Exploring larval food limitation as a probable cause of decline in Iowa populations of a butterfly, *Speyeria idalia* Drury (Lepidoptera: Nymphalidae, Argynninae)

by

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CHAPTER 1. GENERAL INTRODUCTION

The Regal Fritillary, *Speyeria idalia* Drury (Lepidoptera: Nymphalidae: Argynninae) is an example of a prairie endemic species of butterfly that has experienced severe population declines because of habitat destruction. *S. idalia* is one of the best indicators of high quality prairie in North America (Hammond and McCorkle 1983). With the disappearance of prairie habitat, widespread populations of *S. idalia* have declined in numbers and distribution. *S. idalia* was listed as a Category II species under the Endangered Species Act until 1996, when this category of protection was deleted by the U.S. federal government (J. Bade, pers. com.). Category II species were species that were candidates for listing, but there was not sufficient knowledge regarding their status to warrant proposing them for listing as endangered or threatened (USFWS 1996). *S. idalia* also has a special "status of concern" in national grasslands in North and South Dakota, and the Iowa Department of Natural Resources has listed it as a sensitive species. Population estimates of this insect in Iowa and across the nation have declined sharply over the last 50 years, and our documentation with mark-recapture and transect estimates of small population sizes of *S. idalia* in Iowa during 1995 (Table 1) suggests that the insect could go extinct locally.

In 1995, after we found a number of intermediate-sized to small-sized populations of *S. idalia* in Iowa (Table 1), our research began to focus on the causes of population decline of *S. idalia*. We considered many possible reasons for this decline. We did not investigate the issue of inbreeding for two major reasons. First, genetic markers are difficult to obtain for any species, and additionally, the interpretation of their importance to inbred populations is regarded with skepticism at this time. Also, over several years, insect populations fluctuate in size and genetic composition such that it would be unlikely that we could determine whether the effects we would see were caused by random events or an actual inbreeding effect. Assuming these issues could be overcome, a third factor was that the time required to address this issue meaningfully was beyond the scope of a Masters' project.

One potential reason for *S. idalia*'s decline in Iowa is the quality of habitat it occupies Iowa's prairies. Violets (family Violaceae) are the larval hostplants for this insect. We

Table 1. 1995 estimates of *Speyeria idalia* populations at sites in southwestern Iowa.

Prairie name	*Population estimate*	*Method*
Page Private Prairie	2	
Sheeder Prairie	50	mark-recapture
Reichelt Unit of Stephens State Forest	4	
Polk City Prairie	1	
Moeckley Prairie	220	mark-recapture
Ringold Wildlife Area	7	
Doolittle Prairie	2	
Rolling Thunder Prairie	120	mark-recapture
Kalsow Prairie	500	visual estimate[1]
Loess Hills Wildlife Area sect. 9	160	mark-recapture
Loess Hills Wildlife Area sect. 21	2	

[1] P.C. Hammond

summarized records of land management of these prairies from the Iowa Department of Natural Resources and found that many of the prairies we had surveyed had undergone intensive agricultural management in previous years. Some of the management treatments included plowing, burning, tree and brush removal, and grazing. We found that most of these treatments (except in certain areas which had been moderately grazing) had a negative effect on the violet populations surveyed, in contrast to managed areas with virgin prairie (Kelly and Debinski, unpublished). We also examined the availability of mature prairie flowers which would provide food sources (nectar) to adult *S. idalia.* Quality nectar source availability attracts and maintains adult *S. idalia* populations, and if found in areas with large populations of larval hostplants, allows the adult females to lay eggs in areas favorable to the next generation. Limited nectar sources have demonstrated negative effects on the fecundity of adult females in a related species, *Speyeria mormonia* Boisduval (Boggs 1993).

Considering many factors in our study, the natural history of *S. idalia* indicates that limited food availability to the insect in the larval stage could have negative effects on the population sizes in areas with low hostplant density. Because of the small size of *S. idalia* habitat in Iowa and the rather limited violet abundance that we observed, we predicted a hostplant limitation problem involving the availability of violets in these habitat patches. In summary, the single consistent factor apparent in Iowa populations of *S. idalia* insects was the diminished abundance of larval hostplants.

Having hypothesized that the limited larval hostplant resources were causing a decline in Iowa *S. idalia* populations, we looked outside Iowa for a comparison of *S. idalia* populations in areas where violets were in greater abundance. We proposed comparing the sizes of some *S. idalia* populations in Kansas, Nebraska, or South Dakota with those of Iowa populations by using mark-recapture techniques. Hostplant abundance data at each site were also collected. In addition to assessing the effect of larval food limitation on the population size of this insect, we hypothesized that larval food limitation would have detrimental effects on the size and fecundity of the individual insects (see literature review further in this chapter). Here, we refer to food limitation according to the definition given by Eisenberg et al. (1981, 210): "any difference in weight gain between field animals and maximally fed laboratory animals." We wanted to test the hypothesis that larval development under food limitation was delayed using *S. idalia* insects in the field, but collection of larvae in the field was impractical because of the relative rarity of the species and the absence of a precise selective trapping method. Instead, we decided to test the effects of larval food limitation on insect larvae in the laboratory. Our hypothesis was that adult insect body size would be smaller if the larval stage experienced food limitation from an inadequate hostplant population.

We intended to perform laboratory food limitation studies on *S. idalia* larvae, but we had problems in 1995 attempting to keep eggs and larvae alive because of the insect's strict temperature and humidity requirements. We chose to rear the Painted Lady butterfly, *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae), as a comparison to another Lepidoptera, and measure its response to food limitation in the larval stage. Studying *V. cardui* in addition to *S. idalia* has allowed us to progress with laboratory studies by rearing butterflies on food limited diets during the winter months, when collecting wild specimens from the field is impossible. Also, *V. cardui* demonstrates reasonably low mortality in the laboratory and can be reared in successive generations without tedious overwintering conditions.

Because *V. cardui* has been a much less fastidious insect in the laboratory than *S. idalia*, we have been successful in determining its response to larval food limitation. We conducted three experiments testing the effects of food limited diets in its larval stage. These experiments led to some significant progress in our understanding of how lepidopteran larvae might respond to inadequate food resources.

In the field, we were able compare *S. idalia* insects from areas of different hostplant abundance; we compared weights of adults collected from larger prairies to those from smaller, more isolated prairies. At this time, we have some evidence that *S. idalia* from the smaller areas in Iowa expressed smaller adult body size than those from the larger areas in South Dakota and Kansas, although without further laboratory evidence specific to *S. idalia*, we cannot conclusively state that larval food limitation leads to this effect.

Literature Review

We found a great body of literature on other species of arthropods that supported the hypothesis that larval food limitation could be responsible for diminished fitness, fecundity, or smaller populations of *S. idalia* found in Iowa. Many studies implicated food limitation as a cause of both diminished larval and adult body size. Reduced fecundity also was correlated to limited larval, nymphal, or juvenile resource acquisition. The background research presented here primarily focused on studies that considered arthropods in food limitation. We justified limiting our research comparisons to arthropod groups because many arthropod groups have larval or nymphal stages clearly separate from reproductive stages. Separating effects of food limitation between these stages avoids confounding our interpretation of weight gain or loss and its relationship to food limitation versus reproduction. Also, the skeletal structure dictates certain limits on size attainment despite appropriate nutrition, whereas many invertebrates can simply shed an exoskeleton to accommodate growth in body structure (Evans 1984).

Many approaches have been useful in studying the effects of food limitation on arthropods. There have been studies that used experimental field (Durbin et al. 1983, Lenski 1984, Richardson 1991, Ritchie and Tilman 1992, Rossi and Hunt 1988, Wise 1975) and laboratory settings (Slansky, Jr. 1980, Fischer and Moore 1993, Miyashita 1991, Poston et al. 1978, Hainsworth et al. 1991, Hainsworth and Hamill 1993) and some that used both (Juliano 1986, Collins 1980, Eisenberg et al 1981, Backus and Herbers 1992). Designs vary, although the main approach of experimental comparison has been to manipulate food supply through enrichment or reduction (see review by Olson and Olson 1989). We also found some studies that experimentally added more competitors to a system, sometimes referring to this as crowding or density-dependence treatments (e.g., Fischer and Moore 1993, Levitan 1991, Fincke 1994). Another means of studying the effects of food limitation involves the use of an index of food limitation (Olson and Olson 1989) such as mass of food voided, ration indicator indices, or egg mass production. Indirect indices of food limitation that employ a combination of body parameters also have been used. A field and laboratory design demonstrated that the comparison of a *ratio* of two skeletal parameters of sea urchins reared in control and food limited settings provided evidence of food limitation: the demipyramid grows at a reduced rate as the test shrinks from food deprivation (Levitan 1991). Additional studies we examined also addressed one or more broad topics in ecology, such as competition (Wise 1975, Juliano 1986, Ritchie and Tilman 1992), predation (Fischer and Moore 1993), or interspecific competition (Fincke 1994) in addition to food limitation.

In summarizing the literature, we focused on presenting the literature on arthropod species. We divided the arthropod studies by two general criteria, not mutually exclusive: 1) predaceous and phytophagous arthropods and 2) food limitation studied in the adult versus juvenile stage. We compared and contrasted elements of those studies with the goals and design of our research, and we will discuss the implications of our results on previous work in the concluding chapter of this thesis.

Studies of predaceous arthropods indicate that in some groups such as carabid beetles, Linnaeus (Coleoptera: Carabidae) the role of adult food availability is more important in reproduction than larval, nymphal or juvenile stage food availability (Juliano 1986, Wise 1979). Also, in predaceous arthropods, cannibalism is a more common response to food limitation in the immature stage, when there are more individuals in larval vs. adult stages and conspecific encounters are more frequent (Fincke 1994). This may suggest that larvae of predaceous arthropods do not express an effect of food limitation in the larval stage by diminished body mass or delayed adult emergence, but may compensate by eating insects of the same guild. Even though this is speculation, the issue of cannibalism at the larval stage would at the least complicate the interpretation of changes in larval body mass as an appropriate measure of food limitation, because some larvae would gain mass through cannibalism while others would decrease in mass or disappear altogether. In summary, adult food limitation has a much greater documented effect on the reproduction of predaceous arthropods when such nutritional limitation occurs in the adult stage (Juliano 1986 and Eisenberg et al. 1981).

Competition for food resulted in reduced growth of one or both larvae relative to controls in a field setting of Odonate larvae (Odonata: Libellulidae) (Fincke 1994). This study attributed causes of larval mortality in *Megaloprepus coerulatus* Latreille (Odonata: Libellulidae) to cannibalism, although larvae killed by conspecific larvae often were not eaten. The author claimed that cannibalism functions to reduce the number of potential competitors for food in addition to providing nutrition. Further, conspecific killing in *M. coerulatus* functions “to prevent exploitative competition, which is the resource that ultimately limits population size of this insect.” This behavior of killing conspecific larvae is intriguing because the larvae that mature as single occupants in water holes can sustain themselves on a territory size smaller than they will tolerate sharing with another larvae in a larger aquatic area. Another dragonfly larvae study found that density affects feeding activity and growth, but not survivorship (VanBuskirk 1993).

Naturally occurring food levels were insufficient to maximize reproduction in adult bombadier beetles *Brachinus* Weber (Coleoptera: Carabidae) (Juliano 1986). Survival was reduced by moderately low or very low food amounts, and food appeared to be in short supply in field sites. Increased feeding did not lead to earlier timing of reproduction in this study. Laboratory evidence suggested that over the long-term (more than two months) egg production in *B. lateralis* Weber was ten times greater than that by underfed field females, and feeding affected reproduction over several months in this species. The longer feeding season for adults in this species suggests that adequate nutrition in the adult stage is more essential to reproduction, and that spatially variant food limitation can have a greater impact over the long-term than daily and seasonal fluctuations. Although this study demonstrated that feeding rates in nature are probably low enough to reduce reproductive success, no correlation was made between limited population size and competition.

Field manipulations were used to determine whether spiders *Linyphia marginata* C.L. Koch (Araneae: Lynyphiidae) were food limited by adding food to the webs of immature spiders and determining the effect on survival and weight gain (Wise 1975). He found that immature survival was not improved with food supplementation at lower or higher densities, while mature spiders experienced a negative effect of density on both fecundity and survival. In immatures, food supplementation increased the rate at which the spiders gained weight, regardless of density.

Food availability for mantid nymphs affects body length of adults, which in turn affects potential adult weight gain (Eisenberg et al. 1981). In a laboratory experiment that compared the weight of a predaceous adult mantids, *Tenodera ardifolia sinensis* Saussure (Dictyoptera: Mantidae) in the field over several weeks with laboratory reared females fed *ad libitum*, food limitation was evident in the field adults as the weights of laboratory females increased until they oviposited ootheca. The reproductive activity of laboratory females depositing ootheca was documented to reduce weight, and the results of the study showed that females must acquire adequate nymph resources as well as additional adult food in order to produce ootheca. However, feeding males *ad libitum* in the laboratory did not result in any increase in weight.

A combination field and laboratory study of an ant, *Leptothorax longispinosus* Mayr (Hymenoptera: Formicidae) whose diet was supplemented with crickets demonstrated that protein supplementation had no effect on female production by nests; production of queens was insensitive to protein supplementation (Backus and Herbers 1992). However, production of sexually functional members of the colony increased in food rich areas where underfed diploid larvae matured to become workers instead of gynes. Food supplemented nests produced more males than nests in control areas. Despite the original hypothesis that higher density areas rich in ant species would experience greater food limitation (in this particular species *L. longispinosus*, low protein), laboratory or field manipulations of food *quality* did not cause ants to reallocate food resources.

Effects of food limitation on growth rate and body size were observed in the spider *Nephila clavata* Linnaeus (Araneae: Araneidae) (Miyashita 1991). Three natural populations in experimental field settings demonstrated less variation in growth rate and body size of adult females than the laboratory females reared on extreme levels of food supplementation and limitation. Both rate of growth and body size of adult females were increased significantly by food supplementation.

In a comparison of food supplementation between two sympatric beetle species: *Carabus limbatus* Say (Coleoptera: Carabidae) and *C. sylvosus* Linnaeus, supplementation increased larval abundance and initiated earlier emergence in the next generation (up by 77%), as well as greater adult body mass in *C. limbatus* (Lenski 1984). Competition between the two species for naturally occurring food was diminished for *C. sylvosus* when *C. limbatus* received supplementation. No detectable increase in the timing of adult emergence was observed after food supplementation.

In phytophagous arthropods, the larval food stage can provide as much or more nutrition to the individual as it could obtain in the adult insect stage, but some insects do not feed at all in the adult stage (Evans 1984). Certainly, larval food sources of phytophagous species are not more energy rich relative to nectar sources for adult insects. But both larval and adults sources of nutrition available to phytophagous species provide far less energy rich resources than juvenile or adult prey of predaceous arthropods, which we have established earlier have different food limitation issues. The general reduced energy content of hostplant and nectar sources of phytophagous arthropods in contrast to predaceous arthropods suggests greater attention to food limitation as a possible cause for diminished individuals and populations. This is because phytophagous animals have to consume a relative greater mass of their food to obtain the same energy, because cellulose and other indigestible compounds make up a significant proportion of their diet (Evans 1984). While the potential of food limitation is documented for phytophagous arthropod species in both the larval and adult stages, our concern for food limitation in *S. idalia* is directed at the larval stage, because these insects invest a much greater proportion of their life cycle foraging at this developmental stage (Matoon et al. 1971). An additional reason for studying food limitation at the larval stage of arthropods is that there is no reproductive stage taking away larval resources and thus adding variation in the weight gain statistic we are attempting to compare. This decrease in body mass associated with egg or ootheca oviposition can invalidate the method of using different food rations and their consequential change in weight gain in studies of food limitation at the adult stage (Eisenberg et al. 1981, Juliano 1986).

Within comparisons of arthropod research, we found a number of studies that tested food limitation at the larval and adult stage. The effects of larval dipteran food limitation on adult body characteristics were studied with the species, *Ephydra cinerea* Jones (Diptera: Ephydridae) (Collins 1980). The results indicated that *larval* food limitation caused reduced *adult* body size, delayed development time, reduced fecundity, and elevated prereproductive mortality. The other dipteran species examined maintained constant egg size under larval food limited conditions, with no significant difference in volume, fertility, or time to hatching.

The degree of larval food limitation could influence development time, and loss of larvae could be enhanced in food limited environments if smaller larval size or slower behavioral response enhanced vulnerability to predation (Fischer and Moore 1993). This study examined the relationship of food limitation and juvenile mortality caused by predation in a pelagic dipteran larvae, *Chaoborus punctipennis* Lichtenstein (Diptera: Chaoboridae). Percent mortality of *C. punctipennis* was increased five-fold in predator treatments over controls. No significant interaction between food limitation and predation was observed, although predation by copepods may limit recruitment of juvenile *C. punctipennis* in productive lakes.

The effect of increased food availability was studied in many species of coarse particle detritus feeders of montane streams (Richardson 1991). Abundance of individuals, mean adult body mass at emergence, or both, were increased significantly from enriched leaf litter resources in seven of nine species in treatment versus control streams. While both sexes exhibited the increase in adult body mass, females gained proportionally more in some species. In this study, adult emergence times were not significantly faster in the supplemented streams.

Egg production was used as an indicator of food limitation effects in (adult) milkweed bugs, *Oncopeltus faciatus* Dallas (Hemiptera: Rhopalidae, Lygaeinae), laboratory reared on different levels of seeds per week (Slansky 1980). Adult females delayed onset of egg production from three weeks post-eclosion in controls to six weeks post-eclosion in the most severe food limited treatment. These severely food limited females were unable to convert ingested food to egg production efficiently. The rate of dry egg mass produced relative to dry matter of milkweed seeds consumed was about 7.7% in the 25 seeds per week treatment compared to 27.9% (control: 100 seeds per week) and 21.1% (50 seeds per week). Ironically, females that were resource starved lived longer than those with sufficient seeds in the diet. Perhaps these females laid too few eggs to exhaust themselves of resources.

A colonial insect study supplemented the diet of a paper wasp *Polistes metricus* Latreille (Hymenoptera: Vespidae, Polistinae) with honeybee honey (Rossi and Hunt 1988). The supplemented wasps produced offspring earlier than control colonies. There was a shorter time span between founding and emergence of larval colonies. Also, the percent body fat of supplemented offspring was greater than that of foundresses (adults laying the eggs). The greater percent of non-cuticular fat associated with supplemented offspring indicated greater reproductive potential.

A study of adult food limitation with *V. cardui* in a laboratory setting tested sucrose solution assimilation and the efficiencies of mature egg production (Hainsworth et al. 1991). The foraging frequency of these insects decreased with increased sugar concentration, while insects preferred the more concentrated sucrose sources. Although this study found a great deal of variation in how much the insects would forage if allowed to feed *ad libitum,* newly emerged female *V. cardui* expressed a "rapid and direct impact of ingested sugars on potential reproductive performance." This was measured as an increase of mature eggs laid per week. An implication of this study to a field setting is that intense foraging early in the adult stage of this insect permits a shift in energy investment to mating in males and ovipositing in females. A further study found that more highly concentrated energy sources required longer intervals between meals, as sugar concentrations were experimentally modified to affect different energy gains (Hainsworth and Hamill 1993).

In our own research, we considered the influence of adult nectar sources to *S. idalia*, but we had no scientific basis to compare quality of nectar source availability among sites in Iowa. Also, other studies suggested that in spite of limited adult nectar source availability to *S. idalia*, the adults would still lay enough eggs to ensure a population comparable to the last season (Boggs 1988, Hammond and McCorkle 1983). That is to say, one life history trait of most *Speyeria* insect species is that they lay an enormous number of eggs per female in contrast to the number of larvae that survive to adulthood the next season. Because few females were found on Iowa prairies in either season we surveyed, we note the possibility of only a few females genetically contributing to future year's offspring. One obvious element unique to prairies in Iowa is their smaller size and greater isolation. This could potentially be causing inbreeding depression effects relative to similar sites in Kansas, Nebraska, or South Dakota (Falconer 1981). We observed several hundred eggs laid by single female insects in our laboratory, and the species we studied was reported to lay the most eggs per female of any in its genus (Matoon et al. 1971). For these reasons, we more strongly supported the idea that the food limitation effect we hypothesized could be demonstrated in the larval stage of Iowa *S. idalia* populations.

Thesis Organization

This thesis is organized as two separate components: a laboratory and a field study. The laboratory study is written in the format of a manuscript to be submitted to the *American Midland Naturalist.* The field study is written in the format of a manuscript to be submitted to the *Journal of Conservation Biology.* This introduction marks chapter one, while the laboratory and field study are designated chapters two and three respectively. The final chapter of general conclusions attempts to correlate the results of both laboratory and field work with a special emphasis on our inferences for future experimental work and conservation measures that may be undertaken to preserve Iowa populations of the insect we studied.

CHAPTER 2. LARVAL FOOD LIMITATION AS TESTED IN THE LABORATORY WITH THE PAINTED LADY BUTTERFLY *Vanessa cardui* LINNAEUS (LEPIDOPTERA: NYMPHALIDAE)

A paper to be submitted to the *American Midland Naturalist*

Liesl Kelly and Diane Debinski

ABSTRACT: Larvae of the Painted Lady Butterfly *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae), were reared on a commercial diet apportioned to two food limited and one control (*ad libitum*) treatments tested in three trials. Larvae were analyzed for potential differences in both larval and adult weight, and time to pupation and emergence. In all trials, larvae of all diet treatments reached similar maximal weights, but days to pupation and adult emergence were prolonged in the larval food limited treatments across three trials. Higher incubation temperature in Trials II and III confounded treatment effects by accelerating larval development and progression toward the adult stage. Higher incubation temperature also increased maximum larval weights achieved in Trial III. Across three trials, no standard larval, pupal, or adult weight, nor standard time to pupation or emergence was achieved. Although within trials, control insects developed more rapidly and achieved maximal weights more rapidly than food limited larvae.

INTRODUCTION

Food limitation has been studied for a variety of ecological reasons. Arthropod groups are unique in having certain life history similarities. Many arthropod species have larval or nymphal stages clearly separate from reproductive stages, and some seasonally abundant species have only non-overlapping generations. The absence of these discrete life history traits would confound our interpretation of weight gain or loss in the larval or adult stage and its relationship to food limitation. Many studies have implicated food limitation as a cause of both diminished larval and adult body size, as well as having an effect of reduced fecundity correlated to limited larval, nymphal, or juvenile resource acquisition. There is an enormous amount of research that supports the hypothesis that larval food limitation could be responsible for diminished fitness, fecundity or smaller populations of insects.

Previous Research.-- Suboptimal nutrient resources can have a variety of consequences to individuals or populations of insects. When food limitation occurs in the larval stage of an insect, body size can be affected immediately, while reproductive consequences will not be manifest until later life stages when the insect depends on other food resources. Previous research on food limitation therefore distinguishes effects of food limitation by larval, nymphal, or adult life stages in insects and other arthropods.

Food availability in nymphs affected adult body length of mantids (Dictyoptera: Mantidae) (Eisenberg 1981). Production of sexually functional members of the ant colony increased in food rich areas where underfed diploid larvae matured to become workers instead of gynes (Backus and Herbers 1992). Food supplementation increased larval abundance and initiated earlier emergence in the next generation (up by 77%), as well as greater adult body mass in a beetle, *Carabus limbatus* Say (Coleoptera: Carabidae) (Lenski 1984). The degree of larval food limitation and development time were studied in a planktonic copepod (Fischer and Moore 1993). The study found that the loss of larvae could be enhanced in food limited environments if smaller size or slower behavioral response of larvae increased vulnerability to predation.

The effect of increased food availability was studied in many species of coarse particle detritus feeders (Richardson 1991). Abundance of individuals, mean adult body mass at emergence, or both were significantly increased from enriched leaf litter resources in seven of nine species in treatment versus control streams. In this study, adult emergence times were not significantly faster in the supplemented streams.

Egg production in (adult) milkweed bugs, *Oncopeltus faciatus* Dallas (Hemiptera: Rhopalidae, Lygaeinae), laboratory reared on different levels of seeds per week (Slansky 1980). Adult females delayed onset of egg production from three weeks post-eclosion in controls to six weeks post-eclosion in the most severe food limited treatment. These severely food limited treatment females were unable to efficiently convert ingested food to egg production. Feeding efficiency was affected by food limitation: the rate of egg dry mass to dry matter of milkweed seeds consumed was about 7.7% in the 25 seeds per week treatment compared to 27.9% (control: 100 seeds per week) and 21.1% (50 seeds per week).

A colonial insect study supplemented the diet of a paper wasp *Polistes metricus* Latreille (Hymenoptera: Vespidae, Polistinae) with honeybee honey (Rossi and Hunt 1988). The supplemented wasps produced next year's offspring earlier than control colonies. There was a shorter time span between founding and emergence of larval colonies. Also, the percent body fat of supplemented offspring was greater than that of foundresses (adults laying the eggs). The greater percent of non-cuticular fat associated with supplemented offspring indicated greater reproductive potential.

Many experimental designs have been useful in studying the effects of food limitation on arthropods. Of course there have been studies that used experimental field (Durbin et al. 1983, Lenski 1984, Richardson 1991, Ritchie and Tilman 1992, Rossi and Hunt 1988, Wise 1975) or laboratory settings (Slansky, Jr. 1980, Fischer and Moore 1993, Miyashita 1991, Poston et al. 1978, Hainsworth et al. 1991, Hainsworth and Hamill 1993) and some that used both (Juliano 1986, Collins 1980, Eisenberg et al 1981, Backus and Herbers 1992). Designs vary as well, although the main approach of experimental comparison has been to manipulate food supply through enrichment (supplementation) or reduction (Olson and Olson 1989). Some studies experimentally added more competitors to a system, sometimes referring to this as crowding or density-dependence treatments (Fischer and Moore 1993, Levitan 1991, Fincke 1994). Another means of studying the effects of food limitation involves the use of an index of food limitation (Olson and Olson 1989) such as mass of food voided, ration indicator indices or egg mass production. Also indirect indices of food limitation that employ a combination of body parameters have been used.

We designed this laboratory experiment to study a potential field situation with an insect, the Regal Fritillary, *Speyeria idalia* Drury (Lepidoptera: Nymphalidae, Argynninae), whose populations are declining in Iowa. This species has a limited distribution in Iowa on remnant, isolated prairies which may not provide sufficient hostplant availability, either in relative density or absolute abundance, to the larval stage of the insect (Kelly and Debinski, unpublished data). The purpose of our study was to establish results from laboratory research that would increase our understanding of the effects we might expect to see in the field if larval food limitation were applied to *S. idalia.* We were interested also in how other insects would respond to food limitation in the larval stage, so we began rearing larvae of the Painted Lady Butterfly *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae) in food limited treatments in the laboratory. One effect we predicted was that larval food limitation would result in lower *adult* body mass, assuming that some members of a population would survive limited food resource availability. Also, we speculated that emergence dates (from pupal to adult stages) would be delayed in food limited treatments. Finally, we wanted to determine whether the larval stage of food limited treatments would have a decreased rate of body mass accumulation. We used a laboratory setting to provide further evidence for field data we observed in the insect *S. idalia*, such as lower body mass in adult insects and later emergence dates on some Iowa prairies. Our long-term objective is the conservation and restoration of *S. idalia* in prairies in Iowa. Therefore, the research presented here attempts to predict the response of butterflies to lower larval food resources.

The research presented here shows how larval food limitation affects discrete developmental parameters such as maximal larval weight, emergence weight of adults, and time to pupation and emergence. We attempted to avoid confounding results from addressing multiple issues such as competition or nutrient quality by rearing larvae in isolation and using a complete diet medium. Also, we focused on larval food limitation effects on the individual, instead of effects on populations, multiple generations, or the issue of genetic selection.

Gathering insects from the field for the purpose of laboratory rearing can have considerable drawbacks. This may include unknown disease presence or suboptimal fertility of the insects, inability to locate insects, coordinating the collection effort with population emergence and general limited abundance. Limited abundance is especially an issue for species of conservation concern. Many insects, such as *V. cardui*, are available commercially from laboratory supply companies such as Ward's Biological Supply. Because *V. cardui* is a much less fastidious insect in the laboratory than *S. idalia*, we predicted greater success in determining its response to larval food limitation. *V. cardui* has been raised for many generations in laboratory settings with low mortality rates. Also, *V. cardui* can be reared in successive generations without tedious overwintering conditions. For these reasons, we chose to rear *V. cardui* as a taxonomic outgroup comparison of lepidopteran response to food limitation.

Previous studies have examined *V. cardui* larval development under different temperature regimes (Poston et al. 1977) and food limitation in a competition setting (Poston et al. 1978). The results of the Poston et al. (1977) study suggested an optimal temperature for larval growth at approximately 24° C, where maximal pupal weight and minimum mortality (total over all larval instars) were achieved. In a competition study with treatments of different numbers of larvae per container, the same authors were unable to demonstrate a significant difference between food limited treatments in the larval development (days to pupation), larval mortality, or leaf consumption in their 1978 study of groups of one, two and three *V. cardui* insects raised on a given area of soybean leaves. Our objectives were slightly different; our treatments reflected different amounts of food consumed relative to a control response we measured. We designed a protocol that involved treatments of insects reared in isolation, with the purpose of testing food limitation, but without effects of competition.

METHODS

We measured the weight gain of individual *V. cardui* larvae that were fed in separate containers different amounts of a commercial food medium from Ward's Biological Supply. All insects were fed *ad libitum* until the third instar, when we began to measure specific amounts of food eaten by insects assigned to the control treatment. By providing specific rations and measuring amounts remaining after feeding, we calculated 60% and 80% of the amount eaten by the control insects, in grams of food per day, and fed this amount to insects assigned to the two food limited treatments.

We conducted three trials testing food larval limitation in *V. cardui*. Trial I occurred in February, 1996, and Trials II and III occurred in May, 1996. Trial I began with 300 insects spread over three treatments. Trials II and III were intended to occur as *one* set of 300 larvae, but had to be split into two trials because they consisted of groups of larvae hatched on two different days. Thus, Trial II consisted of a set of 120 larvae, while Trial III consisted of 180 larvae, each trial spread over three treatments (60%, 80% and *ad libitum* food ration). Trial I began with 100 insects in each treatment, while Trials II and III included only 40 and 60 insects respectively. Trial I occurred under mean daily temperatures in the laboratory of 18° C (s.e.: 0.3, from ten daily temperatures), while Trials II and III occurred under mean daily temperatures in the laboratory of 22° C (s.e.: 0.4, from nine daily temperatures). Larvae were held in individual containers *en masse* in six large communal chambers. All food was separated into individual containers to avoid feeding interaction between insects. We measured the larval weight gain over the larval stage by weighing the insects to the nearest 0.1 mg every three days in Trial I and II, and every day in Trial III. We also noted the number of days to pupation, the number of days from hatching to adult emergence, and the adult weight to the nearest milligram achieved by each insect. The food limitation design was continued to the stage at which insects matured into the pupal stage. A mean weight of all insects within a treatment was used for comparison of rate of larval growth. Adult weights were measured by voiding the weight of a glassine envelope and recording the weight of adult insects suspended in the envelope.

RESULTS

Trial I.--The insects of all treatments in Trial I attained about the same body mass before reaching pupation. (Means for this statistic, peak larval body mass, are: control, 475.9 mg; 80% limited, 467.5 mg and 60% limited 480 mg, and were not significantly different ($F = 1.87$, $p > 0.05$). However, peak larval weight of *V. cardui* was reached later (3, 6 days respectively) in the 80% and 60% food limited treatments. (See FIG. 1.) In Trial I, the adult emergence weights demonstrated a significant difference among food limited treatments ($F = 8.92$, $p < 0.05$) level for control versus 80%, for control versus 60% ($F = 11.21$, $p < 0.005$), and no significant difference between 60% and 80% food limited treatments. Similarly, both the average time from larval hatching to pupation as well as the average time from hatching

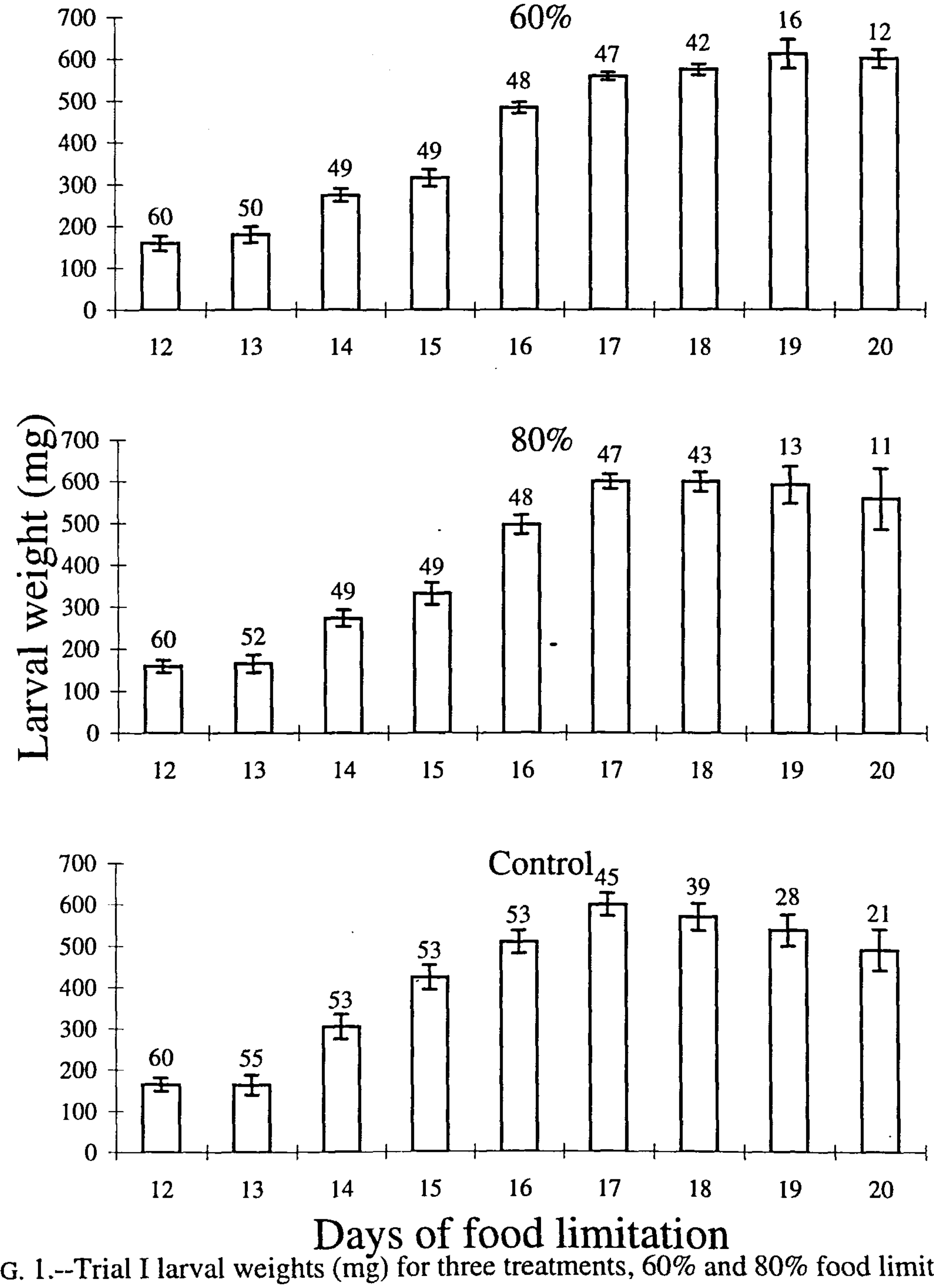

FIG. 1.--Trial I larval weights (mg) for three treatments, 60% and 80% food limited diets and *ad libitum* (control) fed to *Vanessa cardui* larvae in a February 1996 laboratory trial. Bars indicate s.e. Numbers at mean points indicate insects remaining in larval feeding at days after hatching.

to adult emergence showed a significant an effect of food limitation: control versus 80% and 60% food limited treatments were significantly different ($F = 2.81$, 3. $p > 0.05$). (See TABLE 1 for a summary of pupation and emergence data.)

Trials II and III.--As described above, we reared two sets of larvae in the same manner as for Trial I, but the mean temperature in the laboratory was 22° C (s.e.: 0.4, from ten daily temperatures). Trial II consisted of 40 insects in each treatment, while Trial III consisted of 60 insects in each treatment.

From days seven through ten, in Trial II there was no significant difference in the larval weight gain among treatments; differences began to occur on day 13 of food limitation, when control mean larval mass exceeded that of 60% and 80% food limited larvae ($F = 4.23$, $p = 0.05$; FIG. 2). The pattern of increasing controls larval mass continued through pupation in Trial II, although we analyzed this parameter at day 13, because sample size dropped after this time as pupation occurred. Over time, the maximum mean larval weight of each diet treatment occurred in the same order in trials I and II: controls, then 80% then 60% food limited treatments.

The larval weight gains in Trial III showed no significant difference among treatments until day 15 (food limitation began on day 12). On day 15, mean larval weight of the control insects exceeded larval weight in food limited treatments and reached peak larval weight sooner than 60% and 80% levels ($F = 5.86$, $p = 0.005$; 60% and 80% NS difference: $F = 2.12$, $p > 0.05$; FIG. 3). However, in this trial we took measurements daily instead of every three days and recorded the amount of food consumed and the average daily gain of all control insects, FIG. 4. Finally, we compared larval weight gain in control insects with amount of food consumed. Adult emergence times for all treatments in Trials II or III did not differ among treatments ($F = 1.12$, $p > 0.05$; TABLE 1, FIG. 5, and FIG. 6). Insects in the Trial III control treatment increased in weight early on the experiment even though the greatest amount of food consumption occurred in the latest larval instar (FIG. 6).

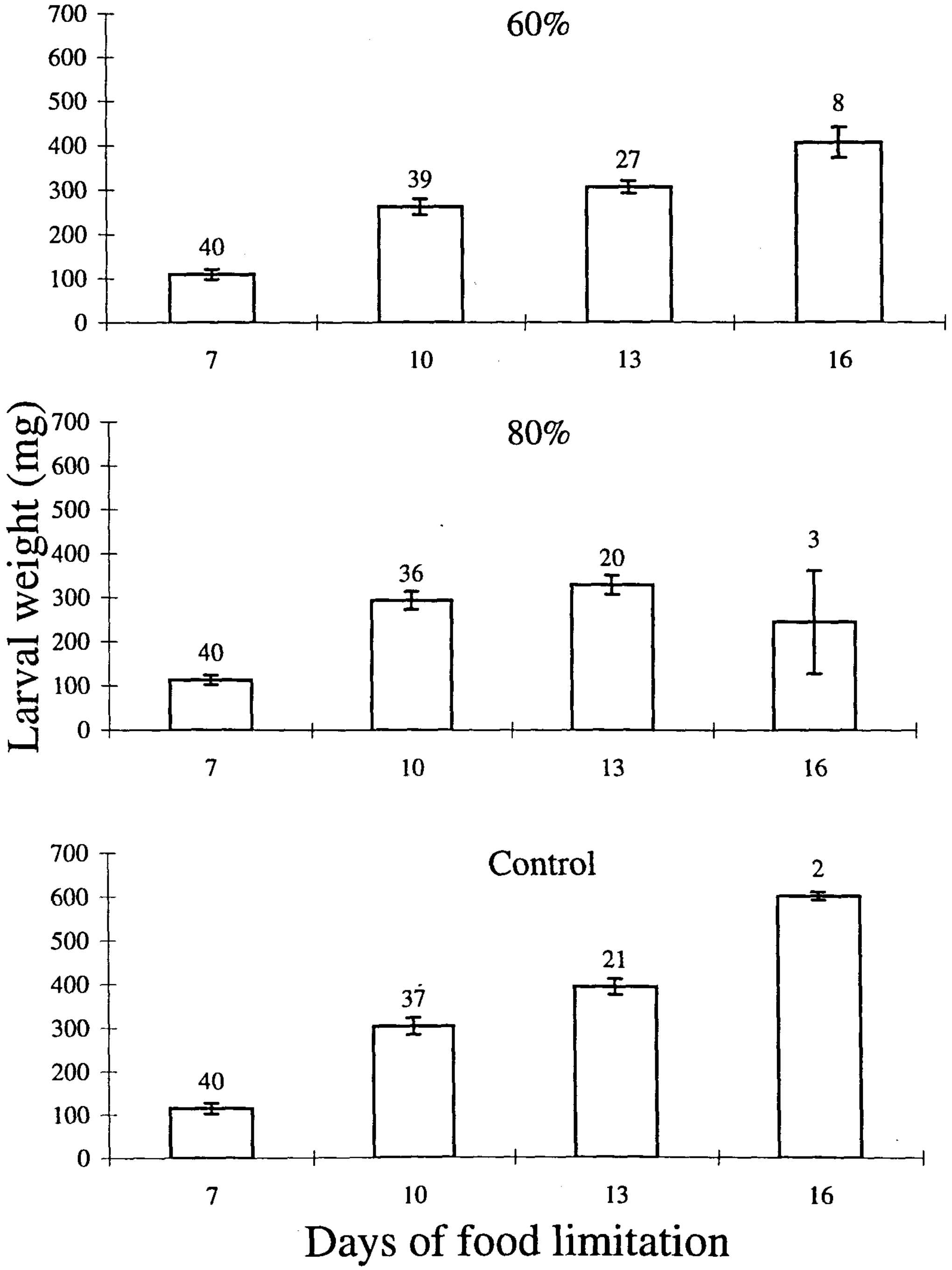

FIG. 2.--Trial II larval weights (mg) for three treatments, 60% and 80% food limited diets and *ad libitum* (control) fed to *Vanessa cardui* larvae in a May 1996 laboratory trial. Bars indicate s.e. Numbers at mean points indicate insects remaining in larval feeding at days after hatching.

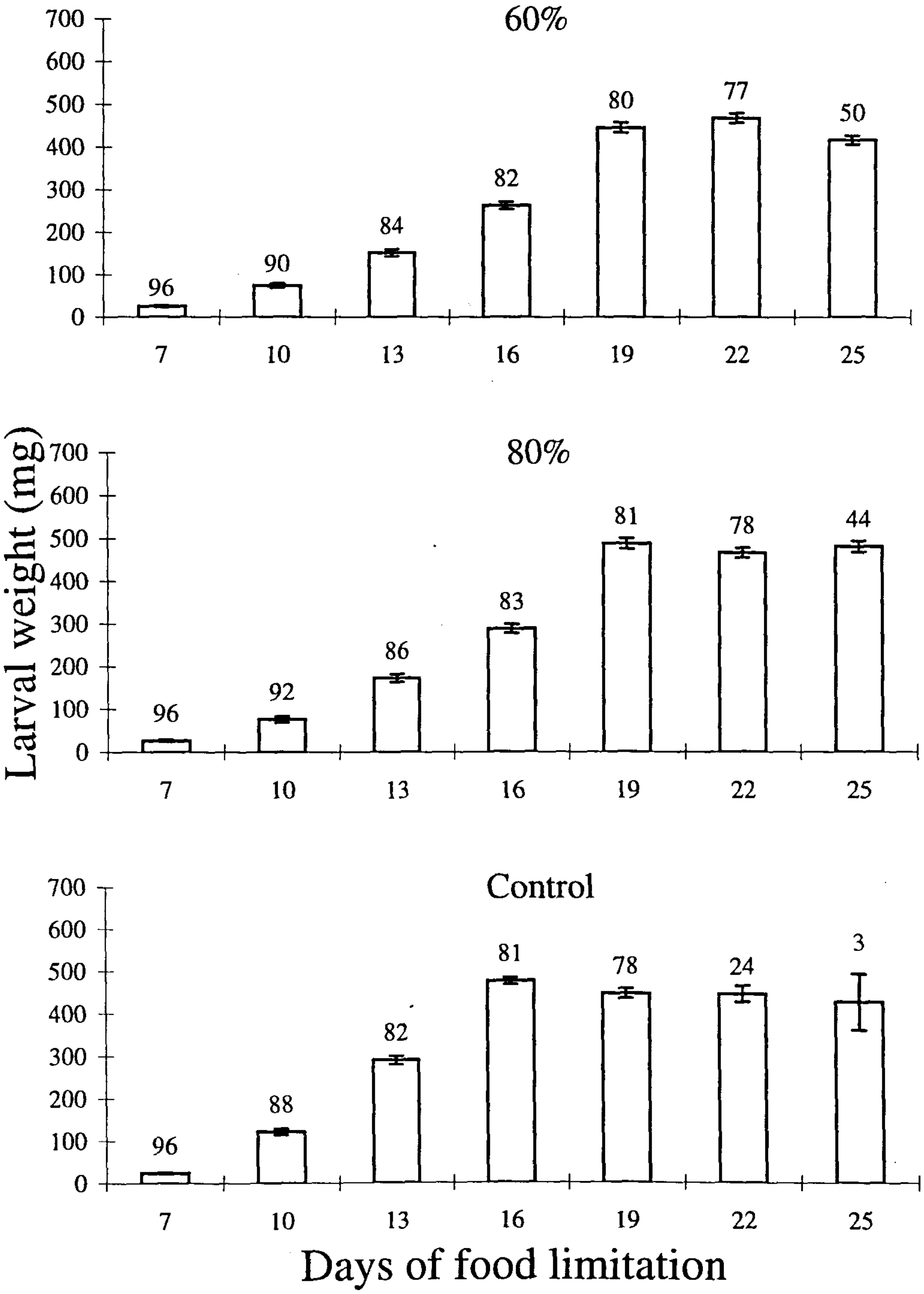

FIG. 3.--Trial III larval weights (mg) for three treatments, 60% and 80% food limited diets and *ad libitum* (control) fed to *Vanessa cardui* larvae in a May 1996 laboratory trial. Bars indicate s.e. Numbers at mean points indicate insects remaining in larval feeding at days after hatching.

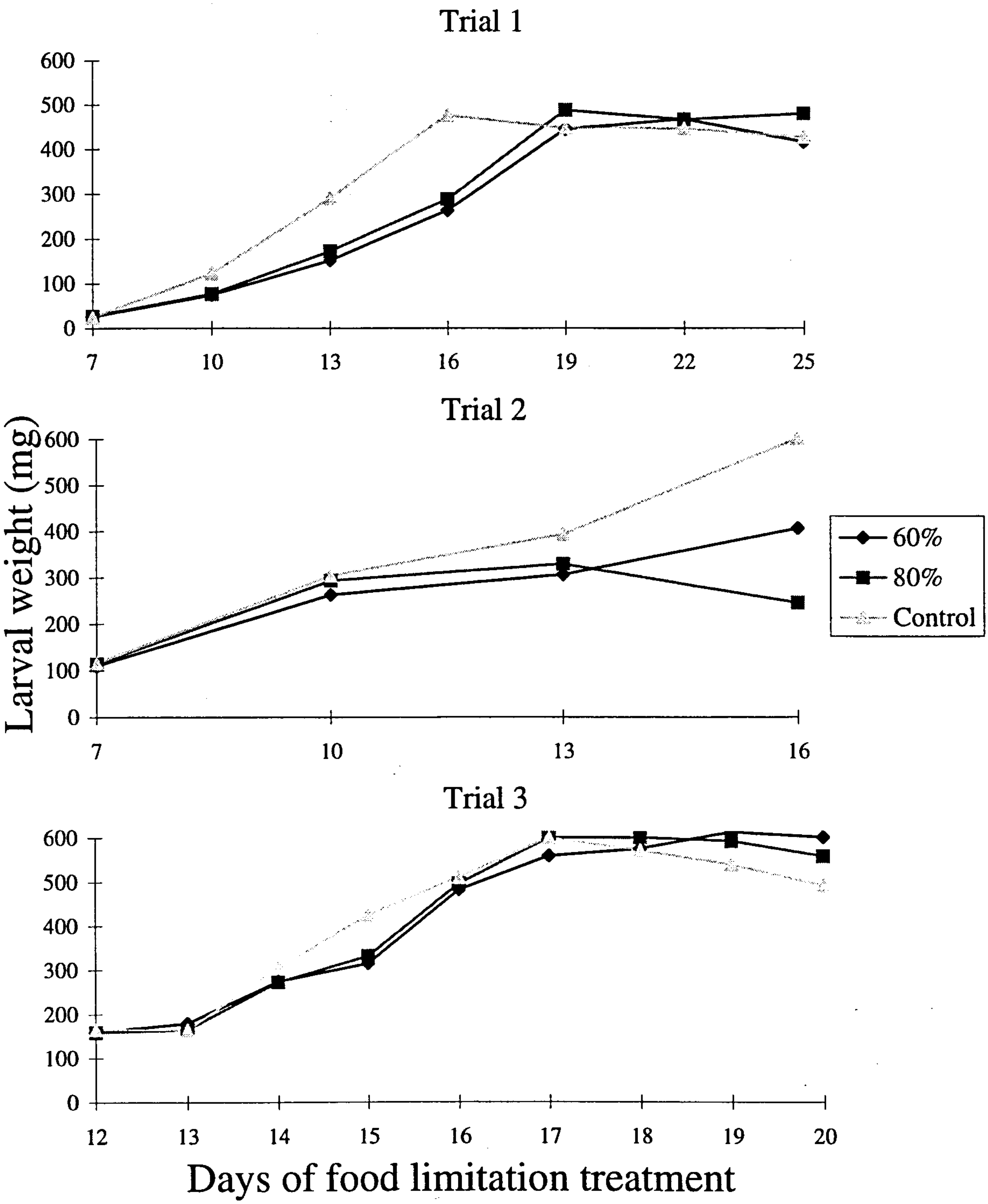

FIG. 4.--Summary of larval weights of *Vanessa cardui*, laboratory reared on 60% and 80% food limited diets and *ad libitum* (control) diet fed to larvae in all 1996 laboratory trials. Diamonds represent 60%, squares represent 80% and triangles represent controls.

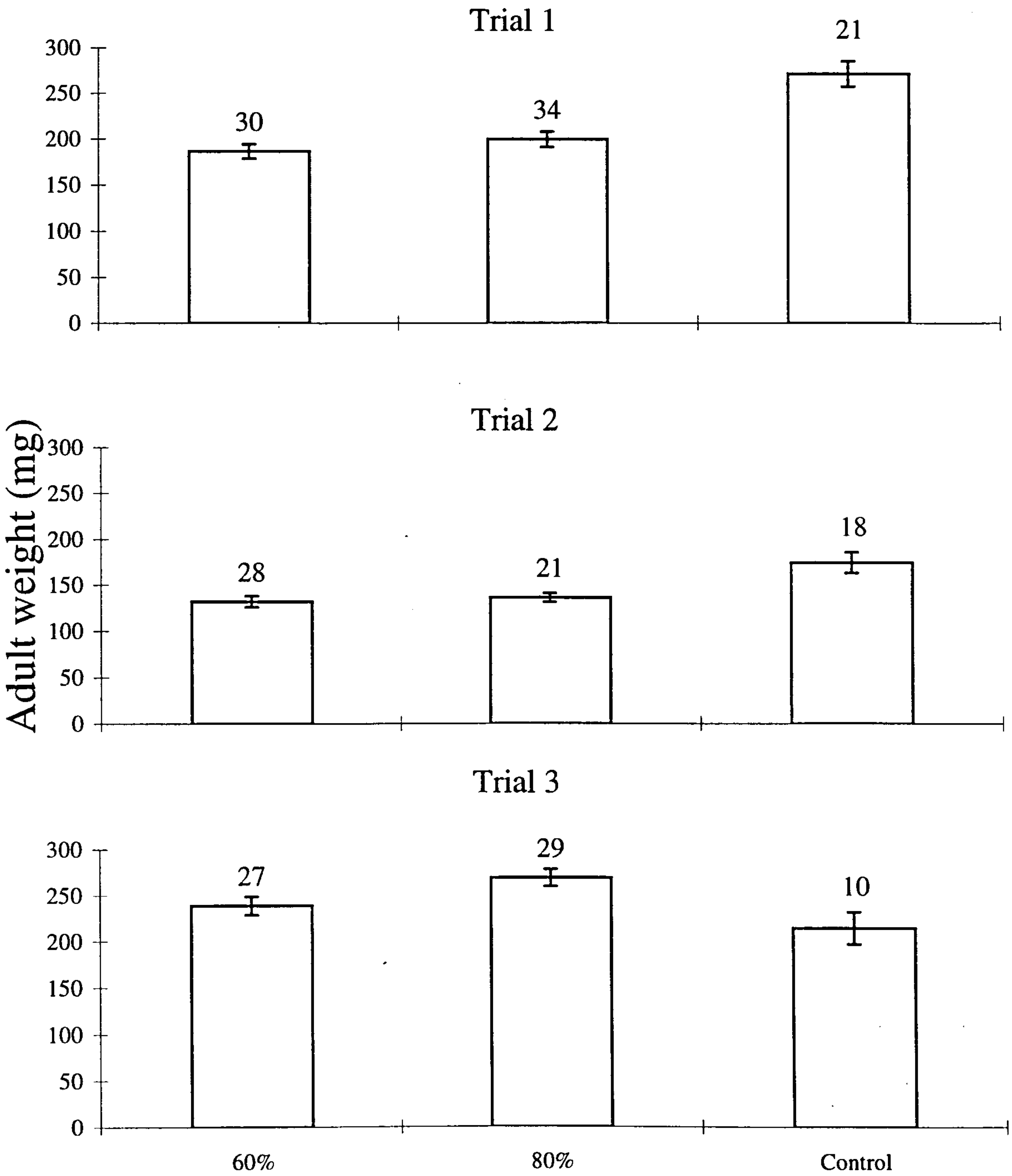

FIG. 5.--Mean adult weights (mg) for three treatments, 60% and 80% food limited diets and *ad libitum* (control) fed to *Vanessa cardui* larvae in all 1996 laboratory trials. Bars indicate s.e.

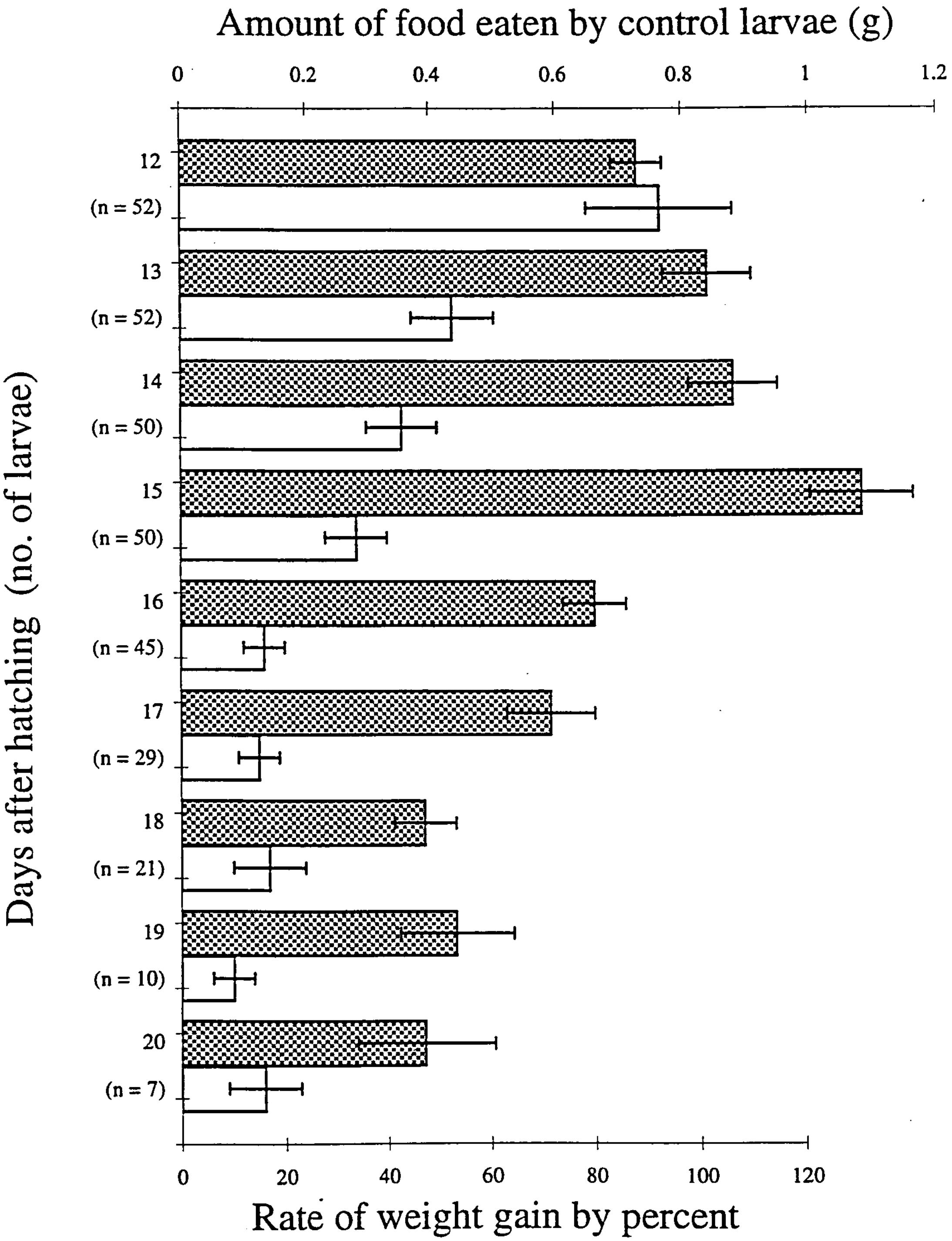

FIG. 6.--Trial three control larvae of *Vanessa cardui* reared *ad libitum*. The graph shows the relationship between the amount of food medium eaten (grams per day) in the checkered mean bars on the upper axis, and weight gain (% increase from previous day's weight) in open mean bars on the lower axis. Bars around the mean indicate s.e. Overall, mortality was low; sample size drops dramatically from day 16 to 17 due to pupation.

TABLE 1. Pupation and emergence weight of all trials, with survival data.

Trial: Treatment	% Larval Survival	Ave. Days to Pupation / SEM	% Pupal Survival	Ave. Days to Emergence / SEM	Ave. Adult Wt. (mg) / SEM
Trial 1:					
60%	41.0	25.6	30.0	34.5	186.1
(n = 100)	(n = 41)	0.2	(n = 30)	0.1	7.8
80%	38.0	23.8	34.0	33.9	199.2
(n = 100)	(n = 38)	0.4	(n = 34)	0.3	8.5
Control	27.0	19.8	21.0	29.2	271.1
(n = 100)	(n = 27)	0.3	(n = 21)	0.3	13.9
Trial 2:					
60%	82.5	15.7	70.0	24.1	131.7
(n = 40)	(n = 33)	0.4	(n = 28)	0.5	6.0
80%	77.5	14.9	52.5	23.1	135.8
(n = 40)	(n = 31)	0.4	(n = 21)	0.5	4.8
Control	75.0	14.4	45.0	23.1	173.9
(n = 40)	(n = 30)	0.2	(n = 18)	0.4	11.3
Trial 3:					
60%	68.3	17.5	45.0	26.5	238.8
(n = 60)	(n = 41)	0.3	(n = 27)	0.2	9.9
80%	55.0	17.7	48.3	26.5	268.8
(n = 60)	(n = 33)	0.3	(n = 29)	0.2	9.3
Control	53.3	17.9	16.7	27.0	214.5
(n = 60)	(n = 32)	0.4	(n = 10)	0.5	17.4

DISCUSSION

As far as measuring the effects of larval food limitation in *V. cardui*, two important concerns are the rearing temperature and the frequency with which larval weights are taken. If the insects are reared at a suboptimal temperature, the rate of gain in body mass will occur more slowly, so this will allow the effect of food limitation, if any, to be more observable.

We noted, as was found in previous studies (Poston and Pedigo 1977, Poston et al. 1978), that temperature greatly influenced the rate of larval development in *V. cardui*. Larvae did not experience dramatic fluctuations in daily temperature, but in this series of experiments, we have noted that the mean daily temperature in the laboratory was 4° C lower in Trial I than in Trials II and III. We assume this was responsible for the unusually lengthy emergence times we observed in the Trial I. Poston et al. (1977) reported a mean of 23.5 days to pupation for insects (food not limited in that study) reared at 24° C. Our protocol of sampling larval weights every three days is sufficient to detect variation in food limited larval development if the temperature is held at 18 °C but not 22°C. The insects' sensitivity to lower temperatures may allow the food limitation to have an effect. Clearly, when we measured larval development every day at 22°C in Trial III in May, this was not sufficient to detect the variation caused by food limitation. In fact, the insects reared at 22°C in Trial III reached the greatest larval weights of all three trials, with maximum weights across food limitation treatments measuring around 600 mg, roughly 100 mg greater than weights achieved in Trials I and II. Insects achieved greater mean adult weights in Trial III than in Trials I and II. However, the adult weights among treatments within Trial III did not differ significantly from one another.

Nonetheless, excessive larval mortality could have a greater effect on mean larval and adult weight than the effect of food limitation. We could offer no explanation for the result that control larval, pupal, and adult mortality rates were consistently greater than those on food limited diets. Even when mortality did not lower sample size, eventually the number of insects remaining in larval development pupated and reduced sample size. This contributed to larger error estimates at the end of Trial III, and caused the final values of larval weights to overlap among treatments. Our three trials demonstrated a wide range of results, and the trials combined did not succeed in arriving at common mean values for control or treatment peak larval mass, days to pupation and emergence, or final adult mass. However, relative to results within trials, control larvae always gained weight faster than either food limited treatment. An issue untested in this study is the effect of larval food limitation on reproductive potential. Testing reproductive potential was not possible with the present design, because no data were collected regarding egg-laying capacity of adult insects that were food limited as larvae. The only indication that larval food limited adults would lay fewer eggs was suggested (as a hypothesis that could be tested future work) as evidenced by adults that weighed less after being reared on food limited diets as larvae.

In an environment of unlimited food supply, there is an advantage to gaining weight faster than competitors: in the event of future limitations to resources, gaining weight faster can bias an individual's survival with respect to other individuals who are not able to obtain sufficient resources and either die prematurely or weigh less. Only Trial I was able to demonstrate that sufficient larval food resources was correlated to a larger adult body mass. In this series of experiments, larval food limitation did not decrease the number of insects that emerged as adults compared to control insects. Food limited insects could not eat the same volume of food as controls, but by design of this experiment, they could continue eating longer until they pupated. In a field setting, food limited larvae might have enough food to eat up to a certain point when the entire population exhausts the food supply. Then, larvae might not survive beyond the average control's time to pupation. Apparently, treatment insects in the laboratory merely changed their growth rate in response to food limitation, but did not (in every trial) exhibit lower peak larval mass or adult mass. Also in the laboratory setting described here, there was no predation, at least not treatment-biased. An extended larval or pupal stage experienced by food limited insects would subject them to greater exposure to predation in a field setting, as well as actually exhausting the larval food supply. In field settings, larvae would have poor escape abilities compared to their adult bodies, so we assume that greater mortality of food limited insects would occur in the larval rather than adult stage. In summary, our design has limited the influence of natural variables which in field settings, would effectively increase the mortality of larvae experiencing food limitation.

Time exposed to predation could be responsible for the unequal sex ratios exhibited by protandrous (male-biased, early adult emergence) insect species. In a study of protandry in insects, Nylin, et al. (1993) stated that no advantage is conferred to an individual that emerges earlier unless it can reproduce earlier. Food limitation could also explain the enormously unbalanced sex ratio of *S. idalia* that we have observed in Iowa populations of the insect. Males outnumber females ten to one in some populations (Kelly and Debinski, unpublished data), whereas the expected sex ratio in *S. idalia* observed in the field without seasonal bias is about four to one (Nagel 1991). We hypothesize a larval hostplant limitation problem that allows some males to emerge, while limiting further the adult female emergence in larval development when females are still searching intensively for hostplants. From the evidence presented in this research, the females could either delay adult emergence in response to larval food limitation or emerge on schedule with males at the expense of reduced body mass. Alternatively, over time, genetic selection to emerge earlier (before larval hostplant resources are exhausted) could possibly provide a basis for larval food limited populations to emerge earlier on average than non-limited populations and thus adult insects would weigh less. However, there is evidence to suggest that female insects cannot gain the extra mass required for egg resources without an extended larval period (Nylin 1993), so it seems unlikely that females would ultimately weigh less (no difference from local males) as a consequence of larval food limitation.

Another consideration regarding larval food limitation is the availability and quality of other hostplants. Violets are the sole hostplants acceptable to larval *S. idalia* and other members of its genus (Opler and Krizek 1984, Schull 1987). However, *V. cardui* is a larval hostplant generalist, the most widespread butterfly in the world, claiming dozens of hostplant species (Scott 1986). For any insect species, the quality of the plants can influence larval to pupal and adult survival. Larvae that choose alternate hostplants do not always derive sufficient nutrition and can accumulate adverse chemical compounds from the available second choice plants (Feeney et al. 1985, Finke and Scriber 1988). Also, the nutritional content of hostplants can cause insects to modify behavior by eating more plant material, which increases their exposure to predation (Fajer 1991). Even though this study introduces a variety of results of food limitation on one lepidopteran species raised in the laboratory, more research is required in order to draw more firm conclusions concerning the effects of larval hostplant limitation on *S. idalia.* One expects different consequences from food limitation treatment of two insects, especially given that insects may select a set of acceptable larval hostplants limited to one genus.

Larval hostplant limitation for insects has important conservation implications. First, conservation efforts should define area and biomass requirements of larval hostplants and consider the abundance and distribution of the larval hostplant within the insect species' habitat. Second, other research points to the quality of hostplants available in the habitat as a determinant of larval success. Third, further investigation is necessary to clarify the potential consequences of delayed adult emergence in insects influenced by larval food limitation. This effect warrants attention as a possible influence on fecundity of wild insect populations, if it is observed as commonly as other potential consequences of larval food limitation such as reduced body mass or mortality. Finally, other ecological effects, such as the natural history of protandry, already a strong factor in the population dynamics of some insect species (Nylin 1993), can have a synergistic negative effect when combined with food limitation to limit populations, especially of females, and could help explain inflated sex ratios observed in the field.

ACKNOWLEDGMENTS

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CHAPTER 3. EXPLORING LARVAL FOOD LIMITATION AS A PROBABLE CAUSE OF DECLINE IN IOWA POPULATIONS OF THE REGAL FRITILLARY BUTTERFLY *Speyeria idalia* DRURY (LEPIDOPTERA: NYMPHALIDAE, ARGYNNINAE)

A paper to be submitted to the *Journal of Conservation Biology*

Liesl Kelly and Diane Debinski

Abstract

Regal Fritillary butterfly, *Speyeria idalia* Drury (Lepidoptera: Nymphalidae, Argynninae), populations were surveyed in Iowa, South Dakota, and Kansas for population size using mark-recapture estimates. Individual insects were weighed to the nearest 10 mg, and abdominal, thoracic, and wing lengths as well as head capsule widths were measured to the nearest 0.1 mm. Violet densities and total biomass estimates were calculated for all sites, and these estimates of the insect's larval hostplant availability were correlated to the size of the insect. Weights of *S. idalia* were significantly less in areas of low hostplant density and abundance; Iowa prairie areas had low *S. idalia* insect sizes and hostplant populations.

Introduction

The Regal Fritillary, *Speyeria idalia* Drury (Lepidoptera: Nymphalidae, Argynninae), is an example of a prairie endemic species of butterfly that has experienced severe population declines because of habitat destruction. *S. idalia* is one of the best indicators of high quality prairie in North America (Hammond and McCorkle 1983). With the disappearance of prairie habitat, widespread populations of *S. idalia* also have declined in numbers and distribution. *S. idalia* was listed as a Category II species until 1996, under the Endangered Species Act (J. Bade, pers. com.), when this category of protection was deleted by the U.S. federal government. Category II species were species that were candidates for listing, but there was not sufficient knowledge regarding their status to warrant proposing them for listing as endangered or threatened (USFWS 1996). *S. idalia* has a special "status of concern" in national grasslands in North and South Dakota (pers. coms., Nat'l. Grasslands Visitor's Center, Murdo, SD), and the Iowa Department of Natural Resources has listed it as a sensitive species (J. Fleckenstein, pers. com., IDNR). Population estimates of this insect in Iowa and across the nation have declined sharply over the last 50 years, and our documentation of small population sizes of *S. idalia* in Iowa during 1995 (Table 1), suggests that the insect could go extinct locally.

Because we found a number of intermediate to small sized populations of *S. idalia* in Iowa in 1995 (Table 1), our research began to focus on the causes of this species' population decline. Considering many factors in our study of the insect, the natural history of the insect indicates that limited food availability to the insect could have negative effects on the population sizes in areas with low hostplant density and few nectar sources available to the adult females. Limited nectar sources have been demonstrated to have negative effects on the fecundity of adult females in another related species, *Speyeria mormonia* Boisduval (Boggs 1993). Because we observed field evidence of rather limited *Viola* populations, the insect's larval hostplant, and we noted the small size of *S. idalia* habitat in Iowa, we hypothesized that the limited availability of violets affected *S. idalia* populations in these habitat patches.

The sites in Iowa are worth comparing as a group to sites outside Iowa because the prairie areas in Iowa are isolated plots of natural areas surrounded entirely by agricultural development. Iowa prairies collectively represent an example where habitat fragmentation has apparently had a negative effect on this species. In contrast, all remaining *S. idalia* populations we surveyed in Kansas, South Dakota, and North Dakota are bordered by habitats that still accommodate *S. idalia* to some extent, albeit in lower observable densities. An exception to this was the Dorothy Akins Memorial Prairie in Douglas County, Kansas where the 17 acres of high quality prairie habitat was surrounded by wooded areas and farm fields. Significant populations of the insect can be found in Nebraska, although the prime survey time for Nebraska populations overlaps closely with our survey effort in Iowa, and thus we could not survey *S. idalia* in both regions at the same time. In summary, the current range of the insect covers the tall grass and mixed grass prairies across the Great Plains, east

Table 1. 1995 estimates of *Speyeria idalia* populations at sites in southwestern Iowa.

Prairie name	*Population estimate*	*Method*
Page Private Prairie	2	
Sheeder Prairie	50	mark-recapture
Reichelt Unit of Stephens State Forest	4	
Polk City Prairie	1	
Moeckley Prairie	220	mark-recapture
Ringold Wildlife Area	7	
Doolittle Prairie	2	
Rolling Thunder Prairie	120	mark-recapture
Kalsow Prairie	500	visual estimate[1]
Loess Hills Wildlife Area sect. 9	160	mark-recapture
Loess Hills Wildlife Area sect. 21	2	

[1] P.C. Hammond

Table 2. 1995 estimates of *Viola pedatifida* populations at sites in southwestern Iowa.

Prairie name	*Violet density estimate plants / m $_2$*	*Violet population estimate*
Page Private Prairie	1.5	95
Sheeder Prairie	1.1	39,200
Reichelt Unit of Stephens St. Forest	2.5	128,000
Polk City Prairie	0.9	310
Hawthorn Wildlife Area	1.9	424,000
Kish-ke-kosh Preserve	9.7	12,900
Raymond-Hilts Private Prairie	4.6	900
Howe Private Prairie	5.2	1,100
Moeckley Prairie	0.7	35,000
Ringold Wildlife Area	0.45	22,600
Doolittle Prairie	0.62	9,200
Rolling Thunder Prairie	1.9	210,000
Kalsow Prairie	0.48	148,000
Loess Hills Wildlife Area sect. 9	2.7	183,000
Loess Hills Wildlife Area sect. 21	1.5	98,000

Note: Sites mentioned in Table 2 but not Table 1 had no *S.idalia* insects seen in 1995.

into prairie areas that have not been completely destroyed by plowing, grazing, spraying and human development.

Speyeria idalia is an insect with a unique natural history. The adult males emerge in late June and search for females, which do not appear in great numbers for at least two weeks (Matoon et al. 1971). Females are then immediately mated. Adult females finish their cycle feeding on nectar throughout the summer until the shortening of photoperiod stimulates their oviposition period. Females then lay eggs near mid-September, with the first instar larvae hatching from these eggs in about two weeks, falling to the ground or burying themselves underground to withstand the harsh prairie winter. In the spring, surviving larvae emerge to break winter diapause and begin eating young violet leaves, their obligate larval hostplant (Schull 1987, Opler 1984).

Tall-grass prairie is deemed the primary habitat of this species (Hammond 1983, Opler 1984, Schull 1987). The range of *S. idalia* in Iowa has been limited to prairie remnants. Logically, the butterfly's presence is correlated with the presence of violets (*Violaceae*). *S. idalia*'s larval host plants include *Viola pedata* (Bird's-foot Violet), *V. pedatifida* (Blue Prairie Violet), *V. papilionacea* (Common Blue Violet), *V. lanceolata* (Lance-leafed Violet) and *V. nuttallii* (Nuttall's Violet) (Schull 1987, Opler and Krizek 1984). Regions in Iowa where *S. idalia* is most abundant contain largely Blue Prairie Violet. The other violet species tend to be found in the more moist habitats of the state, not the typical dry prairies where *S. idalia* is found. Our research suggests that the Blue Prairie Violet is not very abundant in Iowa because of its low rate of reproduction by seed propagation and poor increase in its leaf mass over the growing season. Other violets, such as the Common Blue Violet, quickly gain a much greater leaf mass and produce more viable seeds than the Blue Prairie Violet (pers. obs.), but these violets usually are not found as abundantly in *S. idalia* prairie habitat as the Blue Prairie Violet. Nor do these other violets persist in hot dry areas long into the summer as does the Blue Prairie Violet.

Conservation efforts for Lepidoptera should attempt to address the insect's behavioral as well as physiological habitat preference (Pullin 1996, Ehrlich and Murphy 1987, Weiss and Murphy 1988). In the case of *S. idalia*, males are known to patrol a territory over an open patch, suggesting behavioral reasons for the *S. idalia* preference of prairie habitat over other habitats (Schull 1987, Opler 1984). The prairie does not offer the greatest hostplant density of violets in general, but it does offer the greatest density of Blue Prairie Violets. Although the Blue Prairie Violet is the violet found in greatest density in prairies where *S. idalia* is found, our 1995 data show that the violet resource base on these prairies is not very dense, ranging from 1.7 to 4.6 plants per square meter, spread over only a few acres (Table 2). We also found *S. idalia* only on sites where violets were found, and population sizes of the insects seemed correlated with our estimate of violet densities. These preliminary observations suggested that *S. idalia* populations may be declining from inadequate hostplant abundance, because this insect selects prairie habitat where *V. pedatifida* predominates. In contrast, a woodland species, the Great Spangled Fritillary, *S. cybele* Fabricius, is found throughout Iowa. Its larvae feed on the Common Blue Violet, which is found in densities of up to 20 stems per square meter in Iowa forests (Kelly and Debinski, unpublished data). A fourfold difference in hostplant abundance could explain the relative rarity of *S. idalia* on Iowa prairies. This insect consumes a large biomass of violet leaves during the larval stage; it is one of the largest butterflies in Iowa, and certainly the largest in its genus.

Other research confirms the detrimental effects of food limitation on insects. In the Diptera, reduction in adult body size and fecundity, as well as increased larval mortality, were demonstrated from larval food limitation in a laboratory setting (Collins 1980). Nymphal food limitation was implicated in reducing the fecundity of female mantid (praying mantis) (Dictyoptera: Mantidae) in field populations (Eisenberg et al. 1981). A field study demonstrated that food limitation negatively affected bombardier beetle reproduction, and suggested that such limitation may explain spatial differences in assemblage composition among age classes of these insects (Juliano 1986).

Food limitation investigations meet the objective of determining the importance of host plant abundance to the survival and fecundity of this butterfly. Walnut Creek National Wildlife Refuge, a wildlife refuge in south central Iowa, is the newest large-scale prairie restoration project in the midwest (USFWS 1992). A long-term goal of our research is to restore areas of Walnut Creek as suitable habitat for *S. idalia*. Thus, our research on hostplant limitation is critical to the development of appropriate conservation methods, such as determining an adequate resource base for this insect.

Objectives

We attempt to focus our research on the following questions:

(1) Does the larval hostplant, Blue Prairie Violet (*Viola pedatifida*), serve as a limiting factor for *S. idalia* populations by virtue of its limited abundance in Iowa prairies?

(2) Because body weight positively influences fecundity in this insect, do adult females of *S. idalia* in smaller Iowa prairies weigh less than *S. idalia* females in areas where hostplant abundance is greater? That is, can field data demonstrate that smaller adult females emerge on prairies having lower hostplant abundances?

(3) Could hostplant limitation be a reason why the range of *S. idalia* is declining in Iowa prairies?

The objectives of our laboratory and field work were to measure the extent to which a suboptimal level of resource availability affects the emergence date, individual body weight and overall population size of *S. idalia.* These are potentially critical factors in determining why the *S. idalia* populations are declining in Iowa. As a means of comparison, we investigated these survival and fecundity traits in *S. idalia* from eight sites in Kansas, South Dakota, and North Dakota, where hostplant abundance is greater than in Iowa.

Methods

To establish evidence of the effects of food limitation on the development of *S. idalia* in Iowa's wild populations, we measured length of wings (as in Boggs, 1988), thoracic and abdominal, and head capsule width of individual *S. idalia* adults at eight field sites in Iowa, and compared these with those of adult *S. idalia* adults captured on eight sites in Kansas, South Dakota, and North Dakota. Field sites in Iowa were chosen after the 1995 field season when we surveyed over 50 prairie areas in Iowa to determine which sites had hostplants of violets and of those, which had *S. idalia.* Several sites examined in 1995 reported only a few, or even one, male insects present. Then, we examined prairie areas in some of the states with larger *S. idalia* populations. We chose sites outside Iowa known to have relatively large, regularly present populations of *S. idalia* as comparison sites for Iowa prairies to test our food limitation hypothesis.

Our investigation of hostplant abundance considered the number of violet stems per square meter as well as a measure of violet presence over the whole site. Violet species found in Iowa and Kansas were *V. pedatifida* and occasional small patches of *V. papilionacea*, whereas the predominant species found in the South Dakota and North Dakota prairies was *V. nuttallii.* Stem-per-quadrat measurements of the dominant violet species were recorded in the alternate meter squares of each of five 10 m X 10 m plots (a total of 50, 1 m^2 subplots per 100 m^2). These plots were located in the areas of highest violet density. In Iowa prairie remnants, we identified the extent of violet coverage on all of the sites we visited in order to predict the total biomass of violets available to the *S. idalia* populations present. We determined the presence or absence of violets in 100, 1 m^2 points at each site. We arrived at a rough percent estimate of total violet coverage by evenly spacing the 100 points across the prairies, which ranged from 10 to 200 acres.

From these initial data, we calculated estimated hostplant abundance by multiplying the percent coverage estimate by the hostplant density estimate (the average of five 10 m X 10 m plots) by the number of acres of habitat present or surveyed. We were not able to survey *S. idalia* in areas larger than about 200 acres. We therefore estimated violet populations in the areas surveyed for insects, and also in the total habitat area. In Table 3, "Habitat Hectares by Coverage" reflects the number of violet plants in the entire area of contiguous grassland habitat (areas of 6.9 to 25, 911 ha), while "Survey Hectares by Coverage" reflects the violet population in the area we surveyed for insects (areas of 6.9 to 64.8 ha). In large areas of habitat, we surveyed *S. idalia* populations in areas of 64.8 ha (200 acres) as determined with landmarks and section lines corresponding to detailed maps. In sites of less than 64.8 ha area, we surveyed the entire habitat for *S. idalia.* Ultimately, we used the two violet population estimates to examine the correlation between the hostplant abundance at each site with the *S. idalia* population estimate.

Adult *S. idalia* at each site were captured with a field net, placed in a glassine envelope and weighed using a medium resolution electronic scale with precision to the hundredth of a gram and repeatability to 0.005 g. The weight of the envelope was voided to arrive at the weight of the insect. Other body measurements of abdominal, thoracic, and wing length, as well as head capsule width, were taken with dial calipers to the nearest 0.1 mm. Head capsule width in both larval and adult Lepidoptera have been noted as measures of development and nutrition (Bastian and Hart 1990, Charlet and Gross 1990, McClellan and Logan 1994). All measurements were performed prior to the oviposition season, which begins at the onset of shortening daylight length in early fall (Matoon et al. 1971). Thus, the abdominal length measurements reflect pre-reproductive body dimensions.

On the first day of a mark-recapture exercise, each insect was marked with a Sanford® Sharpie® ultra fine permanent marker and released for potential recapture. These marks may remain on a living insect indefinitely and have been demonstrated not to induce mortality or to limit flight mobility (pers. obs., Nagel 1991). Our sequence of site surveys across the region began with the sites in Kansas, followed by Iowa and South Dakota moving from south to north, so that we could arrive at population size estimates that included both males and females after initial emergence. Each site was surveyed a total of six person-hours per day, usually two consecutive days in the mark-recapture experiment (see results for dates). The mark-recapture effort amounted to only two days per site in order to accommodate 16 sites surveyed by the same research team in the short flight season that *S. idalia* exhibits during the summer.

Results

Violet Density

All areas surveyed for *S. idalia* were surveyed for violets as well (Table 3). Unfortunately for purposes of our comparison, the western areas with greater *S. idalia* populations had a different species of violet, Nuttall's Violet. However, we were interested in total violet biomass in relation to the *S. idalia* populations we found at those sites. Because the total leaf area of plants we counted in sites with the Nuttall's and Blue Prairie

Table 3. 1996 violet density estimates, violet presence over entire area, and violet biomass estimate.

Viola spp.	*Survey Locations*		*Five Plot Density stem/sq.m*	*100 Point Coverage as a percent*	*Hectares of Habitat*	*Hectares Surveyed*	*Habitat Hectares by Coverage (Total number of violet plants, an est.)*	*Survey Hectares by Coverage*	S. idalia *1996 pop. est.*
V.peda.	**Iowa**	Loess Hills, Section 21	1.04	0.32	101.2	20.2	338000	67500	175
V.peda.		Sheeder Prairie	0.52	0.43	10.1	10.1	22800	22600	163
V.peda.		Stephens State Forest	2.38	0.07	404.9	8.1	673000	13500	21
V.peda.		Ringold Wildlife Area	1.02	0.13	485.8	64.8	644000	86000	270
V.peda.		Cayler State Preserve	0.44	0.20	64.8	64.8	57500	57500	319
V.peda.		Anderson State Pres.	0.49	0.18	64.8	64.8	57400	57400	494
V.peda.		Kalsow State Pres.	0.55	0.38	64.8	64.8	136000	136000	224
V.peda.		Rolling Thunder	2.36	0.36	49.8	49.8	423000	423000	60
V.nuttallii	**South Dakota**	Murdo	2.78	0.13	485.8	64.8	1760000	234000	435
V.nuttallii		Wall	6.26	0.28	48.6	64.8	852000	1140000	333
V.nuttallii		Harp Dam	5.52	0.33	323.9	64.8	5900000	1180000	473
V.nuttallii		Richland Dam	5.08	0.24	161.9	64.8	1970000	790000	571
V.nuttallii		Antelope Creek	5.86	0.22	81	64.8	1040000	835000	211
V.nuttallii	**North Dakota**	Sheyenne Nat'l Grass.	5.56	0.65	25910.9	64.8	936000000	2340000	564
V.peda.	**Kansas**	Dorothy Akins	1.60	0.40	6.9	6.9	44300	44300	98
V.peda.		Konza Prairie	3.50	0.53	3481.8	64.8	64600000	1200000	~

~ No population estimate possible without recaptures.

Violets was quite similar (pers. obs.), we grouped the violet statistics for each site as if we were counting the same plant species.

The five plot areas chosen as violet density estimates indicated areas with the greatest density on the whole prairie. When the entire area of the prairie where *S. idalia* was surveyed is included in a comparison between total hostplant and insect abundance, a thinly positive ($r^2 = 0.46$) correlation exists (Table 3: column "*Survey Hectares by Coverage*").

S. idalia Population Sizes vs. Habitat Area

We found large populations of *S. idalia* across tall and mixed prairies of larger areas (Table 4). One relationship we found was the link between the size of *S. idalia* male population estimates and area surveyed. This yielded the greatest correlation we observed between insect and hostplant populations ($r^2 = 0.75$, $P = 0.17$, df = 11). This corresponds to the behavioral observation that the male insects patrol territories across the prairie. Thus, the more area we surveyed, the greater our success in marking male individuals. The females, in contrast, exhibited almost no territorial behavior, and to complicate our efforts to estimate their populations, the females did not appear in sufficient numbers to provide reliable population data in Iowa prairies. We should have encountered more females in the Iowa prairies, because we spent over a month at various locations. At some point during the season, some of the sex ratios should have approached equity, though this never happened at sites we visited in Iowa. Lincoln-Petersen estimates (Table 4) include both male and female insects.

In contrast, we happened to arrive at the peak of female populations in South Dakota and North Dakota in mid-August of 1996. Here, the females outnumbered the males in all the sites we visited. None of the grasslands in either states were isolated patches such as the areas in Iowa, with the exception of the Wall, South Dakota site. This area had many *S. idalia* in a brushy drainage, but not nearly as many were found in the surrounding, shorter grass grazed area that isolated the drainage. This latter site yielded the highest male recapture rate (24%) of all the North Dakota and South Dakota sites. The contrast in quality of surrounding habitat seemed to influence recapture rates and thus population estimates.

Table 4. 1996 Mark-recapture raw data and Lincoln-Petersen estimators of 16 *S. idalia* populations.

Survey locations		Mark, recapture dates	Male first mark n_1	Female	Male second mark n_2	Female	Male recapture m_2	Female	Lincoln-Petersen pop. est.	Hectares surveyed	Insect density est. (insects / ha)
Iowa	Loess Hills, Section 21	24, 25 July	17	4	18	4	3	0	175	20.2	8.7
	Sheeder Prairie	19, 23 July	52	0	32	0	15	0	163	10.1	16.1
	Stephens State Forest	17, 18 July	7	0	6	0	3	0	21	8.1	2.6
	Ringold Wildlife Area	6, 8 July	59	1	42	0	12	0	270	64.8	4.2
	Cayler State Preserve	31 July, 1 August	36	1	60	1	8	0	319	64.8	4.9
	Anderson State Preserve	1, 2 August	65	2	50	1	8	0	494	64.8	7.6
	Kalsow State Preserve	30, 31 July	31	1	30	0	5	0	224	64.8	3.5
	Rolling Thunder	26, 29 July	16	2	11	3	5	1	60	49.8	1.2
South Dakota	Murdo	7, 8 August	32	36	24	30	7	3	435	64.8	6.7
	Wall	9, 10 August	27	34	22	27	7	4	333	64.8	5.1
	Harp Dam	9, 10 August	17	28	14	24	1	3	473	64.8	7.3
	Richland Dam	10, 11 August	6	45	10	41	1	4	571	64.8	8.8
	Antelope Creek	11, 12 August	15	29	12	26	3	7	211	64.8	3.3
North Dakota	Sheyenne Nat'l Grass.	12, 13 August	14	22	10	34	1	2	564	64.8	8.7
Kansas	Dorothy Akins	13, 14 July	49	13	11	7	31	0	98	6.9	14.2
	Konza Prairie	13, 14 July	40	24	38	32	0	0	~	64.8	~

~ No population estimate possible without recaptures.

That is, if very low quality habitat surrounded the patch we surveyed, we were more successful in recapturing insects. Perhaps this result was an effect not only the of total area of habitat used by the insect, but the isolation of that habitat. All analyses of correlation between recapture rates and habitat area or area surveyed yielded only slightly positive values ($r^2 \leq 0.49$, $P = 0.22$, df = 11).

Insect Body Measurements

Insects of Iowa prairies where we considered food limitation to be a problem (Loess Hills, Sheeder Prairie, Stephens State Forest, Ringold Wildlife Area, and other areas surveyed in 1995 that produced few or no *S. idalia* insects) did produce insects of smaller weights (see Fig. 1) among both males and females, in contrast to the prairie areas we surveyed in Kansas, North Dakota, and South Dakota. T-tests were used in statistical analyses and Iowa insects were grouped in comparison to insect populations from the other states. Males and females were analyzed separately because of their invariable difference in mean weight at any given site. Iowa male weights averaged 0.33 g, or less than North Dakota and South Dakota male weights at 0.37 g and Kansas weights at 0.38 g, for a difference significant at the $p < 0.0005$ level (same results with separate t-tests: H_A $\text{weight}_{IA} < \text{weight}_{SD}$, df = 125 and H_A $\text{weight}_{IA} < \text{weight}_{KS}$, df = 149). Iowa female weights averaged 0.50 g, or less than North Dakota and South Dakota female weights at 0.54 g, for a difference significant at the $p < 0.0025$ level, df = 30, and less also than Kansas weights at 0.58 g, for a difference significant at the $p < 0.0005$ level, df = 30. Of the other body measurements, only the male wing length data indicated a pattern among Iowa insects versus insects from sites outside Iowa (Figs. 2 & 3). Iowa male wing lengths averaged 41.1 mm, or less than North Dakota and South Dakota male wing lengths at 42.6 mm and Kansas wing lengths at 43.9 mm, for a difference significant at the $p < 0.0005$ level in both comparisons: H_A $\text{wing length}_{IA} < \text{wing length}_{SD}$, df = 125 and H_A $\text{wing length}_{IA} < \text{wing length}_{KS}$, df = 149.

None of the other insect body measurements yielded significantly different means from sites of each region. The correlation between the insect body mass and any other

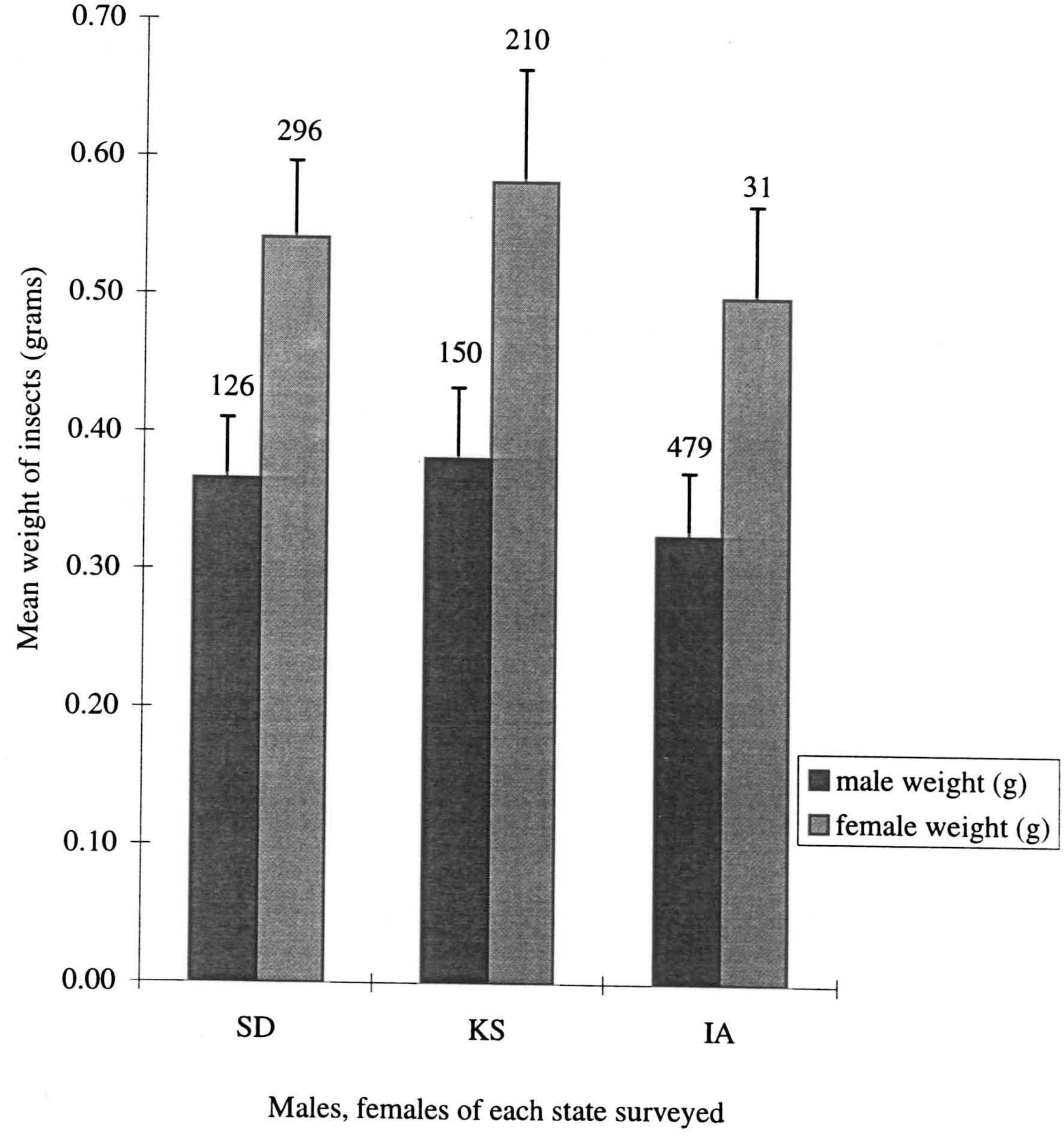

Fig. 1. Male and female mean weights (g) of Regal Fritillary butterflies (*Speyeria idalia*) butterflies from Iowa, South Dakota and Kansas in 1996. Bars indicate standard error and numbers indicate total insects weighed at all sites in that state.

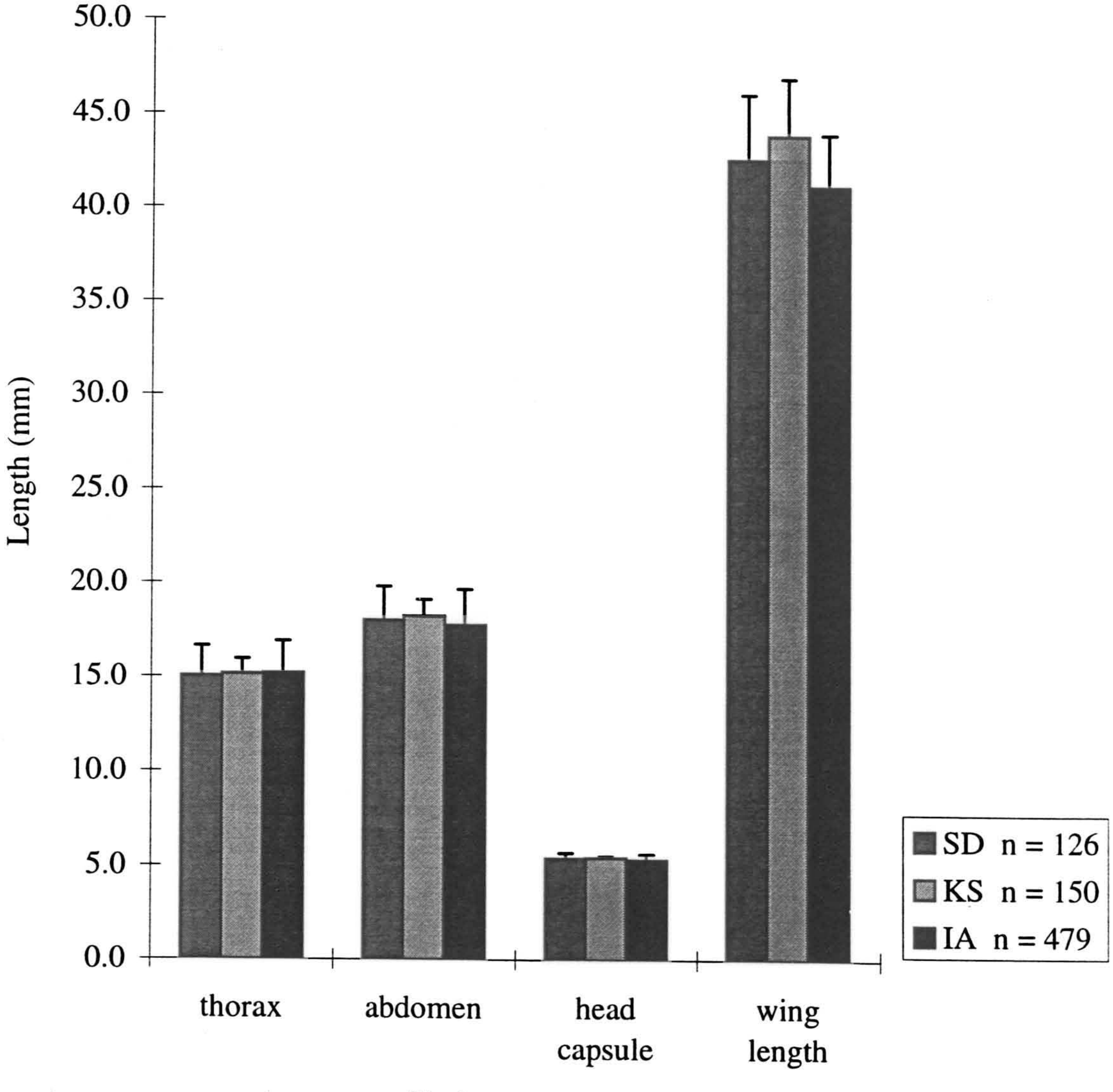

Fig. 2. Male Regal Fritillary butterfly (*Speyeria idalia*) body measurements of insects from Iowa, South Dakota, and Kansas in 1996. Bars indicate standard error. Note sample size in legend.

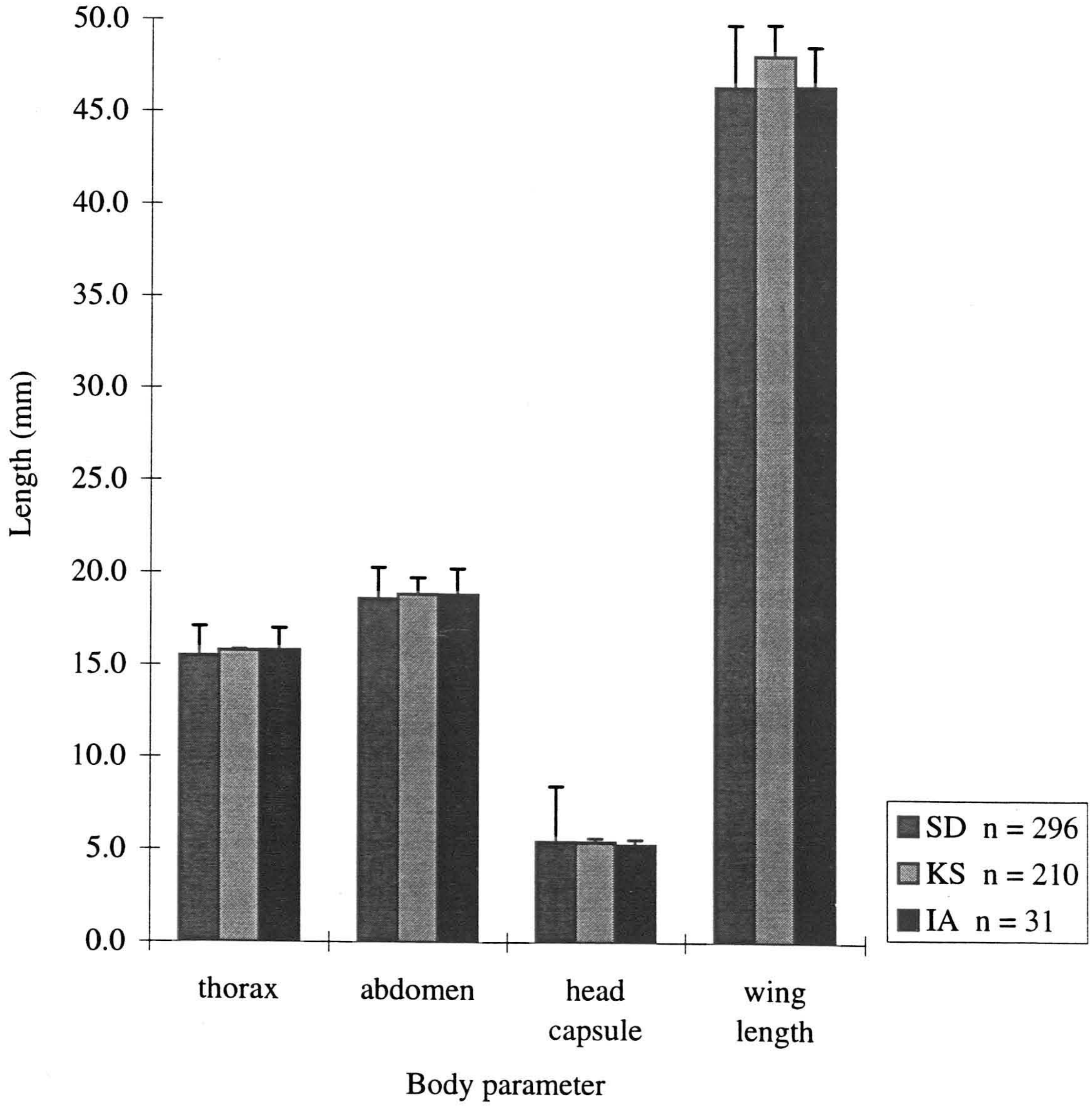

Fig. 3. Female Regal Fritillary butterfly (*Speyeria idalia*) body measurements of insects from Iowa, South Dakota, and Kansas in 1996. Bars indicate standard error. Note sample size in legend.

parameter we measured on the insect was generally between $r^2 = 0.60$ and $r^2 = 0.70$ at all sites surveyed for *S. idalia*.

Discussion

We realize that the estimate of total number of violet plants may not precisely predict the population size of *S. idalia* that may be potentially supported by the habitat. One problem in the violet data analysis is the approach of using the locally greatest densities as an estimate of how dense the coverage is over the entire prairie area (where *S. idalia* is found). A more accurate statistical estimate of the whole violet population over the entire prairie area was desirable, but not practical to obtain. The accuracy of total abundance estimates is challenged by the variation in density of violets across the entire habitat, while our estimate infers that the areas of greatest violet density are representative of the entire habitat. This factor may influence the degree to which our estimates of violet abundance are correlated with *S. idalia* populations.

However, there may be a threshold value of total hostplant abundance necessary to support a *S. idalia* population and this value certainly would provide useful information in conservation decisions. It is worthy to note that three other areas (not included in the 1996 mark-recapture comparisons or insect measurements) in Iowa with violets had only a few *S. idalia* individuals present, with hostplant abundances around 125,000 plants, while two sites with an estimated violet population around 13,000 had no *S. idalia* insects at all.

Nearly all body measurements were predictable from the weight of the insect, although no substantial variation existed in thoracic length, abdominal length, or head capsule width. This may indicate that a genetic mechanism regulates the observable size of the body parameters of thoracic and abdominal length as well as head capsule width. Otherwise, with the exception of wing length and overall insect weight, we could not discriminate the means of other body parameters we measured according to locations or sexes. We attribute the variation in insect weight among sexes to the greater abdominal mass (not necessarily length of body segments) of a female developing eggs. This extra mass associated with females may require them to have larger wings than males in order to fly more effectively. It is not clear how the other parameters of thoracic, abdominal length, or head capsule could contribute to the insect's ability to fly farther or carry more eggs. Therefore it is not surprising that our data show little variation in such measurements.

Reasons for Decline of *S. idalia* in Iowa and Possible Management Solutions

Hammond and McCorkle (1983) attribute the decline of a number of *Speyeria* populations to the extent of detrimental environmental disturbances caused by humans. In Iowa prairies, the erratic distribution as well as low abundance of violets may be responsible for the small *S. idalia* populations. One typical cause of this erratic and often restricted distribution of violets is an episode of great disturbance to the prairie such as plowing. Many of the sites we surveyed in Iowa have extensive areas of invasive Brome Grass (*Bromus spp.*) which have few if any violets in them, but *S. idalia* adults often patrol these areas and feed at the nectar sources present in them. Such areas with no violets may be detrimental to female fecundity if adult females spend significant time searching there in vain for areas with hostplants where they could deposit eggs.

In summary, even 16 sites inclusive of violet and insect data proved insufficient to draw the conclusion that total violet biomass estimate could predict *S. idalia* population size. Other factors obviously enter into the process of predicting an expected population size of this insect. For instance, one site in the Loess Hills Wildlife Area (section 9) in Monona County, Iowa had been burned in early 1996, and the fire may have killed most of the *S. idalia* larvae. This could explain the low population size at that site. A similar explanation may be suitable for the Hawthorn Wildlife Area in Mahaska County, Iowa, where a large *V. pedatifida* population consistently has been exposed to fire as a management tool to limit brush encroachment by *Rubrus spp.* Fire management may explain the low insect population at this site as well. Our estimates indicate that an adequate hostplant population exists.

One cannot rule out the possibility that some habitat areas where *S. idalia* is found in small numbers could be so isolated from an historic distributional range of the insect that only a few males migrating in search of mates would falsely imply evidence of a hostplant limitation. We need a greater number of sample sites to resolve this potentially confounding effect. The extent of *S. idalia*'s decline and population isolation in Iowa already has reached a point where its distributional range could be excluding the insect from otherwise suitable habitat. When one considers that the available hostplant and adult nectar resources of some sites in Iowa are marginal, their isolation from sites with stable *S. idalia* populations may not allow immigration to occur. Genetic analysis may in the future be useful in determining whether small *S. idalia* populations arose from stable adjacent populations of the insect.

Two unanswered questions remain. First, how does the limitation of larval hostplants affect this insect? Quantifying not only the total biomass required to support one insect through its life, but also an entire population, would lend helpful evidence to conservation efforts. Second, knowing specifically how the effects of food limitation are manifested in an individual insect (number of eggs laid, total lifespan, delayed emergence, etc.) versus a population (adverse fluctuation in population size from year to year, or perhaps unequal sex ratios) would help in gathering more meaningful field data and interpreting results with more of a cause than correlation in mind. For example, we know that males emerge sooner (at least two weeks) than females. If males are able to accomplish this by consuming larval hostplant resources faster than females, a hostplant limitation problem could be caused by females lacking sufficient resources to finish their larval stage. This may explain why the Iowa population data we observed had so few females. (At one site, at the peak flight period, we were able to catch 85 males and not a single female in two days. Overall in Iowa, after spending nearly a month surveying areas in the state, we caught 479 males and only 31 females.) We would require laboratory data to justify the hypothesis that males can consume hostplant resources more efficiently than females. Alternately, females could emerge later because of a longer developmental process achieved solely in the chrysalis stage. This would lend little meaning to a larval hostplant limitation effect being responsible for an inflated sex ratio of Iowa *S. idalia* populations.

Ideally, we would use examples of stable *S. idalia* populations to make management decisions regarding restored prairie areas in Iowa. Laboratory data would be helpful in the assessment of how much violet biomass is required to support the insects. Field work suggests that the hostplant density and nectar availability could be just as important to the persistence of *S. idalia* populations as the amount of habitat available. Ultimately, we are interested in a threshold value of violet mass we should consider establishing or preserving to restore prairie areas with *S. idalia* populations. We need to consider also the quality of surrounding habitat available to the insects, as well as their isolation from other *S. idalia* populations which may potentially immigrate to newly established habitat patches. Models may be helpful in determining how likely certain population sizes would go extinct. Of course, more long-term population data would assist this goal as well.

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CHAPTER 4. GENERAL CONCLUSIONS

The two parts of this study have revealed much information concerning the potential for larval food limitation as a negative effect in Iowa populations of *Speyeria idalia* (Drury) (Lepidoptera: Nymphalidae: Argynninae). The field study confirmed larger, denser populations of both the insect and its larval hostplants in more continuous habitat areas outside of Iowa. The laboratory portion of the study suggested different results than expected regarding the effect of food limitation on insect development. This knowledge will be useful in interpreting results of *S. idalia* reared on suboptimal food limitation, a future phase of this research. Three goals remain after completion of these two parts of the research effort: to apply the laboratory and field work, to obtain more long-term research data on *S. idalia* populations, and to restore *S. idalia* populations in Iowa to more stable sizes.

From the experiments involving *Vanessa cardui* (Linnaeus) (Lepidoptera: Nymphalidae), we have established that there is some plasticity of body size in response to larval food limitation, especially when reared at different temperatures. Also, the rate of gain in larval mass occurs more rapidly at non-limited food treatments. Using the knowledge gained from rearing *V. cardui* in the laboratory, we would like to make similar predictions concerning the size of *S. idalia* insects raised in the laboratory. *S. idalia* insects have some unique life history traits that distinguish their laboratory response from that which we observed in *V. cardui*. *S. idalia* must undergo a larval diapause that involves torpor-like conditions before first instar larvae will begin feeding (Matoon et al. 1971). *V. cardui* however, will not tolerate low temperatures at any stage in development (Poston et al. 1977). This difference of temperature influence will likely confound the results of insect body mass of *V. cardui* reared under larval food limitation treatments as observed in the three trials we performed here. Our hypothesis is that *S. idalia* would be less affected from being reared at various temperature regimes in the laboratory because of its adaptation in the wild to variation in temperature and a winter diapause. The chemical basis for this difference may be the contrast in these species' ability to store and utilize fat reserves at lower temperatures. This remains unknown, a result we would seek to establish in future laboratory work. Also in future laboratory studies, we must quantify the extent to which smaller adult insect body size (if indeed this is achieved in *S. idalia*) reduces the number of eggs laid per female compared to females reared *ad libitum* on larval hostplants. Establishing this is critical to demonstrating that larval food limitation has detrimental effects in the adult female populations of *S. idalia* in Iowa.

Recommendations for Future Research

The next step of this effort is to relate the results of the laboratory work to the field work in a broader sense. We have evidence that areas in Iowa are food limited; they have inadequate larval hostplant resources to sustain large populations of *S. idalia*. The *S. idalia* populations are very small in Iowa, and individual insects in Iowa are smaller in body size than their conspecifics in surrounding states. We need to confirm *which* parameters of *S. idalia* life history are most affected by larval food limitation. If body size is one of the parameters, then how much reduction in body size could contribute to infertility, shorter life span, smaller abdomen, and thus reduced number of eggs laid? At this point in the research, no mechanism is outlined for the reduced population sizes of *S. idalia*. One hypothesis is that the females require an extended larval stage to accumulate enough fat reserves to lay eggs (Nylin 1993). If hostplant resources are limited in this insect, and given that males emerge prior to females in this species, it is reasonable to suspect that females are suffering a deficit of larval food availability at a critical point in their development (fifth instar). This would reduce the number of eggs a female from larval food limited areas could lay. The female populations are on an order of magnitude smaller than males at *every* site in Iowa we observed at in the course of a month. We covered the major flight period in Iowa and should have seen more females emerge had more been present. We need to establish laboratory evidence that larval food limited insects weigh less as adults and cannot lay as many eggs as control females.

In addition to the data established from field work in the last two years, *S. idalia* populations in Iowa need to be monitored over the course of several years. This is necessary to establish the cause of small populations and adult insect body size as food limitation, instead of genetic selection, random genetic drift, or environmental influences. In reality, field populations of animals are not infinitely large and sizes are rarely constant (Hartl 1994). Therefore, before making any long-term conservation decisions regarding the restoration of this insect, we should establish long-term data on the size of naturally present populations. The possibility of random genetic drift causing smaller body size in both females and males of Iowa *S. idalia* insects seems an unlikely scenario because each site tested in Iowa had insects of smaller body size than *S. idalia* insects in other states. How then, would all sites of small populations in Iowa randomly have smaller sized insects? Instead, under a random drift scenario, fixation of genes that control body size would lead to some populations of larger and some populations of smaller sized insects within Iowa. Another hypothesis to explain the smaller insect size of *S. idalia* observed in Iowa is the genetic selection of smaller adult body mass. This seems unlikely, and contrary to theory regarding reproductive strategy of this insect. The "sweepstakes" reproductive strategy of *S. idalia* (Wagner et al. 1994) suggests that females lay a considerable number of eggs to compensate for tremendous larval mortality in the next generation. Some insects that experience food limitation in the larval stage will sacrifice adult body size and *number* of eggs laid, but not size of the individual eggs (Collins 1980, Boggs 1989). How then could genetic selection favor the trait of reduced body size which would likely limit rather than increase the number of eggs produced? Rather, we propose that the reduced adult body size of *S. idalia* insects observed in Iowa is caused by an environmental factor of limited food resources. Ideally, with further laboratory evidence, we seek to discern the environmental from genetic effects of larval food limitation in *S. idalia*.

Several areas of the field research require further evidence before more progress can be made in the conservation of *S. idalia*. First, we lack sufficient evidence concerning the extent of dispersal from one study site to another. Some of the sites we surveyed were situated within two miles of other comparable habitat area, although of all study sites where we weighed insects and performed mark-recapture estimates, the nearest distance between sites was 20 miles, and the average site was at least a county away from its nearest neighboring site. We would like to know dispersal distance, *i.e.* how far an insect can fly to a neighboring location and lay eggs. This information would indicate the likelihood that a site where the insect goes extinct could be colonized by a neighboring *S. idalia* population. We could establish this information by conducting a massive release of a marked *S. idalia* population (laboratory reared individuals) and monitoring the surrounding habitat for the appearance of these marked individuals. We could then assess dispersal distance for this insect species. Similar work was performed on the Bay Checkerspot, *Euphydryas editha bayensis* Scudder (Lepidoptera: Nymphalidae), (Harrison 1989). Another concern we have for the quality of the field sites *S. idalia* inhabits is the availability of nectar sources. For instance, what are the nutritional qualities of the flowers in these habitats? Do they provide sufficient sucrose for egg maturation in adult females (studied in Hainsworth et al. 1991, Hainsworth and Hamill 1993)?

Another concern with future field work involving *S. idalia* populations in Iowa is whether the adult weights remain constant over time, or whether insects gain or loose weight over time relative to egg production, nectar availability, etc. At this time we have no evidence supporting or refuting this claim. A longer mark-recapture effort involving fewer sites more intensely surveyed was suggested by colleagues at Iowa State University. This would allow an observation of weight change in individual insects as well as a closer monitoring of the emergence of females, a critical problem in the conservation of Iowa *S. idalia* populations. As mentioned in chapter three, only 31 female insects were captured in Iowa sites where nearly 500 males insects were captured. We did not see many more females that we were not able to capture.

The issue of such a skewed sex ratio is of real concern to conservation efforts. Our data suggest that future populations of this insects depend annually upon the success of less than 100 females in Iowa. This is not exactly an ideally sized gene pool for a rare insect with annually fluctuating population sizes that is also experiencing habitat loss. Habitat loss is a related conservation concern. In future years, we would like to rear adult females in the laboratory for release to restored prairie sites in Iowa to increase population sizes and stability of this insect. It is fundamental that we understand more about the role of female fecundity in declining *S. idalia* populations in Iowa before we can begin to conserve these populations effectively.

We also intend to restore violet populations in Iowa with replantings such as the one underway at Walnut Creek Wildlife Refuge in Jasper County, Iowa. The main goal of violet replantings is to restore natural violet cover to areas with prairie plants similar to current *S. idalia* habitat. Violets were extirpated from many prairie areas that underwent cultivation or an attempt of agriculture in pioneer days. Replanting violets also acknowledges the need for increased biomass availability to larvae, and is the protocol for other reintroductions of threatened species, such as the Oregon Silverspot, *Speyeria zerene hippolyta* Boisduval (Hammond 1993, Hammond and McCorkle 1991) where acres of soil with violet roots have been moved for transplantation to suitable habitat. With future laboratory evidence, we intend to model mathematically how much biomass of larval hostplant is necessary to sustain a population of *S. idalia* in the hundreds or thousands. This is the size population we would ideally like to see established or restored at several prairie sites in Iowa. We can combine our field data estimates of the large populations of violets and *S. idalia* outside Iowa, and by correlating the adult insect density estimate at the same locations, we can predict the total violet biomass necessary to support a larval population of *S. idalia*. In addition to these proactive conservation measures, we would like to promote conservation by discouraging unnecessary collecting of Iowa *S. idalia* insects and habitat destruction and alteration, such as plowing, draining of mesic areas, and insecticidal spraying. This should be a broad obligation to conserve not only the condition of the prairie habitat where *S. idalia* is found, but to conserve the diversity of other insects and wildlife present in this same habitat. While acknowledging our bias, we will utilize the attractiveness of *S. idalia*'s unique beauty to protect the rarity of other prairie plant and animal species which would benefit from conservation protection shared with *S. idalia* (Launer and Murphy 1994). Facing the issue of habitat destruction nationwide, *S. idalia* has become extirpated from much of New England and the Middle States (Wagner et al. 1994) only recently. We must act now, with more informed scientific information, to avoid the same outcome for this special insect in Iowa.

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