A GENETIC BASIS FOR LIFE HISTORY DIFFERENCES AMONG POPULATIONS OF THE MIDGE, *CHIRONOMUS RIPARIUS* (DIPTERA:CHIRONOMIDAE)

by

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ABSTRACT

Laboratory and field experiments were conducted with six populations of *Chironomus riparius* to test for variation among the populations in life history parameters such as growth, mortality and/or reproductive rates. Egg masses of *C. riparius* from five field populations and one laboratory population were obtained and used to initiate laboratory cultures for experimentation. Environmental effects on life history variations could be ruled out because experiments were conducted with larvae that had been reared in the laboratory for several generations under identical conditions. Populations were compared under a variety of experimental conditions to test whether they would respond similarly, if genetically similar, or differently, if their genotypes were different.

The populations were subjected to a variety of experimental conditions in the laboratory in which temperature and food quality were manipulated. The population collected from Strouble's Creek, Virginia demonstrated a pattern of high growth rate and low mortality rate, while the population collected from the final clarifier at the Madison, Wisconsin sewage treatment plant showed a pattern of low growth rate and high mortality

rate. When several populations were transplanted into a high chlorine environment at the Blacksburg, Virginia sewage treatment plant, the population that had been previously exposed to the chlorine showed a significantly lower mortality rate than the other populations. A series of toxicity tests revealed different sensitivities among several populations to an experimental pesticide. Differences among populations were also found in numbers of egg masses produced, numbers of eggs per egg mass, length of time until the onset of adult emergence, and the overall length of the emergence period.

The results of this study show the importance of genetic differences among populations of *C. riparius*. Many commonly measured life history parameters that are usually assumed to vary primarily from differences in environment, have been shown to vary as well from underlying genetic differences between populations. The role of genetics should be assessed, whenever possible, in studies where comparisons between populations of aquatic insects are made, in order to determine the degree to which genetics and environmental variables contribute to observed population differences.

DEDICATION

I dedicate this dissertation to Rathi, Kayla, Mom, and Dad.

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1.0 INTRODUCTION

An unstated and infrequently tested assumption found in the literature on aquatic insect ecology is that life history differences between populations are due primarily to environmental influences such as temperature, food quality, and/or food quantity, with notable exceptions (e.g., Robinson et al. 1992, Hefti et al. 1988, Sweeney et al. 1986, Fairbairn 1984, Martin and Wulker 1971, Khoo 1968). Populations of a species are usually treated as if they did not vary genetically spatially or temporally. Although an examination of recent literature, i.e., mainly subsequent to 1986, indicates that researchers in this field have started to focus on this issue, particularly with respect to genetically-based tolerance of aquatic insect populations to environmental stress (Miller and Hendricks 1996, Postma and Davids 1995, Postma et al. 1995a, Postma et al. 1995b, Hoffman and Fisher 1994, Benton and Guttman 1992a, Benton and Guttman 1990, Jernelov et al. 1981).

The approach used here to ascertain genetic differences in life history traits among populations was a simple but powerful technique and can be used to examine a variety of traits. The basic approach assumes that the effects of genetic makeup on life histories may be studied by comparison of the performance of different populations which are treated identically (Tryon 1940, Ayala 1968, Hedrick and Reichert 1989). Populations respond similarly when genetic makeup is the same or differently if the genetic makeup is not the same. Individuals from test populations are to be reared in the laboratory for at least one generation under identical standard clean water conditions before making

comparisons, in order to eliminate environmental effects, i.e., acclimation differences which may affect performance in field-collected individuals (Bradley 1978b, Levinton and Monahan 1983, Lonsdale and Levinton 1986, Klerks and Weis 1987, Postma et al. 1995a, Miller and Hendricks 1996). In addition, laboratory-rearing for at least one generation under identical, clean water conditions is necessary to rule out maternal effects which may be present in first generation laboratory-reared individuals (Bradley 1978a, Levinton 1980, Fairbairn 1984, Klerks and Weis 1987, Postma and Davids 1995, Postma et al. 1995b). In summary, acclimation effects may confound results when comparisons are made using field-collected organisms and maternal effects may confound results if first generation individuals are compared for genetic differences. Therefore, the proper methodology requires that only individuals from the second or later generations, that have been cultured under identical, clean water conditions, be used when assessing whether differences between populations have a genetic basis. However, the use of second or later laboratory-reared generations has been questioned because of the potential for inbreeding and the accompanying loss of genetic diversity or for the potential for selection to laboratory conditions (Mason et al. 1987, Postma et al. 1995a).

Many studies that have been undertaken to determine the role of genetic differences in producing life history variations between populations or between individuals within a population either do not rule out acclimation effects because they use field-collected individuals for comparisons (e.g., Postma and Davids 1995, Berven 1982) or do not rule out maternal effects because they use first generation offspring of field-collected parents (e.g., Hoffman and Fisher 1994, Fairbairn 1984, Postma et al. 1995a).

To the best of my knowledge only two published studies in the field of aquatic entomology have taken all of the steps required to ensure that the results unequivocally demonstrate that life history variations have a genetic basis and are not caused by the environment, i.e., Postma et al. 1995b, Miller and Hendricks 1996. One reason for the lack of appropriate experimental designs is undoubtedly the difficulty of rearing the test organism in the laboratory for multiple generations.

Enzyme electrophoresis is often used in conjunction with the analysis of life histories and is sometimes erroneously used as evidence of a genetic basis for life history variations. This method is problematic because it is difficult to show that the presence or absence of particular alleles has any connection to the life history trait in question. That is, a cause-and-effect relationship must be established between the resolvable allozymes and the life history trait. Without such information, it is not clear whether the life history trait is varying due to the effects of environmental changes concurrently with another unknown trait that is being selected for under the same varying environmental conditions with the allozymes resolved on the gel actually corresponding to allelic differences being selected for that unknown trait. If this were the case, then the resolvable allozymes might be used as a "marker", but it does not prove that variations in the life history parameter of interest are genetically-based.

Enzyme electrophoresis is useful for measuring certain important components of population genetic structure such as genetic similarity among populations, levels of gene flow between populations, the breeding structure of a population (e.g., high levels of homozygosity would suggest inbreeding), the level of genetic diversity in a population,

and for assessing the *possibility* of selection due to environmental variation (e.g., clines in allozyme frequencies)(Sweeney et al. 1986).

The overall objective of my research was to test for a genetic basis for life history variations among populations of *C. riparius* using experimental procedures that would rule out environmental effects. In support of the overall objective, a series of experiments were conducted with these specific objectives:

1. To test whether under natural (field) conditions, some of the study populations actually differed in life history parameters that would later be studied under laboratory conditions.
2. To test for genetic differences in larval growth and mortality among populations under varying temperature/food quality regimes.
3. To test for genetic differences among populations in certain adult life history parameters such as timing of emergence and fecundity.
4. To test for genetic differences in stress tolerance among populations using transplantation techniques and standard toxicity testing procedures.

2.0 LITERATURE REVIEW

2.1 Biology of *Chironomus riparius* Meigen

Chironomus riparius Meigen is a member of the subfamily Chironominae, in the family of the non-biting midges, Chironomidae. Midges are holometabolous, going through complete metamorphosis consisting of egg, larva, pupa, and adult stages. *C. riparius* mates in aerial swarms (Learner and Edwards, 1966), although in laboratory cultures mating can take place on the walls of the culture container (pers. obs.). After mating, the female deposits the eggs in water. The eggs are contained in a gelatinous matrix which may contain hundreds of individual eggs (Learner and Edwards, 1966). All known chironomids possess four larval instars (Oliver, 1971). Larvae of *C. riparius* are collector-gatherers, feeding mainly on detritus and its associated bacteria and fungi (Coffman and Ferrington, 1984; Pinder, 1986). Larvae of *C. riparius* are tube-builders. The salivary glands secrete a silk-like substance which the larvae use to bind together particles of the substrate (Oliver, 1971). Voltinism varies widely for *C. riparius*. Rasmussen (1984) found the species to be univoltine in a Canadian pond. Gower and Buckland (1978) found a pattern of emergence in a small stream in England beneath a sewage outfall indicating five generations in one year. Learner and Edwards (1966) suggested that seven generations per year occurred in an organically-enriched stream in England, but because the emergence peaks they measured may have been the result of overlapping cohorts, the total number of generations may have been less than seven.

Learner and Potter (1974) found a population of *C. riparius* that produced seven generations in one year in a pond receiving high quality sewage, while another population in an adjacent pond without sewage produced only three generations per year.

2.2 Environmental Variables Affecting Life History Parameters

A wide variety of factors, both biotic (competition, predation, parasitism, nutrition) and abiotic (temperature, nutrition, photoperiod, dissolved oxygen, pH, current, substrate, pollutants) may influence the life history patterns of aquatic insects. It is commonly observed that the life history pattern of a particular species of insect may vary both temporally, from season to season or annually, and spatially, between sites within a particular aquatic habitat or between geographically separate habitats. For example, temperature regime (Butler 1982), food quality (Ward and Cummins 1979) and quantity (Anderson and Cummins 1979), and inter- and intraspecific competition for food and space (Rasmussen 1985, Rasmussen and Downing 1988, Malmqvist and Sjostrom 1989), as well as other factors can contribute to controlling the number of generations a particular population of aquatic insect goes through in one year.

An untested assumption in most studies describing life history differences among populations of aquatic insects is that the variations are due entirely to ecophenotypic variation, and the role of genetics in life history differences is infrequently addressed.

A review of published studies that investigate the role of biotic and abiotic factors on aquatic insect life histories follows.

Hanazato and Yasuno (1989) studied the effect of temperature on the growth of *Chaoborus flavicans*. Egg masses of *Chaoborus flavicans* were collected and allowed to hatch in the laboratory by incubating them at 15, 20, 25 and 30°C. Once 15 larvae hatched at each of the different temperatures they were transferred to dechlorinated water and reared under 14h-10h LD photocycle at again 15, 20, 25 and 30°C. The larvae were fed excess food daily. Food was the rotifer *Brachionus urceolaris* with the addition of the cladoceran *Moina micrura* after the second instar. The development time of early instars was longer at lower temperatures than at higher temperatures. High mortality of fourth-instar larvae and pupae was observed at higher temperatures (25 and 30), whereas their survival markedly increased at lower temperatures (20 and 15). Growth rates of early instar larvae was reduced at lower temperatures whereas the maximum body length of larvae increased with declining temperature. Fourth-instar duration for males was shorter than for females resulting in earlier emergence of males.

Soderstrom (1988) studied the effect of temperature and food quality on survival and growth rates, developmental time adult size, and fecundity in two species of mayflies, *Parameletus minor* and *P. chelifer*. Nymphs were collected in the field and reared in the laboratory. Four different temperatures (5.9, 10.8, 14.6, and 19.8°C) and three types of food (detritus from a seasonal stream, detritus from a river margin, and Tetramin) were used. Excess food was provided for the nymphs. Both temperature and food quality affected growth rates and fecundity. Growth rates were greater at higher temperatures for both species. *P. minor* grew faster than *P. chelifer* at lower temperatures whereas at higher temperatures the reverse was true. Mortality rate was less at lower temperatures

for both species and mortality increased at higher temperatures. Development time was shorter at higher temperatures for both species. Growth rates in both species decreased with an increase in the C/N ratio of the food. In *P.minor* fecundity decreased with increase in temperature. In *P. chelifer* there was no difference in egg production at 10.8 and 14.6 °C, but food quality affected egg production in both species.

Hauer and Benke (1987) studied the effect of river hydrograph and temperature on the growth rates of black fly larvae. Larvae were divided into size classes and introduced into stream chambers. The stream chambers were maintained at ambient water temperature. Growth rate was strongly affected by temperature. There was a strong positive relationship between growth rate and water temperature. At a given temperature however, black fly abundance and growth rates reached their highest value as the river discharge increased and the flooding of a riparian swamp occured. The influence of flooding on growth of black flies is considered to be due to the increase in food quantity and quality. Flooding was associated with an increase in seston quality.

Webb and Merritt (1987) studied the influence of diet on the growth of mayfly *Stenonema vicarium*. Field collected nymphs were reared in stream channels in the laboratory at 11±1°C, under 12:12 h photoperiod on two different types of food: natural stream periphyton (ALG) and leaf detritus devoid of algae (DET). Diet had a significant effect on growth rates with nymphs on ALG growing faster than those on DET.

Huryn and Wallace (1986) studied in-situ growth rates of chironomids in streams. Larvae were sorted into three length classes and introduced into growth chambers. Stream temperature was recorded continuosly. After 7-14 days of incubation, material in

the chambers was removed and the chironomids were counted and measured. Individual larvae were measured before and after incubation and an average length was estimated. Using a length-weight regression, the average ash-free dry mass was obtained and instantaneous growth rate coefficients (IGRs) were calculated. Temperature significantly and linearly affected the IGRs of the larvae in all the three size classes. The range of temperature considered was between 2-15°C.

Lillehammer (1986) studied the effect of temperature on the egg incubation period and nymphal growth of two *Nemoura* species, *N. viki* and *N. arctica*, in the laboratory. Their growth was studied at 4, 8, 12, 16, 20 and 24 °C. The nymphs were fed a mixture of S*alix* and *Betula* leaves. Temperature affected survival and growth of both species. Temperatures above 16°C were lethal. Growth of both species was slow at 4°C with *N.arctica* failing to emerge at 4°C.

Sweeney and Vannote (1986) examined the effects of leaf species and stream temperature regimes on growth and production of the stonefly *Soyedina carolinensis*. Temperature played a more important role than diet in influencing nymphal growth patterns. There was a strong correlation between adult biomass and temperature, regardless of diet, during the months of December, January, and February, when >80% of nymphal biomass was produced. They concluded that small changes in temperature during the three critical months of nymphal growth resulted in significant differences in adult size. The temperature differences they observed between sites over the three month period of maximum growth were approximately 5°C, and resulted in a 32% reduction in adult size at the cooler sites. Diet was seen to influence adult size at one experimental

site. Adult biomass was greatest when nymphs were fed sugar maple leaves, and lowest when nymphs were fed American beech leaves.

Rasmussen (1985) studied the effects of larval density and microdetritus on the growth of two species of chironomid larvae. Different densities of *Chironomus riparius* (CR) and *Glyptotendipes paripes (*GP) mixtures were studied in enclosures placed in a pond. The densities studied were 9:1 CR:GP mixture and 1:9 CR:GP mixture. The effect of microdetritus additions were studied by adding seived pond mud to which Tetramin fish food was added and allowed to decompose. The results showed that density significantly affected growth rates. Reduced growth rates were stronger in intraspecies comparisons whereas weak effects were seen in interspecies comparisons, i.e. the effect was stronger on the most abundant species rather than the least abundant. The addition of microdetritus resulted in increased growth rates for *C. riparius* at both densities, whereas no effect was observed for *G. paripes*. The observed difference was considered to be due to the differences in the feeding habits of the two species, *C. riparius* being a collector-gatherer and *G. paripes* a filter-feeder.

Fuller and Mackay (1981) studied the effect of diet on the growth of third, fourth and fifth instars of three species of *Hydropsyche*. Field collected larvae of *H. betteni*, *H. slossonae* and *H. sparna* were reared in laboratory chambers. The larvae were fed an excess amount of food in relation to gut capacity, twice daily. The diet consisted of leaf detritus, ground up fecal detritus, diatoms and animal material composed of finely chopped terrestrial enchytraeid worms. Greatest weight gains for all species was found to occur on animal diet. Leaf detritus produced the slowest overall growth rates. Growth

for all species was significantly faster on diatoms than on leaves. Comparison of growth on leaf detritus and fecal detritus did not show any difference except for the third instars of *H. betteni*. Large weight gains were associated with diatoms and animal diets with animal diet being more important during summer. Animal material as a diet varied in its impotance with season, species and instar. Even though detritus was found in high percentages in larval guts its contribution to larval growth was found to be of minor importance. Weight gains were always highest in *H. sparna* when compared to the other two species, regardless of the food type. Fuller and Mackay suggested that the ability of *H. sparna* to convert food more efficiently may explain its widespread distribution.

Carter (1980) studied the effect of temperature, oxygen saturation and phytoplankton biomass on an unexpected variation in the length of life cycle of *Chironomus anthracinus*. The cause for the extension of the life cycle from one to two years for some larvae was found to be the weight of the fourth instar larvae. Some fourth instar larvae in 1975 weighed 0.7 mg which was lower than larval weights in the previous years. The failure to gain enough weight prevented pupation and extended the length of life cycle. The relationship between percent oxygen saturation and monthly dry weight increase did not show a significant relationship. He suggested that temperature played a role. It also appeared that both quantity and quality of the standing phytoplankton may be important in affecting larval growth.

Markarian (1980) studied the relationship between water temperature and aquatic insect growth in a stream. Insects belonging to Ephemeroptera, Plecoptera and Trichoptera were studied. Benthic samples of the insects were collected from the stream.

The daily mean temperature was computed using the data obtained from continuous temperature recorders. Degree days were calculated using the formula K = y(t-a), where K= degree days, y=time in days, t=actual environmental temperature and a=critical minimal temperature below which growth ceases. Pronotum widths and body lengths of the insects were measured. Growth rates were also calculated. All species were greatly affected by temperature, with lower specific growth rates at lower temperatures. Between-site temperature differences accounted for the differences in growth for some species more than for others. However, growth was directly proportional to the number of degree-days experienced by the populations.

Sweeney and Vannote (1978) and Vannote and Sweeney (1980) describe the Thermal Equilibrium Hypothesis, a model to explain the relationship between temperature and the geographic distribution and stability of aquatic insect populations. They hypothesized a dynamic equilibrium between temperature and insect growth, metabolism, reproduction and development time. When individuals of a species inhabit an area with an optimal thermal regime, body weight and reproductive potential are maximized. Body weight and fecundity decrease in populations farther away from the thermal optimum because temperature-controlled functions (e.g., efficiency of energy use, developmental processes and generation times) characteristic for the species are in nonequilibrium with the environment. They predicted that as a species moves into water that is warmer than its thermal optimum the following occurs: (1) individuals increase the rate of energy assimilation. (2) the proportion of energy available for growth decreases because maintenance metabolism will require an even higher proportion of total

metabolism, (3) adult tissue begins to form earlier in the development process and proceeds faster, reducing the period of larval growth, and (4) these factors (i.e., reduced growth period, maintenance metabolism as a greater proportion of total metabolism) result in decreased adult body weight and fecundity. When populations occur in habitats with colder than optimal temperatures they predicted: (1) reduced assimilation rates, (2) no disproportional increase in maintenance metabolism, (3) the slower formation of adult tissue, increasing the length of the larval or nymphal development period, (4) lower adult body weight because, although larval and adult tissues both develop slower, larval tissue development is slowed proportionately more than adult tissue, and (5) reduced reproduction rate because of smaller adults and less efficient conversion of stored energy into eggs. The stability of a subpopulation decreases when it occurs in a less-than-optimal thermal regime because its ability to exploit ecosystem resources is reduced compared with that of other species in the community. This lowers the subpopulation in the competitive hierarchy and could lead to extinction when increased environmental stress increases, puts a premium on the efficient use of resources.

Mackey (1977a) studied the development rates, length-age growth rates and length-weight relationships of twelve species of chironomid larvae at three temperatures and different types of food. Development rates were studied at 10, 15 and 20°C in a laboratory setting. Detritivore species were fed epiphytic detritus from which the fauna had been removed. Carnivores were fed *Stentor* along with daily additions of small oligochaetes and detritus. Filter feeders were given a culture of Thames River phytoplankton and detritus. Food type affected growth and development rates for

Ablabesmyia monilis. Most species showed increased growth and development rates with an increase in temperature except for *Cricotopus bicinctus*, which showed only an increase in development rate.

2.3 Genetic Differences Between Populations (No Life History Data)

Jackson and Resh (1992) used allozyme electrophoresis to study populations of the caddisfly *Helicopsyche borealis* within and between drainage basins in California, as well as in three streams in eastern North America. They found fixed allelic differences between populations indicating a lack of interbreeding between populations. The populations were not panmictic even though the sample sites were all within 7-11 km of each other.

Preziosi and Fairbairn (1992) genetically characterized 28 populations of the apterous waterstrider, *Gerris remigis*, from streams throughout North America using starch gel electrophoresis. Their analysis indicated that the greatest differentiation occurs at the level of streams within watersheds. The levels of differentiation among watersheds within regions and among regions within the total were similar and slightly lower than differentiation among streams within a watershed. Only sites within a stream exchanged a sufficient number of individuals to prevent genetic differentiation through the effects of genetic drift. This supports the conclusion of Zera (1981) who found substantial genetic differences between populations of *G. remigis* even over short distances. Preziosi and

Fairbairn (1992) found a high level of fixation of alternative alleles without geographic pattern indicating the effects of genetic drift.

Kambhampati and Rai (1991) compared morphometric traits and allelle frequencies in 19 populations of the mosquito *Aedes albopictus* in the U.S. They found significant variation within and among populations both in morphometric traits and allele frequencies. While the morphometric variations followed a pattern indicating some grouping based on geographic location, allele variations did not and so were thought to be the result of genetic drift.

Woods et al. (1989) studied the genetic variability of nine loci in seven laboratory populations and one wild population of the midge *Chironomus tentans* using starch gel electrophoresis. Three loci showed no variation among the populations tested. Two loci varied only in the wild population. The remaining four loci varied in the wild population and at least one of the six laboratory populations. The wild population had seven unique alleles distributed among eight loci. None of the lab populations had a unique allele. The wild population was significantly more heterozygous than all of the laboratory populations. The lab populations also differed significantly in levels of heterozygosity. The percentage of polymorphic loci was lower among the lab populations compared to the wild population, but not quite significantly so ($p < 0.06$). Of particular interest was the finding of significant differences between the Miami University and University of Michigan populations in the level of heterozygosity even though both were derived from a common source. A decline in genetic variation may be due in part to the more uniform environmental conditions in the laboratory, bottlenecks, nonrandom mating patterns and

founder effects. The authors suggest that when a species undergoes domestication (lab culturing) the loss of alleles and loss of heterozygosity is expected.

Guttman and Weigt (1989) examined microgeographic genetic variation among populations of three species of *Enchenopa binotata* (Homoptera: Membracidae) using starch gel electrophoresis. They found significant heterogeneity among nymphal samples taken from different braches of the same tree as well as between samples from trees within the same local area. Because the nymphs collected on each branch could be offspring from one to a few females (little movement occurs between branches after hatching), the authors suggest that sampling error may be the source of heterogeneity among branches within a tree, i.e., an artifact caused by small sample size.

Guttman and Weigt (1989) showed that tree host species was more important than macrogeographic distance in explaining genetic distance between populations of the treehopper *Enchenopa binotata*. Individuals from the tree host *Ptelea trifoliata* (wafer ash) had the lowest vagility (as demonstrated by mark-recapture studies) and also the greatest genetic distance between populations compared to *E. binotata* found on other host plant species. The authors suggest that genetic drift between the isolated, low vagility populations of *Enchenopa* on *P. trifoliata* may be the cause of the observed differentiation.

Crouau-Roy (1989) used starch gel electrophoresis to describe the genetic structure of populations of highly specialized cave beetles (*Speonomus hydrophilus*). Twenty-three geographically close populations of the beetle occurring both in caves and under the soil in mountains were studied. She found moderate genetic divergence among

the proximate populations of beetles. The author states that the spatial heterogeneity and lack of association between genetic and geographic distances are in agreement with the expectations of a model of differentiation by founder effect with reduced gene flow.

Funk et al. (1988) and Sweeney et al. (1987), using starch gel electrophoresis, conducted allozyme analysis of >2000 individuals from 82 populations of mayflies from the genus *Eurylophella* in eastern North America. They found three morphologically distinct groups of 8 species for which the species within the groups could only be distinguished electrophoretically and concluded that there are probably 15 species of *Eurylophella* in eastern North America, rather than the currently recognized 8 or 9 species.

Dillon (1984) examined correlations among environmental difference, geographic distance, and population divergence between populations of the snail *Goniobasis proxima*. Using starch gel electrophoresis, he found a correlation between genetic distance and geographic distance but none between genetic distance and environmental difference. He theorized that low to no gene flow between populations prevented the establishment of clines in allele frequencies over environmental gradients.

McDonald and Ayala (1974) conducted an experiment similar to that of Powell (1971) and also found that populations exposed to heterogeneous laboratory environments maintained more genetic variation than populations living in uniform environments. Their results also supported the idea that genetic polymorphism can be maintained by selection favoring two or more phenotypes in the same population (multiple niche polymorphisms supported by diversifying selection). They warned.

however, that random genetic drift could be a significant factor on population heterozygosities if the population size were small at the start or at any time during the experiment. They ruled out drift since each experimental population contained a large number of flies (423) descended from a large number of wild parent flies (282).

Powell (1971) collected *Drosophila willistoni* from a site in Brazil and maintained separate experimental populations in the laboratory under conditions of either low, medium, or high environmental variability. Populations in more variable environments had higher genetic variability (heterozygosity per locus and alleles per locus) at the end of the 45 week study as measured by starch gel electrophoresis. This indicates that environmental heterogeneity maintained gene polymorphisms (multiple niche polymorphisms). Average heterozygosity per individual was less in all laboratory populations than that measured in the field, indicating a loss of genetic diversity when field-collected flies were reared in the less variable laboratory environment.

2.4 Life History Differences Between Populations

2.4.1 Life History Differences Between Populations (No Genetic Work Done)

Malmqvist and Sjostrom (1989) studied populations of the perlodid stonefly, *Isoperla grammatica*, in streams in southern Sweden. They found that populations from different streams differed greatly in growth rate and other population parameters. Individuals found in the Fiskaback River grew at a low, but steady rate throughout the

winter season, while growth was halted in other rivers during winter. The winter temperature in the Fiskaback River was relatively warm compared to the other rivers studied. They also reported that the size of last instar nymphs were smaller from the Fiskaback River, presumably a result of colder spring and summer water temperatures, while the largest last instar nymphs were found in the Kruddarebacken River, which was the warmest stream in the spring.

Jop and Stewart (1987) found faster growth rates in stoneflies in spring-fed Battle Branch, Oklahoma than those reported for the same species in streams in more northern regions of the country. They attributed the differences partly to the higher and less variable stream temperatures in Battle Branch.

Perry et al. (1987) found the univoltine, winter-emerging stonefly, *Taenionema pacificum*, emerged earlier in two consecutive years at a site on the Flathead River, Montana, where water released from the Hungry Horse Dam raised the water temperature relative to an upstream site. During the second year of the study, warmer weather resulted in higher water temperatures causing emergence to occur 1-2 months earlier than the previous year at both sites.

Sweeney and Vannote (1986) collected nymphs of the winter stonefly *Soyedina carolinensis* from a spring brook and a spring seep within the White Clay Creek watershed in Pennsylvania. On any given date, the average biomass of individual nymphs from the spring brook was lower than that of nymphs from the spring seep. There was also a delay in the onset of nymphal growth and adult emergence at the spring brook

relative to the spring seep populations. They suggested that the cooler temperature regime of the spring brook site was the cause of the differences.

In six species of ephemerellid mayflies in Oregon, California, and Canada, Hawkins (1986) observed differences between populations in timing of the onset of annual growth and in individual growth rates. Annual growth began in June for Oregon populations of *Drunella doddsi* and *D. coloradensis/flavilinea*, but not until September for populations in Canada. A similar pattern was seen in *Ephemerella infrequens/inermis*. The warmest streams were in Oregon and the coldest in Canada. Hawkins attributed this pattern to an inverse relationship between egg development and temperature. However, for populations within Oregon there was no correlation between the onset of growth and temperature. In fact, the onset of growth varied by as much as four months in some populations of *E. infrequens/inermis* in Oregon, even when stream temperatures were similar, indicating control by a factor other than temperature. In two streams with long periods of ice cover during winter, growth rates were significantly lower than in streams without ice. Hawkins speculated this may have been due to lower food availability as well as low temperatures under the ice.

Humpesch (1980) found that eggs from a river population of the mayfly *Ecdyonurus dispar* in England have a delayed hatching time, while eggs of *E. dispar* from several lake populations do not. He hypothesized that eggs from the river population undergo a type of dormancy. He also found that the hatching time for eggs of two other mayfly species, *E. picteti* and *E. venosus*, also differed between localities, with the

hatching times for populations from cooler environments taking longer than those from warmer environments.

Ward and Cummins (1979) observed that *Paratendipes albimanus* produced one generation per year in Augusta Creek, Michigan. Temperature and food quality were shown to both play important roles in controlling larval growth. Low stream temperatures limited growth during winter, but low food quality limited growth at other times of the year. Laboratory experiments showed significant differences in growth rates when the quality of food varied. When high quality, microbially-colonized, pignut hickory leaves were used as a food source, larvae grew to the fourth instar, but larvae fed natural stream detritus failed to grow at all. The quality of the food source correlated to the amount of microbial biomass present. They were able to observe a second generation develop in the laboratory at temperatures similar to those in Augusta Creek, when the larvae were fed high quality pignut hickory leaves. They concluded that food quality, and not temperature, prevented a second generation from developing in the field.

2.4.2 Life History Differences Supported by Electrophoresis

Robinson et al. (1992) examined the life history and genetic structure of two populations of the mayfly, *Baetis tricaudatus*, and the stonefly, *Hesperoperla pacifica*, occurring in two streams with different flow regimes. Both species had greater genetic variability in the stream with a more variable flow cycle. Their data supported the

premise that higher heterozygosities and polymorphisms are found in populations occurring in more variable environments.

Scott and Koehn (1990) exposed clams of the species *Mulinia lateralis*, collected from Great South Bay, Long Island, New York, to varying combinations of temperature and salinity in the laboratory. Horizontal starch gel electrophoresis was used to identify allozyme genotypes at 11 polymorphic loci. Under experimental conditions where temperature and salinity were at levels considered to be stressful to the clams, there was a significant relationship between the degree of allozyme heterozygosity and growth rates (added shell length), i.e., individuals with more loci in a heterozygous state grew significantly faster under stressful conditions than individuals with fewer heterozygous loci. This was not the case under optimal conditions, where no significant relationship was found between level of heterozygosity and growth rate. The authors concluded that increased fitness (higher growth) derived from a decreased cost in maintenance metabolism in individuals with a higher level of heterozygosity.

The cost of protein synthesis is an important part of maintenance metabolism. When stressed by factors such as temperature, salinity, pollution, etc.., individuals require more energy and respond by increasing protein synthesis and respiration rate; those able to do so most efficiently (heterozygotes) are more fit under these stressed conditions. These differences in fitness are most extreme under stressful conditions and may not be apparent when conditions are optimum.

Tedengren et al. (1990) concluded that physiological differences observed between populations of the blue mussel, *Mytilus edulis*, from the low salinity (7 ppt)

Baltic Sea and the high salinity (28-30 ppt) North Sea were mainly due to phenotypic variation and to a lesser degree to differences in genotype. After one year of acclimatisation, oxygen consumption, ammonia excretion, O/N ratios, and clearance rate of Baltic Sea mussels transplanted to the North Sea approached those of native North Sea mussels, indicating these metabolic parameters were regulated mainly by physiological adaptations to environmental differences. The gene frequencies of *Pgi* and *Pgm*, two loci coding for enzymes which have important roles in controlling metabolic rate, differed between the two populations, probably due to differential selection, and the authors state that part of the observed physiological differences between mussels from the two populations may be determined by these genotypic factors acting directly on metabolic pathways.

Quattro and Vrijenhoek (1989) analyzed the genetic diversity in the endangered Sonoran topminnow, *Poeciliopsis occidentalis occidentalis*. The authors tested the hypothesis that genetic diversity is associated with enhanced mean fitness of the populations. They selected for study three populations, one which was homozygous at all 25 loci surveyed, one with intermediate, and one with high levels of heterozygosity. When the progeny from females collected from the field sites were used in a series of laboratory experiments they found that the homozygous population had the highest mortality, the slowest growth, and the lowest fecundity of the three populations. Conversely, the most heterozygous population had the lowest mortality, highest growth and highest fecundity.

Hefti et al. (1988) used starch gel electrophoresis to compare five populations of *E. dispar*, three from Britain and two from continental Europe. There were differences in the frequencies of polymorphic enzyme loci that indicated the continental populations are separated from the British populations and that, within Britain, the lake populations are closer to each other than to the river populations. In a paper reviewed earlier, Humpesch (1980)(Section 2.4.1), found differences in embryonic development time between river and lake populations of *E. dispar*. The differences found in this study at the genetic level confirm the phenotypic differences reported in Humpesch (1980) and provide evidence that differences in egg development time are under genetic control.

Sweeney et al. (1986) examined the genetic structure of two species of mayfly, *Ephemerella subvaria* and *Eurylophella verisimilis*, using starch gel electrophoresis. For *E. subvaria*, allele frequencies at certain loci varied significantly among sites on the Delaware River. Most of the variance occured at sites located 7 and 14 kilometers below a reservoir where there were no physical barriers to prevent movement of individuals between areas. However, there is a strong gradient in water temperature below the reservoirs which the authors speculate may cause a temporal barrier to gene flow between subpopulations of mayflies on the Delaware River. That is, the onset and duration of emergence in the spring differs by several weeks in stream reaches impacted by the cold-water release from the reservoirs. Temporal segregation during mating could reduce gene flow between subpopulations. Interestingly, they found that there was a cline in allele frequencies for two loci below the reservoirs that paralleled water temperature. They also reported that adult size and fecundity of *E. subvaria* increases almost two-fold at sites

downstream from the reservoirs. With regard to *E. verisimilis*, they found that the subpopulations showed very little genetic difference and approached panmixia. The authors point out that although natural selection can discriminate among allozymes at a given genetic locus, electrophoresis cannot be used to determine whether the variation in life history traits observed below the reservoirs are heritable or even to what degree the life history trait variations are genetic versus ecophenotypic. However, the results of the study do allow the indirect evidence of significant genetic variation to show selection may be responsible for the variation in life history traits.

Hilbish (1985) studied the frequency of the Lap^{94} allele in populations of the mussel *Mytilus edulis*. Each year recruitment from the open ocean into Long Island Sound brings juveniles where the frequency of the Lap^{94} allele is about 0.55. At one site in the Sound, East Marion, there is strong selection pressure directed against juveniles with the allele and it decreases in frequency to about 0.12. The cline in frequency of the Lap^{94} allele is maintained even in the face of strong gene flow from the open ocean. The actual length of the cline is 30 km. The mussels in the East Marion population grow only a negligble degree following recruitment from the open ocean, while other populations grow rapidly. Therefore, selection against juveniles with the Lap^{94} allele is coincident with the period of retarded growth.

Zera (1981) compared patterns of spatial variation of polymorphic enzyme loci and levels of variability in two species of waterstrider with differing degrees of winglessness: the nearly wingless *Gerris remigis* and the wing-polymorphic *Limnporus canaliculatus*. Allele frequencies among populations of *L. canaliculatus* were

homogeneous over large distances and were probably the result of extensive dispersal and gene flow. In contrast, polymorphic loci of the wingless *G. remigis* showed spatial variation in allele frequencies, even over short distances. The authors suggest this is due to reduced gene flow among populations of *G. remigis*.

Varvio-Aho and Pamilo (1980), and Varvio-Aho (1979) used horizontal starch gel electrophoresis to calculate genetic variation within local populations and differentiation of populations for several species of the waterstrider *Gerris*. The least heterozygosity was found in populations of *G. lateralis*, which is characterized by a high proportion of wingless individuals and small isolated populations. These factors reduce gene flow, which tends to increase the effects of genetic drift resulting in a loss of genetic variation within populations (Futuyma 1986). *G. odontogaster* is characterized by monomorphically long-winged (alate) populations, is thus a good disperser and as a result showed the least differentiation among populations.

Singh and Zouros (1978) collected larvae of the American oyster, *Crassostrea virginica*, and maintained them in culture for one year. At this time, the 200 largest and 200 smallest oysters were assayed electrophoretically for five enzyme loci. The largest oysters had higher average heterozygosities, and individual heterozygosity was positively correlated with body weight.

2.4.3 Life History Differences (Genetic Basis - Not Electrophoresis)

Kautsky et al. (1990) transplanted blue mussels, *Mytilus edulis*, reciprocally between North Sea and Baltic Sea populations. The growth rate for mussels in the Baltic Sea is much lower than the North Sea, and the final size of Baltic Sea mussels is only one-third the size of North Sea blue mussels. Reciprocally transplanted mussels grew at rates similar to those of native mussels at each site, indicating that this variation can largely be explained by non-genetic physiological differences due to environmental salinity. When Baltic mussels were transferred to the North Sea initial survival was very high, followed by high mortality in the following late summer. In contrast, immediate mortality was over 90% among North Sea mussels tranferred to the Baltic Sea. The authors concluded the different survival rates probably had genetic causes. In a companion paper, Johannesson et al. (1990) showed that the high initial mortality of North Seas mussels transplanted to the Baltic was selective on some enzyme loci and that only North Sea mussels of Baltic Sea genotype survived. Also, the sudden mortality of Baltic mussels in late summer 1 yr after transplantation to the North Sea seemed to be due to genetic factors, as it was very selective against Baltic genotypes. While the North Sea (28 parts per thousand salinity) mussels were probably killed by direct salinity effects after transfer to the Baltic (7 parts per thousand salinity), the authors speculated that a possible mechanism for death of the Baltic genotypes could have been a genetically determined low resistance to diseases in Baltic mussels.

Lonsdale and Levinton (1986) demonstrated the presence of significant genetically based differentiation in growth rate and reproductive traits (percent reproductive females and mean clutch size) among females of an harpacticoid copepod, *Scottolana canadensis*, taken from a broad range of latitudes and reared in the laboratory under the same conditions. As temperature increases (15-25°C), the growth rate of southern-derived copepods continues to increase, while that of northern-derived copepods levels off or decreases. Southern-derived *S. canadensis* also have a higher percentage of reproducing females at high temperature (25°C) when rations (cells ml^{-1}) are reduced, while northern-derived females are at an advantage at low temperature (15°C). The authors conclude that both life history traits indicate a local adaptation to maximize scope for growth and reproduction at prevailing temperatures.

Hsiao (1985) studied geographic variation among populations of the Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata*. He describes a variety of criteria for discriminating geographic variants including ecological, physiological, and behavioral variations. Additionally, genetic variation was measured by isozyme techniques. Differences in host plant preference were found in several populations. One population from southern Arizona was uniquely adapted to a native host, *Solanum elaeagnifolium*. Individuals from several populations of the CPB collected from cultivated potatoes, *Solanum tuberosum*, were unable to utilize *S. elaeagnifolium*. Similarly, CPB populations from southern Mexico did not survive well on either the cultivated potato or *S. elaeagnifolium*. Different populations of CPB have adapted to seasonal differences throughout their range. Beetle populations from southern Texas to southern Mexico enter

diapause when they sense a change in the quality and/or a shortage of host plants. This condition occurs during the spring and summer drought periods. Diapausing individuals stay above ground and resume feeding and reproduction when favorable moisture and host plants occur. In the temperate regions, where winter temperatures are below freezing, a seasonal adaptation strategy has evolved. CPB react to the reduction of daylength in late summer and enter diapause. These individuals burrow into the ground to hibernate as a protection from freezing temperatures.

Examination of enzyme products of 11 genetic loci from 12 CPB populations using starch gel electrophoresis indicated that 11 of the populations (from U.S.A., Canada, Europe) were genetically similar, while one population from southern Mexico was significantly different from the other 11 populations, indicating it may be a distinct subspecies (mean genetic distance of 0.212).

Fairbairn (1984) compared body size and development times among four closely located populations of the gerrid, *Limnoporus notabilis*, in southwest British Columbia, Canada. Adult females were collected from the field and brought back to the laboratory. Offspring of field-collected females were tested under identical conditions to reduce the phenotypic or environmental components of variation among sites which could contribute to differences seen in laboratory experiments. She found that differences in body size and development times observed in field populations were also seen in laboratory experiments. Body size and development time varied significantly among the four populations. One weakness of the experiments, acknowledged by the author, is that by

using first generation offspring of field collected females, the possibility existed for maternal influence on body size and development time in the laboratory experiments.

Levinton and Monahan (1983) examined individuals of two subspecies of *Ophrytrocha puerilis* (Polychaeta:Corvilleidae) from differing thermal regimes in cultures maintained for over a year. Despite common rearing, the two subspecies showed substantial differences in somatic growth rate. At 15°C the warmwater subspecies grew more slowly, while at 20°C growth for the two subspecies was not significantly different. At 24°C, the warmwater species grew more rapidly and suffered substantially less mortality than the northern subspecies. Levinton and Monahan's results demonstrate a shifting growth advantage consistent with the differing thermal regimes of the two subspecies. In other words, there is a strong genotype-environment interaction. To explain this data they propose the "*Latitudinal Compensation Hypothesis*" which predicts that local evolution should maximize metabolic efficiency and favor maximum growth under local thermal conditions.

Berven (1982) conducted reciprocal transplant experiments with juvenile wood frogs (*Rana sylvatica*) to determine the role of the environment versus genotype in causing differences in life histories of mountain and lowland populations. Wood frogs from the mountain environment (Shenandoah Mountains, Virginia) had lower growth rates and prolonged developmental periods, larger size and delayed reproduction due to the drier and colder conditions found there relative to the lowlands (Prince Georges County, Maryland). Differences in the timing of the breeding period were found to be due solely to ecophenotypic variation, as transplanted individuals bred at exactly the same

time as that of the resident populations into which they had been transplanted. In contrast, differences in egg size and the number of eggs per egg mass were claimed to be due entirely to genetic variation, since transplanted individuals retained the same characteristics for these two traits as the populations from which they were transplanted. The differences between the populations in age and size at first reproduction were found to be controlled by a combination of genetic and environmental controls since transplanted individuals were intermediate between their home population and the resident population into which they were transplanted.

Tauber and Tauber (1981) showed that genetic variation exists between populations of the *Chrysopa carnea* species-complex. The *Chrysopa carnea* species-complex, the common green lacewing, occurs throughout the Holarctic region, where it is typified by diverse species and strains. These species and strains exhibit considerable variation in their seasonal and life history traits. Much, but not all, of the variation appears to be related to variations in local seasonal conditions. The critical photoperiod for diapause induction varies greatly with locality. In general there is a north-south gradient (from long to short) in the length of the critical photoperiod. The authors selected for both shortened diapause and increased response to diapause-inducing photoperiod in the adult stage. Their selection regimens reduced the duration of diapause by 62 days in three generations. Selection for increased adult response to diapause-inducing photoperiods resulted in an increase from 0 to 64% in the proportion of females that entered diapause without ovipositing. Simultaneously, the time to enter diapause in these females decreased from 64 to 11.5 days. Therefore, both characters were amenable

to rapid alterations by artificial selection. There is considerable variability in the thermal requirements for growth and development of preimaginal stages. The authors analyzed the thermal requirements for development in six populations from various localities in North America. Each population was tested under a range of temperatures from 15.6 to 26.7°C. Data indicated considerable genetic variability in the thermal requirements for growth and development among populations.

Stearns and Sage (1980) identified two populations of mosquito fish (*Gambusia affinis*) living in a freshwater stream and in a brackish estuary of Armand Bayou, Texas. Female fish collected from the freshwater site had fewer young than females from the brackish water site located several hundred meters downstream. Following a series of laboratory experiments they concluded that the fish from the freshwater site were physiologically maladapted to that habitat and life history differences between the field populations were not the result of local adaptation to each habitat or developmental sensitivity to early environment conditions. They suspected either fish movement into the freshwater stream at times of high water is providing enough gene flow to prevent local adaptation, or the freshwater population is the result of a recent colonization event.

Michailova and Fischer (1975) analyzed chromosomes from larvae of the midge *Chironomus plumosus* reared from egg masses collected from sites in Europe and North America. The adults and larvae from the natural populations could not be distinguished morphologically. They found that each of the natural populations had characteristic patterns of chromosome inversions and other rearrangements, making them clearly distinguishable. Hybridization was not possible between the Canadian and a European

population; normal synapsis between chromosomes in the hybrids was not possible. Other differences in the Canadian population such as dissimilar arrangement of the heterochromatin and different homozygous inversions provide the basis for the separation of the Canadian population into a separate species.

Martin and Wulker (1971) examined inversion polymorphisms in chromosome arms of the midge *Chironomus staegeri* from different populations. They found a close association between some inversion types and water depth of the habitat from which the larvae were collected. The shallow water forms appeared to have two generations per year with adults emerging in early May and again in August. The deep water forms had only one generation a year with peak emergence in late May. The authors speculated that the difference in emergence times may limit gene flow between the populations.

Ayala (1968) described an experiment highlighting the role of genetic variation in producing life history differences between populations. Several geographic strains of *Drosophila serrata* from New Guinea and Australia were grown in the laboratory for multiple generations at two temperatures, 25°C and 19°C. The mean population size was strikingly different for flies from different sources. At 25°C the New Guinea and Australia strains had approximately equal population size, but the Australian population was larger at 19°C, which, he suggested, may mean that the Australian flies are adapted to live in a climate colder than that of New Guinea.

McNeilly and Antonovics (1968) studied closely adjacent mine and pasture populations of the grass species, *Agrostis tenuis* and *Anthoxanthum odoratum*. Mine populations of both species flowered about one week earlier than pasture populations and

the difference was maintained under identical conditions in the laboratory. The differences are genetically determined and are the result of natural selection. They concluded the difference in flowering time was an adaptation to local ecological conditions (tolerance to metals in the soil), as a method to reduce the deleterious effects of gene flow. Divergence has been followed by reproductive isolation.

Khoo (1968) showed that adults of the stonefly *Diura bicaudata* from Wales lay two different types of eggs. Adults from the stream Afon Hirnant lay only eggs that undergo a diapause of about 9-11 months and require a long period of chilling to break the diapause. Adults from Lake Bala lay both diapause and non-diapause eggs, with a higher percentage of the non-diapause type that hatch 2-3 months after oviposition. *D. bicaudata* is found predominantly in the northern Eurasia and North America. The populations in Wales are thought to be glacial relicts. Therefore, Khoo hypothesized that the diapause character is an adaptation to cold temperatures. A long diapause ensures that the eggs do not hatch at the end of the short northern summer, just before the onset of winter. The temperatures are generally low throughout the year in the Afon Hirnant, so there would not be selection pressure to modify this ancient strategy. However, the water temperature of Lake Bala can become quite warm during the summer, allowing longer periods of favorable growth to a population able to break the obligate period of diapause. Therefore, the occurence of non-diapausing eggs in the Lake Bala population is thought to be of recent evolutionary origin. The diapausing populations are thought to undergo a two-year life cycle, while the Lake Bala population is probably univoltine. Khoo states that the differences between the populations are sufficient to classify them as separate

subspecies. Crosses between the populations indicated that the diapause trait is probably genetically controlled, with the type of egg laid determined by the female parent.

Birch et al. (1963) measured the innate capacity for increase (r_m) as a means to distinguish between the fitness of geographic races *of Drosophila serrata*. Laboratory studies were conducted with either the first or second generations of flies collected from populations in New Guinea and Australia. In the subspecies *D. serrata birchii* the value of r_m increased from north to south. In the subspecies *D. serrata serrata* r_m decreased from north to south. The adaptive value for this clinal genetic variation in r_m was unknown.

2.5 Genetic Differences in Resistance to Toxicants/Stress

Miller and Hendricks (1996) cultured *Chironomus riparius* for 18 months in water containing low levels of Zn (10 ug/L). To determine whether there was a genetic basis for Zn resistance, adults were allowed to breed in clean water conditions for one generation and larvae from the second generation were exposed to Zn and the response compared with experimental populations that had not been exposed to Zn. When growth rate was measured, Zn tolerance did not increase relative to the control population, but a genetic basis for increased tolerance (at least 8-fold) was indicated when the respiration rate of instar IV larvae was measured.

Postma and Davids (1995) exposed *Chironomus riparius* to a range of cadmium concentrations for nine consecutive generations in the laboratory. A 96-hour test of

growth conducted at the end of the experiment showed increased resistance to cadmium in comparison with a control population. However, maternal effects conferring acclimation to cadmium could not be ruled out as the source of resistance because at no time during the sequence of experiments were the larvae removed from the cadmium-contaminated water.

Postma et al. (1995a) collected individuals of the species, *Chironomus riparius*, from two cadmium-polluted sites and two unpolluted sites, rearing them in the laboratory *under clean conditions*, and using the first generation offspring in laboratory experiments with cadmium. They found that populations from the polluted and unpolluted sites differed in their response to cadmium in the laboratory. Growth rates increased for individuals from all populations when exposed to cadmium, but was significantly longer for the populations from the unpolluted sites compared with individuals from the unexposed population. Hatchability of eggs was decreased in all populations but was significantly less for individuals from the unpolluted sites compared with the polluted sites. Mortality increased significantly under cadmium exposure in the laboratory and there was apparently no difference between the populations for this trait. Their results showed that, individuals from cadmium-polluted sites were less affected by cadmium in the laboratory than individuals from clean sites, i.e., individuals from polluted sites had faster growth rates and higher egg hatchability compared to individuals from unpolluted sites. Because larval development times increased (decrease in growth rate) for all populations when exposed to cadmium, the authors suspect a "cost of tolerance" in metal-tolerant populations. This is made clear when comparisons were made under clean

conditions and mortality rate and larval development time were significantly higher in individuals from the exposed populations compared with individuals from unexposed populations. Although the authors attributed the differences in cadmium-tolerance between populations to genetic differences, the fact that they used first generation offspring of field collected individuals does not rule out the possibility that maternal effects induced by the environment may have played a significant role in the supposed genetic differences.

Postma et al. (1995b) reared cadmium-tolerant larvae of *Chironomus riparius* under clean conditions in the laboratory and compared their life history parameters with those of nontolerant larvae. In contrast to lab studies described in another work by Postma et al. (1995a), cadmium-tolerant larvae in this study had been reared under identical, clean conditions for *two* generations before use in order to ascertain whether differences between cd-tolerant and reference larvae had a genetic basis and were not attributable to maternal effects. Under clean conditions the Cd-tolerant midges experienced increased larval mortality, reduced larval growth rate, and a reduced number of egg masses per female, compared with reference larvae. When both the Cd-tolerant and reference larvae were exposed to a range of zinc concentrations, increased fitness was found for the Cd-tolerant midges in terms of higher growth and reproduction but not survival relative to the reference larvae. The authors speculated that metallothionein-like proteins, which had been selected for when cadmium was present, enabled the Cd-tolerant midges to do well when exposed to zinc, but the extra energy needed to maintain this tolerance mechanism resulted in lower fitness under clean conditions because of the

"costs of tolerance", i.e., the extra energy needed for physiological maintenance of the tolerance mechanism.

Hoffman and Fisher (1994) looked for genetic differences between larvae of *Chironomus riparius* collected from a sewage treatment plant (STP) and larvae from a laboratory population that had been cultured for approximately 16 years. They compared aspects of fitness (female fecundity, male and female pupal weight, and larval developmental period), response to insecticides (comparison of LC50s), and biochemical parameters (mixed function oxidase, general esterase, acetylcholinesterase and glutathione-*S*-transferase activity). Field-collected larvae were reared in the lab for one generation and F1 generation offspring were used as experimental animals. Significant genetic differences between the populations were found for nearly every parameter measured, although, since they used first generation offspring of field-collected organisms for these tests, they cannot rule out maternal effects on the results. The STP population was 13-250x more tolerant to insecticides than the lab population. Under normal lab conditions, significant differences were found for pupal dry weight and development time (first instar to pupation); there was no difference in female fecundity (# eggs per egg mass) between populations. Increased glutathione-*S*-transferase activity in the STP population was thought to represent a potential detoxification mechanism for the DDT and organophosphate insecticides used in the study resulting in the much higher LC50s for the STP population. In addition, higher mixed-function oxidase activity in the STP population was thought to also play a role in greater oxidative metabolism of toxicants and so served as another detoxification strategy in that population.

Benton and Guttman (1992a) exposed larvae of *Nectopsyche albida* (Leptoceridae:Trichoptera), collected from Lakengren Lake, Ohio, to a solution of mercury nitrate. Horizontal starch gel electrophoretic techniques were used for the resolution of genetic loci. The time to death differed significantly among genotypes and levels of heterozygosity. As the number of heterozygous loci present increased from zero to four, toxicant resistance increased. The authors speculate that as tolerant genotypes are selected for and sensitive genotypes eliminated from pollution-impacted populations, the overall genetic variability decreases, possibly leaving the population vulnerable and unable to respond to future environmental stresses.

Benton and Guttman (1990) looked at the relationship between genotype and time to death of the mayfly *Stenonema femoratum* exposed to copper. Individuals were collected from Indian Creek, Ohio. Horizontal starch gel electrophoresis showed that survival time differed significantly among allozyme genotypes for one of three polymorphic loci analyzed. They theorized that copper may have inactivated the less tolerant enzyme systems (allozymes).

Chagnon and Guttman (1989) examined the differential survivorship of allozyme genotypes in the mosquitofish (*Gambusia affinis*) exposed to copper and cadmium. Allozymes were resolved using starch gel electrophoresis. They found that both Cu and Cd had significant effects on the survival of *G. affinis* with different allozyme genotypes. Copper significantly decreased the overall survival of heterozygotes at the GPI-2 locus. Fish with genotypes homozygous for a specific allele at the GPI-2 locus survived at significantly higher rates when exposed to cadmium. They refer to a number of

mechanisms of metal-enzyme interaction that may be responsible for the observed effects of these metals on allozyme variants, i.e., "heavy metals may influence enzyme activity through selective chemical modification of affinity labeling, metal chelation, noncovalent binding of inhibitors and analogs, and nonspecific association with complex ions".

Gillespie and Guttman (1989) found that allele and genotype frequencies of the polymorphic gene loci phosphoglucomutase (PGM) and malate dehydrogenase (MDH) differed significantly between populations of the central stoneroller (*Campostoma anomalum*) above and below an area receiving industrial runoff. A distance of less than 500 m separated populations with significant differences in frequencies of allozyme genotypes. At pollutant-impacted sites the frequencies of the PGM-A allele and PGM-AA and PGM-AB genotypes were significantly less than in nonimpacted sites. In the laboratory, individual stonerollers with the above-mentioned sensitive PGM genotypes and also with the MDH-BB genotype were also more sensitive to solutions of copper sulfate, giving added support to the idea that certain genotypes are more sensitive to environmental toxicants than other genotypes. The authors state that the reduced genetic diversity in populations in which selection favours toxicant-tolerant individuals and eliminates nontolerants results in populations less able to deal with further stress.

Luoma et al. (1983) conducted laboratory toxicity tests with bivalves (*Macoma balthica*) collected from sites contaminated with different levels of copper within San Francisco Bay. The LC50s for copper were six-fold higher for individuals collected from the area where environmental concentrations of copper were the highest. The authors speculated that either copper stress was exerting selective pressure on *M. balthica*

(genetic basis for differential tolerance) or inducing a physiological adaptation to stress (ecophenotypic response). Because they used field-collected organisms for the laboratory experiments, the authors could not rule out acclimation to copper and physiological adaptation.

Lavie and Nevo (1982) investigated the effects of zinc and copper pollution on the allozyme variation of phosphoglucose isomerase (Pgi) gentypes in the marine gastropods *Monodonta turbinata* and *M. turbiformis*. They found differential survivorship of Pgi allozyme genotypes for both metals. Using starch gel electrophoresis, two alleles (S, M) and three genotypes (SS, SM, MM) were resolved at the Pgi locus for *M. turbinata*. Two alleles (M, F) and three genotypes (MM, MF, FF) were also resolved for *M. turbiformis*. When exposed to zinc, the allozyme genotype SS was selected against in *M. turbinata*, but no differential survival in genotypes was observed in *M. turbiformis* which does not have the S allele. When exposed to copper, the MM genotype was selected against in both species. The authors conclude the results support the adaptive nature of some Pgi genotypes in these species of gastropods.

Bradley (1982) examined the degree of physiological and genetic adaptation of the copepod *Eurytemora affinis* to areas with different thermal regimes in the Chesapeake Bay region. Field populations occurred in habitats where temperatures ranged from near 0°C to 30°C and above. Generation time varied from 3 months at the lowest temperature to 10 days at the highest temperature. Temperature tolerance is therefore an important trait in *Eurytemora*. Previous studies by Bradley (1978a,b) demonstrated both physiological and genetic variation in temperature tolerance. In this study (Bradley 1982)

he addressed the question of genetic variation in *E. affinis* populations. To measure genetic differences between treatments and between areas in the field the general approach he used was to raise progeny from corresponding samples of animals, all under the same conditions, usually 15°C. Any differences observed between treatments or areas was assumed to be genetic. Bradley (1978a) found maternal affects to be absent so he used F_1 progeny. The main life history trait he measured was temperature tolerance. Tolerance was measured by lowering a vial containing a single copepod into hot water at 32°C. The temperature was then raised 0.5°C at 5-minute intervals. Tolerance was defined as the number of minutes it took before the copepod became immobile. Males from station 2, the immediate discharge area, had markedly higher tolerances than those from station 1 (cooling water intake site). The differences are assumed to be genetic because progeny were all raised at 15°C.

Jernelov et al. (1981) investigated why larvae of the midge, *Chironomus riparius*, were the dominant bottom fauna in highly acidic ponds (pH 3-5) in the Smoking Hills region of the Canadian Northwest Territories. Nearby neutral or alkaline ponds were dominated by other species of chironomids. They concluded it was the buffering capacity of hemolymph rather than the oxygen binding capacity of the hemoglobin found in this species that gave it the ability to tolerate low pH. They compared individuals from the Smoking Hills population with individuals from a population in Scandinavia and found that the hemoglobin content was twice as high in the Canadian population. Under standard laboratory conditions, using field-collected larvae, the differential tolerance to low pH was maintained.

Nevo et al. (1981) exposed shrimp (*Palaemon elegans*) to a range of mercury concentrations and determined the frequency of allozyme genotypes at the phosphoglucomutase (PGM) locus. Five alleles were found (S^-, S, M, F, F^+). They found differential survival of two genotypes (MS and MM) at different mercury concentrations. The MS heterozygote exhibited superior survival at low and intermediate concentrations, and the MM homozygote was the genotype found to best tolerate the highest mercury concentrations tested. The results indicated that certain genotypes have an adaptive role in mercury tolerance.

Sankurathri and Holmes (1976) studied the effects of thermal effluents on the population dynamics of the snail, *Physa girina*, in Lake Wabamun, Alberta. In the area unaffected by thermal effluents, the population overwintered in the deep part of the lake as either mature or immature snails, i.e., no eggs or hatchlings were found. The snails migrated back into the shallows as the aquatic macrophytes developed in the spring. Reproduction took place during in late spring-early summer and the population remained stable until ice covered the lake in November. In contrast, in the areas heated by thermal effluents, growth and reproductive activity are continuous, not seasonal, since mature snails, egg masses and hatchlings are found throughout the year. There was no movement back into deeper water during the winter, although population densities were lower, probably because of reduced stands of macrophytes even in the heated area during the winter. Several overlapping generations were apparently present at all times.

Walley et al. (1974) showed that rapid development of metal tolerance could be achieved by the grass, *Agrostis tenuis*, when seeds were sown onto soil containing high

levels of copper. In normal nontolerant populations they estimate 1-2% of individuals can survive in copper contaminated soil. These survivors can leave offspring that inherit copper tolerance. In this way, natural selection can rapidly change the genetic characteristics of the population from a normal population with a low frequency of individuals with copper tolerance, to one in which all plants carry the genes necessary to survive in the contaminated soil..

Bryan and Hummerstone (1973), working with the polychaete, *Nereis diversicolor*, showed that individuals collected from estuarine sediments contaminated with high levels of zinc could regulate the level of zinc in body tissues by decreasing absorption and increasing excretion of the metal. The ability to regulate body levels of zinc was considered a genetic adaptation as the worms were able to perform the function under standard laboratory conditions and worms from other non-polluted conditions were not.

2.6 Theories of Stress Resistance

Several authors have recently developed theories describing how organisms cope with stress in the environment (Calow and Sibley, 1990; Holloway et al., 1990; Koehn and Bayne, 1989). These theories assume a considerable amount of genetic heterogeneity within populations, and that some individuals are better adapted to cope with environmental stress, and therefore have a higher fitness, than other individuals in the

same population. A description of these theories is given below, followed by a comparison of the major points in them.

I. Principle of Allocation (Calow and Sibley, 1990)

Calow and Sibley (1990) describe a "Principle of Allocation" with a tradeoff between the scope for growth (Sfg) and the scope for metabolism (M). Under the stress of a toxic environment some individuals may sacrifice Sfg by increasing energy input into a higher M, thereby increasing their overall fitness. The authors use the term Sfg to mean production rate, or the difference between total production (P_t) and the energetic costs of production (C_p): $Sfg = P_t - C_p$. They assume that C_p represents a constant proportion of Sfg so that P_t is linearly proportional to Sfg. Scope for metabolism (M) is total metabolism minus the costs of production: M = total metabolism - C_p.

Individuals that have the ability to resist stress are classified as tolerant and others are nontolerant. Further, the tolerant individuals fall into two categories: fixed tolerant forms, which are those individuals that express their stress-resisting mechanisms under both clean and polluted conditions, and facultative tolerant forms, which express their stress-resisting mechanisms only under polluted (stress) conditions.

Stress-resisting mechanisms are energy expensive. Therefore, under clean conditions, the nontolerant and facultatively tolerant forms have the highest fitness because they have more energy available for Sfg. The fixed tolerant form gains no survival benefits in clean conditions; the higher rate of metabolism necessary to maintain

its stress-resisting mechanisms takes energy away from growth and, by the Principle of Allocation, it has a lower Sfg and overall lower fitness than the other forms.

Under polluted conditions the higher metabolism of the fixed and facultatively tolerant forms causes them to have less energy available for growth (Principle of Allocation), but because of their high survival rate (metabolically expensive stress-resisting mechanisms), they have an overall higher fitness. The intolerant forms have lower fitness due to low survival, notwithstanding that Sfg may still be higher than in the tolerant forms.

II. Genetic Options Set (Holloway et al., 1990)

Holloway et al. (1990), describe a "Genetic Options Set" that involves a tradeoff between growth and survival. A resistant genetic option consists of individuals that place energy resources into detoxification, not growth. Resistant individuals are characterized by high survival, but low growth rates because the energy required for maintaining detoxification mechanisms takes energy away from growth. Alternatively, under the susceptible option, individuals place most of their energy into growth, not detoxification. Susceptible individuals are characterized by higher growth rates but relatively low survival rates. The authors assume that growth and survival rates are genetically determined. In an environment under constant conditions, with relatively low levels of toxins, both susceptible and resistant individuals are present; all have equal fitness, but maintain different genotypes defined by the susceptible and resistant genetic option sets.

Under constant conditions, alleles resulting in genetic options with lower fitness are weeded out until the population is composed primarily of individuals with equal fitness.

Upon the introduction of a strong toxin into the stable environment, many of the genotypes will prove to be maladaptive and will confer low fitness to individuals possessing them. Some individuals will die while others will suffer only sublethal effects. Individuals with the highest fitness continue to optimize energy allocation between growth and mortality. Therefore, after an initial increase in mortality rate, selection eliminates the less-fit genotypes, eventually returning the population to equally fit susceptible and resistant individuals. However, the authors point out that some of the genes expressed in the new environment may be different than those expressed in the previously stable environment.

When the authors conducted an experiment to test this theory things did not occur exactly as expected. After transplanting a stable population of weevils into a high toxin environment, they observed that overall mortality rates did increase as predicted, but after five generations the population was composed of individuals either with high growth/low mortality rates or low growth/high mortality rates. There was no tradeoff between growth and mortality rates in the genotypes as predicted. Since both fitness functions were low in the latter group, extinction would result unless there was rapid selection for faster growth and reduced mortality in their offspring. These results led them to theorize about the existance of another genetic options set, i.e., resistant individuals with a high growth/low mortality rates.

III. Heterozygote Advantage (Koehn and Bayne, 1989)

Koehn and Bayne (1989), working with the mussel *Mytilus edulis*, have described a theory emphasizing the role of energy balance in coping with stress. They identify two groups of individuals within populations which vary in the efficiency of their maintenance metabolism (R_m). These differences have important implications for individual fitness when the population becomes stressed. Maintenance metabolism is the balance between protein synthesis and protein breakdown (=protein turnover). According to their theory, individuals with a high rate of protein turnover, which is correlated with a high rate of protein synthesis, allocate a much higher percentage of their total energy expenditure into maintenance metabolism, and so have less energy available to deal with stress. In other words, individuals for which R_m is a higher proportion of R_t (total metabolism) are less able to cope with stress. The authors maintain that R_m is genetically determined.

Several other characteristics identify these two groups. Individuals with R_m as a low percentage of R_t grow faster because the extra energy is used to maintain a higher consumption rate which increases somatic growth; i.e., they have a greater Sfg. Analysis of polymorphic gene loci indicate that individuals with higher growth also have a higher level of heterozygosity.

In summary, *faster growing individuals* are marked by: high heterozygosity, low maintenance metabolism, a low rate of protein synthesis, high synthesis efficiency, and

overall higher fitness. *Slower growing individuals* are marked by: low heterozygosity, high maintenance metabolism, a high rate of protein synthesis, low synthesis efficiency, and overall lower fitness. Under optimal conditions, where food is abundant and there is a low premium for the efficient use of energy, the differences in Rm between the two groups of individuals are not reflected in differences in growth rates. That is, these underlying genetic differences result in only slight phenotypic variations in the ability to utilize energy that do not affect fitness. However, when the population becomes stressed, the ability to use available energy resources efficiently is put at a premium and the individuals with high heterozygosity have a significant fitness advantage.

IV. Comparison of Theories

All three theories are in agreement that extra metabolic energy is needed for organisms to cope with stress. However, there are differences as to the particular fitness function that energy is placed into when dealing with stress. In the theory of Calow and Sibley, individuals "divert" metabolic energy into stress-resistance, resulting in lower growth but overall higher fitness because survival rate increases in polluted conditions.

The Genetic Options Set of Holloway et al. differs from Calow and Sibley in that some individuals (susceptible option) cope with stress by allocating energy into increased growth rate rather than by reducing mortality. However, other individuals (resistant option) behave similarly to the "tolerants" in Calow and Sibley's theory. The "resistants"

divert energy into detoxification, thereby reducing mortality but sacrificing growth rate to do so, which is how Calow and Sibley's "tolerants" deal with stress.

In Koehn and Baynes's theory, individuals with maintenance metabolism as a lower proportion of total metabolism (heterozygotes) have greater fitness in polluted conditions because more energy is available for stress resistance. This theory holds that higher growth rates result from the efficient use of energy under stressful conditions making individuals similar to the "susceptibles" in the theory of Holloway et al. except that there is no tradeoff with survival as there is with the "susceptibles". Successful individuals in Koehn and Baynes's theory also differ from the successful individuals in the theory of Calow and Sibley because they increase survival without sacrificing growth rate.

3.0 MATERIALS AND METHODS

3.1 Origin of Cultures

Cultures of *Chironomus riparius*, obtained from five field populations and one laboratory population, were used for this research. Egg masses were collected from three sites in Virginia, one site in Kansas, one site in Wisconsin, and from a laboratory culture at the University of Connecticut. In July, 1988 egg masses were collected from the secondary clarifier (VA1) and from the chlorine contact tanks (VA2) at the Blacksburg, Virginia sewage treatment plant. In August, 1988 egg masses were collected from Strouble's Creek (VA3) on the Virginia Tech campus. In July, 1988 egg masses were collected from Mill Creek, in Johnson County, Kansas (KS) and transported by car to Virginia Tech packed in moist cotton. In September, 1988 egg masses were collected from the final clarifiers at the Madison, Wisconsin sewage treatment plant (WI) by research biologist Jeffrey C. Steven of the Madison Metropolitan Sewerage District. The egg masses were placed in vials containing moist cotton and shipped via overnite mail. In February, 1989 several egg masses were collected from laboratory cultures at the University of Connecticut (CN) by Dr. Hans Laufer and mailed overnite in vials of moist cotton. The University of Connecticut cultures were originally from the Netherlands and have been maintained in this country by Dr. Laufer for thirty years (pers. comm.).

3.2 Confirmation of Species Identification

Adult males and females, pupal exuviae and larvae from each culture were sent to taxonomic experts for confirmation of species identification. Species identifications were provided by Dr. John H. Epler, Research Associate, Florida Agricultural and Mechanical University, Tallahassee, Florida and M.E. Dillon, Agriculture Canada, Biosystematics Research Centre, Ottawa, Ontario, Canada. Voucher specimens are with M.E. Dillon.

3.3 Culture Maintenance

Each population was maintained in three separate two-gallon glass aquaria. Culture water was obtained from a clean stream, Sinking Creek, in Montgomery County, Virginia. Water was added to a depth of approximately five inches. Each aquarium was aerated and covered with tight fitting aluminum window screen to prevent adults from escaping. Brown paper towels that had been boiled for twenty minutes and rinsed three times in stream water were used as substrate for the larvae. The towels were cut into one cm wide strips and added to each aquarium until they formed a loose layer on the bottom approximately three cm deep. The cultures were kept at room temperature (20-24°C) and on a 16-hour light:8-hour dark photoperiod. Larvae were fed a suspension of Tetragrowth^{tm} larval fish food made by blending 10 g of fish food in 100 ml of distilled water. Approximately 10 ml of the suspension was added to the cultures each week.

3.4 Obtaining Organisms For Experimentation

Mating and egg-laying took place inside the culture tanks. Egg masses were removed from the sides of the tanks using fine-tipped forceps. If Instar I larvae were required for an experiment, single egg masses were placed in small plastic cups containing stream water. The hatching time for larvae was 2-3 days. Newly-hatched Instar I larvae were counted under a dissecting microscope at 12X magnification, then transferred by means of a glass pipet either directly to the test containers or to small plastic bottles containing stream water for transport to the field. For the series of toxicity tests, Instar II larvae were used. Instar II larvae were allowed to develop by transferring the newly-hatched Instar I larvae into aquaria and allowing them to develop for 3-4 days. Instar determination was made by measuring larval head-capsule widths.

3.5 Instar Determination

Larval instars were determined using an ocular micrometer to measure the larval head capsule width. Table 1 shows the head capsule width for the four larval instars.

3.6 Determination of Larval Dry Biomass

Larval dry masses were obtained from larvae that had been preserved in 10% formalin (Donald and Paterson, 1977). Larvae were dried for 24 hours at 65°C and then cooled in a dessicator for 24 hours (Leuven et al. 1985) before being weighed to the nearest microgram on a Cahn Model 28 balance.

3.7 Calculation of Instantaneous Growth Rates

Instantaneous growth rates were calculated using the formula:

$$IGR = \ln Mf - \ln Mi / t$$

where Mf = mean dry mass of larvae on the sample date, Mi = mean dry mass of larvae at test initiation, and t = number of days from test initiation to sample date (Huryn and Wallace 1986).

3.8 Statistical Analysis

All statistical analyses were performed using the Number Cruncher Statistical System computer program, Version 5.0. A one-way analysis of variance (ANOVA), followed by a Newman-Keuls multiple comparison test were used to compare the populations. The Newman-Keuls test makes all possible comparisons between means

and identifies those that are significantly different from one another. In some instances, the nonparametric Kruskal-Wallis test, and the Mann-Whitney-U test were used.

3.9 Description of Cages Used In Field Experiments

Cages were used to expose larvae in the field. The cages were designed to be at the surface of the water, with most of the cage beneath the water surface. This was accomplished by inserting the cages into holes cut in styrofoam floats. The cages were prevented from slipping through the holes by protruding wire arms, attached with duct tape, near the top of each cage. Various types of substrate were placed inside the cages depending on the particular field experiment. Replicate cages were randomly arranged in the styrofoam floats. Four-inch diameter pvc pipe was used for main portion of the cage. A bottom was created by sealing a four-inch plexiglass disc with rubber silicone. To prevent the entry of flying insects into the cage, a piece of nylon window screen was secured across the top with rubber bands. Three openings were cut into the sides of the cage, near the bottom, to allow water to circulate freely through the cage. These openings were spaced evenly around the pipe one-half inch above the bottom, the dimensions of each opening was 2 X 4 inches; each opening was covered with 64 micrometer Nitex^{tm} screen, secured with rubber silicone.

3.10 In-Situ Field Growth Experiment

The purpose of the in-situ experiment was to establish whether there were significant differences in growth rates between populations of *C. riparius* in the field. Laboratory tests would then be conducted to determine whether any significant differences in growth between populations measured in the field would also be found with the same populations under identical laboratory conditions.

Larvae were tested in the same habitats from which they were originally collected (e.g., larvae from the Strouble's Creek culture were placed into cages in Strouble's Creek). The in-situ experiment was conducted from 9-21-88 to 10-21-88 with populations VA1 and VA2, and from 9-7-88 to 10-2-88 with VA3. Larvae from each of the three populations were obtained from cultures maintained in the laboratory. Six cages were placed at each of the three test sites: (1) the secondary clarifier, and (2) chlorine contact tanks at the Blacksburg sewage treatment plant, and (3) Strouble's Creek. Each cage contained fifty first-instar larvae. One cage was sampled without replacement from each site every five days until emergence was complete or 30 days passed. Substrate and food were obtained from the site where each test was conducted. The substrate was autoclaved before being added to the cages in order to kill any midge larvae present. The food/substrate used for the two sites at the sewage treatment plant consisted of filamentous algae and sludge, collected from the sides of the tanks adjacent to where larvae occurred. The material collected from the walls of the secondary clarifier had a high percentage of algae in it, while the material collected from the walls of the chlorine

contact tank was mainly bleached-looking sludge with very little algae. The main material used as food/substrate in the Strouble's Creek cages was well-colonized leaves. Temperatures were monitored daily with a min/max thermometer. An analysis of variance (ANOVA) followed by a Newman-Keuls multiple comparison test compared the mean dry weights of larvae from each site on corresponding sample days (e.g., weight of larvae on day 5).

3.11 Laboratory Growth and Mortality Experiments

A series of laboratory experiments identified as 1A-1C, 2A-2C and 3A-3D compared the effects of temperature and food type on the growth and mortality of different *C. riparius* populations. Statistical analysis of growth and mortality rates (when possible) were made for the populations tested in each experiment. The analysis consisted of comparing the dry mass and numbers surviving at each of the time intervals when larvae were sampled.

In experiments 1A, 1B, and 1C, growth rates were compared among five populations (VA1, VA2, VA3, KS, WI) at the end of each experiment (a total of three comparisons). In experiments 2A, 2B, and 2C, growth rates were compared among six populations (VA1, VA2, VA3, KS, WI, CN) at three time intervals in each experiment (a total of 9 comparisons). In experiments 3A, 3B, 3C, and 3D, comparisons among three populations (VA3, CN, WI) were made at three time intervals in each experiment (a total of 12 comparisons). Statistical analyses compared growth rates for a total of 24 time

intervals in this series of experiments. A statistical comparison of mortality could only be conducted for experiments 3A-3D, because earlier experiments were not replicated.

3.11.1 Laboratory Experiments 1A-1C

These experiments were initiated on 1-26-89. Experiment 1A was conducted at 11°C and ran 26 days to 2-22-89. Both experiment 1B and 1C ran for 6 days to 2-2-89 and were conducted at 18°C and 27°C, respectively. Five populations were used for these three experiments: VA1, VA2, VA3, KS and WI. Fifteen Instar I larvae from each population were tested at the three temperatures. Test containers were not replicated. The larvae were added to 200 ml of stream water in a 250 ml flask and aerated. Each flask contained 12 cm^2 of paper towel substrate. Two ml of the Tetragrowth^{tm} feeding solution were added to each flask at test initiation; an additional two ml was added weekly in experiment 1A. On the final test day of each experiment, the contents of the flasks were poured into a white enamel pan for sorting and the larvae preserved in 10% formalin. An analysis of variance followed by a Newman-Keuls multiple comparison test was performed for each experiment, comparing the mean dry biomass of the larvae on the final day. A statistical comparison of mortality could not be made since test containers were not replicated.

3.11.2 Laboratory Experiments 2A-2C

These experiments were initiated on 3-20-89. Larvae from all six laboratory populations were tested (VA1, VA2, VA3, KS, WI, CN). During each experiment one flask per population was removed at three sampling intervals and the larvae counted and preserved. Experiment 2A was conducted at 14°C and ran 34 days; larvae were sampled on days 10, 20 and 34. Experiment 2B was conducted at 18°C and ran 23 days; larvae were sampled on days 7, 12 and 23. Experiment 2C was conducted at 27°C and ran 12 days; larvae were sampled on days 4, 9 and 12.

Fifteen Instar I larvae were added to each test container. Test containers were 250 ml flasks containing 200 ml of stream water. The flasks were aerated. Each flask contained 12 cm^2 of paper towel as substrate. Two ml of the Tetragrowth^{tm} feeding solution was added to each flask at test initiation. An additional two ml of food was added weekly to each remaining flask. On each sample day, the contents of the sampled flasks were sorted in a white enamel pan and the larvae preserved. Preserved larvae were later weighed. An analysis of variance followed by a Newman-Keuls multiple comparison test was performed comparing the mean dry biomass of the larvae from each population on each sample day.

3.11.3 Laboratory Experiments 3A-3D

Three populations were tested in this series of experiments: VA3, CN and WI. Experiment 3A was conducted at 11°C and ran 52 days from 2-10-90 until 4-3-90; larvae were sampled on days 13, 27 and 52. Experiment 3B was conducted at 11°C and ran 31 days from 2-10-90 until 3-13-90; larvae were sampled on days 11, 24 and 31. Experiment 3C was conducted at 27°C and ran 15 days from 1-30-90 until 2-14-90; larvae were sampled on days 5, 10 and 15. Experiment 3D was conducted at 27°C and ran 9 days from 1-30-90 until 2-8-90; larvae were sampled on days 3, 6 and 9.

Ten instar I larvae were initially added to each beaker. The test containers were 250 ml Nalgene^{tm} beakers containing 200 ml of stream water. On each sampling date three replicate beakers were removed from each population (e.g., on day 13, in experiment 3A, a total of nine beakers were removed, three beakers from each of three populations). The contents of each beaker were poured into a white enamel pan and the larvae sorted and preserved.

Larvae were fed leaves in experiments 3A and 3C and a solution of Tetragrowth^{tm} larval fish food in experiments 3B and 3D. The leaf species used as both food and substrate in experiments 3A and 3C was Red Maple (*Acer rubrum*). The leaves were picked from a tree while still green and put into a mesh bag in Sinking Creek for eight weeks during the fall of 1989 so that they could become well-colonized with aquatic fungi and bacteria. When the leaves were removed from the stream they were approximately five percent skeletonized. The leaves were gently rinsed and stored in the

freezer until used in the laboratory experiments. In experiments 3A and 3C, five grams of leaves were added to each beaker. In experiments 3B and 3D, two ml of the Tetragrowth^{tm} feeding solution was initially added to each beaker and one ml weekly, thereafter. In experiments 3B and 3D, 12 cm^2 of paper towel was also added to each beaker as substrate.

A separate analysis of variance, followed by a Newman-Keuls multiple comparison test, or Mann-Whitney-U tests were performed comparing mean numbers surviving and mean larval dry biomass of each population on each sampling day (e.g., on day 13 in experiment 3A, the mean dry biomass and mean number surviving would be compared between VA3, CN and WI).

3.12 Transplant Experiments

3.12.1 First Transplant Experiment

The purpose of the first transplant experiment was to test whether individuals originally collected from the chlorine contact tanks (VA2) would show increased tolerance to the presence of chlorine compared to individuals from other local populations (VA1 and VA3). Individuals had been cultured in the laboratory for one year before initiation of the first transplant experiment.

The first transplant experiment ran 15 days from 9-8-89 until 9-23-89. The populations tested were: VA1, VA2 and VA3. There were eight replicate field cages used

per population. Thirty-five Instar I larvae were added to each cage. Each cage received 20 cm^2 of paper towel as substrate and 10 ml. of Tetragrowth^{tm} larval fish food. On the final day, the contents of each cage were poured into a white enamel pan and the larvae counted. An analysis of variance and Mann-Whitney-U tests were performed comparing the mean number surviving in each population.

3.12.2 Second Transplant Experiment

The purpose of the second transplant experiment was to determine whether reintroducing larvae from laboratory culture VA2 to the chlorine contact tanks would produce individuals exhibiting increased tolerance to the conditions there compared to other local populations, i.e., select for chlorine-tolerance. The experiment was designed to ensure that any increased tolerance observed would be attributable to genetic makeup and not acclimation effects.

The second transplant experiment ran 15 days from 8-15-90 until 8-30-90. The populations tested were the same as the first transplant experiment except for the addition of a strain referred to as "VA2b". The strain VA2b arose in the following way: larvae from the laboratory culture of population VA2 (collected from the chlorine contact tanks two years earlier) were loaded into field cages and placed into the chlorine contact tanks at the Blacksburg STP. The larvae were allowed to develop to the prepupal stage (indicated by the swollen thorax of the Instar IV larvae), at which time they were brought back to the lab and allowed to pupate, emerge, mate and lay eggs. The resulting Instar I

larvae were also loaded into the field cages and placed into the chlorine contact tanks until they developed into prepupae. As before, these larvae were brought back to the lab to finish their development and lay eggs. In order to rule out acclimation effects, three more generations were allowed to develop in the laboratory before initiating the second transplant experiment. Therefore, the strain VA2b, used in the second transplant experiment, is the result of rearing two generations of larvae from VA2 lab cultures in the chlorine contact tanks.

The mean number surviving was determined on days 7 and 15. On day 7, three replicate cages per population were removed and the larvae counted On day 15, six replicate cages per population were removed. Twenty-five Instar I larvae were initially added to each cage. An analysis of variance followed by a Newman-Keuls multiple comparison test was performed comparing the numbers of surviving larvae in each population on days 7 and 15.

3.13 Emergence and Egg Mass Study (Laboratory Experiment 4)

The purpose of experiment 4 was to compare the six populations for one full life cycle, including adult emergence and egg-laying. This study provided information on life history parameters beyond growth and mortality data obtained from earlier experiments.

The starting date for the study using VA3, CN and WI was 5-28-90; the study using VA1, VA2 and KS began 7-12-90. All studies continued until adult emergence was complete. One-hundred Instar I larvae were added to each of three replicate containers

per population. Only two replicate containers were used for the KS population because of an accident with one of the test containers. The test containers were plastic storage boxes 13" long, 8" wide and 3.5" deep containing stream water to a depth of 2.5". Two brown paper towels were cut into 1" wide strips and added to each container. Ten ml of the Tetragrowth^{tm} feeding solution were initially added to each container; an additional 4 ml was added after one week. Test temperature was 22°C . The containers were gently aerated. Tight-fitting aluminum window screen was used to cover each container. Each container was checked daily for fresh pupal exuviae, adults and egg masses. The number of eggs in an egg mass were counted by placing small weights on each end of a coverslip in order to flatten out the egg mass. A dissecting microscope at 12.5X magnification was used to count the eggs.

Populations were compared for the following life history parameters: number of days until the onset of emergence, number of days from the beginning to the end of emergence, number of test days required to reach 50% emergence, number of days from the beginning of emergence until 50% emergence, number of adults emerging, number of egg masses produced, and number of eggs per egg mass. Statistical analyses used were: analysis of variance, Newman-Keuls multiple comparison test, and paired t-tests.

3.14 Toxicity Tests

The purpose of the toxicity tests was to determine whether there were any differences in sensitivity between four populations to a pesticide.

A summary of the test conditions is in Table 2. Eight day, static-renewal tests were performed using Instar II larvae. The populations tested were: VA1, VA2, VA3 and WI. The toxicant was an experimental organic pesticide (CT-1) for controlling larvae of the Asiatic clam and was provided by Dr. Donald Cherry. Dilution water was dechlorinated tap water. Laboratory cultures were acclimated to the test dilution water for two weeks prior to test initiation. Significant differences in larval biomass compared to the control were analyzed using an ANOVA followed by Dunnet's Test. LC50's were calculated with a computer program using probit analysis and the binomial test.

4.0 RESULTS

4.1 Field Growth Experiment

The in-situ experiment established that growth rates were significantly different between three populations when larval growth was measured in cages placed at sites where the larvae were originally collected (Figure 1, Table 3). Larvae in the secondary clarifier (VA1) weighed significantly more than the other two populations (VA2, VA3) on all sampling dates except day 15 when the dry mass of larvae from VA1 was not significantly different from the larvae in VA2; however, larvae from both VA1 and VA2 weighed more than larvae growing in Strouble's Creek (VA3). By day 20, the last date in which larvae were still present at all three sites, larvae from VA1 weighed significantly more than larvae from VA2, which, in turn, weighed significantly more than larvae from VA3. By day 25, only adults were present at the secondary clarifier (VA1) and chlorine contact tanks (VA2), while at Strouble's Creek (VA3) most individuals were present as fourth instar larvae with some adults found. By day 30, most individuals at Strouble's Creek were either pupae or adults, but some fourth instar larvae were still found.

The number of accumulated degree days were similar at the three field sites (Figure 2). By day 20, the number of degree days were 414, 410 and 382 at the secondary clarifier, chlorine contact tank, and Strouble's Creek, respectively.

4.2 Summary of Laboratory Growth and Mortality Experiments (1A-1C, 2A-2C, 3A-3D)

To summarize data from the series of laboratory experiments identified as experiments 1A-1C, 2A-2C, and 3A-3D, the populations were compared based on the number of times that they were found in a lowest, highest, or middle group; e.g., based on the results of a Newman-Keuls test, a high, middle, and low group can be seen in the following example using mean dry mass results from experiment 1B: WIaVA1bKScVA3cVA2c. WI is in the lowest weight group, VA1 is in the middle group, and KS, VA3, VA2 are in the highest weight group.

There were 24 time intervals when growth comparisons could be made in this series of laboratory experiments: 1A-1C (3 times, only at the end of each experiment), 2A-2C (9 times, i.e., at 3 time intervals during each experiment), 3A-3D (12 times, i.e., at 3 time intervals during each experiment). Since some populations were not tested in every experiment, and no significant differences were found in other experiments, the number of times that a particular population was included in these comparisons varies (Table 4).

Mortality rates could only be statistically analyzed for experiments 3A-3D, because no replication was used in earlier experiments. However, certain trends in mortality were evident in the earlier experiments and they are discussed below.

Several generalizations can be made concerning the results of these laboratory growth/mortality experiments:

1. The population collected from the final clarifiers at the Madison, Wisconsin sewage treatment plant (WI) consistently exhibited the lowest growth rate. Table 4 shows that of 12 time intervals where WI was compared to other populations in which significant differences were found, it was included in the group (either alone or with other populations) with the lowest mean dry mass 100% of the time.

2. The Wisconsin population (WI) consistently exhibited the highest mortality rate. In 19 out of 24 (79%) time intervals, WI had the highest mortality of any population.

   In experiments 3A-3D, when mortality could be statistically analyzed because the treatments were replicated, WI had significantly higher mortality in 6 out of 12 comparisons with VA3 and CN. The only population other than WI that produced a significantly high mortality rate was CN, which had significantly higher mortality than VA3 for one time interval out of 12 comparisons (exp. 3A, day 52, Table 19a) - even in this instance, the mean mortality rate of WI (57%) was higher than CN (50%), but WI was not statistically different from VA3 because of high variability among replicates.

3. The population originally collected from Strouble's Creek (VA3), on the Virginia Tech campus, generally exhibited significantly higher growth rates than the other populations tested. Table 4 shows VA3 was included in the group with the

highest mean dry mass (either singly or with other populations) 9 times, or 81.8% of 11 time intervals when VA3 was compared to other populations in which significant differences were found.

Table 5 shows that at no time during the entire series of laboratory experiments (1A-1C, 2A-2C, and 3A-3D) did larvae from the Wisconsin population ever weigh significantly more than other larvae. Table 5 also shows that two of the faster-growing populations, VA3 and CN, never weighed less than larvae from either KS or VA2.

4. VA3 exhibited relatively low mortality. While mortality rates of VA1, VA2, and KS were comparable to VA3 in experiments 1A-1C and 2A-2C, VA3 showed markedly lower overall mortality rates than CN and WI in experiments 3A-3D. For experiments 3A-3D, VA3 had 20% mortality or less at all 12 sampling intervals. In 9 out of 12 time intervals (75% of the time), VA3 mortality was 10% or less. In contrast, CN had greater than 20% mortality at 5 out of 12 sampling intervals (42% of the time), and only 3 instances (25% of the time) when mortality was less than 10%. Mortality was greater than 20% for WI at all 12 sampling intervals, and was 50% or higher 8 out of 12 times (67% of the time).

4.2.1 Laboratory Experiments 1A-1C

Experiment 1A

In experiment 1A (Tables 6 and 7, Figures 3 and 4), no significant differences in growth rates were observed. Mean dry mass after 26 days ranged from a high of 0.140 mg (VA3, instantaneous growth rate (IGR) = 0.190 $mgmg^{-1}day^{-1}$) to a low of 0.089 mg (VA1 and VA2, IGR = 0.173 $mgmg^{-1}day^{-1}$). Percent mortality ranged from a high of 87% in WI to a low of 0% in VA2.

Experiment 1B

In experiment 1B (Tables 8 and 9, Figures 5 and 6), WI weighed significantly less than all other populations after six days of growth, the mean dry mass was 0.006 mg (IGR = 0.299 $mgmg^{-1}day^{-1}$). At the high end of the range were KS, VA3 and VA2 with mean dry masses of 0.038, 0.038 and 0.041 mg (IGR = 0.606, 0.606 and 0.619 $mgmg^{-1}day^{-1}$), respectively. Percent mortality was highest in WI (53%) and lowest in VA2 (0%).

Experiment 1C

In experiment 1C (Tables 10 and 11, Figures 7 and 8), after six days of growth, WI had a mean dry mass of 0.124 mg (IGR = 0.803 $mgmg^{-1}day^{-1}$) and weighed

significantly less than all other populations. Larvae from VA3 had a mean dry mass of 0.675 mg (IGR = 1.08 $mgmg^{-1}day^{-1}$) and weighed significantly more than all other populations. Percent mortality ranged from a high of 47% in WI to a low of 13% in both VA3 and KS.

4.2.2 Laboratory Experiments 2A-2C

Experiment 2A

In experiment 2A (Tables 12 and 13, Figures 9 and 10), larvae were sampled at three time intervals. On day 10, larvae from CN had a mean dry mass of 0.014 mg (IGR = 0.264 $mgmg^{-1}day^{-1}$) and weighed significantly more than larvae from all other populations. The lowest mean dry mass on day 10 was for VA2 at 0.006 mg (IGR = 0.179 $mgmg^{-1}day^{-1}$). The highest percent mortality on day 10 was 60% (WI and KS), while the lowest was 13% (VA1).

On day 20, larvae from VA3 had a mean dry mass of 0.335 mg (IGR = 0.291 $mgmg^{-1}day^{-1}$), which was significantly higher than all other populations. The population with the lowest mean dry mass on day 20 was VA2 at 0.157 mg (IGR = 0.253 $mgmg^{-1}day^{-1}$). Larvae from WI had the highest percent mortality on day 20 (87%), while the populations with the lowest mortality were VA1 and KS (0%).

There were no significant differences between the populations in mean dry mass on day 34. CN had the highest mean dry mass at 2.03 mg (IGR = 0.224 $mgmg^{-1}day^{-1}$),

while WI was the lowest at 0.959 mg (IGR = 0.202 $mgmg^{-1}day^{-1}$). On day 34, WI had the highest percent mortality (93%), while the lowest was VA2 (0%).

Experiment 2B

In experiment 2B (Tables 14 and 15, Figures 11 and 12), larvae were sampled at three time intervals. On day 7, VA3 and CN had the two highest mean dry masses, each at 0.015 mg (IGR = 0.387 $mgmg^{-1}day^{-1}$). Larvae from VA3 and CN did not weigh significantly more than larvae from VA1 (0.013 mg, IGR = 0.366 $mgmg^{-1}day^{-1}$), but they did weigh more than larvae from the other populations.

On day 7, larvae from WI had the lowest mean dry mass at 0.005 mg (IGR = 0.229 $mgmg^{-1}day^{-1}$) and weighed significantly less than VA1, CN and VA3. The population with the highest percent mortality on day 7 was WI (67%), while the lowest was VA1 (13%).

On day 12, larvae from VA3 weighed significantly more than all other populations and had a mean dry mass of 0.229 mg (IGR = 0.453 $mgmg^{-1}day^{-1}$). The lowest mean dry mass on day 12 was WI at 0.096 mg (IGR = 0.380 $mgmg^{-1}day^{-1}$). Larvae from WI weighed significantly less than larvae from VA1 and VA3. The highest percent mortality was WI (47%), while the lowest was VA1 and VA2 (0%).

On day 23, there were no significant differences in mean dry mass amongthe populations. The highest mean dry mass was KS (2.14 mg, IGR = 0.333 $mgmg^{-1}day^{-1}$) and the lowest was VA1 (1.15 mg, IGR = 0.306 $mgmg^{-1}day^{-1}$). The populations with the

greatest percent mortality on day 23 were WI and CN (both 53%), while the lowest was VA1 (0%).

Experiment 2C

In experiment 2C (Tables 16 and 17, Figures 13 and 14), larvae were sampled at three time intervals. On day 4, larvae from CN weighed significantly more than larvae from all other populations. The mean dry mass of CN was 0.018 mg (IGR = 0.722 $mgmg^{-1}day^{-1}$)(larvae from VA3 were not sampled until day 5, and so were not included in the analysis of the first sampling interval in experiment 2C). Larvae from VA2 and WI weighed significantly less than all other populations on day 4 at 0.005 mg and 0.006 mg (IGR = 0.402 and 0.448 $mgmg^{-1}day^{-1}$), respectively. The percent mortality on day 4 ranged from a high of 27% (CN) to a low of 7% (VA2).

On day 9, VA1 had the highest mean dry mass at 0.885 mg (IGR = 0.754 $mgmg^{-1}day^{-1}$), which was significantly higher than all other means. The smallest mean dry mass was 0.508 mg for KS (IGR = 0.692 $mgmg^{-1}day^{-1}$). On day 9, the highest percent mortality was 53% (CN) and the lowest was 0% (VA3).

By day 12, larvae from only three populations were present because complete emergence had occurred for VA1, CN and WI, leaving only adults present. There were no significant differences in mean dry masses of larvae from the three remaining populations: VA2, VA3 or KS. Percent mortality on day 12 ranged from highs of 67% in CN and 47% in WI, to a low of 0% in both VA1 and VA3.

4.2.3 Laboratory Experiments 3A-3D

Summary of experiments 3A-3D

In this series of four experiments, larvae from three populations (VA3, CN and WI) were compared at two temperatures (11°C and 27°C), and using two types of food. One food was of high quality (Tetragrowth^{tm} larval fish food) and the other of lower quality (stream-conditioned leaves of Red Maple, *Acer rubrum*) (Tables 18-21 and Figures 15-22). For each combination of temperature and food the larvae were sampled at three time intervals. Finding significant differences in growth rate depended on the type of food given to the larvae. In experiments 3B and 3D, when larvae were fed Tetragrowth^{tm}, there were four time intervals, out of a possible six, when significant differences in growth rate were found. When larvae were fed conditioned leaves in experiments 3A and 3C, no significant differences in growth were found at any time interval.

In every case except one, the difference in growth rate was due either to VA3 or CN being greater in mass than WI. The exception was day 31 in experiment 3B, when the growth of VA3 was higher than CN.

With respect to mortality, there were seven time intervals, out of a possible twelve, when significant differences in mortality were found. Again, in all cases but one, the differences were due to either VA3 or CN having a higher survival rate compared to

WI. The exception was day 52 in experiment 3A, when CN had a significantly higher mortality rate than VA3. In contrast to growth rate differences between the three populations, significant differences in mortality occurred at both temperatures and for both food types.

Experiment 3A

Tables 18a, 19a and Figures 15, 16 show the results for experiment 3A.

No significant differences in mean dry mass were found between the three populations during experiment 3A. On day 13, average larval biomass ranged from a high of 0.005 mg (IGR = 0.124 $mgmg^{-1}day^{-1}$) for both VA3 and CN, to a low of 0.003 mg (IGR = 0.084 $mgmg^{-1}day^{-1}$) for WI. On day 13, the percent mortality of larvae from WI was 90%, which was significantly greater than the 20% mortality seen for both VA3 and CN.

On day 27, average larval biomass ranged from a low of 0.030 mg (IGR = 0.126 $mgmg^{-1}day^{-1}$) for VA3, to a high of 0.050 mg (IGR = 0.145 $mgmg^{-1}day^{-1}$) for WI. On day 27, WI had the highest mortality at 57%, which was significantly greater than VA3 (7%) or CN (23%).

On day 52, average larval biomass ranged from a low of 0.074 mg (IGR = 0.083 $mgmg^{-1}day^{-1}$) for VA3, to a high of 0.135 mg (IGR = 0.094 $mgmg^{-1}day^{-1}$) for CN. On day 52, percent mortality ranged from a low of 7% in VA3, to 50% in CN, to a high of 57% in WI. Mortality in CN was significantly higher than that in VA3; however,

mortality in WI was not significantly higher than VA3 because of the large variation for WI replicates.

Experiment 3B

Tables 18b, 19b and Figures 17, 18 show the results for experiment 3B.

In experiment 3B no significant differences in growth were seen on day 11. Average larval biomass ranged from a high of 0.006 mg (IGR = 0.163 $mgmg^{-1}day^{-1}$) for VA3 to a low of 0.003 mg (IGR = 0.100 $mgmg^{-1}day^{-1}$) for WI. On day 11, WI had significantly higher average mortality (80%), than either VA3 (0%) or CN (20%).

On day 24, the average larval biomass of both VA3 and CN, 0.174 mg (IGR = 0.215 $mgmg^{-1}day^{-1}$) and 0.171 mg (IGR = 0.214 $mgmg^{-1}day^{-1}$), respectively, were higher than WI which was 0.061 mg (IGR = 0.171 $mgmg^{-1}day^{-1}$). On day 24, WI had a significantly higher average mortality at 80%, than both VA3 (0%) and CN (7%).

On day 31, average larval biomass of VA3 (0.836 mg, IGR = 0.217 $mgmg^{-1}day^{-1}$) was significantly greater than CN (0.371 mg, IGR = 0.191 $mgmg^{-1}day^{-1}$), which in turn, was significantly greater than WI (0.140 mg, IGR = 0.159 $mgmg^{-1}day^{-1}$). On day 31, WI had a significantly higher average mortality at 77% than both VA3 (0%) and CN (0%).

Experiment 3C

Tables 20a, 21a and Figures 19, 20 show the results for experiment 3C.

No significant differences in mean larval biomass were found between the three populations during experiment 3C. On day 5, the highest mean biomass was CN at 0.016 mg (IGR = 0.554 $mgmg^{-1}day^{-1}$) and the lowest was WI at 0.011 mg (IGR = 0.480 $mgmg^{-1}day^{-1}$). On day 5, mortality in WI was 53%, significantly higher than both VA3 (3%) and CN (7%).

On day 10, the highest mean biomass was VA3 at 0.081 mg (IGR = 0.439 $mgmg^{-1}day^{-1}$) and the lowest was CN at 0.053 mg (IGR = 0.397 $mgmg^{-1}day^{-1}$). The highest mortality on day 10 was CN at 37% and the lowest was VA3 at 10%.

On day 15, the highest mean biomass was CN at 0.149 mg (IGR = 0.334 $mgmg^{-1}day^{-1}$) and the lowest was WI at 0.128 mg (IGR = 0.323 $mgmg^{-1}day^{-1}$). The highest mortality on day 15 was WI at 37% and the lowest was VA3 at 0%.

Experiment 3D

Tables 20b, 21b and Figures 21, 22 show the results for experiment 3D.

There were no significant differences in mean larval biomass on day 3 in experiment 3D. The highest mean biomass was WI at 0.016 mg (IGR = 0.924 $mgmg^{-1}day^{-1}$) and the lowest was VA3 at 0.012 mg (IGR = 0.828 $mgmg^{-1}day^{-1}$). The highest mortality on day 3 was WI at 23% and the lowest was VA3 at 10%.

On day 6, both CN (0.298 mg, IGR = 0.950 $mgmg^{-1}day^{-1}$) and VA3 (0.273 mg, IGR = 0.935 $mgmg^{-1}day^{-1}$) had a significantly higher mean larval biomass than WI (0.162 mg, IGR = 0.848 $mgmg^{-1}day^{-1}$). The highest mortality on day 6 was WI at 43% while VA3 and CN both had 20% mortality.

On day 9, the mean larval biomass of VA3 at 1.15 mg (IGR = 0.783 $mgmg^{-1}day^{-1}$) was significantly greater than WI at 0.743 mg (IGR = 0.734 $mgmg^{-1}day^{-1}$). The highest mortality on day 9 was WI at 50% and the lowest was VA3 at 20%.

4.3 Transplant Experiments

4.3.1 First Transplant Experiment

The first transplant experiment was initiated in September, 1989 using laboratory-reared larvae from the three Virginia populations that had been cultured in the laboratory for approximately one year (VA1, VA2, and VA3). The results (Table 22, Figure 23) showed no significant difference between the three populations in the number of larvae surviving after 15 days exposure in the chlorine contact tanks of the Blacksburg sewage treatment plant.

The larvae originally collected from the chlorine contact tanks (VA2) had the highest mean number surviving at 5.75 larvae out of an original 35 larvae (16%). The mean number surviving for VA3 was 2.75 (7.8%) and for VA1 it was 3.12 (8.9%).

4.3.2 Second Transplant Experiment

The second transplant experiment was conducted from 8/15/90 to 8/30/90, using the same three laboratory populations which were used in the first transplant experiment, plus an additional strain, VA2b. There were significant differences in larval survival between these populations after 7 and 15 days of exposure in the chlorine contact tanks (Table 23, Figure 24). On day 7, VA2b had the highest mean survival with 13 out of an initial 25 larvae (52%) surviving. Survival in the other populations was significantly lower with mean numbers surviving of 4.7 (19%), 5.7 (23%) and 8.3 (33%) for VA3, VA2a and VA1, respectively. On day 15, VA2b had a mean number surviving of 4.8 (19%). Survival in the other populations was significantly lower with mean numbers surviving of 0.5 (2.0%), 0.67 (2.6%) and 1.3 (5.2%) for VA1, VA2a and VA3, respectively.

4.4 Emergence and Egg Mass Study (Laboratory Experiment 4)

Total adults emerging

The mean number of emerging adults was significantly greater from VA1 (88.3), VA2 (86.3), and VA3 (96.0), than from KS (74.5), CN (69.3) and WI (47.3)(Table 24,

Figure 25). The number of emerging adults from WI was significantly less than all other populations.

Number of days until the onset of emergence

The mean number of days until the onset of emergence was significantly less for VA3 (11.7) than for KS (13.0) and CN (13.0). The mean number of days required for VA1, WI and VA2 was 12.0, 12.3 and 12.7, respectively (Table 24, Figure 26).

Length of adult emergence period

WI had the shortest mean emergence period at 4.7 days (Table 24, Figure 27). The mean length of the emergence period for WI was significantly less than for KS (8.5), VA2 (9.3) and CN (8.7). The mean emergence period for VA3 was 8.7 days.

Number of test days to reach 50% emergence

Both VA3 and WI took significantly less time to attain 50% emergence than the other populations, with 14.0 days needed. The greatest mean number of days required were 16.3 and 16.5 days for VA2 and KS, respectively. VA1 and CN required 15.0 and 15.7 days, respectively (Table 24, Figure 28).

Number of emergence days to reach 50% emergence

WI required a mean of only 1.7 emergence days to reach the 50% emergence level, significantly less than both KS and VA2, which required means of 3.5 and 3.7 days, respectively. VA3, CN and VA1 required means of 2.3, 2.7 and 3.0 days, respectively (Table 24, Figure 29).

Emergence curves

Figure 30 shows emergence curves for the six populations during laboratory experiment 4.

Number of egg masses produced

The mean number of egg masses produced per 100 initial larvae was significantly higher in VA1 (27) and VA2 (35) than in WI (15). Adults from VA1 also produced significantly more egg masses than adults from KS (14) (Table 25, Figure 31). To some extent, the number of egg masses produced depended on the number of adults that emerged, i.e., the greater the number of adults that emerged, the larger the number of egg masses produced.

In general, the number of egg masses produced per emerging adult was approximately 0.3. However, adults from KS produced less egg masses than expected, while adults from VA2 produced more than expected as seen in Table 26.

Number of eggs per egg mass

Adults from WI produced significantly more eggs per egg mass than adults from all other populations (Table 25, Figure 32). The mean number of eggs per egg mass for WI was 596, while the means for KS, CN, VA2, VA1, and VA3 were 453, 468, 484, 500 and 530, respectively. Adults from WI produced the only egg masses that contained more than 700 eggs, and they produced at least twice as many egg masses containing more than 600 eggs than adults from any other population (Table 27).

4.5 Toxicity Tests

Under low aeration, WI was the least sensitive population tested, as it weighed significantly less at the 8 mg/L CT-1 treatment level, while VA1, VA2 and VA3 were significantly different at the 2 mg/L level (Table 28). Under high aeration, WI and VA3 were the least sensitive populations tested since they both weighed significantly less than the control at the 8 mg/L treatment level; while VA1 and VA2 weighed significantly less than the control at the 4 mg/L treatment level.

There was essentially no difference between the LC50's for the four population tested. The 95% confidence limits overlapped considerably, obscuring any differences between the populations (Table 29).

5.0 DISCUSSION

This research provided evidence for a genetic basis to various life history parameters among populations of *C. riparius* and that differences in genetic makeup may result in significant divergence between population life histories under laboratory conditions. A genetic component was found to exist for larval growth, development and mortality rates. In addition, it was demonstrated using artificial selection and transplant experiments, that there is a genetic basis for chlorine-tolerance in this species.

5.1 In-Situ Field Growth Study

Prior to investigating whether genetic differences influenced life histories of the study populations, an in-situ study was conducted with three local populations of *C. riparius* (VA1, VA2, VA3) to establish the occurrence of significant differences in life histories under field conditions, i.e., rates of larval growth and development. Although the two STP populations (VA1, VA2) experienced identical water temperatures (Figure 2), larvae grown in the chlorine-contact tank (VA2) were significantly smaller on three out of four sample dates compared to larvae in the secondary clarifier (VA1)(Table 3). This difference was probably due to chlorine-stress and perhaps lower food quality experienced by VA2 since no algae was seen growing in the chlorine-contact tanks and the larvae had to subsist on a pale, bleached-looking form of sludge. Larvae in Strouble's Creek (VA3) experienced lower water temperatures throughout the field study and

appeared to subsist primarily on organic detritus originating from decomposing plant litter. VA3 larvae consistently weighed less than VA1 larvae and also weighed less than VA2 larvae from day 15 until the end of the study. Thus, it appears that differences in growth rates were due to a combination of different water temperatures, food quality and chlorine stress between the three sites.

Development rates also differed between the sites and could not be accounted for solely by differences in water temperature. Pupae first appeared at VA1 on day 15 when 326 degree days had accumulated; at VA2 on day 20 after 410 degree days; and at VA3 on day 25 after 474 degree days. In addition, no more larvae were found at the two STP sites after 20 days, while larvae were found at VA3 until the end of the study on day 30 (Figure 1). Therefore, it appears that factors such as food quality and chlorine stress, as well as temperature differences may have had an important role in producing developmental differences at the three sites.

5.2 Laboratory Comparisons of Growth

The results of the laboratory studies identified as 1A-1C, 2A-2C, and 3A-3D, positively demonstrated genetic differences between these research populations; and strongly support the premise of the study, i.e., that populations of *C. riparius* have underlying genetic differences that result in significant variations in life histories. Significant genetic-based differences in mean dry mass (growth) were found in all experiments except for 1A, 3A, and 3C.

Genetic-based differences in growth rates, using individuals from different populations that had been cultured under identical lab conditions for one or more generations have been shown by other researchers. Lonsdale and Levinton (1986) demonstrated genetic differences between populations of the copepod *Scottolana canadensis* under different laboratory temperature regimes. Postma et al. (1995b) found that Cd-tolerant *C. riparius* grew significantly slower than reference larvae under clean conditions. However, when exposed to Zn, the Cd-tolerant midges grew significantly faster than the reference larvae. Using offspring from individuals reared for one generation in clean conditions, Miller and Hendricks (1996) did not see significant growth differences between Zn-tolerant *C. riparius* and a reference population. Levinton and Monahan (1983) showed that individuals from two subspecies of the polychaete, *Ophryotrocha puerilis*, exhibited significant differences in growth. At a cold test temperature, the northern subspecies grew faster, while at a warm test temperature the southern subspecies grew faster. There were no growth differences seen at an intermediate temperature.

Several studies have implicated population genetic differences as the cause for different growth rates, but either due to maternal or acclimation effects on test organisms, the data is equivocal. Postma and Davids (1995) found that after rearing *C. riparius* for nine generations in cadmium-contaminated water, that growth rates were reduced significantly less than a nonexposed reference population during an acute cadmium toxicity test. However, the Cd-exposed population was not reared under clean conditions before the toxicity test so acclimation effects may have played an important role in the

different responses of the two populations. Postma et al. (1995a) used first generation larvae of field-collected *C. riparius* from two cadmium-polluted sites and two unpolluted sites and found that growth rates were decreased for all larvae when exposed to cadmium in the laboratory, but not as much so for the individuals from polluted sites. Hoffman and Fisher (1994) used first generation offspring of field-collected larvae and found significant differences in pupal dry weight between a population of *C. riparius* from a sewage treatment plant and another population that had been cultured in the lab for 16 years; maternal effects may have played a role. Maternal effects could not be ruled out by Fairbairn (1984) since she also used offspring of field-collected females. She showed that significant differences in female growth rates observed in the field between four populations of the gerrid, *Limnoporus notabilis*, persisted in the laboratory. Males from one population grew faster while in the field but showed no difference from other males when reared in the lab. The females that grew faster in the field also grew faster in the lab. Berven (1982) discussed how environmental differences between populations in the field may mask underlying genetic differences and when the populations are reared in the lab under identical conditions the pattern of life history variations may run counter to the trend seen in the field. During the in-situ growth study, larval growth was much lower in VA3 compared to VA1 and VA2, but under identical lab conditions VA3 always grew faster than VA2 and grew at a higher rate than VA1 in four out of five comparisons.

A number of studies have used electrophoretic techniques to show correlations between growth rates and some aspect of genetic structure. However, electrophoresis does not unequivocally show that the observed growth differences are genetically-based.

i.e., a cause and effect relationship cannot be established (Sweeney et al. 1986, Singh and Zouros 1977). Scott and Koehn (1990) found a significant relationship between allozyme heterozygosity and growth rate in the clam, *Mulinia lateralis*. Under stressful conditions of salinity and temperature, individuals with more loci in a heterozygous state grew significantly faster than individuals with fewer heterozygous loci. This did not occur when the clams were grown under optimal conditions. Quattro and Vrijenhoek (1989) studied three populations of the Sonoran topminnow, *Poeciliopsis occidentalis occidentalis*. The population homozygous for all loci surveyed had the slowest growth rate while the most heterozygous population had the highest growth rate. Hilbish (1985) identified strong selection pressure directed against juvenile mussels (*Mytilus edulis*) with the Lap^{94} allele. A population with individuals possessing the Lap^{94} allele grow only a small degree following recruitment from the open ocean, while other populations grow rapidly. Singh and Zouros (1978) found that higher average heterozygosities in individuals of the American oyster, *Crassostrea virginica*, was positively correlated with body weight.

5.3 Laboratory Comparisons of Survival

The results of the ten laboratory studies identified as 1A-1C, 2A-2C, and 3A-3D, support that significant genetic-based differences in mortality rate existed between the study populations. Because treatments were not replicated in experiments 1A-1C or 2A-2C, it was not possible to show statistically significant differences in survival rate, but a

clear trend was evident, i.e, that WI had substantially higher mortality than the other populations in all six of these studies. Statistical analysis of survival in experiments 3A-3D showed significant differences between populations in all experiments except for 3D.

Levinton and Monahan (1983) cultured two subspecies of the polychaete, *Ophrytrocha puerilis*, through several generations under identical conditions and found that at warm test temperatures the warmwater subspecies suffered less mortality than the coldwater subspecies. The differences in mortality between subspecies were unequivocally genetic-based. Postma et al. (1995b) found that Cd-tolerant *C. riparius,* reared for two generations in clean water, experienced increased larval mortality compared to reference larvae when both were exposed to clean water conditions. When both the Cd-tolerant and reference larvae were exposed to a range of zinc concentrations, there was no difference in survival rate between the two populations.

A variety of studies using electrophoresis, show correlations between genetic composition and mortality rates. Quattro and Vrijenhoek (1989) found that field-collected Sonoran topminnows (*Poeciliopsis occidentalis occidentalis*) from populations that differed in the degree of heterozygosity also showed differences in mortality under standard laboratory conditions, i.e., high heterozygosity correlated with low mortality. Hilbish (1985) found that selection pressure against juvenile blue mussels, *M. edulis*, with the Lap^{94} allele results in high mortality at one site in Long Island Sound. Kautsky et al. (1990) and Johannesson et al. (1990) reported differential survival among populations of blue mussels, *Mytilus edulis*, from the North Sea and Baltic Sea using reciprocal transplant experiments. In the former study they found significant mortality in

individuals transplanted from both the North Sea to Baltic Sea and vice versa. While they attributed some of the mortality to salinity shock, they speculated that genetic differences may also have played a role. However, reciprocal transplant experiments using field-collected organisms cannot unequivocally differentiate between acclimation, maternal, or genetic causes producing differential responses (Kautsky et al. 1990). In the latter study (Johannesson et al. 1990), electrophoresis was performed on resident and transplanted individuals to look for correlations between survival and genotype. They found that in transplants from the Baltic to North Sea that selective mortality occurred against Baltic individuals with characteristic Baltic sea genotypes at two loci (Pgi and Pgm). Similarly, high mortality occurred against mussels with characteristic North sea genotypes when transplanted to the Baltic Sea; the only survivors had characteristic Baltic genotypes at the Pgm and Pgi loci. Benton and Guttman (1992) found that time to death for the caddisfly, *Nectopsyche albida*, exposed to mercury nitrate differed among genotypes and levels of heterozygosity. Benton and Guttman (1990) showed that time to death for the mayfly, *Stenonema femoratum*, exposed to copper differed among allozyme genotypes at one of three loci examined. Chagnon and Guttman (1989) found that mosquitofish (*Gambusia affinis*) heterozygous at the GPI-2 locus had significantly higher mortality rates when exposed to copper and that fish homozygous for a certain allele at that locus had significantly lower mortality rates when exposed to cadmium. Lavie and Nevo (1982) found that sensitivity to zinc increased for the marine gastropod, *Monodonta turbinata*, when it was homozygous for a particular allele at the Pgi locus and sensitivity to copper increased when it was homozygous for another allele at the Pgi locus. Nevo et al. (1981)

identified five alleles at the PGM locus in the shrimp, *Palaemon elegans*. The heterozygote, MS exhibited lower mortality at low and intermediated concentrations of mercury while MM homozygotes had the lowest mortality at the highest mercury concentrations tested.

5.4 Emergence and Egg Mass Study (Laboratory Experiment 4)

The results of experiment #4 described here show significant genetic differences between populations in a number of aspects related to emergence. Differences were found in the mean number of days until the onset of emergence, mean number of days from the beginning until the end of emergence, mean number of days required for 50% emergence, and mean number of days from the beginning of emergence until 50% emergence (Table 24).

Although the literature on aquatic insect ecology contains many studies examining emergence patterns of species and the factors governing those patterns, few if any, studies have undertaken to determine whether differences in population emergence patterns have a genetic basis. In their study on the population genetics of mayflies, Sweeney et al. (1986) cite unpublished data while noting they have observed significant differences in the timing of adult emergence above and below reservoirs on the Delaware River. Martin and Wulker (1971) found an "association" between some types of chromosome polymorphisms in the midge, *Chironomus staegeri*, and individuals living in shallow water which were bivoltine and deep water forms which were univoltine. The bivoltine

individuals emerged in May and August, while the univoltine individuals emerged in May. As is the case with electrophoresis, it is difficult to say whether the chromosome polymorphisms have anything to do with the emergence patterns at all, i.e., it may not be a cause-and-effect relationship and the differences in emergence patterns may be due soley to environmental differences.

Experiment #4 also provided evidence showing that some measures of fecundity differed between the laboratory populations. The total number of egg masses laid per initial 100 larvae varied significantly (Table 25). Of course, this parameter is strongly affected by the number of larvae that lived to the adult stage. When the number of egg masses laid per adult was measured, there were no significant differences (Table 26). Interestingly, there was a profound difference in the number of eggs per egg mass between the populations. Six of the seven populations showed no difference from one another in this parameter, but WI laid egg masses with a significantly higher number of eggs. Postma et al. (1995b) found no difference between Cd-tolerant *C. riparius* and reference larvae in the number of eggs/egg mass when both populations were exposed under clean conditions. The Cd-tolerant midges did lay 50% less egg masses than the reference population under clean conditions. Hoffman and Fisher (1994) did not find any difference in the #eggs/egg mass when comparing *C. riparius* larvae from a sewage treatment plant and reference larvae under clean conditions. Lonsdale and Levinton (1986) reported significant genetic differences in mean clutch size between geographic populations of the copepod, *Scottolana canadensis*, which had been reared in the laboratory for multiple generations. Berven (1982) claimed that differences in the

number of eggs per egg mass between mountain and lowland populations of the wood frog, *Rana sylvatica*, were due entirely to genetic variation since transplanted individuals retained the same characteristics for these two traits as the populations from which they were transplanted. However, such an experimental approach, i.e., the use of field-collected test organisms in conducting the transplants, does not rule out either acclimation or maternal effects.

5.5 Transplant Experiments

An initial attempt was made to show significant genetic-based differences in survival between larvae from VA1, VA2, and VA3 while exposed to conditions in the Blacksburg, STP chlorine-contact tank. If increased tolerance to this environment had a genetic basis then larvae originally collected from that tank (VA2) should exhibit lower mortality than the other populations. Results of the first transplant experiment indicated a nearly significant difference in survival between VA2 and VA3 ($p = 0.07$, Table 22).

A second transplant experiment was initiated one year later using larvae from the original chlorine-contact tank population (VA2), but which had been selected for chlorine-tolerance by reexposing them for two generations to the chlorine-contact tanks. Following reexposure, the new strain, VA2b, was cultured under clean conditions for three generations to ensure differences in chlorine-tolerance between exposed populations were due to genetic differences and not acclimation or maternal effects. The results showed a high degree of genetic-based tolerance by VA2b compared to both VA1 and

VA3 (Table 23). These results demonstrated unequivocally that differences in chlorine-tolerance had a genetic basis in these test populations.

The lack of significant differences in the first transplant experiment may have been due to the loss of alleles conferring chlorine-tolerance in VA2 during the one year of laboratory rearing prior to conducting the experiment. Several studies have shown that laboratory culturing often results in a loss of genetic variability. A loss of alleles and decreases in heterozygosity and % polymorphic loci can occur (Woods et al. 1989), sometimes within a few generations (Mason et al. 1987). In some cases, these changes have correlated to alterations in mating frequency, fertility, and various other life history parameters (Mason et al. 1987). Selection for genotypes adapted to a constant temperature and artificial diet may eliminate some variability (Mason et al. 1987). It was shown by McDonald and Ayala (1974) and Powell (1971) with *Drosophila spp.*, that there is a positive relationship between environmental heterogeneity and genetic variation in laboratory cultures. Flies raised in more heterogenous environments were characterized by higher levels of heterozygosity and a greater number of alleles per locus.

Alterations in genetic constitution of cultured populations can also result from random genetic drift in small cultures, nonrandom mating such as inbreeding which decreases heterozygosity, and founder effects caused by starting cultures with small numbers of individuals.

5.6 Toxicity Tests

The LC50s calculated for the four test populations using the molluscicide CT-1 were extremely similar (Table 29). Therefore, no differential sensitivity between populations was indicated for this endpoint. However, a fourfold difference in the lowest observed effect concentration for growth under low aeration suggests that this enpoint may indicate genetic differences in sensitivity between populations (Table 28). Because the data are based on only two toxicity tests it may be premature to generalize about the significance of these results. Although maternal effects cannnot be ruled out, Hoffman and Fisher (1994) found that *C. riparius* collected from a sewage treatment plant was 13-250x more tolerant to insecticides than a reference laboratory population.

6.0 CONCLUSIONS

The data collected during this research provided strong supporting evidence that variations in important life history parameters among populations of *C. riparius* had a genetic basis. A genetic component was found for larval growth, development and mortality rates. In addition, it was demonstrated using artificial selection and transplant experiments, that there is a genetic basis for chlorine-tolerance in this species.

1. Larvae from three populations of *C. riparius*, collected from the Blacksburg vicinity (VA1, VA2, VA3), exhibited significant differences in growth and development rates when exposed in-situ (at their original collection sites) to different environmental conditions (e.g., food and temperature). The direction of these differences was reversed when larvae from these same populations were exposed in the laboratory under identical conditions.

   Life history differences measured during this in-situ field study resulted primarily from the strong environmental differences between the sites that these three populations inhabit. Whereas, life history differences measured in the laboratory were unequivocally the result of differences in the genetic makeup of the test organisms.

2. A series of laboratory studies, conducted under identical conditions with larvae from up to seven different populations, showed that genetic differences among the

populations resulted in significant variations in life history parameters, e.g., growth, mortality, development, and emergence rates.

The Wisconsin population (WI) is characterized by significantly lower growth rates and significantly higher mortality rates in the overwhelming majority of comparisons made in the laboratory experiments (i.e., 1A-1C, 2A-2C, and 3A-3D). Conversely, the Strouble's Creek population (VA3) was characterized generally by high growth and low mortality rates in comparison to the other test populations during this same series of experiments.

3. In experiment 4, the seven populations were allowed to develop through the period of adult emergence until the time that oviposition was complete. Significantly fewer adults emerged from WI than the other six populations and VA3 fell in the group with the greatest number of emerging adults, supporting the data gathered from the earlier series of experiments (1-3) in which the same pattern was seen for larvae.

   From experiment 4, further conclusions can be made, i.e., that genetic-based differences existed among the test populations with regard to the number of days until the onset of emergence, the mean number of days from the beginning until the end of emergence, and the number of eggs per egg mass.

4. Genetic-based tolerance to chlorine was shown in transplant experiments conducted in the Blacksburg STP chlorine-contact tanks. Larvae from VA2 were selected for tolerance to conditions in the chlorine-contact tanks for two generations and, after being reared under clean conditions for two more

generations in the laboratory, were found to exhibit significantly lower mortality upon reexpore to the chlorine-contact tanks compared to individuals from two other populations.

5. Some data was collected suggesting differences in sensitivity among four of the test populations to the pesticide CT-1. Under low levels of aeration during an eight day toxicity test, the concentration of CT-1 producing a significant effect on growth was fourfold higher (two test concentrations) for WI compared to three other populations. In a second toxicity test, under high aeration, a similar pattern was observed although the difference in the effect concentration was only two-fold (one test concentration). Additional toxicity tests could have provided stronger evidence for this apparent differential sensitivity among populations.

7.0 SUMMARY

Laboratory and field experiments were conducted with six populations of *Chironomus riparius* to test for variations among the populations in life history parameters such as growth, mortality and/or reproductive rates. Egg masses of *C. riparius* from five field populations and one laboratory population were used to initiate laboratory cultures for experimentation. Environmental effects on life history variations could be ruled out because experiments were conducted with larvae that had been reared in the laboratory for several generations under identical conditions. Populations were compared under a variety of experimental conditions to test whether they would respond similarly, if genetically similar, or differently, if their genotypes were different.

The populations were subjected to a variety of experimental conditions in the laboratory in which temperature and food quality were manipulated. The population collected from Strouble's Creek, Virginia demonstrated a pattern of high growth rate and low mortality rate, while the population collected from the final clarifier at the Madison, Wisconsin sewage treatment plant showed a pattern of low growth rate and high mortality rate. When several populations were transplanted into a high chlorine environment at the Blacksburg, Virginia sewage treatment plant, the population that had been previously exposed to the chlorine showed a significantly lower mortality rate than the other populations. A series of toxicity tests revealed different sensitivities among several populations to an experimental pesticide. Differences among populations were also

found in numbers of egg masses produced, numbers of eggs per egg mass, length of time until the onset of adult emergence, and the overall length of the emergence period.

The results of this study show the importance of genetic differences among populations of *C. riparius*. Many commonly measured life history parameters that are usually assumed to vary primarily from differences in environment, have been shown to vary as well from underlying genetic differences between populations. The role of genetics should be assessed, whenever possible, in studies where comparisons between populations of aquatic insects are made, in order to determine the degree to which genetics and environmental variables contribute to observed population differences.

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9.0 APPENDICES

Table 1. Larval head-capsule widths.

Instar	Head Capsule Width (mm)
I	0.10 - 0.12
II	0.18 - 0.20
III	0.30 - 0.40
IV	0.52 - 0.65

Table 2. Toxicity test conditions.

Effects Measured: Growth and mortality.

Endpoints: Lowest observed effect concentration (LOEC) for growth. LC50 for mortality.

Renewals: Static-renewal, eighty-percent of the test solution renewed every two days.

Test Duration: Eight days.

Test Organism: *Chironomus riparius*.

Age: Instar II larvae (3-4 days old).

Populations Tested: VA1, VA2, VA3 and WI.

Test Substance: CT-1, an experimental pesticide for controlling larvae of the Asiatic clam.

Test Concentrations: Control, 1, 2, 4, 8, 16, 32 mg/L (ppm).

Replicates: Three replicates per treatment level and the control.

Test Volume: 200 ml.

Test Container: 250 ml Erlenmyer flask.

Number of Organism/Container: Ten larvae per replicate flask.

Substrate: Sieved and washed sand. Sand diameter 180-300 µm.

Temperature: 22 ± 1°C.

Feeding: 0.5 ml. Tetragrowth^{tm} solution every two days.

Acclimation Period: Cultures acclimated to dilution water for two weeks prior to test initiation.

Dilution Water: Dechlorinated tap water, pH 7.8-8.1, hardness 68-70 mg/L as CaCO3, alkalinity 40-48 mg/L as CaCO3, specific conductivity 200-267 µmhos/cm.

Aeration: Two levels tested: high = 100 bubbles/minute
low = 30 bubbles/minute.

Statistical Analysis: ANOVA followed by Dunnet's Test on dry mass of larvae after eight days of growth. LC50's calculated by probit analysis and binomial test.

Table 3. Mean dry mass (mg)(SE) of larvae during in-situ growth experiment.

Days of Growth	Population[1]		
	VA1	VA2	VA3
5	0.077 (0.017)	0.012 (0.001)	0.013 (0.001)
10	0.703 (0.061)	0.166 (0.022)	0.111 (0.006)
15	0.964 (0.048)	0.977 (0.115)	0.384 (0.019)
20	1.68 (0.063)	1.12 (0.094)	0.736 (0.026)
25	---[2]	---[2]	1.18 (0.054)
30	---[2]	---[2]	1.21 (0.107)

[1] Days 5 and 10; VA2aVA3aVA1b; ANOVA, $p < 0.001$.
Day 15; VA3aVA1bVA2b; ANOVA, $p < 0.001$.
Day 20; VA3aVA2bVA1c; ANOVA, $p < 0.001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2]Present only as adults.

Table 4. Summary of findings from experiments 1A-1C, 2A-2C, and 3A-3D.

	Number of Times Found in Lowest Weight Group		Number of Times Found in Middle Weight Group		Number of Times Found in Highest Weight Group	
Population	#	Percent	#	Percent	#	Percent
VA1	2	25.0	5	62.5	1	12.5
VA2	4	50.0	3	37.5	1	12.5
VA3	2	18.2	0	0	9	81.8
KS	3	37.5	4	50.0	1	12.5
WI	12	100	0	0	0	0
CN	2	20.0	3	30.0	5	50.0

Table 5. Comparisons between populations for which significant differences in growth rates were never observed during laboratory experiments.

WI > VA1
WI > VA2
WI > VA3
WI > KS
WI > CN
VA3 < VA2
VA3 < KS
CN < VA2
CN < KS

Table 6. Mean dry mass (mg) of larvae in laboratory experiment 1A.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	11	26	0.089 (0.030)	0.173
VA2	11	26	0.089 (0.010)	0.173
VA3	11	26	0.140 (0.011)	0.190
KS	11	26	0.101 (0.012)	0.178
WI	11	26	0.126 (0.016)	0.186

[1]There were no significant differences in weight; ANOVA, $p = 0.06$.
[2]IGR = instantaneous growth rate.

Table 7. Percent mortality of larvae in laboratory experiment 1A.

Population	Temperature (°C)	Initial Number	Final Number	Percent Mortality
VA1	11	15	5	67
VA2	11	15	15	0
VA3	11	15	10	33
KS	11	15	12	20
WI	11	15	2	87

Table 8. Mean dry mass (mg) of larvae in laboratory experiment 1B.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	18	6	0.021 (0.003)	0.507
VA2	18	6	0.041 (0.005)	0.619
VA3	18	6	0.038 (0.006)	0.606
KS	18	6	0.038 (0.003)	0.606
WI	18	6	0.006 (0.002)	0.299

[1]WIaVA1bKScVA3cVA2c; ANOVA, $p < 0.001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2]IGR = instantaneous growth rate.

Table 9. Percent mortality of larvae in laboratory experiment 1B.

Population	Temperature (°C)	Initial Number	Final Number	Percent Mortality
VA1	18	15	12	20
VA2	18	15	15	0
VA3	18	15	14	7
KS	18	15	15	0
WI	18	15	7	53

Table 10. Mean dry mass (mg) of larvae in laboratory experiment 1C.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	27	6	0.478 (0.065)	1.03
VA2	27	6	0.520 (0.035)	1.04
VA3	27	6	0.675 (0.042)	1.08
KS	27	6	0.365 (0.044)	0.983
WI	27	6	0.124 (0.017)	0.803

[1]WIaKSbVA1bVA2bVA3c; ANOVA, $p < 0.001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2]IGR = instantaneous growth rate.

Table 11. Percent mortality of larvae in laboratory experiment 1C.

Population	Temperature (°C)	Initial Number	Final Number	Percent Mortality
VA1	27	15	11	27
VA2	27	15	11	27
VA3	27	15	13	13
KS	27	15	13	13
WI	27	15	8	47

Table 12. Mean dry mass (mg) of larvae in laboratory experiment 2A.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	14	10	0.007 (0.001)	0.194
		20	0.210 (0.016)	0.267
		34	1.64 (0.152)	0.218
VA2	14	10	0.006 (0.001)	0.179
		20	0.157 (0.014)	0.253
		34	1.27 (0.159)	0.210
VA3	14	10	0.008 (0.002)	0.208
		20	0.335 (0.027)	0.291
		34	1.59 (0.165)	0.217
KS	14	10	0.007 (0.001)	0.194
		20	0.198 (0.010)	0.264
		34	1.64 (0.247)	0.218
WI	14	10	0.007 (0.001)	0.194
		20	0.194 (0.006)	0.263
		34	0.959 (---[3])	0.202
CN	14	10	0.014 (0.001)	0.264
		20	0.210 (0.014)	0.267
		34	2.03 (0.412)	0.224

[1] Day 10: VA2aVA1aKSaWIaVA3aCNb; ANOVA, $p < 0.001$.
Day 20: VA2aWIaKSaVA1aCNaVA3b; ANOVA, $p < 0.001$.
Day 34; ANOVA, $p = 0.22$ n.s.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2]IGR = instantaneous growth rate.
[3]No SE could be calculated because only 1 larva was found.

Table 13. Percent mortality of larvae in laboratory experiment 2A.

Population	Temperature (°C)	No. Days Growth	Initial Number	Number Surviving	Percent Mortality
VA1	14	10	15	13	13
		20	15	15	0
		34	15	13	13
VA2	14	10	15	11	27
		20	15	11	27
		34	15	15	0
VA3	14	10	15	11	27
		20	15	11	27
		34	15	11	27
KS	14	10	15	6	60
		20	15	15	0
		34	15	8	47
WI	14	10	15	6	60
		20	15	2	87
		34	15	1	93
CN	14	10	15	12	20
		20	15	11	27
		34	15	10	33

Table 14. Mean dry mass (mg) of larvae in laboratory experiment 2B.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	18	7	0.013 (0.001)	0.366
		12	0.174 (0.014)	0.430
		23	1.15 (0.016)	0.306
VA2	18	7	0.009 (0.001)	0.314
		12	0.147 (0.009)	0.416
		23	1.52 (0.280)	0.318
VA3	18	7	0.015 (0.002)	0.387
		12	0.229 (0.035)	0.453
		23	1.72 (0.154)	0.324
KS	18	7	0.009 (0.001)	0.314
		12	0.113 (0.008)	0.394
		23	2.14 (0.227)	0.333
WI	18	7	0.005 (0.002)	0.229
		12	0.096 (0.009)	0.380
		23	---[3]	---[3]
CN	18	7	0.015 (0.002)	0.387
		12	0.108 (0.008)	0.390
		23	1.29 (0.191)	0.311

[1] Day 7: WIaKSabVA2abVA1bcCNcVA3c; ANOVA, $p < 0.001$.
Day 12: WIaCNabKSabVA2abVA1bVA3c; ANOVA, $p < 0.001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2] IGR = instantaneous growth rate.
[3] All individuals in pupal stage.

Table 15. Percent mortality of larvae in laboratory experiment 2B.

Population	Temperature (°C)	No. Days Growth	Initial Number	Number Surviving	Percent Mortality
VA1	18	7	15	13	13
		12	15	15	0
		23	15	15	0
VA2	18	7	15	11	27
		12	15	15	0
		23	15	12	20
VA3	18	7	15	12	20
		12	15	13	13
		23	15	14	7
KS	18	7	15	12	20
		12	15	11	27
		23	15	12	20
WI	18	7	15	5	67
		12	15	8	47
		23	15	7	53
CN	18	7	15	10	33
		12	15	9	40
		23	15	7	53

Table 16. Mean dry mass (mg) of larvae in laboratory experiment 2C.

Population	Temperature (°C)	No. Days Growth	Mean Mass (mg)[1] (SE)	IGR[2] ($mgmg^{-1}d^{-1}$)
VA1	27	4	0.012 (0.001)	0.621
		9	0.885 (0.042)	0.754
		12	---[3]	---[3]
VA2	27	4	0.005 (0.001)	0.402
		9	0.550 (0.028)	0.701
		12	1.13 (0.187)	0.586
VA3	27	5*	0.057 (0.008)	0.809
		9	0.597 (0.044)	0.710
		12	1.12 (0.080)	0.585
KS	27	4	0.013 (0.001)	0.641
		9	0.508 (0.032)	0.692
		12	0.878 (0.072)	0.565
WI	27	4	0.006 (0.001)	0.448
		9	0.536 (0.042)	0.698
		12	---[3]	---[3]
CN	27	4	0.018 (0.002)	0.722
		9	0.534 (0.035)	0.698
		12	---[3]	---[3]

[1] Day 4: WIaVA2aVA1bKSbCNc; ANOVA, $p < 0.001$.
Day 9: KSaWIaCNaVA2aVA3aVA1b; ANOVA, $p < 0.001$.
Day 12; ANOVA, $p = 0.21$ n.s.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[2]IGR = instantaneous growth rate.
[3]Only pupae or adults present.

*VA3 weighed on day 5.

Table 17. Percent mortality of larvae in laboratory experiment 2C.

Population	Temperature (°C)	No. Days Growth	Initial Number	Number Surviving	Percent Mortality
VA1	27	4	15	13	13
		9	15	14	7
		12	15	15	0
VA2	27	4	15	14	7
		9	15	14	7
		12	15	14	7
VA3	27	4	15	12	20
		9	15	15	0
		12	15	15	0
KS	27	4	15	13	13
		9	15	14	7
		12	15	13	13
WI	27	4	15	12	20
		9	15	14	7
		12	15	8	47
CN	27	4	15	11	27
		9	15	7	53
		12	15	5	67

Table 18a. Mean dry mass (mg) of larvae in laboratory experiment 3A.

Population	Temperature (°C)	Food[1]	No. Days Growth	Mean Mass (mg)[2] (SE)	IGR[3] ($mgmg^{-1}d^{-1}$)
VA3	11	L	13	0.005 (0.001)	0.124
			27	0.030 (0.003)	0.126
			52	0.074 (0.006)	0.083
CN	11	L	13	0.005 (0.0002)	0.124
			27	0.042 (0.011)	0.138
			52	0.135 (0.055)	0.094
WI	11	L	13	0.003 (---[4])	0.084
			27	0.050 (0.009)	0.145
			52	0.115 (0.034)	0.091

[1]Larvae were fed conditioned leaves (L) in experiment 3A.
[2]No significant differences in dry mass were found between populations in experiment 3A.
[3]IGR = instantaneous growth rate.
[4]No standard error could be calculated because only one larva was found alive.

Table 18b. Mean dry mass (mg) of larvae in laboratory experiment 3B.

Population	Temperature (°C)	Food[1]	No. Days Growth	Mean Mass (mg)[2] (SE)	IGR ($mgmg^{-1}d^{-1}$)
VA3	11	T	11	0.006 (0.0003)	0.163
			24	0.174 (0.006)	0.215
			31	0.836 (0.080)	0.217
CN	11	T	11	0.004 (0.001)	0.126
			24	0.171 (0.019)	0.214
			31	0.371 (0.013)	0.191
WI	11	T	11	0.003 (0.0001)	0.100
			24	0.061 (0.002)	0.171
			31	0.140 (0.025)	0.159

[1]Larvae were fed Tetragrowth[tm] (T) fish food in experiment 3B.
[2]Day 11; No significant differences were found.
Day 24; WIaCNbVA3b; ANOVA, $p = 0.004$.
Day 31; WIaCNbVA3c; ANOVA, $p = 0.0002$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

Table 19a. Percent mortality of larvae in laboratory experiment 3A.

Population	Temperature (°C)	Food[1]	No. Days Growth	Initial No.	Mean No. (SE)[2] Surviving	Percent Mortality
VA3	11	L	13	10	8.0 (1.5)	20
			27	10	9.3 (0.3)	7
			52	10	9.3 (0.3)	7
CN	11	L	13	10	8.0 (2.0)	20
			27	10	7.7 (1.4)	23
			52	10	5.0 (1.5)	50
WI	11	L	13	10	1.0 (1.0)	90
			27	10	4.3 (1.8)	57
			52	10	4.3 (2.6)	57

[1]Larvae were fed conditioned leaves (L) in experiment 3A.
[2]Day 13; WIaCNbVA3b; ANOVA, $p < 0.05$.
Day 27; VA3 > WI; Mann-Whitney U Test, $p < 0.05$.
Day 52; VA3 > CN; Mann-Whitney U Test, $p < 0.05$.

Table 19b. Percent mortality of larvae in laboratory experiment 3B.

Population	Temperature (°C)	Food[1]	No. Days Growth	Initial No.	Mean No. (SE)[2] Surviving	Percent Mortality
VA3	11	T	11	10	10.0 (0)	0
			24	10	10.0 (0)	0
			31	10	10.0 (0)	0
CN	11	T	11	10	8.0 (1.5)	20
			24	10	9.3 (0.7)	7
			31	10	10.0 (0)	0
WI	11	T	11	10	2.0 (1.7)	80
			24	10	2.0 (1.2)	80
			31	10	2.3 (0.9)	77

[1]Larvae were fed Tetragrowth[tm] fish food in experiment 3B.
[2]Day 11; WIaCNbVA3b; ANOVA, $p < 0.01$.
Day 24; WIaCNbVA3b; ANOVA, $p < 0.001$.
Day 31; WIaCNbVA3b; ANOVA, $p < 0.001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

Table 20a. Mean dry mass (mg) of larvae in laboratory experiment 3C.

Population	Temperature (°C)	Food[1]	No. Days Growth	Mean Mass (mg)[2] (SE)	IGR[3] ($mgmg^{-1}d^{-1}$)
VA3	27	L	5	0.014 (0.004)	0.528
			10	0.081 (0.009)	0.439
			15	0.133 (0.029)	0.326
CN	27	L	5	0.016 (0.004)	0.554
			10	0.053 (0.010)	0.397
			15	0.149 (0.040)	0.334
WI	27	L	5	0.011 (0.002)	0.480
			10	0.064 (0.003)	0.416
			15	0.128 (0.060)	0.323

[1]Larvae were fed conditioned leaves (L) in experiment 3C.
[2]No significant differences in dry mass were found between populations in experiment 3C.
[3]IGR = instantaneous growth rate.

Table 20b. Mean dry mass (mg) of larvae in laboratory experiment 3D.

Population	Temperature (°C)	Food[1]	No. Days Growth	Mean Mass (mg)[2] (SE)	IGR[3] ($mgmg^{-1}d^{-1}$)
VA3	27	T	3	0.012 (0.001)	0.828
			6	0.273 (0.033)	0.935
			9	1.15 (0.042)	0.783
CN	27	T	3	0.014 (0.004)	0.880
			6	0.298 (0.029)	0.950
			9	0.975 (0.119)	0.765
WI	27	T	3	0.016 (0.001)	0.924
			6	0.162 (0.008)	0.848
			9	0.743 (0.066)	0.734

[1]Larvae were fed Tetragrowth™ (T) fish food in experiment 3D.
[2]Day 3; No significant differences found.
Day 6; WIaVA3bCNb; ANOVA, $p = 0.02$.
Day 9; WIaCNabVA3b; ANOVA, $p = 0.04$.

[3]IGR = instantaneous growth rate.

Table 21a. Percent mortality of larvae in laboratory experiment 3C.

Population	Temperature (°C)	Food[1]	No. Days Growth	Initial No.	Mean No. (SE)[2] Surviving	Percent Mortality
VA3	27	L	5	10	9.7 (0.33)	3
			10	10	9.0 (1.0)	10
			15	10	10.0 (0)	0
CN	27	L	5	10	9.3 (0.67)	7
			10	10	6.3 (0.67)	37
			15	10	7.7 (1.3)	23
WI	27	L	5	10	4.7 (1.4)	53
			10	10	7.7 (1.4)	23
			15	10	6.3 (1.8)	37

[1]Larvae were fed conditioned leaves (L) in experiment 3C.

[2]Day 5; WIaCNbVA3b; ANOVA, $p = 0.01$.
Days 10 and 15; no significant differences were found.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

Table 21b. Percent mortality of larvae in laboratory experiment 3D.

Population	Temperature (°C)	Food[1]	No. Days Growth	Initial No.	Mean No. (SE)[2] Surviving	Percent Mortality
VA3	27	T	3	10	9.0 (0.6)	10
			6	10	8.0 (1.0)	20
			9	10	8.0 (1.0)	20
CN	27	T	3	10	8.7 (1.3)	13
			6	10	8.0 (1.5)	20
			9	10	7.0 (1.5)	30
WI	27	T	3	10	7.7 (1.2)	23
			6	10	5.7 (0.9)	43
			9	10	5.0 (1.7)	50

[1]Larvae were fed Tetragrowth[tm] (T) fish food in experiment 3D.
[2]No significant differences in mortality were found between populations in experiment 3D.

Table 22. Survival of three populations exposed to chlorine in-situ during the first transplant experiment[1].

Population	Initial No. Per Replicate	Final No. Surviving Mean (SE)[2]	Percent Survival
VA1	35	3.12 (0.79)	8.9
VA2	35	5.75 (1.06)	16
VA3	35	2.75 (1.29)	7.8

[1]Experiment conducted from 9/8/89 to 9/23/89.

[2]No significant differences in mortality between the populations were found in first transplant experiment:
VA2 > VA3, p = 0.07 n.s., Mann-Whitney-U test.
VA2 > VA1, p = 0.13 n.s., Mann-Whitney-U test.
ANOVA, p = 0.12 n.s.

Table 23. Percent survival of three populations exposed to chlorine in-situ during the second transplant experiment[1].

Population	Initial No. Per Replicate	Final No. Surviving[2] Day 7 Mean (SE)	Day 7 Percent Survival	Day 15 Mean (SE)	Day 15 Percent Survival
VA1	25	8.3 (0.9)	33	0.5 (0.5)	2.0
VA2a	25	5.7 (1.3)	23	0.7 (0.2)	2.6
VA2b[3]	25	13.0 (0)	52	4.8 (0.4)	19
VA3	25	4.7 (2.3)	19	1.3 (0.9)	5.2

[1]Experiment conducted from 8/15/90 to 8/30/90.
[2]Day 7; VA3aVA2aaVA1aVA2bb; ANOVA, p = 0.007
Day 15; VA1aVA2aaVA3aVA2bb; ANOVA, p = 0.0001.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

[3]VA2b is derived from VA2a (the original VA2 culture) after having been reexposed to chlorine for two generations.

Table 24. Comparison between six populations of experimental emergence data.[1]

Pop.	Mean Number of Days Until the Onset of Emergence[2]	Mean Number of Days From the Beginning To the End of Emergence[3]	Mean Number of Days Required For 50 Percent Emergence[4]	Mean Number of Days From Beginning of Emergence Until 50 Percent Emergence[5]	Mean Number of Adults Emerging[6] (out of 100 initial larvae)
VA1	12.0 (0)	7.7 (1.2)	15.0 (0)	3.0 (0)	88.3 (2.7)
VA2	12.7 (0.33)	9.3 (0.33)	16.3 (0.33)	3.7 (0.33)	86.3 (3.0)
VA3	11.7 (0.33)	8.7 (1.8)	14.0 (0)	2.3 (0.33)	96.0 (2.6)
KS	13.0 (0)	8.5 (0.5)	16.5 (0.5)	3.5 (0.5)	74.5 (0.5)
WI	12.3 (0.33)	4.7 (0.33)	14.0 (0)	1.7 (0.33)	47.3 (3.2)
CN	13.0 (0)	8.7 (0.33)	15.7 (0.33)	2.7 (0.33)	69.3 (3.2)

[1]Number in parentheses denotes standard error.

[2]VA3aVA1abWIabVA2abKSbCNb; ANOVA, $p = 0.02$.

[3] WI < VA1, VA2, CN; Mann-Whitney-U tests, all $p < .05$.

[4]WIaVA3aVA1bCNbcVA2cKSc; ANOVA, $p < 0.0001$.

[5]WIaVA3abCNabVA1abKSbVA2b; ANOVA, $p = 0.009$.

[6]WIaCNbKSbVA2cVA1cVA3c; ANOVA, $p < 0.0001$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

Table 25. Comparison between six populations of number of egg masses laid and number of eggs per egg mass.

Population	No. Egg Masses Laid per 100 Initial Larvae Mean (SE)[1]	No. Eggs per Egg Mass Mean (SE)[2]
VA1	27 (2.3)	500 (12)
VA2	35 (6.0)	484 (24)
VA3	27 (7.8)	530 (26)
KS	14 (2.5)	453 (12)
WI	15 (2.3)	596 (16)
CN	20 (4.4)	468 (34)

[1]WI < VA1,VA2: Mann-Whitney-U tests, all $p < 0.05$.

[2]KSaCNaVA2aVA1aVA3aWIb; ANOVA, $p = 0.01$.

The means of populations with the same subscript letter are not significantly different (Newman/Keuls multiple comparison test).

Table 26. Number of egg masses per adult.

Population	Mean Number of Adults	Mean Number of Egg Masses	Number of Egg Masses Per Adult
KS	74.5	14.5	0.19
VA3	96.0	27.3	0.28
CN	69.3	20.3	0.29
VA1	88.3	26.7	0.30
WI	47.3	14.7	0.31
VA2	86.3	35.3	0.41

Table 27. A comparison of the number of egg masses with large numbers of eggs in laboratory experiment 4.

Population	No. egg masses with greater than 600 eggs	No. egg masses with greater than 700 eggs
VA1	3	0
VA2	1	0
VA3	7	0
KS	0	0
WI	14	3
CN	1	0

Table 28. Results of Dunnet's Procedure comparing mean dry mass of larvae in treatments to the control. Significance tested at $\alpha = .05$.

	Lowest Concentration In Which Larvae Weighed Significantly Less Than The Control[1]	
Population	Low Aeration[2]	High Aeration[3]
WI	8	8
VA3	2	8
VA1	2	4
VA2	2	4

[1]Treatments are: control, 1, 2, 4, 8, 16 and 32 mg/L (ppm).
[2]Low aeration was 30 bubbles/minute.
[3]High aeration was 100 bubbles/minute.

Table 29. LC50's (mg/L) in 8-day, static-renewal toxicity tests conducted with four populations of the midge, *Chironomus riparius*, using the molluscide, CT-1.

	Aeration Level[1]			
	High		Low	
	statistical method		statistical method	
Population	Binomial	Probit	Binomial	Probit
VA1	17.4 (8-32)	15.0 (--[2])	22.2 (16-32)	20.7 (--[2])
VA2	13.6 (8-32)	13.4 (11-16)	15.2 (1-32)	11.0 (--[2])
VA3	21.6 (16-32)	22.4 (18-27)	25.4 (16-32)	28.3 (--[2])
WI	13.2 (--[2])	13.8 (6-21)	19.7 (16-32)	13.8 (--[2])

[1]High aeration was 100 bubbles/min; low aeration was 30 bubbles/min.
[2]No confidence limits could be calculated.

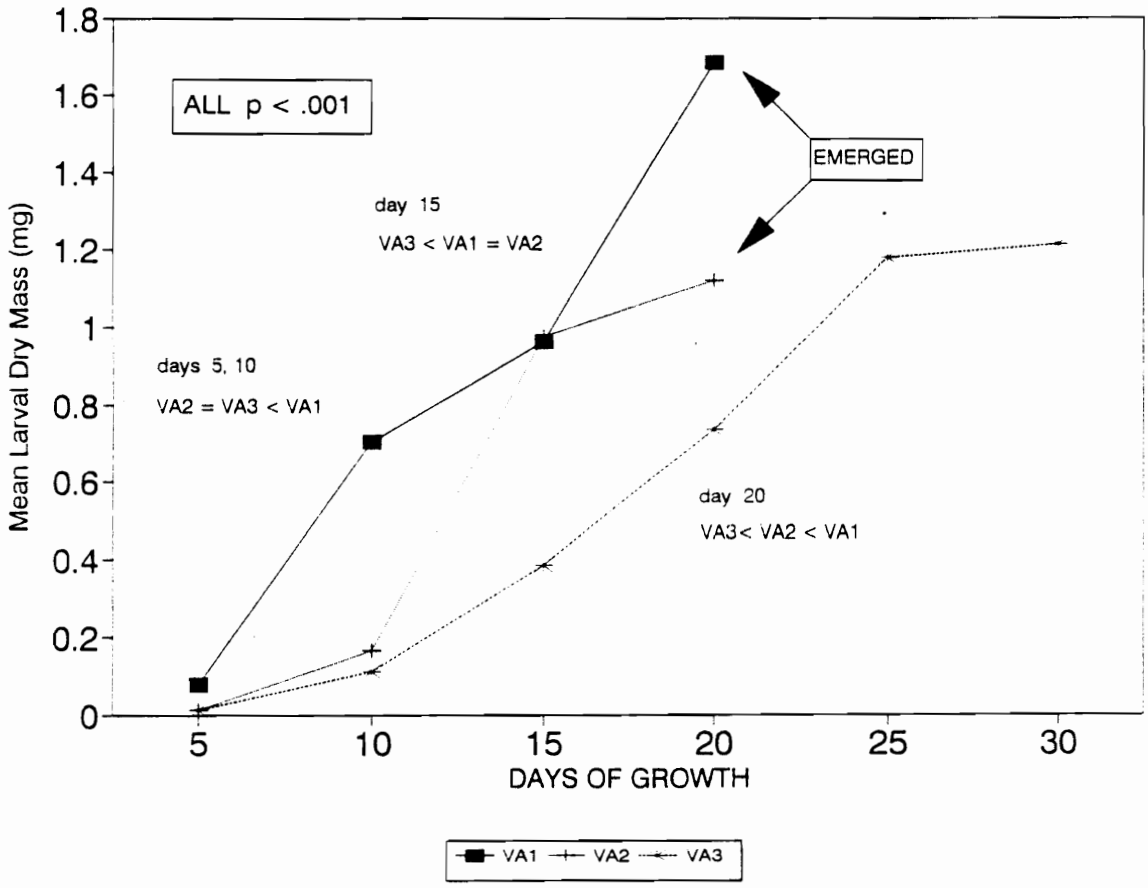

Figure 1. In-situ larval growth curves for three populations.

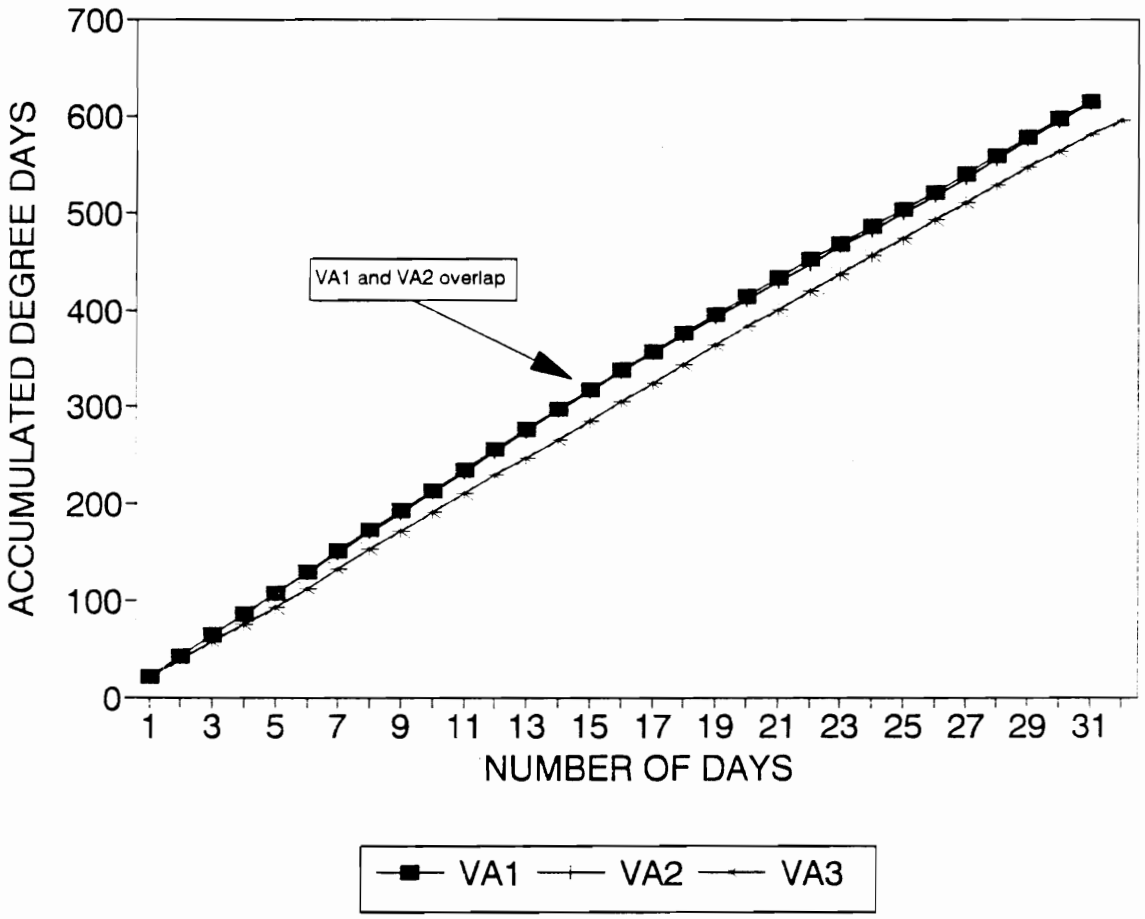

Figure 2. Accumulation of degree days at the secondary clarifier (VA1), chlorine contact tanks (VA2), and Strouble's Creek (VA3) during the in-situ field experiment.

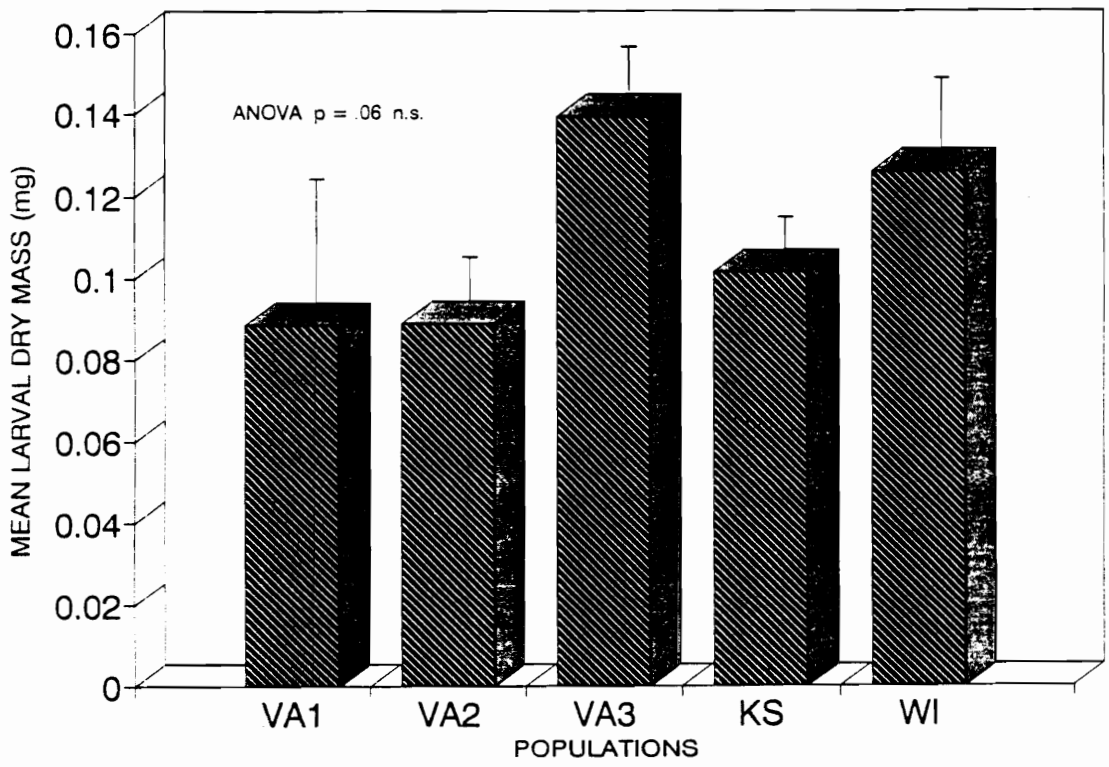

Figure 3. Larval mean dry mass on day 26 in laboratory experiment 1A. Test temperature was 11°C.

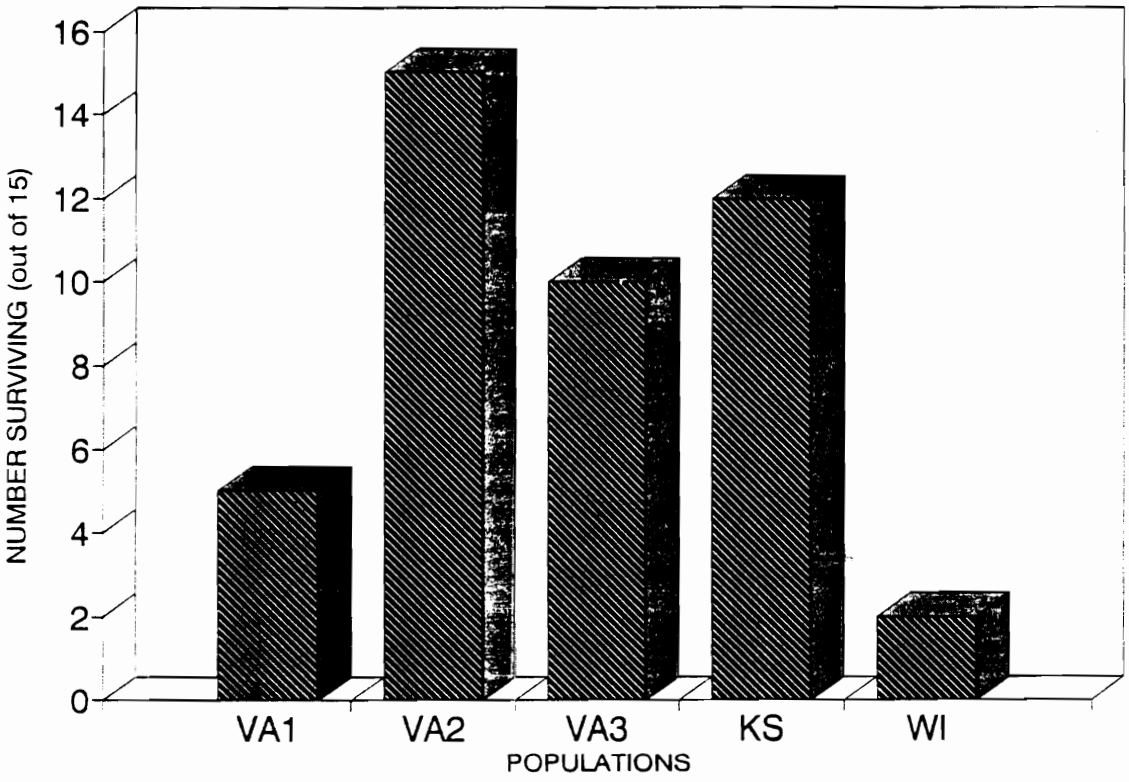

Figure 4. Larval survival in laboratory experiment 1A. Test temperature was 11°C.

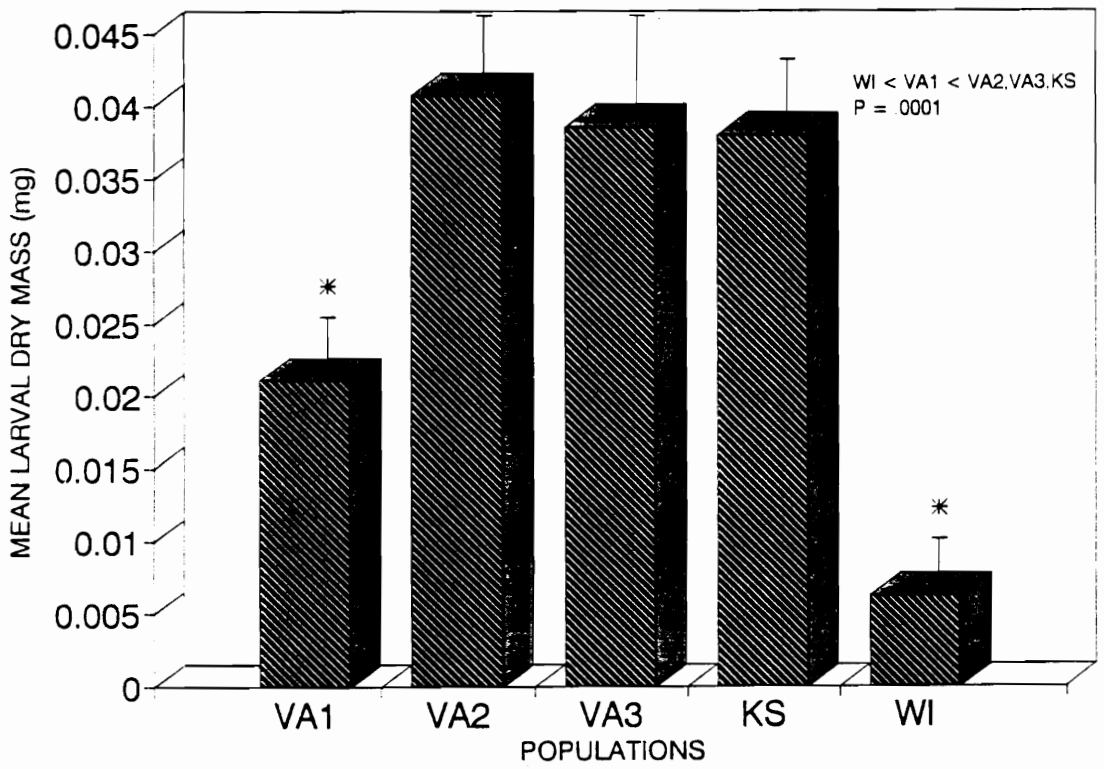

Figure 5. Larval mean dry mass on day 6 in laboratory experiment 1B. Test temperature was 18°C.

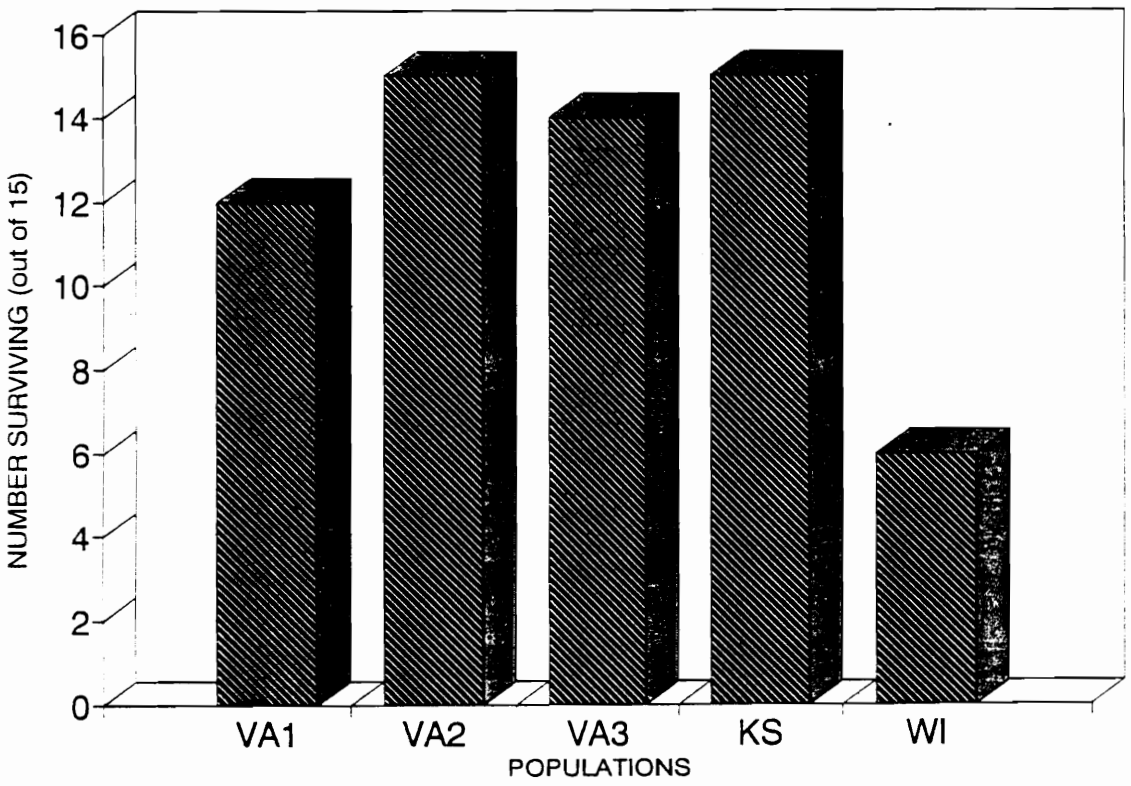

Figure 6. Larval survival in laboratory experiment 1B. Test temperature was 18°C.

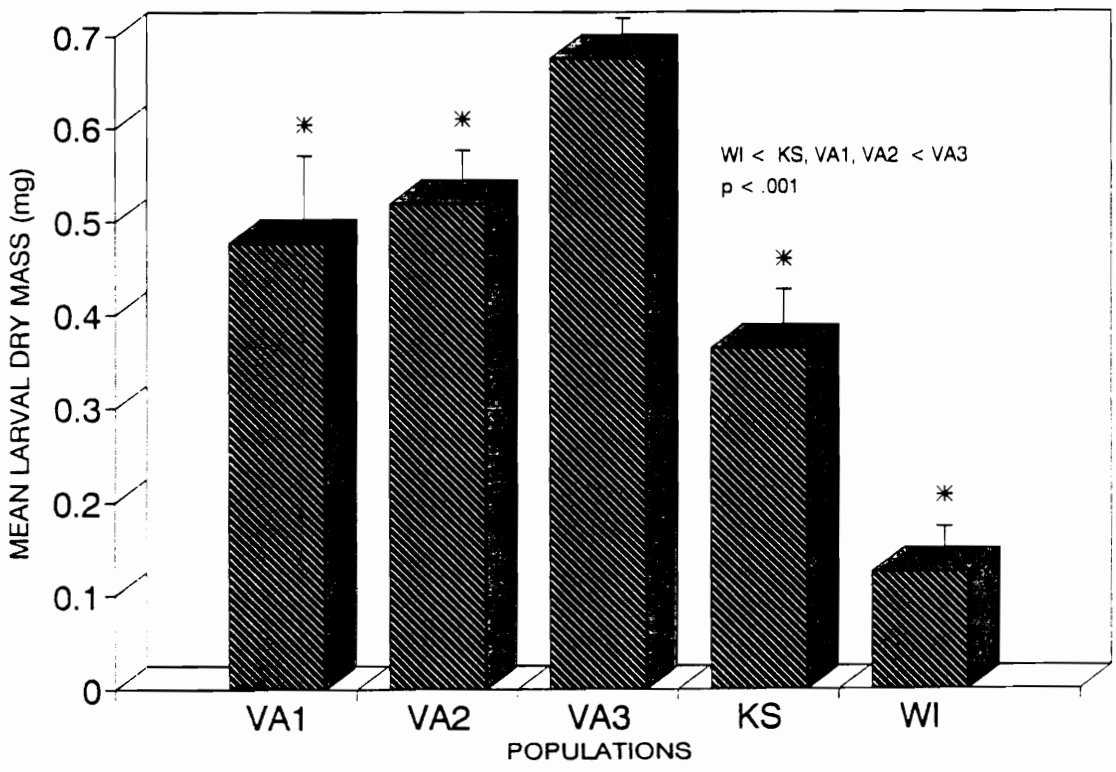

Figure 7. Larval mean dry mass on day 6 in laboratory experiment 1C. Test temperature was 27°C.

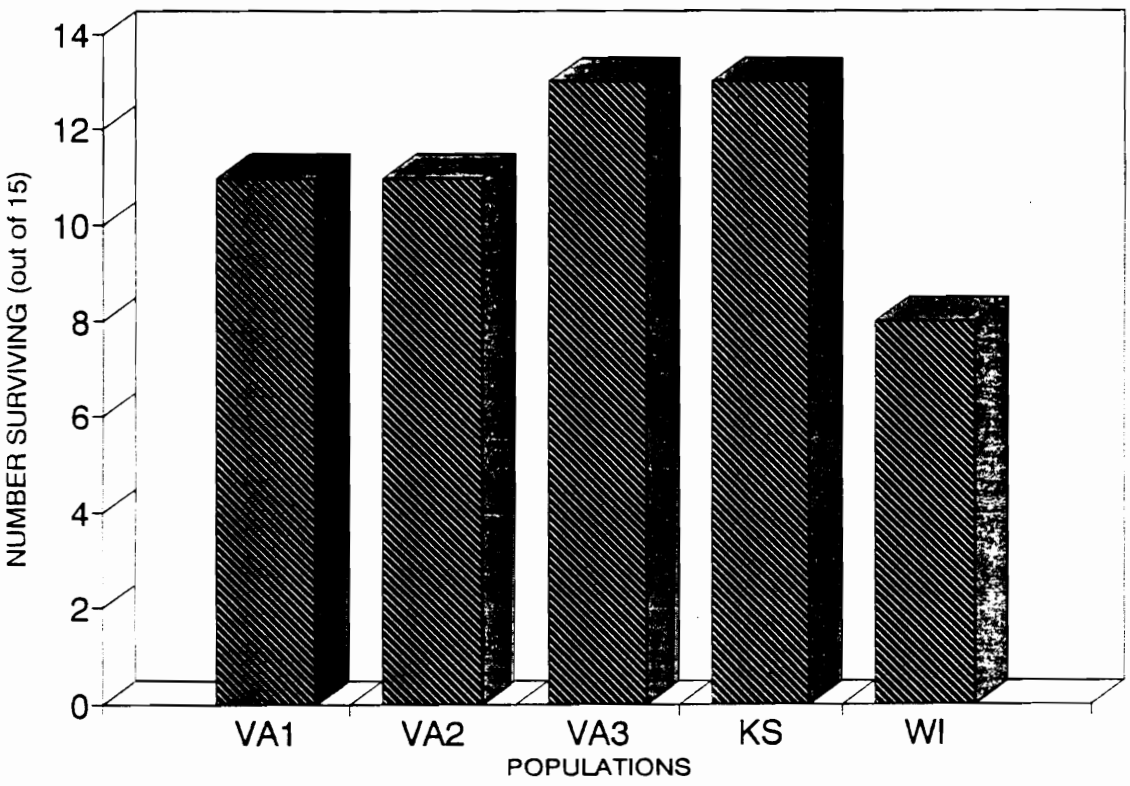

Figure 8. Larval survival in laboratory experiment 1C. Test temperature was 27°C.

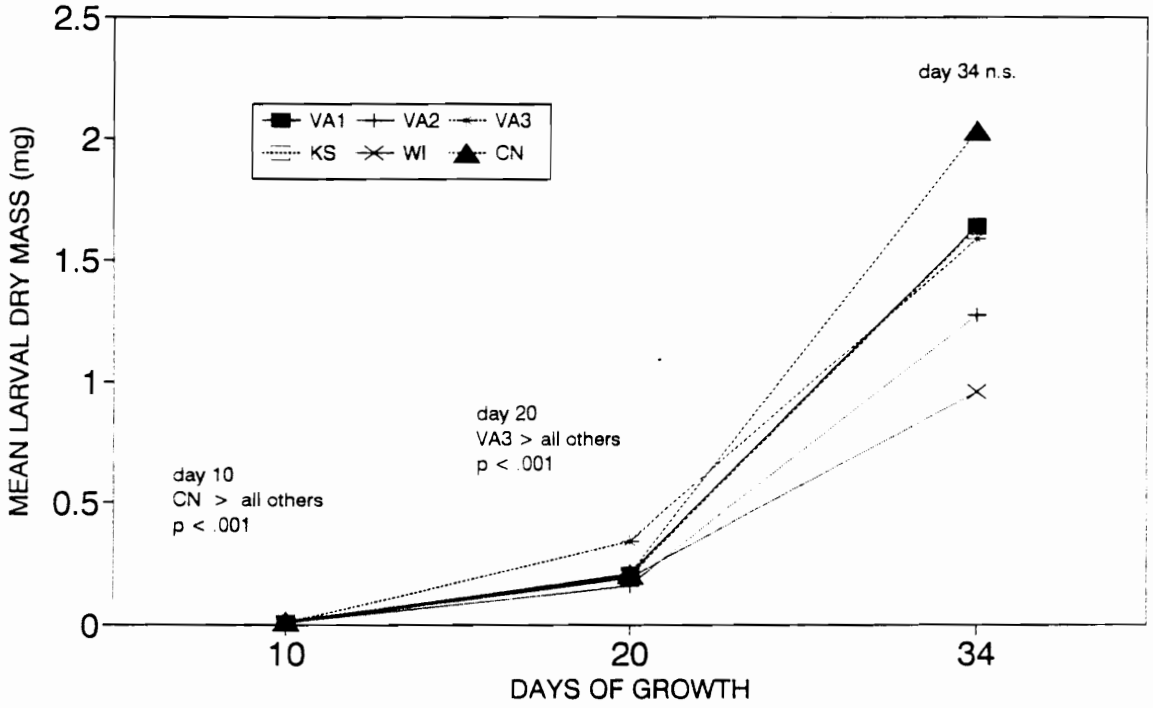

Figure 9. Larval growth curves for laboratory experiment 2A. Test temperature was 14°C.

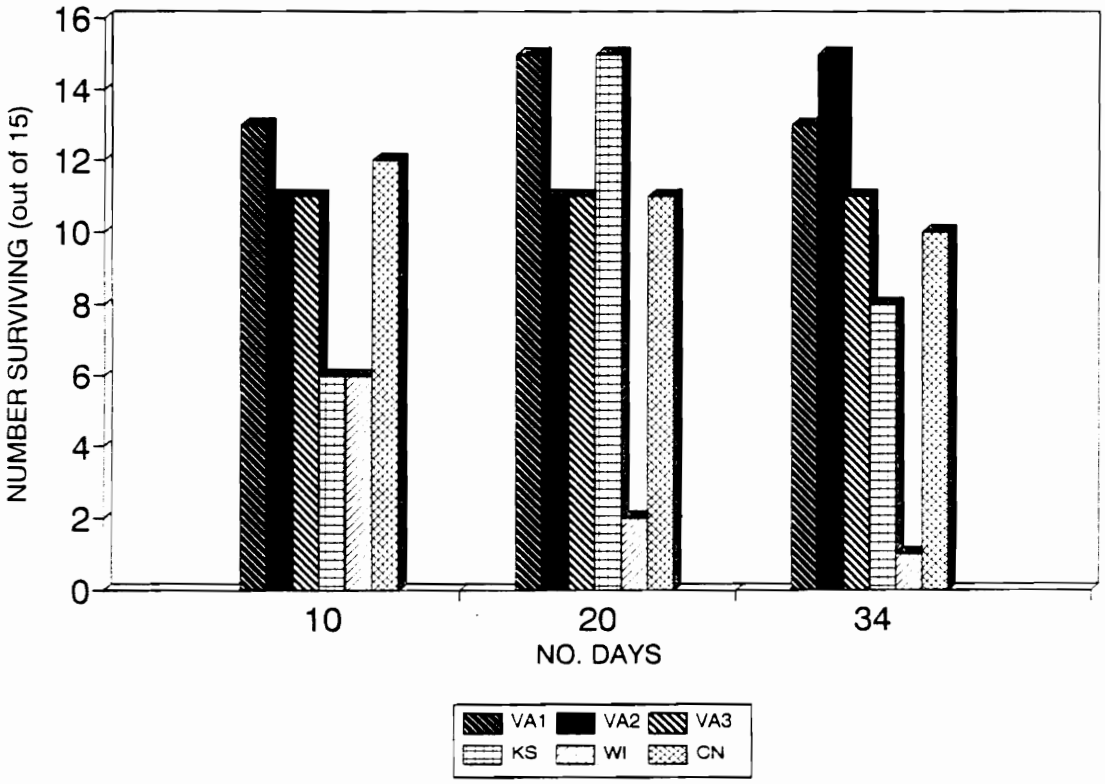

Figure 10. Larval survival in laboratory experiment 2A. Test temperature was 14°C.

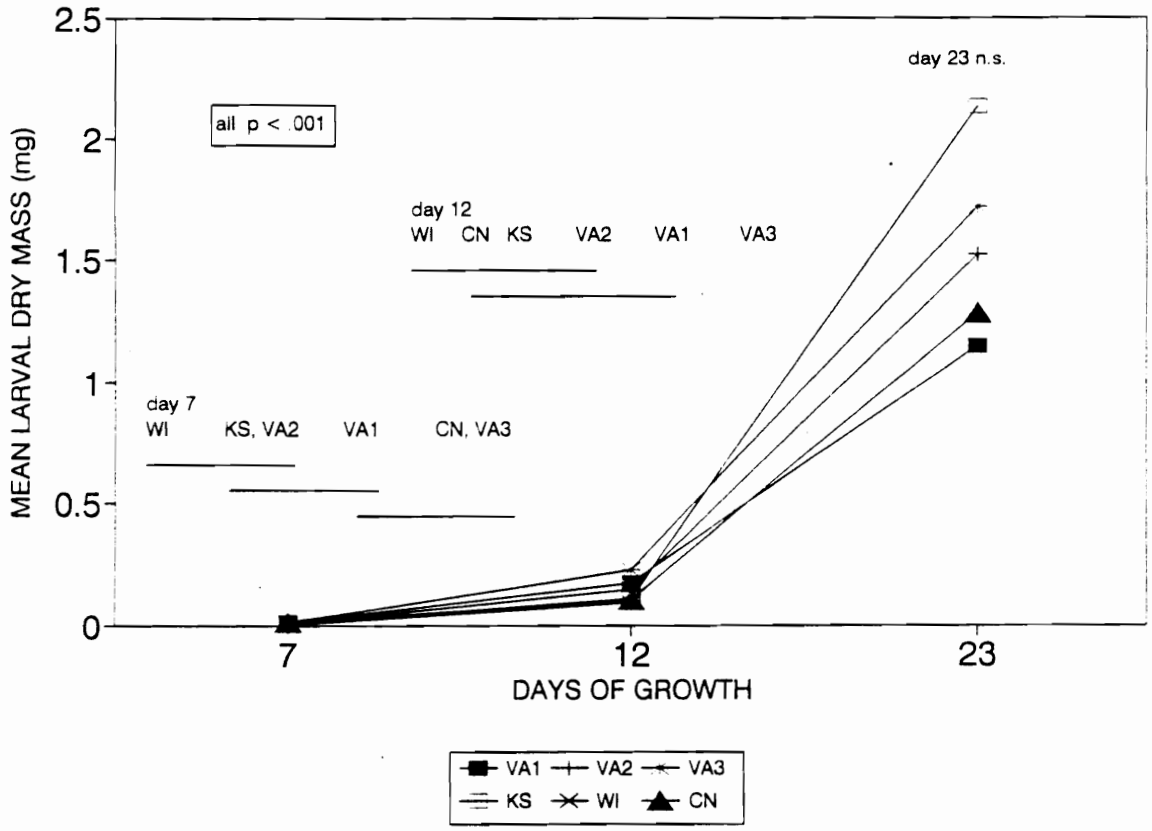

Figure 11. Larval growth curves for laboratory experiment 2B. Test temperature was 18°C.

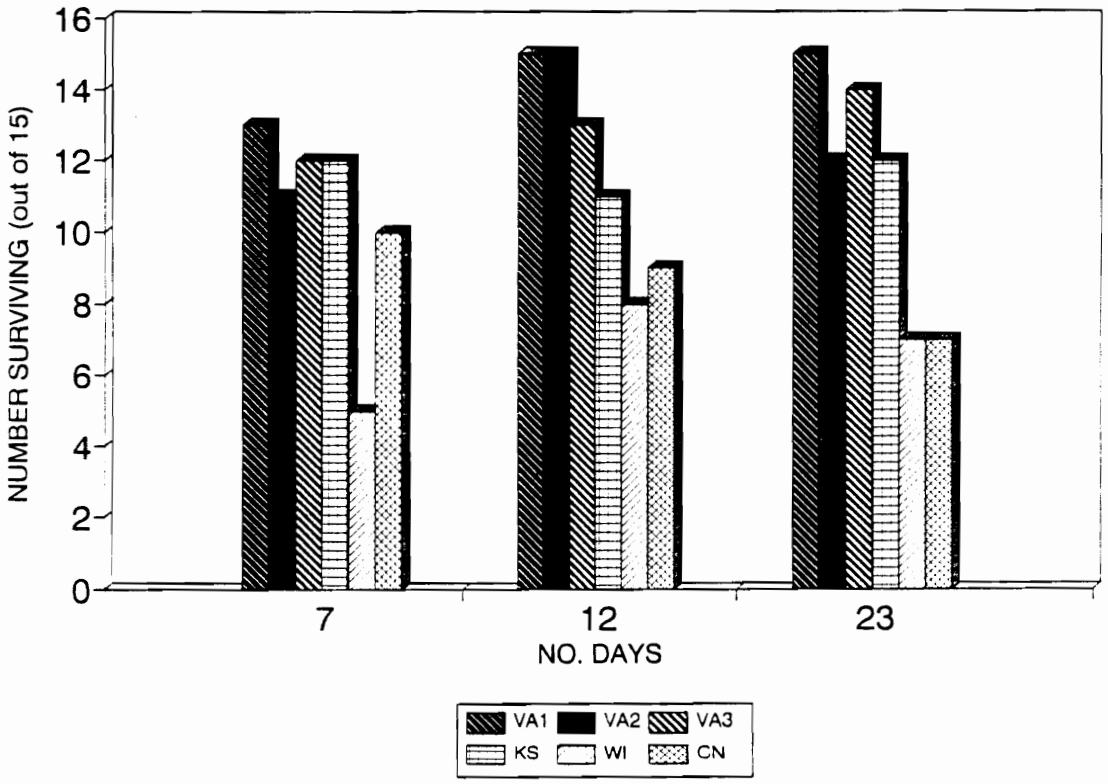

Figure 12. Larval survival in laboratory experiment 2B. Test temperature was 18°C.

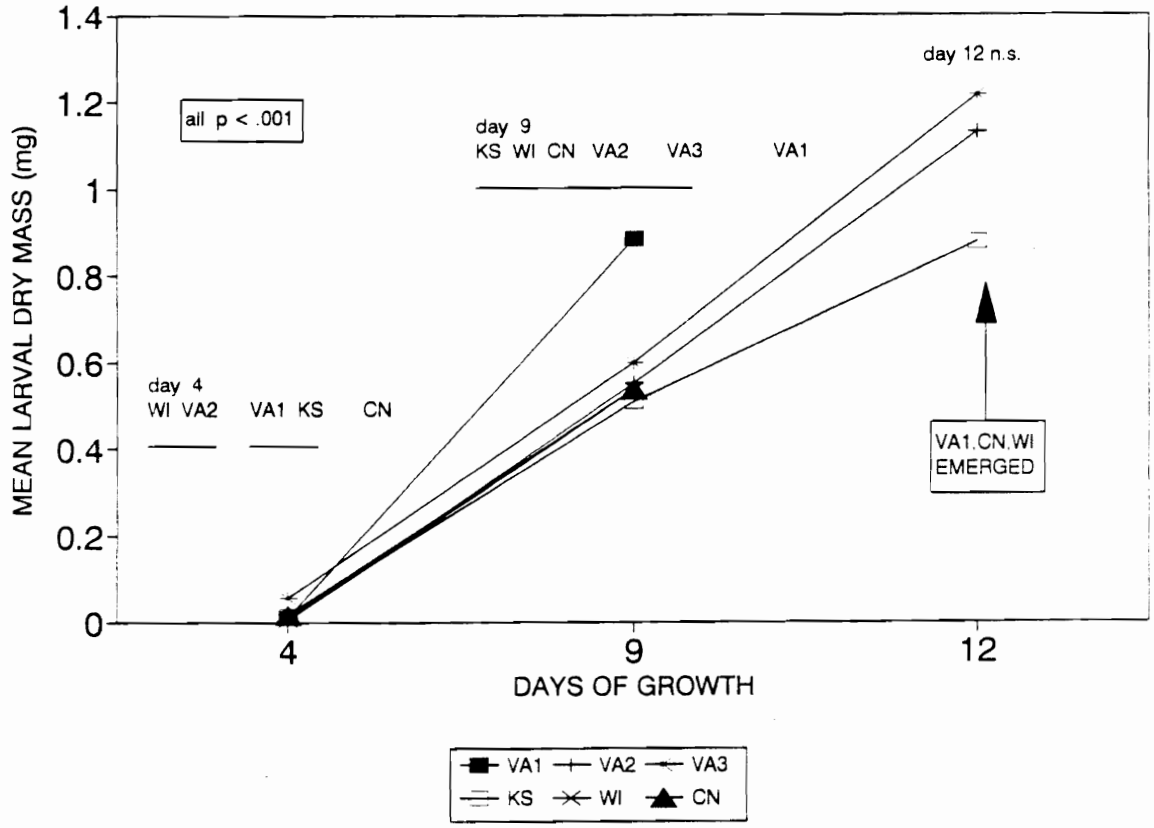

Figure 13. Larval growth curves for laboratory experiment 2C. Test temperature was 27°C.

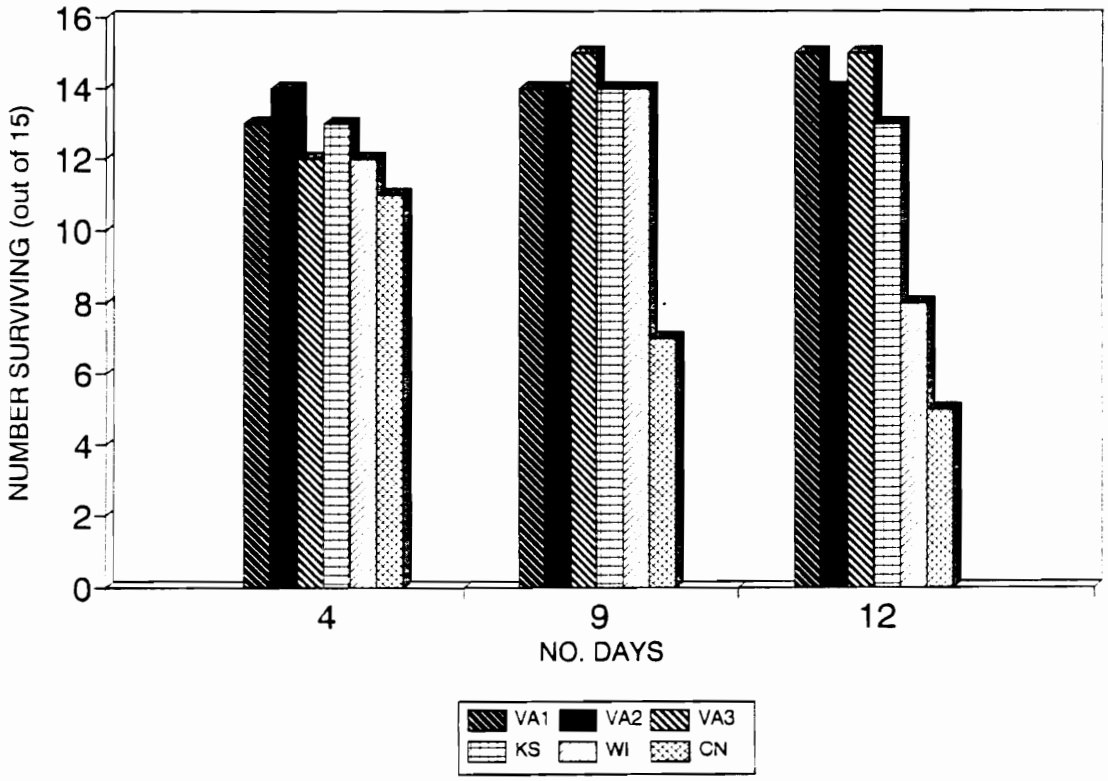

Figure 14. Larval survival in laboratory experiment 2C. Test temperature was 27°C.

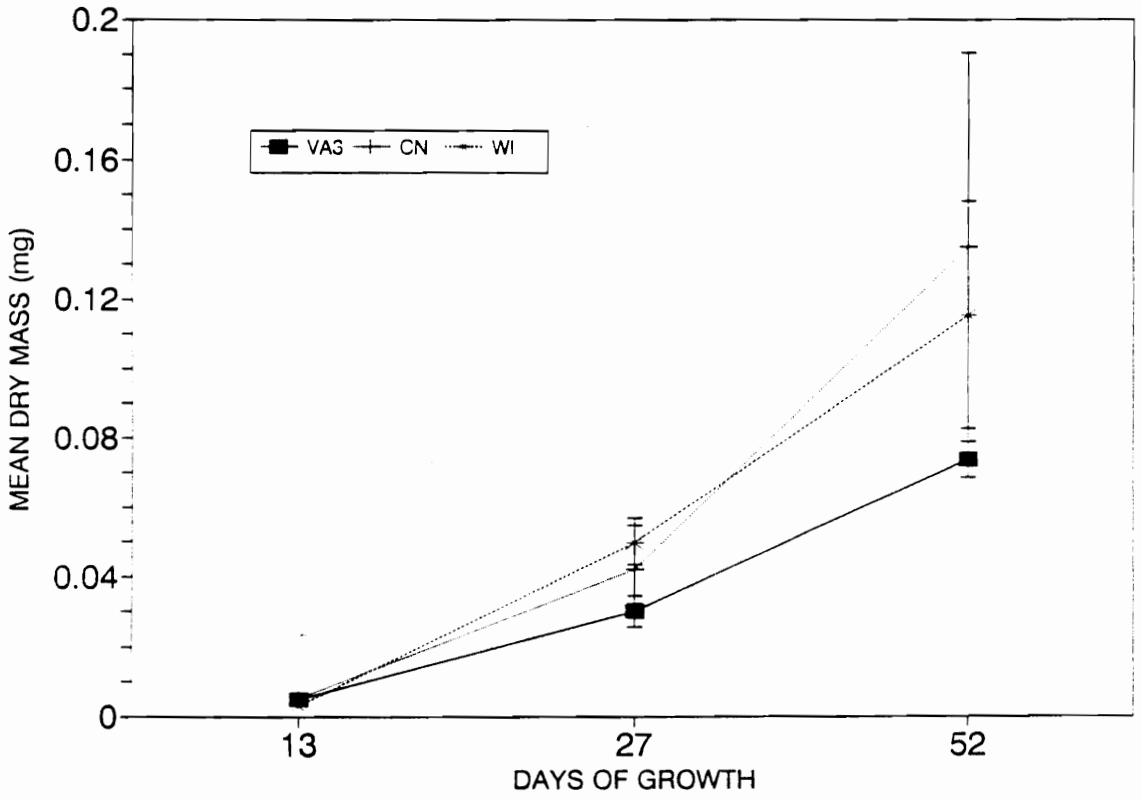

Figure 15. Larval growth curves for laboratory experiment 3A. Temperature was 11°C. Larvae fed conditioned leaves.

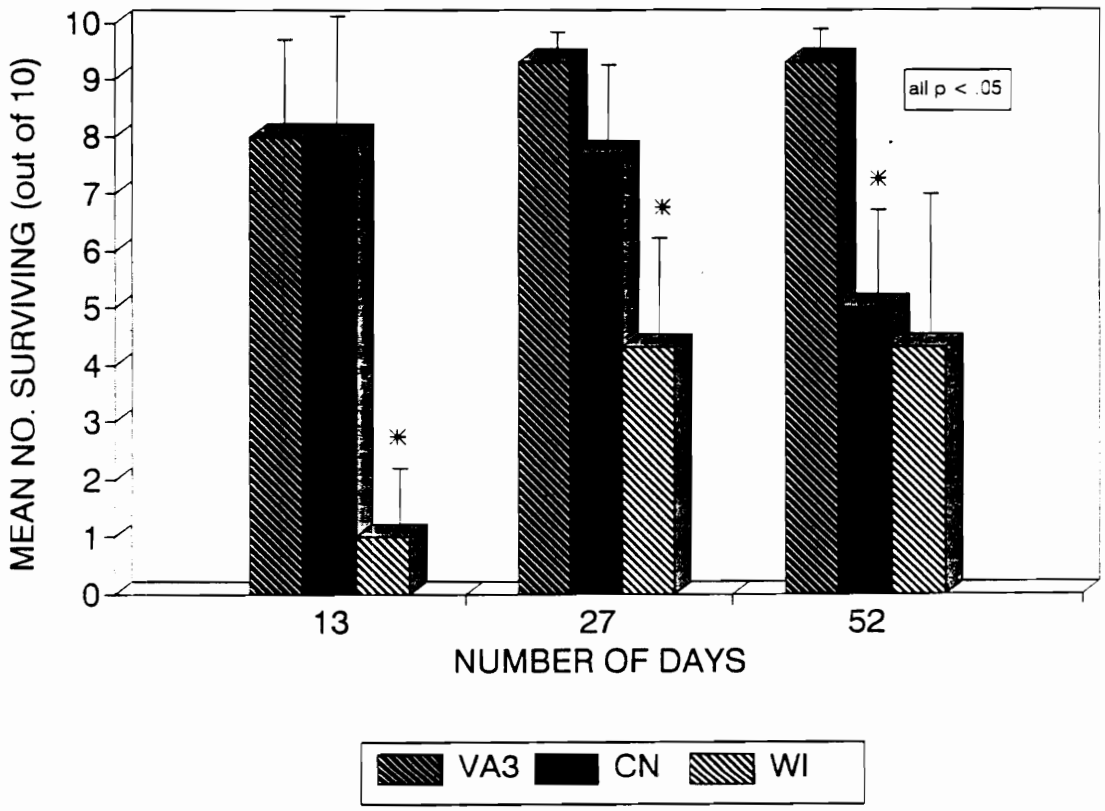

Figure 16. Larval survival in laboratory experiment 3A. Temperature was 11°C. Larvae fed conditioned leaves.

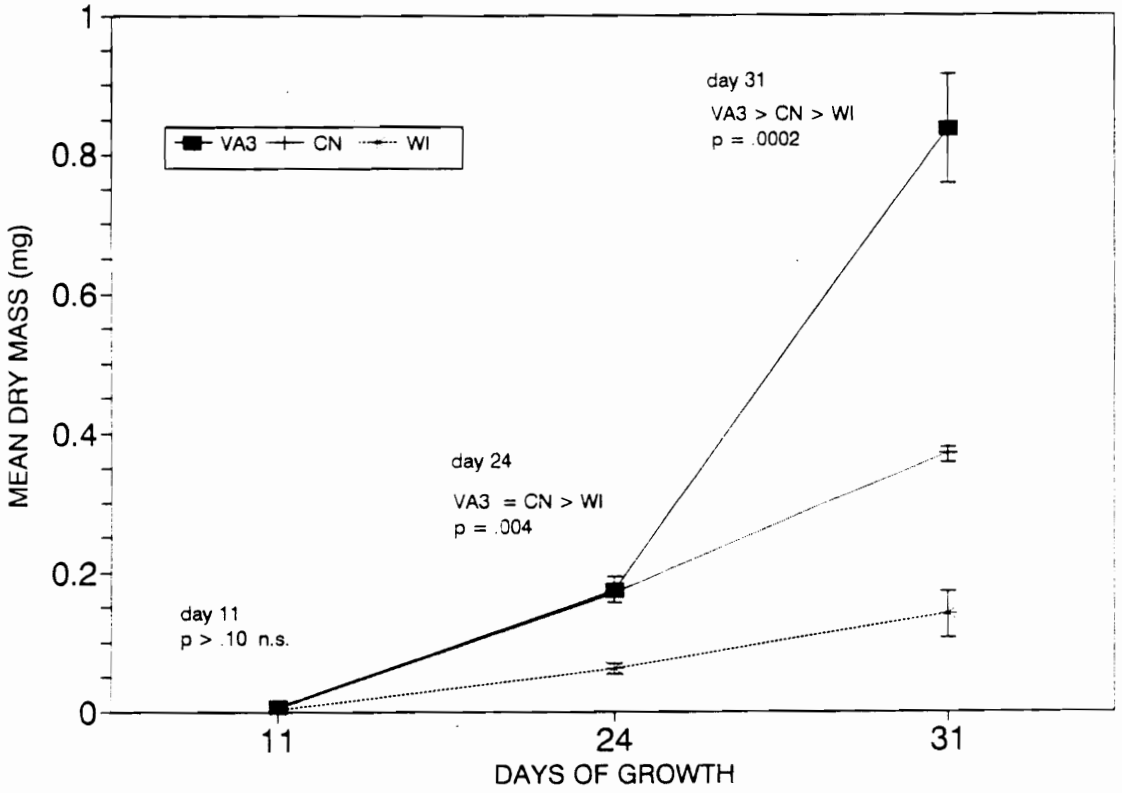

Figure 17. Larval growth curves for laboratory experiment 3B. Temperature was 11°C. Larvae fed Tetragrowth fish food.

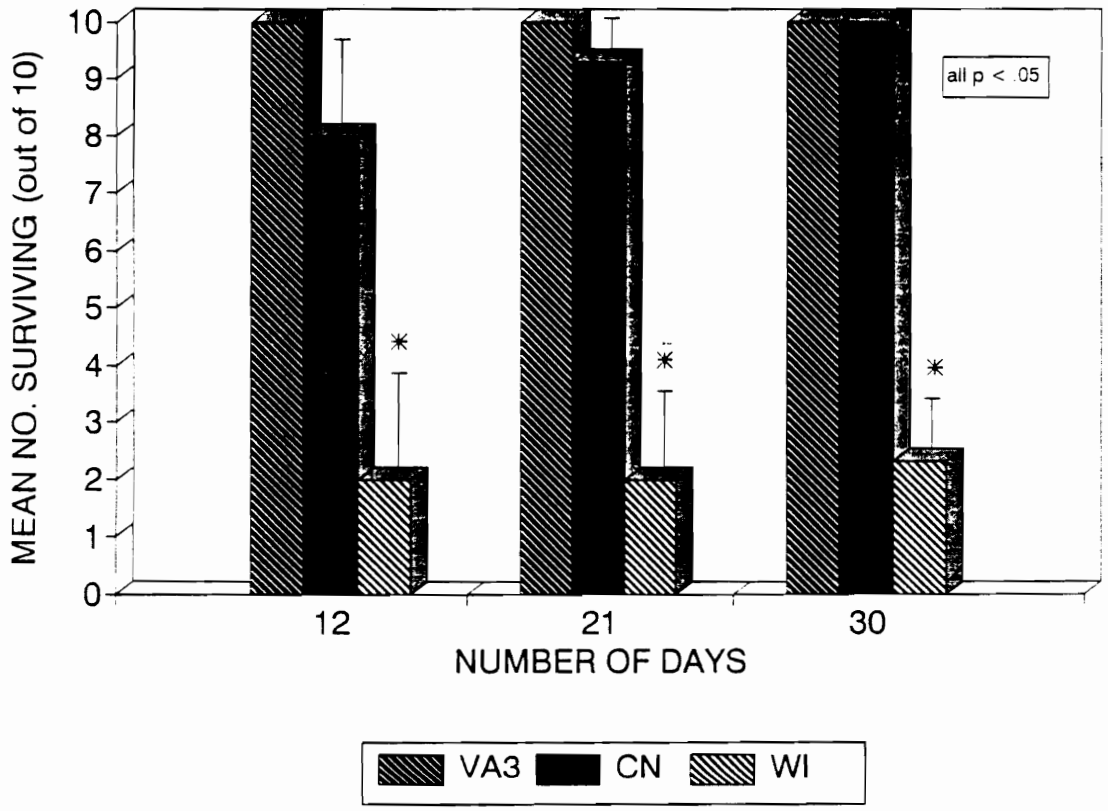

Figure 18. Larval survival in laboratory experiment 3B. Temperature was 11°C. Larvae fed Tetragrowth fish food.

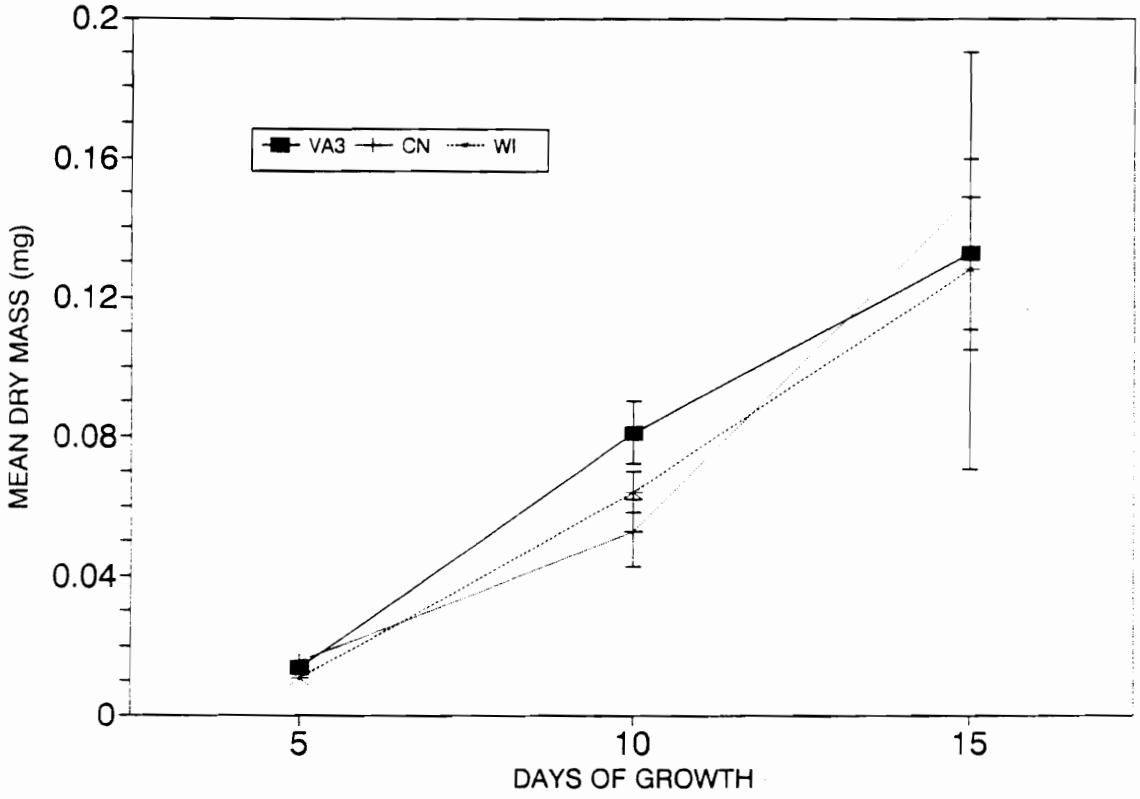

Figure 19. Larval growth curves for laboratory experiment 3C. Temperature was 27°C. Larvae fed conditioned leaves.

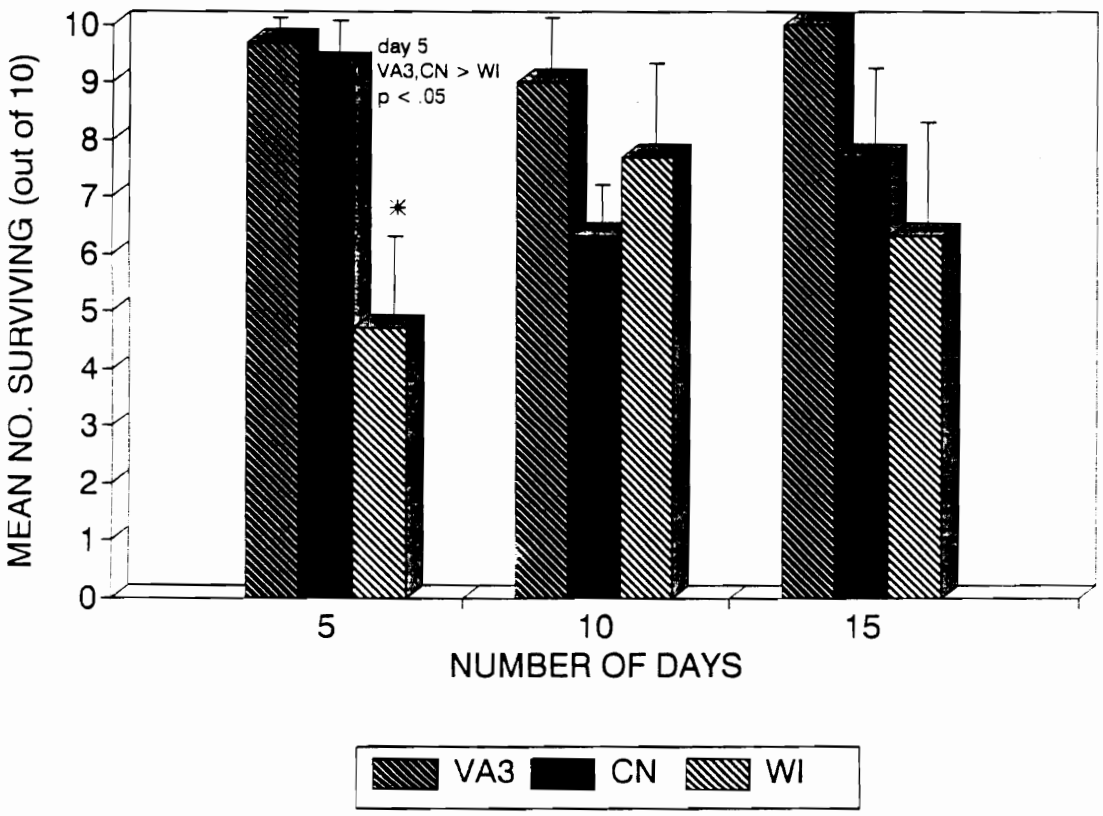

Figure 20. Larval survival in laboratory experiment 3C. Temperature was 27°C. Larvae fed conditioned leaves.

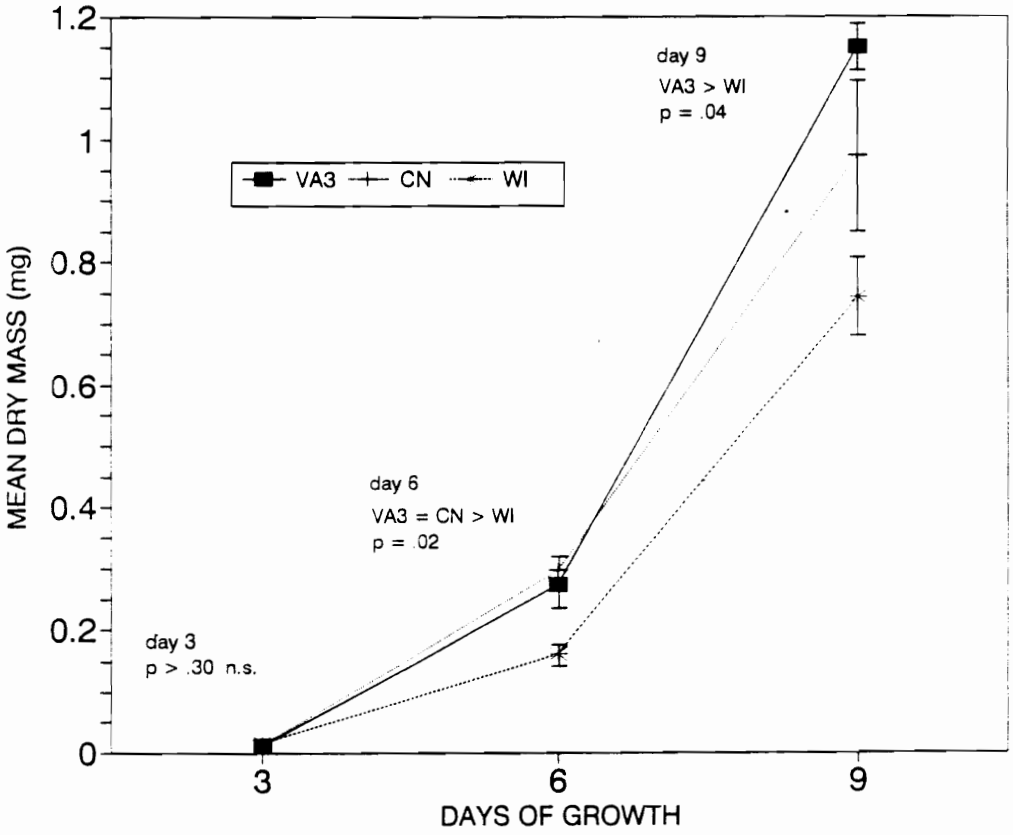

Figure 21. Larval growth curves for laboratory experiment 3D. Temperature was 27°C. Larvae fed Tetragrowth fish food.

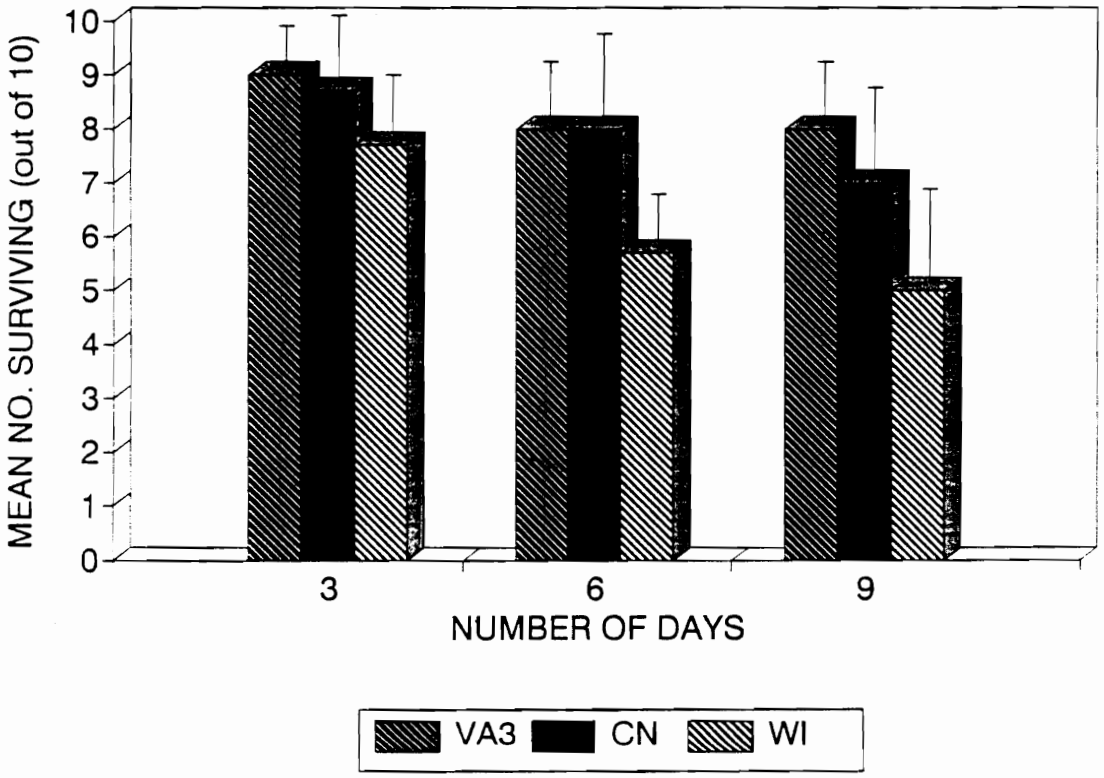

Figure 22. Larval survival in laboratory experiment 3D. Temperature was 27°C. Larvae fed Tetragrowth fish food.

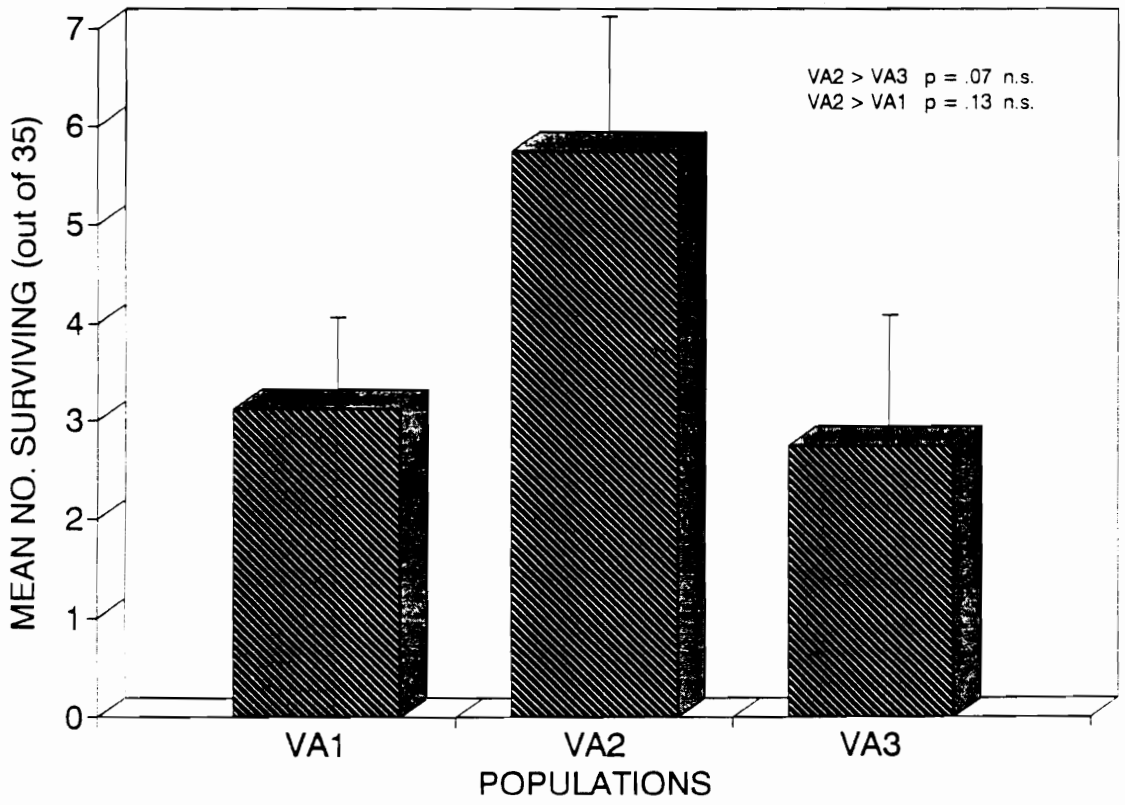

Figure 23. Larval survival after first transplant experiment into chlorine contact tanks.

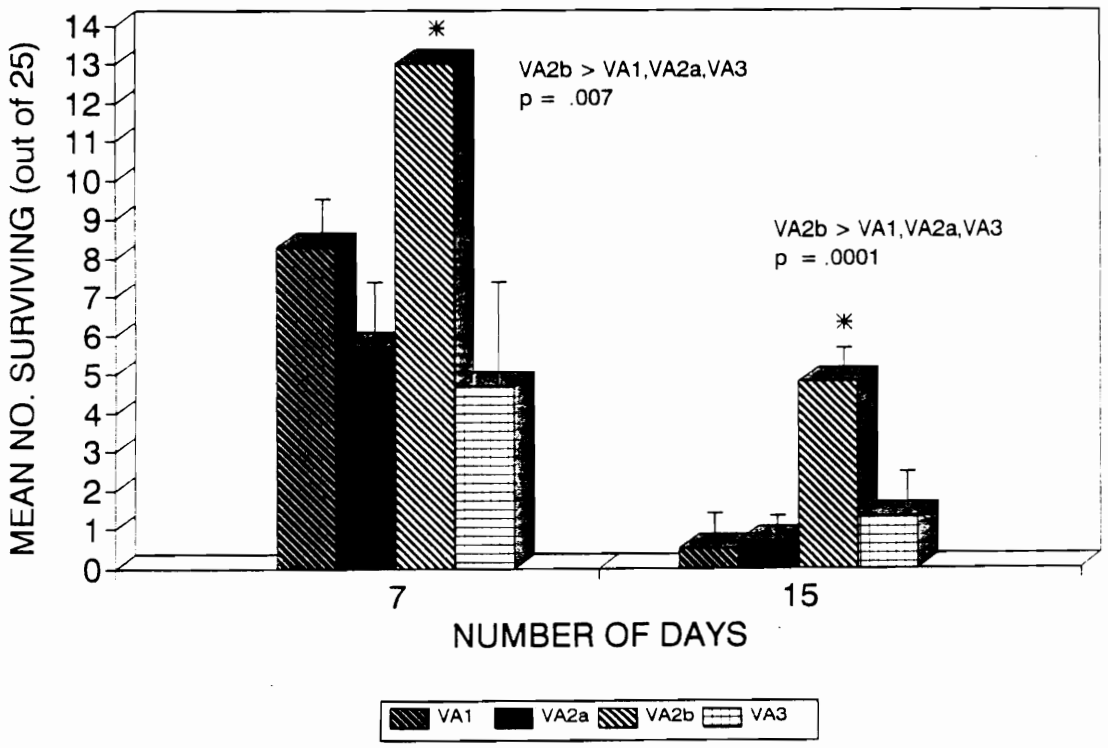

Figure 24. Larval survival after second transplant experiment into chlorine contact tanks.

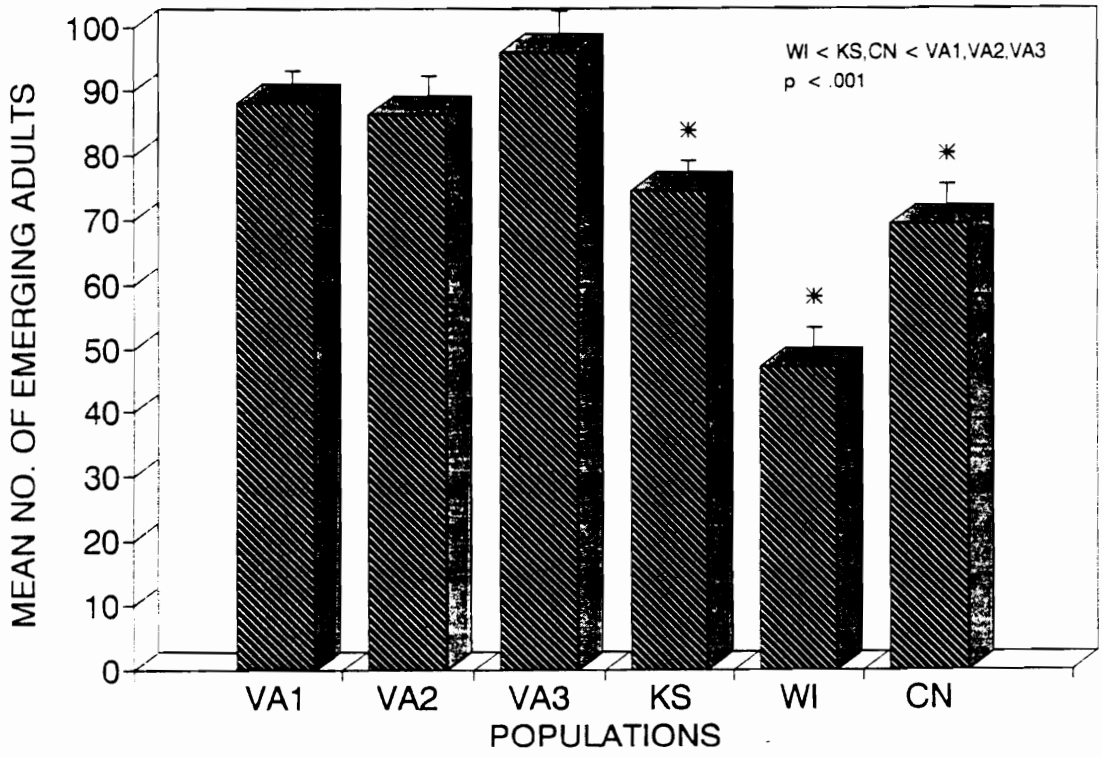

Figure 25. The number of emerging adults in laboratory experiment 4.

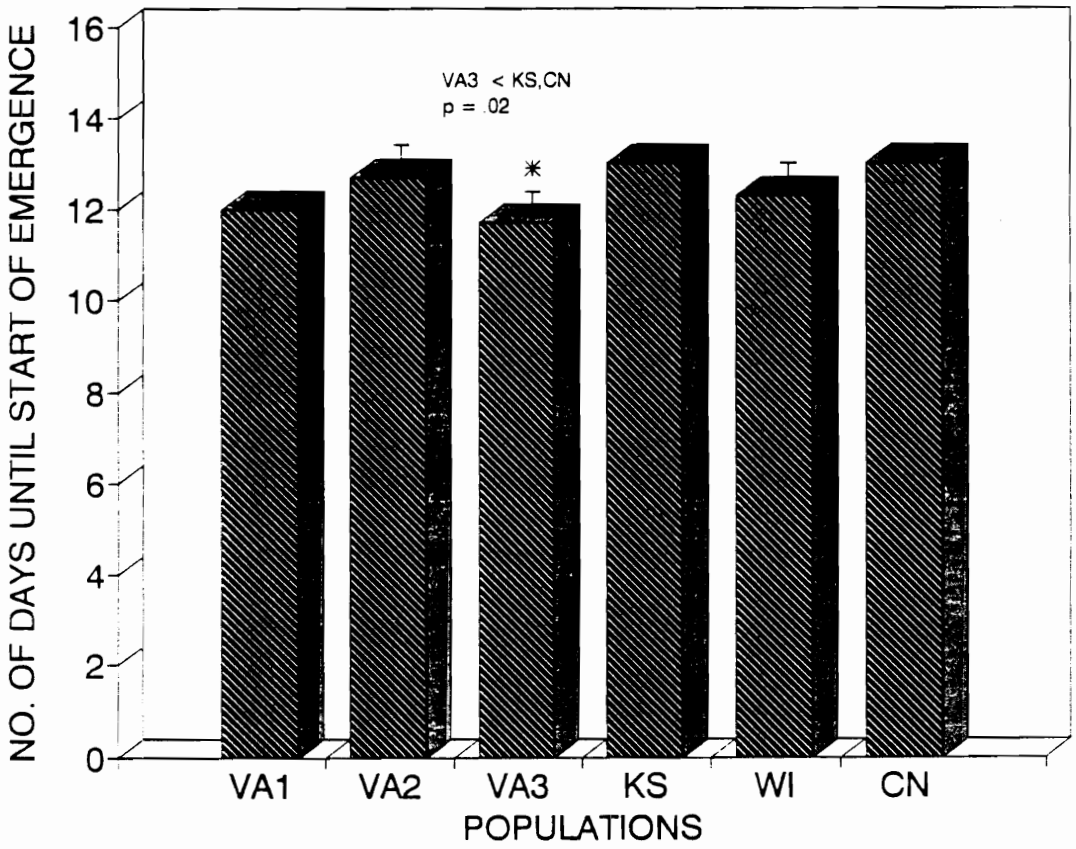

Figure 26. Number of test says before the onset of emergence in laboratory experiment 4.

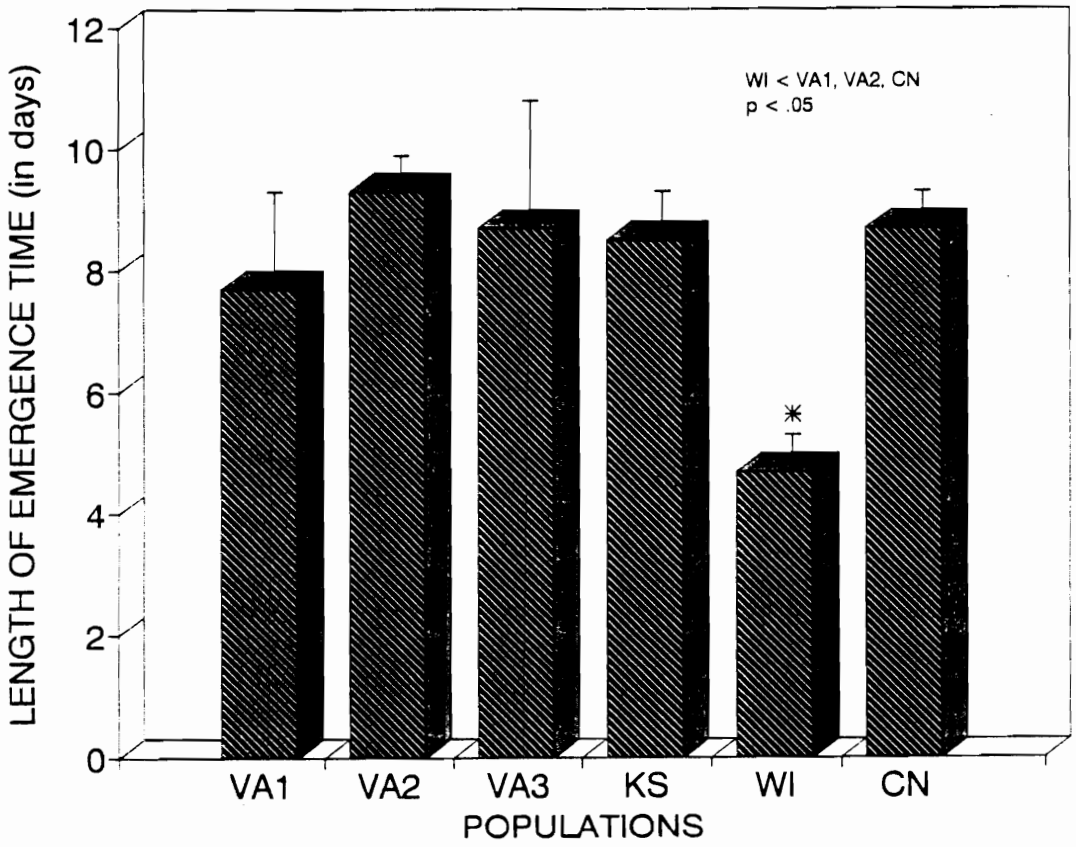

Figure 27. Length of the emergence period, from the beginning to the end of emergence, in laboratory experiment 4.

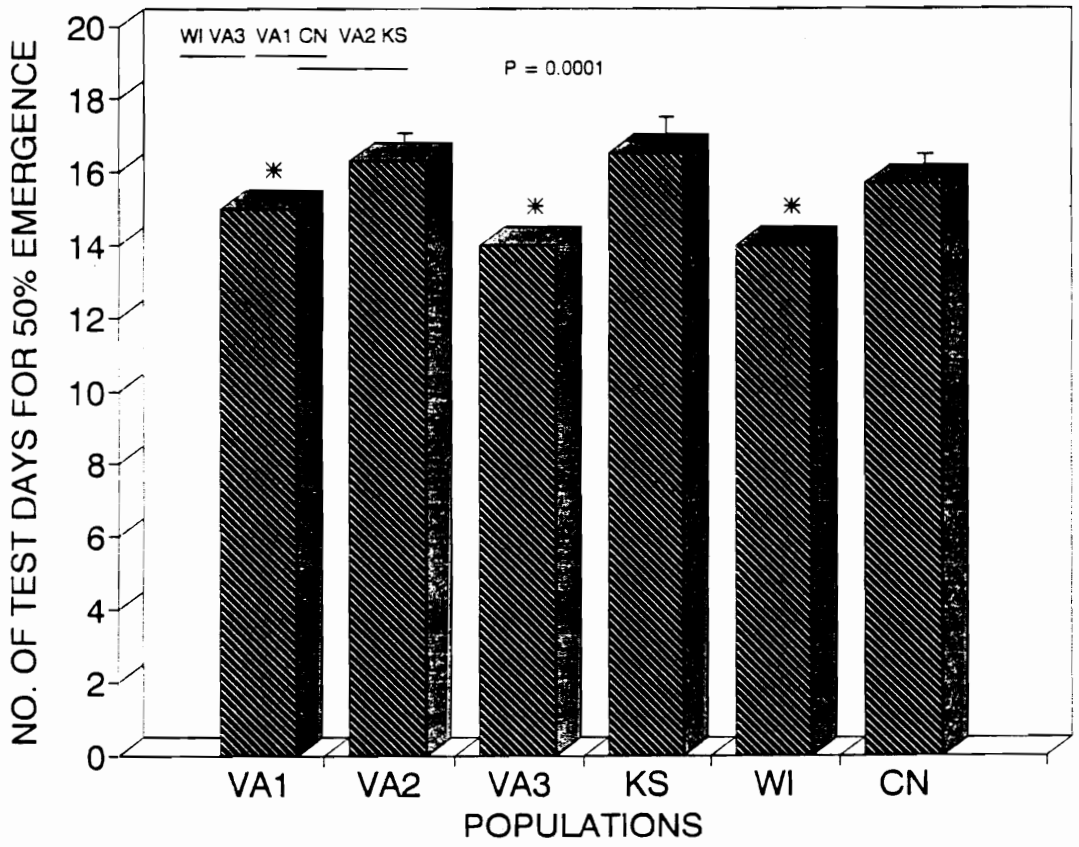

Figure 28. Number of test days until 50 percent emergence, in laboratory experiment 4.

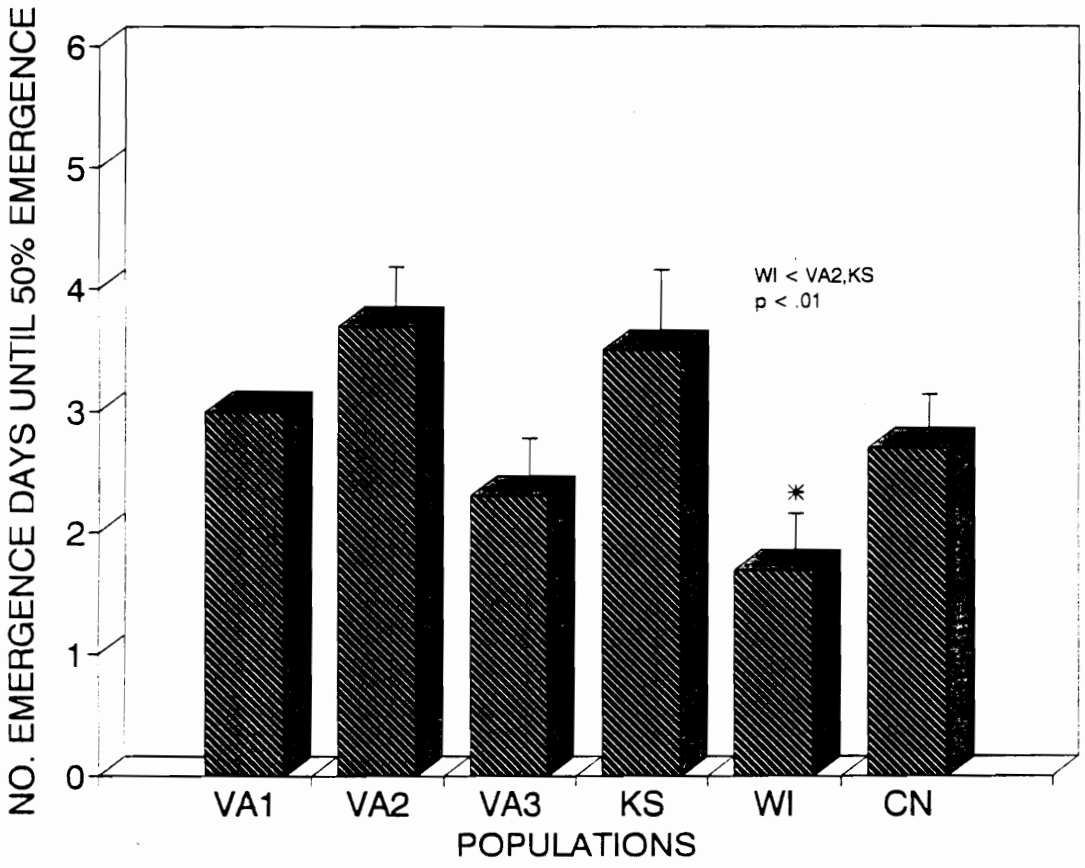

Figure 29. Number of emergence days required to reach 50 percent emergence in laboratory experiment 4.

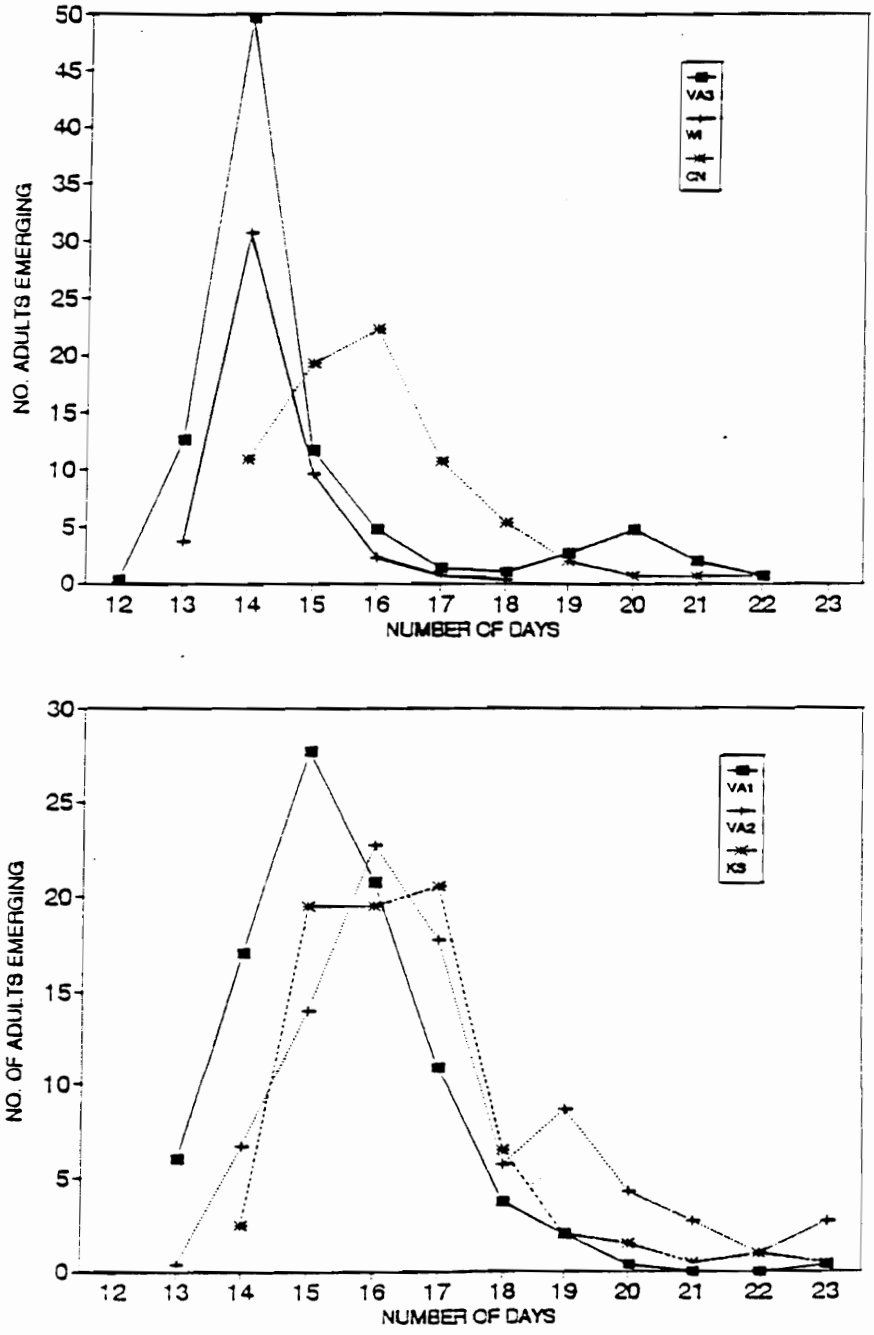

Figure 30. Emergence curves for laboratory experiment 4.

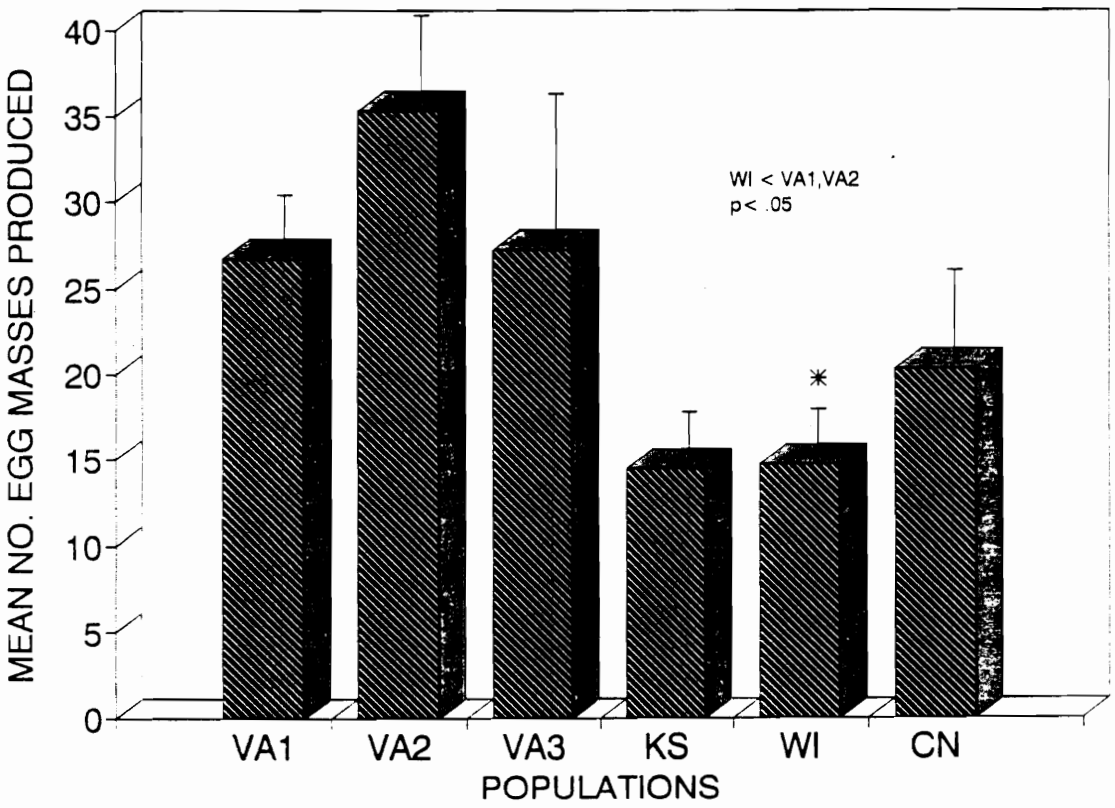

Figure 31. Number of egg masses produced by each population in laboratory experiment 4.

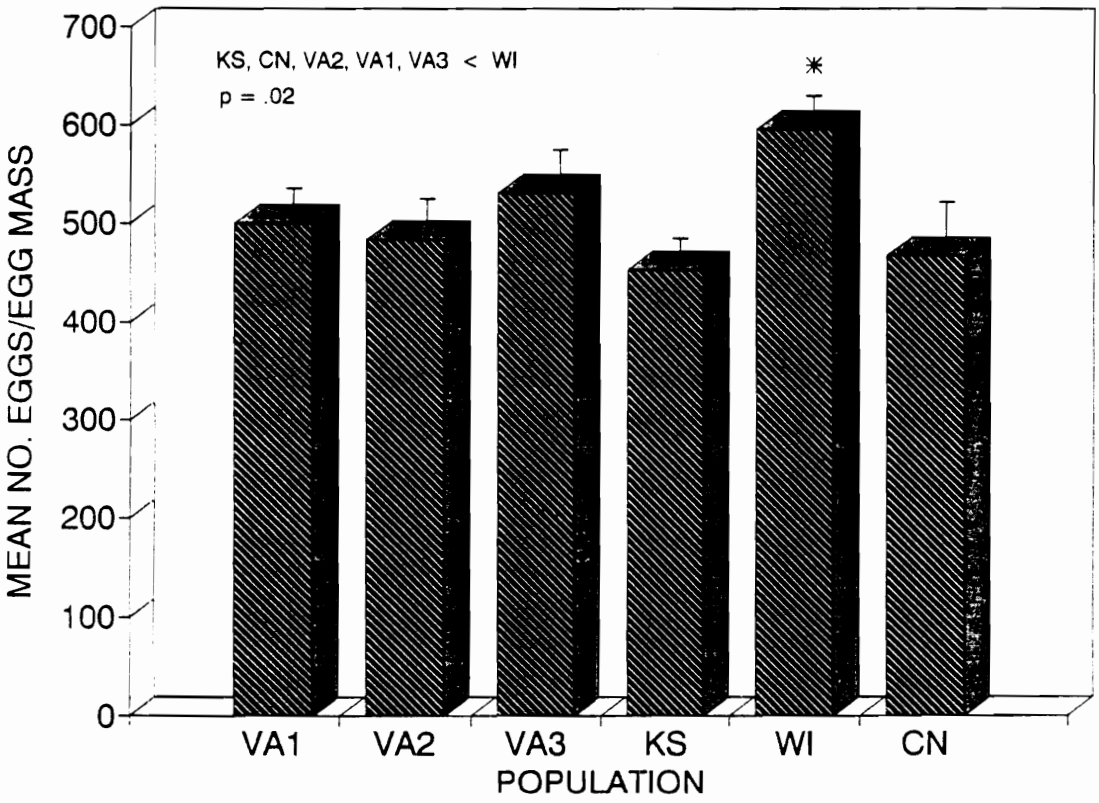

Figure 32. The number of eggs per egg mass produced by each population in laboratory experiment 4.

10.0 VITA

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