fitness on a set of standardized traits (Chapter 1), and are the multivariate equivalent of the selection intensity. The genetic variance-covariance matrix contains the additive genetic variances for the traits and the genetic covariances among them. A covariance is a measure of association which is related to a correlation by the equation (Sokal and Rohlf, 1995, p. 559):

$$r_{XY} = \frac{\mathrm{cov}_{XY}}{\sigma_X \sigma_Y}$$

where r_{XY} is the Pearson product-moment correlation coefficient between X and Y, cov_{XY} is the covariance between X and Y, and σ is the standard deviation for X or Y.

These equations illustrate how genetic correlations can play a role in determining the net fitness function for a trait, through the effect of indirect selection. Estimating genetic correlations has been largely restricted to the laboratory, but Lynch (1999) proposed a new statistical approach for estimating genetic correlations from simple measurements of individuals from the field. In this chapter, we attempt to estimate genetic correlations among body size components in each sex of *Aquarius remigis*, using the method of "estimation in the absence of pedigree information" of Lynch (1999). The utility of this untested method is discussed.

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Ferguson, Ian M. and Daphne J. Fairbairn. Estimating genetic correlations from measurements of field-caught waterstriders.

Abstract

Lynch (1999) proposed a method ("estimation in the absence of pedigree information") for estimation of genetic correlations from phenotypic measurements of individuals for which no pedigree information is available. This method assumes that shared environmental effects do not contribute to the similarity of relatives, and is expected to perform best when sample sizes are large, many individuals in the sample are paired with close relatives, and heritability of the traits is high. We tested the practicality of this method for field biologists by using it to estimate genetic correlations from measurements of field-caught waterstriders *Aquarius remigis*. Results for sample sizes of less than 100 pairs were often unstable or undefined, and even with more than 500 pairs only half of those correlations that had been found to be significant in standard laboratory experiments were statistically significant in this study. Statistically removing the influence of environmental effects (shared between relatives) weakened the estimates, possibly by removing some of the genetic similarity between relatives. However, the method did generate statistically significant estimates for some genetic correlations. Lynch (1999) anticipated the problems found, and proposed another method that uses estimates of relatedness between members of pairs (from molecular marker data) to improve the estimates of genetic correlations, but that approach has yet to be tested in the field.

Introduction

Endler (1986) describes the three requirements for evolution by natural selection: variation in a trait, fitness differences associated with that variation, and inheritance of the trait. The first two have been estimated for many wild populations, usually without any estimate of the last. This has been because the first two are necessary and sufficient for natural selection, as distinct from the evolutionary response to that selection (Arnold and Wade, 1984; Manly, 1985). Nevertheless, natural selection will have no evolutionary consequences unless the trait in question is at least partly inherited, and studies of natural selection usually assume that the trait studied is heritable.

The situation becomes more complex when the evolutionary response to multivariate selection is considered. Not only is selection on, and heritability of, each trait of interest, but also correlational selection and the genetic correlations between traits (Lande and Arnold, 1983; Lynch, 1999). Once again, genetic correlations are usually assumed but rarely reported with multivariate selection gradients.

There are two general approaches to estimating genetic correlations: using the resemblance between known relatives, or the correlated response to known selection (Stearns, 1992). Using the resemblance between known relatives requires detailed knowledge of the relationship between individuals in large samples (Falconer, 1989; Roff, 1997), data that are usually impossible to acquire in a wild population. Using the correlated response to selection requires that the response to selection be known (Stearns, 1992), which may be confounded by environmental effects in wild populations. The estimation of genetic correlations by these methods, therefore, is generally restricted to the laboratory (Lynch, 1999).

There are two major problems with laboratory estimates of genetic correlations. The first is that the values of these correlations depend on the conditions under which they are measured (Falconer, 1989), and therefore the values measured under laboratory conditions may be different than those experienced by wild populations. However, Roff

(1995) and Simons and Roff (1996) demonstrated that laboratory estimates of genetic correlations of morphological traits may be reasonable indicators of genetic correlations under natural conditions. Nevertheless, extrapolations from the laboratory to the field must be made with caution.

The second problem is that estimation procedures in the laboratory are logistically demanding and expensive. The large sample sizes required, combined with the need to raise young, identify relatives, and control matings, make such studies extremely space- and labour-intensive. For some species, laboratory rearing may be impractical or even impossible.

Lynch (1999) proposes two new approaches to the estimation of genetic correlations. We are concerned with the first ("Estimation in the absence of pedigree information"), which uses simple data from a wild population. Individuals in the data set are first organized into pairs. By making the assumption that "shared environmental effects do not contribute to the phenotypic resemblance between relatives," Lynch (1999, p. 256) developed the following formula for a genetic correlation:

$$\hat{\rho}_G(x,y) = \frac{Cov[z_i(x); z_j(y)]}{\sqrt{Cov[z_i(x), z_j(x)] * Cov[z_i(y), z_j(y)]}} \qquad 1$$

where $\hat{\rho}_G(x, y)$ is the estimated genetic correlation between traits x and y,

$Cov[z_i(x); z_j(y)]$ is the phenotypic covariance between trait x in the first individual of a pair (i), and trait y in the second individual of the pair (j),

$Cov[z_i(x), z_j(x)]$ is the phenotypic covariance between trait x in the first individual of a pair (i), and trait x in the second individual of the pair (j),

and $Cov[z_i(y), z_j(y)]$ is the phenotypic covariance between trait y in the first individual of a pair (i), and trait y in the second individual of the pair (j).

There is no inherent order in the pairs, so the numerator may also be calculated as $Cov[z_i(y); z_j(x)]$. In practice, the average of the two may be used. The quantities in this equation are easily estimable from measurements of field-caught organisms, making this approach very attractive to biologists studying natural selection. However, the assumption that shared environmental conditions do not contribute to the similarity of relatives is problematic. Lynch (1999) points out that organisms that are geographically close are typically closely related and share similar environmental conditions. Where the biology of the organism is well known, an investigator may attempt to correct for shared environmental effects, but it is probably impossible in most cases to remove all environmental effects. Even if such a correction is successful, it may be difficult to remove environmental effects without also removing some of the genetic effects that are responsible for genetic correlations.

While Lynch (1999) presents simulations to test the accuracy and precision of this method, given known degrees of relationship between sampled pairs, the method has not yet been tested with field data. We apply the method to data from a field study of the waterstrider *Aquarius remigis*, to discover how practical it is for biologists who are studying natural selection in the wild, and compare the results to those obtained by standard laboratory methods.

Genetic correlations among morphological traits in *Aquarius remigis* have been estimated in the laboratory from both half-sib and full-sib experimental designs (Preziosi and Roff, 1998; Fairbairn and Reeve, unpublished). Both experiments indicate significant positive genetic correlations among most body size components and a strong correlation between phenotypic and genetic correlations. Generalities obtained from these data provide a good basis for comparison with field estimates obtained using Lynch's (1999) method.

Methods

The waterstrider *Aquarius remigis* is a true bug (Hemiptera: Gerridae) that lives on the surface of small rivers and streams across most of temperate and subtropical North America (Preziosi and Fairbairn, 1992). In the northern part of their range, adults diapause over the winter on shore before becoming reproductively active in the spring, mating and laying eggs until death before midsummer. The eggs hatch and develop through 5 nymphal instars, then eclose into adults. Depending on when the eggs are laid and on environmental conditions, eclosion to adult occurs between July and October (Fairbairn, 1985a; Blanckenhorn, 1994; Ferguson and Fairbairn, 2000).

The two study populations of *A. remigis* are found on South Creek and West Creek respectively, on Mont St.-Hilaire, Quebec, Canada, about 35 km SE of Montreal. As part of a mark-recapture experiment, each creek was searched weekly for adult *A. remigis* during the summer and fall of 1993 and 1994 (see Ferguson and Fairbairn, 2000).

All newly-eclosed adults within a 100-metre study area on South Creek and a 200-metre study area on West Creek were captured using hand nets, then photographed (see Preziosi and Fairbairn, 1996). The photographic negatives were later digitized, and the following body size measurements were taken using the software Measurement TV © (Updegraff, 1990): genital length (the length of the external genitalia), abdomen length, thorax length, mean mesofemur length, and mean hind-femur length (see Preziosi and Fairbairn, 1996, 1997 for definitions of these measurements). Date of eclosion (DOE) was recorded as the Julian date on which an individual was first captured. The study areas were marked off in 5-metre sections, and the section in which an individual was captured was recorded as location.

Lynch (1999) suggests that his estimate of genetic correlation may be biased if too few pairs are made up of closely-related individuals. The biology of *A. remigis* is such that we can be reasonably sure that this is not a problem in our study. The study populations are genetically isolated from one another, with the number of migrants per generation $N_m = 0.29$ (Preziosi and Fairbairn, 1992), indicating about 1 migrant per 3.4 generations (out of 250-1,000 adults per generation). Dispersal among populations ("migration") occurs in early spring (Fairbairn, 1985b, 1986; Fairbairn and Desranleau, 1987), and therefore the newly eclosing generation in the late summer consists of individuals that have developed within this population.

Within the streams, these study populations are spatially substructured, with allele frequencies differing significantly between sites less than 100 m apart, and the neighbourhood size estimated from allozyme data is only 170 adults (Preziosi and Fairbairn, 1992). Mark-recapture data have confirmed that adults generally move less than 10 m before the winter (Fairbairn, 1985b; I. M. Ferguson, unpublished data). Thus, adults captured in the late summer and fall are much more likely to be closely related to each other if they are found in the same location.

We also suspect that individuals with the same DOE (eclosing at the same time) are more likely to be closely related, because adults reproducing (in the spring) in a particular location may move or die, resulting in different parentage from week to week in that location. In order to "enrich" the relatedness of pairs (Lynch, 1999), we paired individuals as much as possible with those likely to be close relatives. For each population, generation, and sex, individuals were sorted by location and by DOE within location. Adjacent individuals were then assigned to pairs, insuring that as much as possible members of pairs come from the same location and DOE.

We estimated genetic correlations between all pairs of traits separately for each sex, population, and generation. Each estimate is the average of two estimates, one using $Cov[z_i\,(x);\, z_j\,(y)]$ and the other using $Cov[z_i\,(y);\, z_j\,(x)]$ in the numerator of Equation 1. When these two estimates differ in sign, or by a factor of at least ten, we regard the averaged estimate as 'unstable.'

The number of pairs available for each estimate depended on a number of factors. Samples were generally larger for South Creek than West Creek, for the 1993-94 generation than for the 1994-95 generation, and for females than for males (the sex ratio was not significantly different from 1:1 on the streams, but fewer females were discarded for missing measurements). Sample sizes also varied among traits because some measurements were not available for a few individuals (some individuals were photographed in positions that made certain measurements impossible to take accurately). The sample sizes that we used ranged from 59 pairs (most West Creek 1994-95 male traits) to 278 pairs (some South Creek 1993-94 male traits). Lynch (1999) found that samples with 100 pairs tend to underestimate genetic correlations unless the proportion of pairs that are closely related is high. To avoid this problem, we combined standardized data from different populations and generations (see below) and estimated the genetic correlations between all traits from this combined data set.

The assumption that shared environmental effects do not contribute to similarity between relatives might not hold in these populations. Individuals with similar DOE are more likely to be related, but there is also a strong relationship between DOE and body size (Ferguson and Fairbairn, 2000): adults that eclose later tend to be smaller, probably due to phenotypic responses to shorter days and less food. We removed the influence of DOE by subtracting the weekly mean from each body size measurement, resulting in a mean for each week (for each body size measurement) of zero. The result is a standardized measurement that expresses size as a deviation from the weekly mean for that measurement in that sex, population, and generation. This standardization also allows us to combine the data from the different populations and generations and estimate the genetic correlations using larger sample sizes.

All genetic correlations were estimated using the jackknife, as recommended by Roff and Preziosi (1994). Some of the distributions of the jackknife estimates were skewed, apparently because of a few outliers. Skewed distributions tend to bias the mean (Zar, 1984). We therefore omitted any jackknife pseudovalues that were at least 5 standard deviations from the mean, based on a linear extrapolation of Grubb's table in Rohlf and Sokal (1991, p. 179). The jackknife means are reported. Even after omitting the outliers the distributions remained leptokurtic, therefore significance tests for the jackknife estimates were carried out by randomization of the pseudovalues using the software RT 1.02 (Manly, 1992), with 10,000 randomizations for each estimate. All other statistics were calculated using spreadsheet functions in Microsoft ® Excel 97 SR-1 (Microsoft, 1997).

Results

For two populations, two generations, and two sexes, with ten correlations each, we estimated a total of 80 genetic correlations. We report only the correlations for the combined data (Tables 3.1 and 3.2), but summarize the results for the separate data sets below. The samples from the West Creek population were smaller (59 – 115 pairs), and yielded highly variable estimates: the estimated genetic correlations ranged from –4.76 to 9.01 (median = 0.88). Ten were undefined (the two within-trait covariances in the denominator of Equation 1 were of opposite signs, making their product negative and the square root undefined), and one was disregarded because both within-trait covariances (which estimate trait variances) were negative. Another eight were unstable (the two estimates that were averaged for one genetic correlation differed by a factor of at least ten, or were of opposite sign). The results were less variable for the South Creek samples (with 206 – 278 pairs): the estimated genetic correlations ranged from –0.08 to 1.42 (median = 0.90), none were undefined, and only two were unstable.

The effects of correcting for DOE in the South Creek first generation sample were typical. This correction resulted in lower estimated phenotypic correlations between all traits, but these correlations remained significantly positive and were all higher than the phenotypic correlations in the laboratory reported in Preziosi and Roff (1998). Four of the genetic correlations estimated from the DOE-corrected data were undefined, and three more were unstable. The remaining three estimated genetic correlations ranged from –6.62 to 1.07. In contrast, before correction for DOE the estimated genetic correlations

Table 3.1. Estimates of phenotypic (above the diagonal) and genetic (below the diagonal) correlations for various body size components in male *Aquarius remigis* at Mont St.-Hilaire. All traits have been standardized to a mean of 0 (see text). ^{u} indicates an unstable estimate, in which the two values that are averaged to give the estimate differ in sign or by a factor of at least ten. **Bold** indicates P<0.05. Undefined estimates are left blank.

	Abdomen length	Thorax length	Genital length	Hind-femur length	Mesofemur length
Abdomen length		**0.35**	**0.10**	**0.39**	**0.39**
Thorax length	0.59		**0.20**	**0.53**	**0.58**
Genital length		0.37		**0.28**	**0.28**
Hind-femur length	-0.23 ^{u}	**0.74**	**0.83** ^{u}		**0.83**
Mesofemur length	**0.77**	**0.83**	**1.11** ^{u}	**0.99**	

Table 3.2. Estimates of phenotypic (above the diagonal) and genetic (below the diagonal) correlations for various body size components in female *Aquarius remigis* at Mont St.-Hilaire. All traits have been standardized to a mean of 0 (see text). ^{u} indicates an unstable estimate, in which the two values that are averaged to give the estimate differ in sign or by a factor of at least ten. **Bold** indicates P<0.05. Undefined estimates are left blank.

	Abdomen length	Thorax length	Genital length	Hind-femur length	Mesofemur length
Abdomen length		**0.39**	0.00	**0.42**	**0.46**
Thorax length	**0.76**		0.00	**0.48**	**0.52**
Genital length	-0.32^{u}	-0.12^{u}		**0.08**	**0.09**
Hind-femur length	0.35		**9.99**		**0.74**
Mesofemur length	**1.24**	-0.17	-0.14	0.19	

ranged from 0.71 to 1.18, and none were undefined or unstable. We examined the covariances that are used to estimate genetic correlations, and found that all these covariances are significantly lower for the DOE-corrected data than for the uncorrected data (Sign test, all two-tailed $P < 0.002$). Thus, correction for DOE reduced the magnitude and stability of the estimates of genetic correlations.

Combining the data from different populations and generations resulted in sample sizes of 581 – 677 pairs (the sample size differed between sexes, and depended on which pair of traits was being examined because some measurements were not available for some individuals). All phenotypic correlations between traits in males are significantly positive (Table 3.1), though the correlations between genital length and other traits are weak (maximum $r = 0.28$). In females (Table 3.2) the phenotypic correlations with genital length are even weaker (maximum $r = 0.08$).

In spite of the large sample sizes, two of the twenty genetic correlations examined are undefined, and another five are unstable (Tables 3.1 and 3.2). Most of these involve genital length, and the estimated genetic correlation between genital and hind-femur lengths for females is far outside the normal bounds for a correlation (Table 3.2). However, all significant estimates of genetic correlations in both sexes are positive and greater than the corresponding phenotypic correlations.

We calculated the correlations between our estimates of genetic correlations and those of Preziosi and Roff (1998) and Fairbairn and Reeve (unpublished), using standard Pearson Product Moment Correlation Coefficients. We then used the Mantel test (Manly, 1985, p. 176) to assess whether the sets of estimated genetic correlations differ between these studies. For males, our estimates were uncorrelated with the estimates from the laboratory studies (r = -0.04, Mantel Z = 8.63, P = 0.23 vs Preziosi and Roff [1998]; r = –0.02, Mantel Z = 2.65, P = 0.35 vs Reeve and Fairbairn [unpublished]). For females, the correlation of our estimates with those of Reeve and Fairbairn (unpublished) was small (r = 0.28, Mantel Z = 1.31, P = 0.81), and with those of Preziosi and Roff (1998) was negative (r = -0.68, Mantel Z = 2.57, P = 0.83). The correlation between the two laboratory studies was relatively large but nonsignificant for both males (r = 0.42, Mantel Z = 2.58, P = 0.35) and females (r = 0.81, Mantel Z = 2.27, P = 0.29).

Discussion

The simulations presented in Lynch (1999) suggest that estimates of genetic correlations generated by this method may tend to underestimate the true values when sample size is small (≤100 pairs). The smaller samples that we used (from West Creek) fall into this category. Many estimates from these samples are well outside of the –1 to 1 limits for correlations, and one quarter of the estimates are undefined. The larger samples from South Creek fared better, and are perhaps near the minimum sample sizes required to obtain stable estimates, given the (unknown) "fraction of related pairs" (Lynch, 1999) in this study. This "fraction of related pairs" is not usually known, making the identification of minimum sample sizes for estimating genetic correlations using this method difficult.

Although the phenotypic correlations remain significant (but smaller) after correction for DOE, most of the estimates of genetic correlations became undefined or unstable. Those estimates that are undefined probably reflect near zero covariances between members of pairs for the same trait (Lynch, 1999). Small estimation error may result in covariances of opposite signs in the denominator of Equation 1, resulting in the estimate being undefined. Similarly, where these small covariances have the same sign, the magnitude of the estimated genetic correlation might be exaggerated by having a near-zero denominator in Equation 1, resulting in unstable estimates.

The covariances between members of pairs for the same trait are lower for the DOE-corrected data than for the uncorrected data, leading us to conclude that the correction for DOE has reduced the similarity between members of pairs. One possibility is that the similarity between relatives in the raw data is largely phenotypic, and that correction for DOE has removed most of it. This would imply that the traits have low heritabilities, a conclusion that contradicts Preziosi and Roff (1998) and Fairbairn and Reeve (unpublished), who found moderate to high heritabilities for these traits in laboratory assays. A second possibility is that the correction for DOE has removed both phenotypic and genetic similarities between relatives. If this is the case, the correction may well have removed the very correlations that we seek to estimate. A third possibility is that the incidence of related individuals in the pairs is low. If this is the case, then little of the similarity between members of pairs would be due to genetic influences, despite there being substantial heritability. Although we have done everything possible with these data to maximize the probability that members of a pair are related, we have no way of estimating the "fraction of related pairs."

Larger samples are less prone to difficulties caused by a low "fraction of related pairs" (Lynch, 1999), and therefore combining the data from our four samples into one large data set for each sex is expected to minimize the number of undefined and unstable estimates. Although the estimated genetic correlations for the combined data are more stable than those for the separate data sets, about one-third of the estimates are undefined or unstable (Tables 3.1 and 3.2). Most undefined/unstable estimates involve genital length. The phenotypic correlations with genital length are much lower than those among the other traits (Tables 3.1 and 3.2), and may reflect low genetic correlations. However, some significant genetic correlations reported in Preziosi and Roff (1998) and Fairbairn and Reeve (unpublished) were not found in this study, even though significant heritabilities were found for all of the traits.

If laboratory estimates using half-sib (Preziosi and Roff, 1998) and full-sib (Fairbairn and Reeve, unpublished) designs reflect the genetic correlations in these field populations, then even with sample sizes well above 500 pairs the "estimation in the absence of pedigree information" of Lynch (1999), after correction for DOE, has been unable to detect some strong genetic correlations. It is still possible that the correction for DOE has weakened the genetic correlation, but not correcting for an environmental source of similarity between relatives would invalidate the method (Lynch, 1999). Lynch (1999) states that this method has "much greater sample size requirements" than standard laboratory methods, but even the approximately 2400 individuals measured for this experiment (581-677 pairs in two sexes) does not appear to have been sufficient to detect all of the genetic correlations evident in the laboratory assays.

The method of "estimation in the absence of pedigree information" proposed by Lynch (1999) did produce significant estimates of genetic correlations, particularly for the male traits. This is encouraging, and perhaps better results might be possible for other species, but *A. remigis* is an excellent candidate for this approach. Large numbers of this waterstrider may be collected, the "fraction of related pairs" is probably high, there is ample information on its biology, and the measured traits are known to have high heritability and genetic correlations in laboratory assays. The relatively high frequency of undefined or unstable estimates in the field assays does not bode well for the utility of this method in general.

Lynch (1999) recognized the potential problems associated with this method, and presented a second method that may generate estimates with more precision. This second method requires using polymorphic molecular markers to estimate of the relatedness of members of each pair. When the fraction of related pairs is low Lynch (1999) showed that the standard deviations of the estimated genetic correlations may be lower for the marker-assisted estimates. Thus, the best hope for estimating genetic correlations from field data may lie in studies combining extensive knowledge of the ecology of the populations concerned with molecular data from multiple marker loci.

General Conclusions

This thesis provides new insight into the operation of natural selection in wild populations. This has been possible because of the utility of *A. remigis* as a model organism for studying natural selection and microevolutionary processes at the population level. Technical considerations make this species easy to work with, and its biology is well-suited to studies of adaptation. The rich literature on this and related species provides a solid foundation on which to build. I have been able to measure selection on specific traits, estimate spatial and temporal heterogeneity in fitness functions, examine selection through different life history stages in the adult life cycle, assess the contribution of each stage to variance in lifetime fitness, and estimate quantitative genetic parameters necessary for selection to lead to adaptation, all in natural populations.

The spatial and temporal heterogeneity in fitness functions demonstrated in Chapter 1 suggests that variability in environmental conditions will result in trait distributions never achieving a true evolutionary equilibrium. Instead, changing fitness functions and phenotypic distributions may result in a dynamic equilibrium, with the mean trait value sometimes higher, sometimes lower, and sometimes near the optimum. This would result in selection that sometimes favours decreased trait values, sometimes favours increased trait values, and sometimes is stabilizing. The net effect over many generations or populations is that an overall long-term optimal trait value may exist, but the trait distribution will not necessarily be at that optimum and will tend to be moved around in response to the variable selection. For the evolutionary biologist, this means that measures of selection in any one population or generation may fail to detect the long-term or broad-scale fitness functions actually driving trait evolution in the species of interest.

Multivariate estimates of selection have allowed me to identify specific body size components in *A. remigis* that are under direct selection. The results in Chapter 1 suggest that total length itself has little direct influence on fitness, but fitness is often correlated with components of body size. The adaptive significance of SSD in total length can therefore best be understood as largely the net result of selection on body size components that are correlated with total length. Another factor that must be considered is that selection may occur at different stages in the life history of the organism. Chapter 2 indicates that prereproductive survival is the stage with the largest contribution to variance in net adult fitness in *A. remigis*, but the strongest selection found in this species has occurred in the reproductive season. The influence of selection in the reproductive season on net adult fitness functions may be relatively small due to the variance in fitness contributed by prereproductive survival. An understanding of the adaptive significance of a trait may be best accomplished by the study of selection on the trait and any correlated traits through each life history stage and for net selection.

Although laboratory studies have concluded that the body size components that we have studied in this thesis are genetically correlated, an estimate of genetic correlations in the field would have confirmed that these correlations reflect those that may be constraining the evolution of body size in these populations. In addition, the relative ease of employing the method of "estimation in the absence of pedigree information" proposed by Lynch (1999) would make it a practical alternative to the labour-intensive half-sib and full-sib experiments that are typically used. However, our results suggest that Lynch's (1999) method may not be generally useful, as many estimates were undefined or unstable. Lynch (1999) was aware of the potential limitations of this method, and his second method, which uses polymorphic molecular markers to estimate of the relatedness of members of each pair, may generate more stable estimates (Lynch, 1999).

The theory of evolution by natural selection is an explanation for the adaptive nature of observed traits. However, current trait distributions should not be assumed to be at a stable evolutionary equilibrium within local populations. My results suggest that evolutionary equilibrium may be dynamic due to interactions between phenotypic distributions and locally variable fitness functions.

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Appendix 1. Assessment of the effect of enamel paint marks on *Aquarius remigis*.

Introduction

Our mark-recapture experiment assumes that selection is acting on the marked individuals just as is would were those individuals not marked. Using enamel paint to mark waterstriders is a standard method, and two studies report that such marking had no effect on survivorship in *A. remigis* (Matthay, 1974; Cooper, 1984). Butler (1987) found that survival in the laboratory was significantly increased for marked individuals compared to unmarked ones in one of two related species. However, experimental evidence from the field is lacking. The following experiment was designed to determine whether or not survival of *A. remigis* in the wild is affected by enamel paint marks such as we used in the mark-recapture experiment for this thesis.

Methods

This experiment was conducted on South Creek from the bottom of the lower recapture-only area (see Chapter 1) to a culvert about 250 m downstream. In this area 110 females and 109 males were captured with hand nets on 12 May 1995. We haphazardly chose 55 females and 54 males and marked each of them with 4 transverse yellow stripes which resembled the numbers on the waterstriders in the mark-recapture experiment. The remaining 55 females and 55 males were each marked with a small dark blue dot on the mesonotum. The blue dot covered about 0.5 mm^2, about 2% of the area covered by the yellow stripes, and was difficult to see unless the individual was captured. All marked waterstriders were then released where they were captured. Each week this section of the creek was carefully searched and all waterstriders, marked or not, were captured. This was to ensure that yellow-striped individuals were not being preferentially captured. We decided a priori that we would compare survival times ("age at death" of Krebs, 1989, p. 416) in the two groups once fewer than 10% of all marked individuals remained, which occurred after 5 weeks. The Mann-Whitney U and Kolmogorov-Smirnov Z were computed using SPSS 8.0.0 (SPSS Inc., 1997).

Results

Survival time did not differ between males and females in the yellow striped treatment ($U = 1477$, $P = 0.95$), blue dot treatment ($U = 1373$, $P = 0.31$), or both treatments combined ($U = 5714$, $P = 0.46$); therefore the sexes were combined for comparing the treatments. The survival times were highly positively skewed, with a mean and median of 13.58 and 8.39 days for the yellow-striped striders, 12.25 and 8.31 days for the striders marked with a small blue dot. There was no significant difference in survival time ($U = 5851$, $P = 0.70$) or in the distribution of survival time ($Z = 0.617$, $P = 0.84$) between the two treatments.

Discussion

We found that marking *A. remigis* with enamel paint had no significant effect on survival through the six week experiment. Previous laboratory tests suggested that this marking technique has no toxic effects on waterstriders, and this experiment confirms that lifespan is not shortened due to the marking. Waterstriders are black on the dorsal surface, and we might have expected the presence of yellow marks to make them more obvious to visually-oriented predators. However, mean and median survival for the yellow-marked individuals were slightly higher than that for the blue dot-marked individuals. Probabilities for the statistical tests are nowhere near marginal, and we are confident that this marking technique did not influence survival in our study animals.

Appendix 2. The time lag between a change in food availability and a change in daily fecundity in *Aquarius remigis*.

Introduction

Blanckenhorn (1991c) and Blanckenhorn et al (1995) report that the daily fecundity of the waterstrider *Aquarius remigis* is influenced by the amount of food available to the female. It is not known, however, how quickly a female's rate of egg-laying will respond to a change in food abundance. Preziosi and Fairbairn (1997) captured females in the field and confined them individually in plastic buckets for one day while feeding half of them: no significant difference in fecundity was found between the fed and unfed females. However, Preziosi and Fairbairn (1997) used fecundity over three days in estimates of selection on body size, during which time the females were provided with ample food. Preziosi and Fairbairn (1997) detected no significant difference in fecundity between the first and last days of the experiment, but we are concerned that providing food to the females may have altered their fecundity and perhaps the relationship between fecundity and body size. Our experiment is designed to determine whether the fecundity of female *A. remigis*, estimated over three days, depends on the amount of food provided to the female during the experiment.

Methods

On 9 June 1996, we captured 196 adult *A. remigis* near Morin Heights, Quebec, Canada, about 80 km northwest of Montreal. The following day, we haphazardly placed approximately equal numbers into three initial treatments in large stream tanks in the laboratory, with a sex ratio of 1:1. Each initial treatment received a different amount of food: 2 *Drosophila melanogaster* per male plus 3 *D. melanogaster* per female per day (low food), 4 *D. melanogaster* per male plus 6 *D. melanogaster* per female per day (moderate food), or 0.5 *Acheta domestica* per individual per day (high food). The first initial treatment is the minimum required to maintain adult *Aquarius remigis* (Blanckenhorn et al., 1995). The second initial treatment is double that amount, while the third is more than they can possibly eat (*Acheta domestica* mean mass used in this study: 0.134 g, c.f. *Aquarius remigis* males 0.04 g and females 0.05 g).

After one week, we haphazardly selected 24 females from each initial treatment for use in the final treatments. Females were distributed one to a cage. Each cage was 27 x 40 x 14 cm and contained 5 cm deep water, an air stone to provide bubbles, and a styrofoam oviposition site. We assigned these cages at random to each of the two final treatments, insuring that half of the waterstriders from each initial treatment were assigned to each final treatment. The final treatments were high food (one *Acheta domestica* per cage per day) or no food. Thus there were six different treatments, three initial treatments (low/moderate/high food) x two final treatments (no/high food), with 12 female waterstriders in each.

We arranged the cages in a large growth chamber, blocking the treatments so that none was on average higher, nearer the front, or more shaded than any other. Temperature was maintained at 22°C with a 16L:8D light cycle. For the next three days, we daily replaced the oviposition site and counted the eggs laid.

Within each of the six treatments we averaged the number of eggs laid by individual females each day (mean daily fecundity). This allowed us to analyze the data with a repeated measures ANOVA: mean daily fecundity for each treatment was repeated for each of the three days. We compared days (within-subjects effect), as well as Initial Treatments and Final Treatments (between-subject effects). Fecundity selection has also been studied using daily fecundity over two days (Ferguson and Fairbairn, 2000), and we therefore repeated our analysis omitting the data from the third day. All statistics were calculated using SPSS for Windows 8.0.0.

Results

The repeated measures ANOVA found a significant difference in daily fecundity among the initial food levels (Table A2.1), which appears to be due to the high values for females from the high food initial treatment (Figure A2.1). Daily fecundity differed significantly among days and there was a significant interaction between day and initial food level (Table A2.1). This interaction appears to reflect two things: daily fecundity increased from the first to the second day for the high and moderate initial treatment females but remained stable for the low food females, and daily fecundity increased from the second to the third day for the moderate and low initial food females but decreased for the high food females (Figure A2.1). None of these patterns suggest differences between the females receiving no food and those receiving high food in the final treatment, and indeed, there was no significant effect of final treatment (Table A2.1). However, the statistical probability for this test (0.09) is marginal, and fecundity is higher in the high food final treatment in all cases except days one and two of the low food initial treatment. Thus, there may be a slight immediate effect (i.e. within three days) of food level, but in our analysis, this is swamped by the much larger effect of initial (i.e. previous) food level. Repeating the analysis with only the first two days finds significant differences among initial food levels and days, but not at all between females in the no food and high food final treatments (Table A2.2).

Table A2.1. Repeated measures ANOVA results for daily fecundity in *Aquarius remigis* over 3 days. Day is the time since the Final Treatment has begun (Day 1, 2 or 3), Initial Treatment is the food level before fecundity is assessed (low, moderate or high), and Final Treatment is the food level while fecundity is being assessed (none or high).

	F	df	P
Day	26.33	2,4	0.0050
Day x Initial Treatment	10.76	4,4	0.020
Day x Final Treatment	0.80	2,4	0.51
Initial Treatment	106.26	2,2	0.0093
Final Treatment	9.26	1,2	0.093

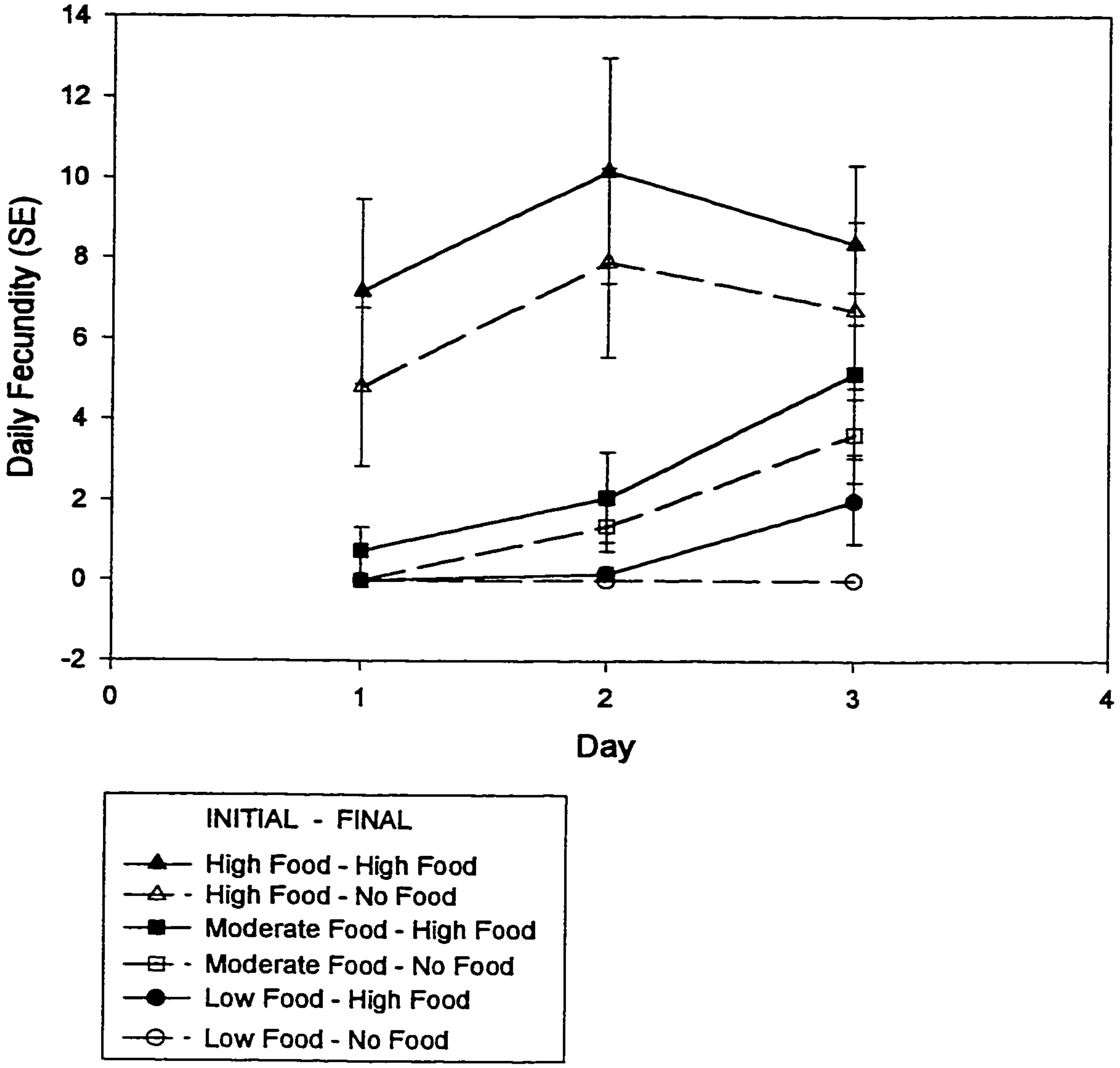

Figure A2.1. Mean daily fecundity in *Aquarius remigis* at Mont St.-Hilaire as a function of day for each initial/final food treatment.

Table A2.2. Repeated measures ANOVA results for daily fecundity in *Aquarius remigis* over 2 days. Day is the time since the Final Treatment has begun (Day 1 or 2), Initial Treatment is the food level before fecundity is assessed (low, moderate or high), and Final Treatment is the food level while fecundity is being assessed (none or high).

	F	df	P
Day	1473.76	1,2	0.00068
Day x Initial Treatment	487.23	2,2	0.0020
Day x Final Treatment	0.038	1,2	0.86
Initial Treatment	49.91	2,2	0.020
Final Treatment	2.48	1,2	0.26

Discussion

The highly significant differences in number of eggs laid among the three initial treatments demonstrate that the experimental design and the sample sizes used are sufficient to detect differences in daily fecundity associated with the range of food levels used. After being subjected to these initial treatments for seven days, egg production remained markedly different for at least three days. We are therefore confident that changes in egg production caused by different food levels will be detectable.

This experiment demonstrates that the amount of food available to female *Aquarius remigis* does not significantly influence their daily fecundity within three days. Although there was no general pattern of daily fecundity increasing in fed females relative to starved females, the increase in mean daily fecundity among high food final treatment females from the low food initial treatment suggests that food-stressed females may begin to increase their daily fecundity in response to an increase in food availability by the third day (Figure A2.1). The borderline significance for the effect of final food treatment (Table A2.1) appears to be due to this increase. There is certainly no suggestion of any such pattern within the first two days (Table A2.2, Figure A2.1).

It may be that food-deprived females (such as those from the low food initial treatment) change their daily fecundity in response to a sudden increase in food availability earlier than females that, for example, experience a sudden decrease in food availability (e.g. females from the high or moderate food initial treatment which were subjected to the no food final treatment). Even if this were to prove true, it is unlikely to be a concern when estimating daily fecundity for female *A. remigis* in the field from experiments of three days or less. Females collected from the field were found to lay a mean 4-5 eggs per day (Preziosi and Fairbairn, 1997; Ferguson and Fairbairn, 2000), indicating that they experience much higher food availability than the females from the low food initial treatment which laid few or no eggs over the first two days. Blanckenhorn et al (1995) also found that females being fed the same as our low food initial treatment laid very few eggs (median lifespan ~ 40 days, median total fecundity in that time ~ 16 eggs). Thus, estimates of daily fecundity over three days or less for *A. remigis* in the field are unlikely to be influenced by the amount of food provided to the females during the experiment.

Appendix 3. Standardized episodic selection coefficients for components of body size in *Aquarius remigis.*

Using the methods presented in Chapter 1, selection coefficients were estimated for components of body size for each episode of selection. n = sample size. * indicates statistical significance at $\alpha = 0.05$ (uncorrected for multiple tests). The sample size for male reproductive lifespan on West Creek in the 1994-95 generation was insufficient to calculate non-linear and correlational selection gradients.

Table A3.1. Standardized multivariate linear selection gradients for male body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for mating frequency DOE was not available.

* $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 466	n = 427	n = 151	n = 121
Genital length	-0.12 (0.14)	-0.15 (0.10)	-0.18 (0.13)	0.08 (0.28)
Abdomen length	0.12 (0.16)	-0.12 (0.12)	-0.22 (0.15)	-0.12 (0.29)
Thorax length	-0.27 (0.21)	-0.14 (0.13)	-0.29 (0.17)	-0.33 (0.32)
Mesofemur length	0.20 (0.25)	0.20 (0.15)	0.53 (0.19)*	-0.15 (0.34)
Reproductive Lifespan	n = 56	n = 82	n = 43	n = 13
Genital length	0.17 (0.18)	0.02 (0.11)	0.03 (0.13)	-0.47 (0.21)
Abdomen length	-0.15 (0.21)	-0.12 (0.14)	0.11 (0.15)	-0.63 (0.27)
Thorax length	-0.01 (0.28)	0.00 (0.17)	0.10 (0.16)	0.32 (0.29)
Mesofemur length	-0.09 (0.33)	0.01 (0.19)	-0.06 (0.16)	-0.16 (0.31)
Mating Frequency	n = 152	n = 246	n = 89	n = 101
Genital length	0.23 (0.10)*	0.10 (0.05)	0.23 (0.16)	0.06 (0.15)
Abdomen length	-0.18 (0.12)	-0.06 (0.06)	0.12 (0.18)	-0.11 (0.15)
Thorax length	-0.16 (0.14)	-0.19 (0.08)*	0.13 (0.19)	0.00 (0.18)
Mesofemur length	-0.01 (0.16)	0.09 (0.09)	0.07 (0.19)	0.14 (0.18)

Table A3.2. Standardized multivariate linear selection gradients for female body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for daily fecundity DOE was not available.

* $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 476	n = 484	n = 209	n = 146
Genital length	0.23 (0.12)*	0.15 (0.09)	0.03 (0.12)	0.02 (0.15)
Abdomen length	0.18 (0.16)	0.00 (0.13)	0.14 (0.13)	-0.04 (0.17)
Thorax length	-0.14 (0.17)	0.03 (0.13)	0.03 (0.13)	0.00 (0.18)
Mesofemur length	0.15 (0.21)	-0.07 (0.14)	0.03 (0.15)	0.32 (0.19)
Reproductive Lifespan	n = 64	n = 89	n = 57	n = 34
Genital length	0.16 (0.13)	0.07 (0.08)	-0.12 (0.13)	0.25 (0.16)
Abdomen length	0.15 (0.16)	0.14 (0.10)	-0.08 (0.14)	-0.36 (0.19)
Thorax length	-0.27 (0.18)	0.04 (0.10)	-0.04 (0.14)	0.33 (0.16)*
Mesofemur length	0.30 (0.25)	-0.13 (0.11)	0.09 (0.16)	0.15 (0.18)
Daily Fecundity	n = 35	n = 60	n = 36	n = 48
Genital length	0.22 (0.18)	0.10 (0.10)	0.07 (0.16)	0.03 (0.18)
Abdomen length	0.12 (0.20)	0.11 (0.14)	-0.14 (0.18)	-0.27 (0.22)
Thorax length	0.00 (0.24)	0.02 (0.17)	0.01 (0.20)	0.18 (0.23)
Mesofemur length	-0.24 (0.22)	-0.04 (0.15)	0.18 (0.22)	-0.01 (0.24)

Table A3.3. Standardized multivariate non-linear selection gradients for male body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for mating frequency DOE was not available.
* $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 466	n = 427	n = 151	n = 121
Genital length	-0.04 (0.06)	0.03 (0.07)	0.20 (0.10)*	-0.09 (0.25)
Abdomen length	-0.16 (0.12)	-0.05 (0.07)	-0.03 (0.13)	0.49 (0.31)
Thorax length	0.38 (0.28)	0.09 (0.09)	0.05 (0.18)	-0.21 (0.28)
Mesofemur length	0.15 (0.32)	0.02 (0.16)	0.31 (0.22)	-0.04 (0.33)
Reproductive Lifespan	n = 56	n = 82	n = 43	n = 13
Genital length	0.28 (0.19)	0.08 (0.10)	0.16 (0.24)	
Abdomen length	0.44 (0.27)	-0.34 (0.28)	-0.04 (0.22)	
Thorax length	0.27 (0.53)	-0.58 (0.29)	0.28 (0.23)	
Mesofemur length	0.28 (0.63)	-0.45 (0.35)	0.27 (0.23)	
Mating Frequency	n = 152	n = 246	n = 89	n = 101
Genital length	0.07 (0.09)	-0.02 (0.04)	-0.20 (0.12)	-0.06 (0.10)
Abdomen length	0.02 (0.08)	0.05 (0.04)	0.16 (0.17)	0.11 (0.10)
Thorax length	-0.13 (0.15)	0.06 (0.10)	0.22 (0.18)	-0.35 (0.22)
Mesofemur length	-0.22 (0.19)	0.05 (0.12)	0.26 (0.21)	0.01 (0.25)

Table A3.4. Standardized multivariate non-linear selection gradients for female body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for daily fecundity DOE was not available.
* $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 476	n = 484	n = 209	n = 146
Genital length	0.16 (0.07)*	-0.03 (0.06)	-0.10 (0.05)*	-0.03 (0.10)
Abdomen length	0.24 (0.17)	-0.18 (0.12)	0.28 (0.11)*	0.14 (0.12)
Thorax length	-0.04 (0.18)	0.04 (0.12)	0.05 (0.11)	0.22 (0.17)
Mesofemur length	-0.05 (0.28)	-0.02 (0.14)	0.07 (0.12)	0.12 (0.17)
Reproductive Lifespan	n = 64	n = 89	n = 57	n = 34
Genital length	-0.03 (0.13)	-0.04 (0.07)	0.09 (0.11)	0.15 (0.28)
Abdomen length	-0.15 (0.16)	0.24 (0.14)	-0.23 (0.19)	-0.15 (0.27)
Thorax length	0.32 (0.27)	-0.10 (0.11)	-0.05 (0.16)	0.00 (0.22)
Mesofemur length	-0.03 (0.39)	0.12 (0.12)	0.08 (0.19)	0.21 (0.21)
Daily Fecundity	n = 35	n = 60	n = 36	n = 48
Genital length	0.59 (0.25)*	0.03 (0.08)	-0.26 (0.17)	0.00 (0.14)
Abdomen length	-0.13 (0.33)	-0.16 (0.15)	-0.01 (0.31)	0.10 (0.32)
Thorax length	-0.24 (0.49)	0.69 (0.29)*	0.02 (0.39)	-0.38 (0.27)
Mesofemur length	0.23 (0.30)	0.29 (0.23)	0.34 (0.37)	-0.45 (0.26)

Table A3.5. Standardized correlational selection gradients for male body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for mating frequency DOE was not available. * $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 466	n = 427	n = 151	n = 121
Genital x Abdomen	0.08 (0.19)	0.04 (0.14)	-0.10 (0.19)	-0.40 (0.30)
Genital x Thorax	-0.20 (0.30)	0.02 (0.17)	-0.27 (0.26)	-0.45 (0.33)
Genital x Mesofemur	-0.29 (0.33)	-0.08 (0.20)	-0.08 (0.30)	-0.27 (0.41)
Abdomen x Thorax	0.27 (0.20)	-0.22 (0.11)	0.01 (0.23)	0.34 (0.35)
Abdomen x Mesofemur	-0.11 (0.30)	0.20 (0.18)	-0.23 (0.25)	0.14 (0.48)
Thorax x Mesofemur	-0.07 (0.46)	0.00 (0.17)	-0.58 (0.32)	0.34 (0.48)
Reproductive Lifespan	n = 56	n = 82	n = 43	n = 13
Genital x Abdomen	0.25 (0.28)	0.13 (0.18)	0.05 (0.43)	
Genital x Thorax	0.56 (0.58)	-0.07 (0.26)	-0.40 (0.37)	
Genital x Mesofemur	-0.81 (0.69)	-0.08 (0.31)	-0.12 (0.38)	
Abdomen x Thorax	-0.60 (0.52)	-0.79 (0.37)*	0.07 (0.27)	
Abdomen x Mesofemur	0.80 (0.55)	0.66 (0.47)	-0.21 (0.28)	
Thorax x Mesofemur	-0.48 (0.81)	1.08 (0.48)*	-0.51 (0.37)	
Mating Frequency	n = 152	n = 246	n = 89	n = 101
Genital x Abdomen	-0.06 (0.15)	0.06 (0.08)	-0.22 (0.24)	-0.04 (0.19)
Genital x Thorax	-0.04 (0.20)	0.10 (0.09)	0.50 (0.34)	0.06 (0.22)
Genital x Mesofemur	0.14 (0.22)	-0.19 (0.13)	-0.32 (0.25)	0.07 (0.21)
Abdomen x Thorax	0.09 (0.15)	-0.02 (0.08)	0.31 (0.27)	0.20 (0.23)
Abdomen x Mesofemur	-0.04 (0.24)	-0.16 (0.12)	-0.36 (0.27)	0.17 (0.19)
Thorax x Mesofemur	0.31 (0.28)	-0.04 (0.20)	-0.31 (0.34)	0.08 (0.38)

Table A3.6. Standardized correlational selection gradients for female body size (SE). For prereproductive survival and reproductive lifespan the coefficients were calculated with DOE in the model, but for daily fecundity DOE was not available. * $\alpha < 0.05$

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Prereproductive Survival	n = 476	n = 484	n = 209	n = 146
Genital x Abdomen	0.11 (0.17)	0.01 (0.13)	0.10 (0.08)	-0.10 (0.21)
Genital x Thorax	0.17 (0.18)	0.11 (0.15)	0.07 (0.20)	0.17 (0.20)
Genital x Mesofemur	-0.13 (0.22)	-0.19 (0.15)	-0.07 (0.24)	-0.06 (0.23)
Abdomen x Thorax	0.12 (0.24)	0.01 (0.14)	-0.12 (0.16)	0.10 (0.27)
Abdomen x Mesofemur	-0.57 (0.30)	0.14 (0.21)	-0.34 (0.19)	0.08 (0.26)
Thorax x Mesofemur	0.06 (0.33)	-0.16 (0.21)	0.15 (0.20)	-0.57 (0.26)*
Reproductive Lifespan	n = 64	n = 89	n = 57	n = 34
Genital x Abdomen	-0.14 (0.18)	0.18 (0.15)	0.03 (0.20)	0.01 (0.38)
Genital x Thorax	0.28 (0.23)	-0.19 (0.16)	-0.03 (0.24)	0.11 (0.23)
Genital x Mesofemur	0.02 (0.39)	0.01 (0.17)	0.19 (0.28)	0.01 (0.40)
Abdomen x Thorax	0.07 (0.30)	0.17 (0.13)	-0.25 (0.24)	-0.49 (0.34)
Abdomen x Mesofemur	-0.34 (0.40)	-0.34 (0.22)	0.49 (0.37)	-0.72 (0.45)
Thorax x Mesofemur	-0.04 (0.53)	-0.06 (0.24)	-0.02 (0.36)	0.45 (0.24)
Daily Fecundity	n = 35	n = 60	n = 36	n = 48
Genital x Abdomen	0.47 (0.32)	0.13 (0.16)	0.13 (0.26)	0.29 (0.28)
Genital x Thorax	-0.08 (0.35)	-0.31 (0.23)	-0.13 (0.27)	0.16 (0.29)
Genital x Mesofemur	-0.32 (0.33)	0.19 (0.19)	0.27 (0.34)	-0.44 (0.34)
Abdomen x Thorax	0.47 (0.31)	-0.12 (0.30)	0.29 (0.30)	-0.15 (0.42)
Abdomen x Mesofemur	0.29 (0.45)	0.34 (0.27)	-0.38 (0.48)	-0.12 (0.57)
Thorax x Mesofemur	-0.35 (0.33)	-1.01 (0.40)*	-0.44 (0.62)	0.64 (0.49)

Appendix 4. Summary statistics for body size in two populations of waterstiders *Aquarius remigis* through two generations.

While the statistical analyses presented in this thesis contribute to our understanding of microevolutionary processes in *Aquarius remigis*, relating these analyses to the biology of this species may be aided by knowledge of the actual body sizes of the organisms under selection. In this appendix I present the mean and standard error for each of the body size measurements used in the analysis of selection.

Table A4.1. Mean (SE) for each body size measurement (in mm) for *Aquarius remigis* at Mont St.-Hilaire. Measurements taken from prereproductive adults (see Chapter 1).

	South Creek		West Creek	
	1993-94	1994-95	1993-94	1994-95
Male	n = 466	n = 427	n = 151	n = 121
Total Length	12.730 (0.028)	13.115 (0.024)	12.478 (0.030)	12.684 (0.035)
Genital Length	2.413 (0.008)	2.551 (0.007)	2.452 (0.012)	2.571 (0.015)
Abdomen Length	3.403 (0.010)	3.515 (0.011)	3.277 (0.017)	3.315 (0.015)
Thorax Length	5.487 (0.015)	5.660 (0.014)	5.362 (0.021)	5.497 (0.020)
Mesofemur Length	9.197 (0.026)	9.418 (0.021)	8.919 (0.033)	9.080 (0.030)
Female	n = 476	n = 484	n = 209	n = 146
Total Length	13.771 (0.030)	14.141 (0.026)	13.454 (0.031)	13.632 (0.033)
Genital Length	0.729 (0.006)	0.730 (0.006)	0.770 (0.012)	0.754 (0.011)
Abdomen Length	5.839 (0.015)	6.007 (0.014)	5.663 (0.019)	5.755 (0.018)
Thorax Length	5.771 (0.015)	5.987 (0.013)	5.684 (0.017)	5.812 (0.015)
Mesofemur Length	9.093 (0.024)	9.318 (0.017)	8.752 (0.032)	8.936 (0.027)