

## ABSTRACT

Title: NUTRIENT LIMITATION AND ITS  
CONSEQUENCES FOR  
PERFORMANCE AND THE  
HOMEOSTATIC REGULATION OF  
MACRONUTRIENT COMPOSITION  
IN TWO PHYTOPHAGOUS INSECTS  
WITH DIVERGENT LIFE-HISTORY  
STRATEGIES

Andrea F. Huberty, Ph.D., 2005

Directed By: Professor Robert F. Denno, Department of  
Entomology

Insect herbivores have a decidedly higher nitrogen and phosphorus than their host plants, an elemental mismatch that places severe constraints on their ability to meet nutritional demands. This study examined the consequences of macronutrient (nitrogen and phosphorus) limitation for two wing-dimorphic, phloem-feeding planthoppers with very different life-history strategies: the sedentary *Prokelisia dolus* and the migratory *Prokelisia marginata*. As a consequence of the constraints dispersal imposes on ingestion capacity, I argue that species using dispersal as a strategy for acquiring limiting nutrients are unable to adequately obtain nutrients when dispersal is not an option. The effect of nitrogen and phosphorus limitation for planthopper performance (Chapter 1) and homeostatic regulation of macronutrient composition (Chapter 2) were determined, as

were the constraints dispersal places on other traits (ingestion capability) used to cope with nutrient limitation (Chapter 3).

The planthopper species responded differently to nutrient limitation. The survival, body size, and development rate of both species was adversely affected on nutrient-deficient host plants, but *P. marginata* was more negatively affected than *P. dolus*. Nitrogen was more limiting than phosphorus for both species. *Prokelisia marginata* was also less able to regulate its macronutrient composition (C:N:P) and incurred greater growth penalties than *P. dolus*. Overall, the migratory species was far more sensitive to nutrient limitation.

Divergent life-history strategies (migratory versus sedentary) and the differential muscle allocation patterns associated with such strategies provide the mechanism underlying the consistently different performance responses of the two planthopper species on nutrient-deficient host plants. Morphometric and gravimetric measures of investment in flight versus feeding indicate that the sedentary *P. dolus* allocates more muscle mass to feeding whereas *P. marginata* invests more heavily in flight. Due to its greater investment in feeding musculature and associated enhanced ingestion rate, the immobile *P. dolus* is better equipped to meet macronutrient demands when faced with nutrient-poor food than the migratory *P. marginata*. Results of this research demonstrate the importance of considering life-history strategies, and associated constraints imposed on ingestion, when assessing how the macronutrient stoichiometry of plants (C:N:P content) interfaces with the nutritional requirements of phytophagous insects to affect their growth and performance.

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LIFE-HISTORY STRATEGIES

By

Andrea F. Huberty

Dissertation submitted to the Faculty of the Graduate School of the  
University of Maryland, College Park, in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
2005

Advisory Committee:  
Professor Robert F. Denno, Chair  
Professor Larry Douglass  
Associate Professor Irwin N. Forseth Jr.  
Associate Professor William O. Lamp  
Professor Charles Mitter

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## Acknowledgements

I would like to thank my committee members for all their time and help with this project. I extend my gratitude to my advisor and friend, Dr. Bob Denno, for all his advice and support during the last 10 years. I also thank my labmates for all the help in the field, especially Debbie Finke for her continued support and laughter throughout this degree (now it's your turn!). I also want to give thanks and love to my husband, Eric Olson and my parents, John and MaryLynn Huberty. I couldn't have finished without their love and faith. I also want to thank my babysitters Kelly, Sara, and my mom. And finally, I want to thank my beautiful daughter, Anika, for her laughter, smiles, and poopy diapers. You kept me going, and I love you.

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## Chapter 1: Nitrogen requirement is greater than phosphorus for two phytophagous insect species with divergent life-history strategies

### **Introduction**

Insect herbivores have a decidedly higher nitrogen (~9% N) and phosphorus (~0.5% P) content than their host plants (~1.5% N, ~0.05%P) (Elser et al. 2000a, Fagan et al. 2002). In the context of maintaining body elemental concentrations (Chapter 2), this stoichiometric mismatch places severe constraints on the ability of insect herbivores to meet their nutritional demands (Mattson 1980, Strong et al. 1984, Elser et al. 2000a). Thus, it comes as no surprise that nutrient limitation for phytophagous insects has been widely documented in an endless number of systems (see reviews by (McNeill and Southwood 1978, Mattson 1980, Waring and Cobb 1992, White 1993, Awmack and Leather 2002, Huberty and Denno 2004).

The importance of nitrogen and phosphorus for the growth of invertebrates has been demonstrated by experiments that either limit or remove the nutrient in question (Busch and Phelan 1999, Bentz and Townsend 2001) or increase its concentration in food resources (Chen and Welter 2002, Richardson et al. 2002). Physiologically, nitrogen is essential for growth (Kainulainen et al. 1996, Rossi et al. 1996, Kerslake et al. 1998), survival (Salim and Saxena 1991, Rossi et al. 1996, Ayers et al. 2000, De Bruyn et al. 2002) and reproduction (van Emden 1966, Rossi and Strong 1991, Bentz and Townsend 2001) due to its role in amino acid production and protein synthesis (Sternner and Elser 2002). Collectively, these studies demonstrate that nitrogen is consistently limiting for

most invertebrates including terrestrial species (Slansky and Feeny 1977, McNeill and Southwood 1978, Mattson 1980, White 1993, Elser et al. 2000a) and aquatic taxa (Urabe and Watanabe 1992, Downing 1997, Hassett et al. 1997, Elser et al. 2000a), and it has been championed as the most limiting macronutrient for phytophagous insects (McNeill and Southwood 1978, Mattson 1980, Strong et al. 1984, White 1993, Polis 1999).

For phytophagous insects in particular, phosphorus has not been considered as important or limiting as nitrogen, perhaps due to the paucity of research focus on this element. However, for the few studies that have investigated the effects of phosphorus limitation on insects, there is evidence showing that it can be an important determinant of survivorship (Clancy and King 1993, Ayers et al. 2000), fecundity (Popp et al. 1989), body size (Janssen 1994, Busch and Phelan 1999), oviposition (Skinner and Cohen 1994), growth rate (Perkins et al. 2004) and population density (Schade et al. 2003). By contrast, phosphorus limitation has been documented widely in many species of aquatic invertebrates, particularly in *Daphnia* (Urabe et al. 1997, Elser et al. 2001). Low levels of ambient phosphorus can result in protracted growth rates for many species of aquatic invertebrates (Urabe and Watanabe 1992, DeMott et al. 1998, Sterner and Schulz 1998, Elser et al. 2001, Makino et al. 2002, Urabe et al. 2002, Xenopoulos et al. 2002) as well as changes in competitive superiority in zooplankton and phytoplankton species (Andersen 1997). The relationship between phosphorus concentration, cellular function, and growth rate has been developed by Elser and colleagues and is termed the growth rate hypothesis (Hessen and Lyche 1991, Elser et al. 1996, Elser et al. 2000b). This hypothesis assumes that phosphorus, because it is required for RNA synthesis, can

impose severe consequences for the growth rate of consumers when it is limiting (Sterner and Elser 2002).

Stoichiometric reasoning suggests that phosphorus may be a much more important component for the growth of phytophagous insects that was previously thought, due to its important link to growth rate in aquatic invertebrates (Sterner and Elser 2002). *Daphnia* for example, when faced with low levels of phosphorus in its food exhibits slow growth, an effect attributed to the lesser amount of RNA available for protein synthesis (Sterner and Elser 2002). Moreover, phosphorus limitation is also strongly implicated for phytophagous insects because the stoichiometric mismatch in food quality is even greater than that for aquatic invertebrates. For example, even though the body N:P ratio for aquatic and terrestrial invertebrates is similar the elemental content of their food differs dramatically with terrestrial plants having significantly higher Carbon:N and C:P ratios than aquatic plants (Elser et al. 2000a). Because phosphorus limitation is well documented for aquatic invertebrates and because terrestrial insects face similar stoichiometric mismatches in both N and P body content compared to their food resources, not only should nitrogen limitation exist for phytophagous insects as previously demonstrated but phosphorus limitation should occur as well (Elser et al. 2000a).

To date, no study has simultaneously investigated the consequences of nitrogen and phosphorus limitation for the performance of terrestrial herbivorous insects. Using a combination of factorial experiments in the laboratory and field, whereby levels of soil nitrogen and phosphorus are manipulated in a crossed design, this study tests the

hypothesis that both macronutrients will limit the performance and population size of phytophagous insects via their cascading effects on host plant quality.

For my investigation, I selected two species of phloem-feeding herbivores, namely the salt marsh-inhabiting planthoppers *Prokelisia dolus* and *P. marginata* (Hemiptera: Delphacidae). These species were purposefully chosen because of dramatic differences in their sensitivity to variation in host-plant nitrogen (Cook and Denno 1994, Denno et al. 2002), competitive ability (Denno et al. 2000), population dynamics (Denno et al. 1996), and risk of predation (Denno et al. 2003). Most notable is a dramatic difference in dispersal capability between the two species, with *P. marginata* being an exceedingly migratory species and *P. dolus* being comparatively sedentary (Denno et al. 1996, Denno and Peterson 2000). Facilitating the assessment of migratory capability in planthoppers is the fact that they are wing-dimorphic with both flight-capable adults (macropters) and flightless adults (brachypters) present in the same population (Denno et al. 1989, Denno et al. 1996, Zera and Denno 1997). Thus, a rough assessment of dispersal ability can be obtained by comparing the fraction of macropters in populations, which is high for *P. marginata* (>90%) and low for *P. dolus* (<20%) (Denno et al. 1996). Inversely associated with dispersal capability in these two species is their ability to tolerate low plant nitrogen via feeding compensation (Denno et al. 2000, Chapter 3). This relationship derives from an apparent trade-off between flight musculature and the cibarial musculature associated with the ingestion of phloem sap and thus their ability to enhance food intake on nutrient-deficient host plants (Chapter 3). Thus, these two species apparently cope with deteriorating host-plant quality in two fundamentally different ways, *P. marginata* by migrating to better quality host plants in other habitats

and *P. dolus* by tolerating periods of low plant quality until nutrient conditions improve (Cook and Denno 1994, Denno et al. 2000, Chapter 3).

Given the fundamentally discrepant mechanisms that underlie the ability of these two species to cope with deteriorating plant quality, I anticipated that nitrogen and phosphorus limitation would affect them differently. Based on differences in feeding compensation, I predicted that when confined to nitrogen-poor and phosphorus-deficient host plants that the sedentary species (*P. dolus*) and wing-form (brachypterous adults of both species) would incur fewer performance penalties than their long winged counterparts (*P. marginata* in general and macropterous adults of both species).

This research aims to elucidate the singular and interactive effects of nitrogen and phosphorus limitation on the performance of two abundant phytophagous insects and to examine the interface between life-history strategy and the consequences of macronutrient limitation. Moreover, this work will contribute to a broader need to understand the relative roles of nitrogen and phosphorus limitation in the nutritional ecology of phytophagous insects, two macronutrients that have important consequences for species interactions, food-web dynamics, and ecosystem function (Coll and Guershon 2002, Sterner and Elser 2002, Vanni et al. 2002, Denno and Fagan 2003, Denno and Kaplan in press.).

## **Study Site and System**

### **Study Site**

The effects of nitrogen and phosphorus limitation on the performance and elemental composition of *Prokelisia* planthoppers and their cordgrass host plant *Spartina*



*alterniflora* were studied in the field on an expansive intertidal marsh in the Great Bay-Mullica River estuarine system at the end of Great Bay Boulevard and just north of the Rutgers University Marine Station, Tuckerton, Ocean County, New Jersey (39° 30.8' N, 74° 19.0' W) The vegetation of this marsh and other mid -Atlantic marshes is dominated by the perennial cordgrass *Spartina alterniflora*, where it grows in extensive pure stands within the intertidal zone (Denno 1983, Gallagher et al. 1988, Mendelsohn and Morris 2000).

#### Evidence for macronutrient variability and limitation in *Spartina alterniflora*

Within the intertidal zone, the structure and nutritional content of *Spartina alterniflora* varies dramatically with elevation due to differences in nutrient subsidy by tides/groundwater and salinity that alters nutrient uptake (Denno 1983, Webb 1983, Gallagher et al. 1988, House et al. 1998). Moving up the elevational gradient from low-marsh habitats (tidal creek banks) to high-marsh plateaus (meadows), *Spartina* plants generally decrease in nutrient content (nitrogen and phosphorus) and height (Denno 1983, Ornes and Kaplan 1989). In particular, nitrogen uptake can be limited on the high marsh under conditions of elevated salinity that arise from evaporation (Mendelsohn 1979, Webb 1983). However, salt stress in *Spartina* promotes increases in the concentration of nitrogen-containing osmoprotectants that may benefit phytophagous insects (Cavalieri and Huang 1981, Bacheller and Romeo 1992, Naidoo et al. 1992). However, under conditions of increased soil salinity cell turgor is adversely affected in *Spartina* (Drake and Gallagher 1984), which may reduce a phloem feeder's access to elevated nitrogen (Huberty and Denno 2004). Thus, natural variation in the nitrogen and phosphorus

content of *Spartina* occurs in the field presenting potential local problems to plant-feeding insects that must meet their nutrient demands.

Nitrogen-limitation has been documented extensively in *Spartina alterniflora*, particularly in high-marsh habitats dominated by short-form plants (Smart and Barko 1980, Broome et al. 1983, Osgood and Zieman 1993). Phosphorus limitation, although it has been suggested for *Spartina alterniflora* (Broome et al. 1975, Broome et al. 1983), is much less likely given that salt-marshes and estuaries have been espoused as long-term phosphorus sinks (Billen and Lan 1991, House et al. 1998). Nonetheless, there are somewhat mixed results for phosphorus limitation in *Spartina* marshes (Patrick and Delaune 1976, Broome et al. 1983, Osgood and Zieman 1993). At the very least, adding phosphorus to *Spartina* elevates its concentration in plant tissues even though plant biomass does not necessarily increase (Patrick and Delaune 1976, Boyer et al. 2001), a result that may benefit phosphorus-limited insects.

#### Evidence for macronutrient limitation in *Prokelisia planthoppers*

*Prokelisia marginata* and *P. dolus* are the most abundant herbivores on Atlantic coastal marshes where they feed exclusively on *Spartina alterniflora* (Denno 1977, Denno et al. 2002). Responses of the two *Prokelisia* species to the nitrogen content of *Spartina* suggest that both species are nitrogen limited, but that *P. marginata* is more sensitive to fluctuating plant nitrogen than its congener (Cook and Denno 1994, Denno et al. 2002, Denno et al. 2003). For example, although both species select the most nitrogen-rich plants or plant parts on which to feed and oviposit, *P. marginata* colonizes nitrogen-enriched plants at tremendously high densities whereas *P. dolus* does not

(Denno 1983, Roderick 1987, Denno et al. 2002). Moreover, what little data exists suggests that both species exhibit enhanced survival and performance on nitrogen-fertilized *Spartina*, but that *P. marginata* shows a stronger response (Denno 1985, Denno and McCloud 1985, Denno et al. 1996, Olmstead et al. 1997). *Prokelisia* also has the ability to withstand nitrogen-deficient plants better than *P. marginata*. For instance, previous feeding by *Prokelisia* planthoppers significantly reduces the amino nitrogen content of their *Spartina* host (Olmstead et al. 1997), an effect that has far greater fitness consequences for *P. marginata* than *P. dolus* (Denno et al. 2000). The ability of *P. dolus* to better compensate for nutrient-deficient phloem sap by increasing its ingestion rate likely underlies its reduced sensitivity to depleted plant nitrogen (Chapter 3). Altogether, existing observations and data suggest that nitrogen is likely limiting for both planthopper species. Notably, nothing is known about phosphorus limitation in these planthoppers and how it might interact with plant nitrogen to affect their performance.

## Methods

Factorial experiments in the laboratory and field were conducted to test the effects of variable nitrogen and phosphorus subsidies on (1) the nitrogen and phosphorus contents of *Spartina* and both *Prokelisia* species, (2) the biomass and mortality of *Spartina*, and (3) the performance (body size and development time), survival and population density of both planthoppers raised on *Spartina* that was grown under the various macronutrient treatment conditions.

### Laboratory Experiment

A 4x4 full factorial experiment was initiated in May 2000 to test the direct and interactive effects of nitrogen and phosphorus subsidies on the growth, performance, and elemental composition (N and P content) of *Spartina* and both *Prokelisia* species. Potted *Spartina* plants (3-5 plants per pot grown in sand substrate) were greenhouse grown in flats (80 pots per flat in each of two flats) exposed to four levels of nitrogen subsidy (0,10,30,60g/m<sup>2</sup>) and four levels of phosphorus addition (0,2,6,12g/m<sup>2</sup>), all crossed. To achieve the desired treatments, plants in each treatment combination were fertilized every three weeks from May until July. Nitrogen was applied as ammonium nitrate (0, 5g, 16g, 32g / flat / application) and phosphorus was supplied as triple super phosphate (0g, 0.8g, 2g, 5g / flat / application). The macronutrient treatments are hereafter referred to as 0N, 10N, 30N and 60N for nitrogen and 0P, 2P, 6P and 12P for phosphorus. The range of N and P fertilization rates was chosen to bracket those used in previous laboratory experiments and to include the spectrum of *Spartina* nutrient contents that occurs naturally in the field (Denno et al. 2000). Plants for this experiment were grown from seed obtained from Environmental Concern, Inc. St. Michaels, MD.

#### Effect of Nutrient Subsidies on *Spartina* Mortality, Biomass and N and P Content

Treatment effects on *Spartina* mortality (assessed on 13 July) and *Spartina* biomass and nutrient content (N and P) (measured on 26 July) were determined on plants that were never exposed to planthoppers. Nutrient subsidy effects were determined at the pot level by measuring plant mortality (number of dead plants per pot/total number of plants per pot) and biomass (g dry mass of *Spartina* per pot) in 25-30 randomly chosen

pots within each treatment. Effects of the nutrient treatments on the nutrient content of *Spartina* (%N and %P of plants per pot) were determined from 6 randomly chosen pots per treatment. Foliar N content was used as an index of the phloem nitrogen available to planthoppers because the two plant nitrogen variables are related (Youssefi et al. 2000). For biomass and nutrient determination, plants were harvested, oven dried for 48 hours at 60°C, and weighed. Subsequently, plant leaves were ground in a Wiley Mill and analyzed for %N using a Perkin-Elmer 2400 CHN analyzer, and for %P by persulfate digestion (Clesceri et al. 1998). The grand mean of all experimental treatment plants was used to assess the overall N and P content of *Spartina*, a statistic that was used ultimately to assess the mismatch in (N and P) stoichiometry between plants and planthoppers.

#### Effect of Nutrient Subsidies on Planthopper Performance, Survival and N and P Content

Cohorts of 3 to 5 first instar nymphs of each *Prokelisia* species were placed separately onto caged treatment plants (20 initial replicate cohorts per treatment) on 17 July (see Denno et al. 2000 for cage design). Nymphs were obtained from lab cultures initiated from ovipositing adults collected from the Tuckerton field site. There was severe plant mortality in the 30N and 60N treatments, resulting in the loss of these treatments. Thus, the effect of the nutrient treatments on planthopper performance was assessed using a 2x4 factorial design (0N and 10N and 0P, 2P, 6P, and 12P). Plant death in the remaining treatments resulted in uneven replication such that 3-12 replicates remained per treatment per species. The survival (%), development time (days to adult molt), and tibia length (mm, a surrogate for body size (Denno and McCloud 1985)) for both *Prokelisia* species was determined at the time of adult emergence. Emerging adult

planthoppers of each species were pooled by replicate (all sexes and wing forms) and oven dried prior to analysis for N and P content as above (Clesceri et al. 1998).

### Statistical Analyses

Treatment effects on *Spartina* biomass, mortality, %N, and %P were each analyzed as a 4x4 factorial mixed-model ANOVA with nitrogen (4 levels) and phosphorus (4 levels) as fixed effects (SAS 2002). Treatment effects on planthopper survival (arcsin squareroot transformed) were analyzed for each species separately using 2x4 mixed-model ANOVA (sexes and wing forms pooled). For each species, treatment effects on development time (days to adult) and body size (tibia length in mm) were each assessed using a 2x4x2 factorial (2 levels of N, 4 levels of P and two wing forms (brachypter and macropter) respectively) mixed-model weighted analysis of variance. For the analyses on development time and body size, only females were used, because few male brachypters of *P. marginata* were produced. Because multiple insects were placed onto caged treatment plants, the mean development time or mean body size per pot was used for each analysis, and means were weighted by the number of observations per pot. A mixed-model ANOVA was also used to compare the average nitrogen and phosphorus contents of *Spartina*, *P. dolus*, and *P. marginata* (organisms were pooled across all treatments for this analysis).

Prior to analysis, data (residuals) were assessed for normality and homogeneity of variances (SAS 2002). If residual variances were heterogeneous, variance partitioning was conducted and the best model was chosen using Bayesian Information Criterion. The degrees of freedom were calculated using the Kenward-Roger method. All means

comparisons were conducted using a Tukey adjustment to account for inflated comparison-wise error rates. If data was transformed for statistical analysis, means and standard errors are presented as untransformed data.

### Field Experiment

A 2x2x2 full factorial experiment was conducted in May 2000 at Tuckerton to test the direct and interactive effects of nitrogen fertilization (0, 80g/m<sup>2</sup>), phosphorus addition (0, 80g/m<sup>2</sup>), and habitat (meadow and tidal creek) on the biomass of *Spartina*, the population density of both *Prokelisia* species, and the N and P content of *Spartina* and both planthoppers. The design was a randomized complete double-block design with two sites (marsh areas) each containing 2 habitat types and each of these containing 2 blocks of the 4 treatment combinations. Thus, each nutrient treatment combination was replicated 8 times for a total of 32 plots. Fertilizer treatments (0N0P, 0N+P, +N0P, +N+P) were randomly assigned to a single plot (2x2m<sup>2</sup>) within each block. Nitrogen was applied as ammonium nitrate (0 or 236g / plot / application) and phosphorus was added as triple super phosphate (0g or 176g / plot / application) on four dates (May 9 and 21, June 4 and 17). This range of N and P fertilization rates was selected because it includes rates used by others to achieve *Spartina* nutrient contents that occur naturally in the field (Patrick and Delaune 1976, Denno et al. 2000). Treatments were established in both habitat types to ensure the presence of both *Prokelisia* species; *P. marginata* occurs abundantly in low-marsh *Spartina* whereas *P. dolus* predominates in *Spartina* meadows (Denno et al. 1996). Habitat type was also included in the design because ambient nutrient availability decreases from tidal creek habitat to meadow habitats, *Spartina* is

relatively more nutrient limited on the high marsh (Ornes and Kaplan 1989), and thus *Spartina* may respond differently to fertilization in the two habitats with regard to nutrient uptake and biomass.

#### Effect of Nutrient Subsidies on *Spartina* Biomass and N and P Content

*Spartina* biomass was determined twice during the time course of the experiment, once on 9 May prior to fertilizer application and again at the end of the experiment on 22 July. Live biomass was assessed by clipping all above-ground vegetation within a 0.047m<sup>2</sup> wire frame (Denno et al. 2002) and one sample was taken from each plot on each date. *Spartina* leaves were subsequently dried at 60°C for 48 hours before weighing. The N and P content of *Spartina* was assessed 6 times (May 9 and 21, June 4, 17, and 24, and July 22) during the experiment by taking 5 snippets (leaves of 5 different *Spartina* stems) per plot per date. Snippet samples were oven dried, ground in a Wiley Mill, and the powder was analyzed for N and P content as above (Clesceri et al. 1998). The grand mean of all experimental treatment plants was used to assess the overall N and P content of *Spartina*, a statistic that was used ultimately to assess the mismatch in elemental composition (N and P) between plants and planthoppers.

#### Effect of Nutrient Subsidies on Planthopper Density and N and P Content

Planthopper density was determined by sampling once in each plot on 22 July using a D-vac suction sampler (2, 8-second placements of the sampling head per plot) (see Denno et al. 2002). Planthoppers were placed into 70% alcohol, sorted to species and wing form, and counted. The N and P content of the two *Prokelisia* species was



determined from samples taken in each plot on 24 June. Insect collections were kept on ice until reaching the laboratory where they were stored at -20°C. Subsequently planthoppers were sorted to species, wing form, and sex and then dried. Three individuals of each species, wing form, and sex were taken from each plot and analyzed for their N and P content as above (Clesceri et al. 1998).

### Statistical Analyses

Treatment and habitat effects on *Spartina* biomass were analyzed as a 2x2x2 ANCOVA (2 levels of N, 2 levels of P, and 2 habitat types) with initial plant biomass (May 9) as the covariate (SAS 2002) and site, block and site\*block as random effects. The effect of treatment and habitat on the nutrient content (%N and %P) of *Spartina* was analyzed as a repeated measures 2x2x2x6 factorial (2 levels of N, 2 levels of P, 2 habitat types, and 6 sampling dates) mixed-model analysis of variance, with the site, block and site\*block as random effects. A separate mixed-model factorial analysis of variance (2 levels of N, 2 levels of P, 2 habitat types, and with site, block, and site\*block as random effects) was run to assess treatment effects on the N and P content of *Spartina* on June 24, the same date on which planthoppers were collected for elemental analysis.

Treatment effects on the density of each *Prokelisia* species (log transformed) were analyzed as 2x2x2x2x2 factorial (2 levels of N, 2 levels of P, 2 habitat types, 2 wing-forms, and 2 sexes) mixed-model ANCOVAs, with plant biomass as the covariate and site, block and site\*block as the random effects. A mixed-model ANOVA was used to compare the average nitrogen and phosphorus contents of *Spartina*, *P. dolus*, and *P. marginata* on June 24 (organisms were pooled across all treatments for this analysis).

Prior to analysis, residuals were assessed for normality and homogeneity of variances, variance partitioning was conducted and the best model was chosen using Bayesian Information Criterion, degrees of freedom were calculated using the Kenward-Roger method and means were compared using a Tukey adjustment to account for inflated comparison-wise error rates (SAS 2002).

## Results

### Laboratory Experiment

#### Effect of Nutrient Subsidies on *Spartina* Mortality, Biomass and N and P Content

In general, enhancing nitrogen and phosphorus subsidies resulted in increases in the nitrogen and phosphorus content of *Spartina* (Figure 1.1). Increasing the nitrogen subsidy resulted in a remarkable increase in the nitrogen content (%) of *Spartina* that ranged from approximately 1% N in the 0N treatment to 8% N in the 60N treatment (main effect of N fertilization,  $F_{3, 126} = 843.30$ ,  $P < 0.0001$ ) (Figure 1.1A). The addition of phosphorus also significantly affected the %N content of *Spartina* ( $F_{3, 130} = 12.28$ ,  $P = 0.0001$ ). However, this effect was slight and was apparently driven by the higher than expected nitrogen content of *Spartina* in the 10N0P treatment. Fertilizing with either P ( $F_{3, 131} = 158.67$ ;  $P < 0.0001$ ) or N ( $F_{3, 136} = 20.65$ ;  $P < 0.0001$ ) significantly increased the phosphorus content (%) of *Spartina* (Figure 1.1B). However, adding phosphorus fertilizer alone (0N with either 2P, 6P or 12P treatments) resulted in a significantly lower P content of *Spartina* than adding P fertilizer along with the N treatments, suggesting that

phosphorus uptake is positively influenced by N fertilization (significant N\*P interaction,  $F_{9, 132} = 12.19$ ;  $P < 0.0001$ ).

Fertilization with both N and P resulted in an increase in *Spartina* biomass, but biomass did not increase linearly with fertilizer application rate (Figure 1.2A) (significant N\*P interaction,  $F_{9, 176} = 23.3$ ;  $P < 0.0001$ ). For example, nitrogen fertilization at the 10N level resulted in the greatest *Spartina* biomass, followed by the biomass achieved at the 30N, 60N and 0N treatments (significant N main effect on biomass;  $F_{3, 176} = 65.04$ ;  $P < 0.0001$ ). Also, *Spartina* exhibited significantly greater biomass when phosphorus fertilizer was added at rates of 2P, 6P, 12P compared to when no fertilizer was added (0P) (significant P main effect on biomass;  $F_{3, 176} = 75.48$ ,  $P < 0.0001$ ), but there was no significant increase in *Spartina* biomass from the 2P to the 12P treatment levels (Figure 1.2A).

The nitrogen subsidy treatments also had non-linear effects on *Spartina* mortality with the least mortality occurring in the 10N, significantly higher mortality occurring in the 0N and 30N treatments, and the highest mortality arising in the 60N treatment (significant main effect on mortality;  $F_{3, 250} = 14.11$ ;  $P < 0.0001$ ) (Figure 1.2B). There was no significant effect of phosphorus fertilization on *Spartina* mortality ( $F_{3, 297} = 0.57$ ;  $P = 0.6$ ) (Figure 1.2C). The high mortality of plants in the 60N treatment, and the eventual damping off of plants in the 30N treatment, precluded the use of these treatments for assessing fertilization effects on *Prokelisia* performance.

### Mismatch in N and P content between *Spartina* and *Prokelisia*

There was a striking difference in the nitrogen (%) and phosphorus (%) content of *Spartina* when compared to both *Prokelisia* species (Figure 1.3). The nitrogen content of *Spartina* was 3 times less than that for either planthopper species ( $F_{2,110} = 304.10$ ;  $P < 0.0001$ ), suggesting that *Spartina* presents a low-nitrogen food source relative to the inherent nitrogen demands of *Prokelisia* (Figure 1.3A). Similarly, the phosphorus content of *Spartina* was also three times less than that for either *Prokelisia* species ( $F_{2,110} = 310.15$ ;  $P < 0.0001$ ), a mismatch that further highlights the poor nutrient quality of *Spartina* for planthoppers (Figure 1.3B). Notably, *P. dolus* had a slightly higher body phosphorus content than *P. marginata*, although their nitrogen contents did not differ (Figures 1.3A and B).

### Effect of Nutrient Subsidies on Planthopper Performance and Survival

Overall, the nutrient mismatch between *Spartina* and the *Prokelisia* species (Figure 1.3) translated into survival and performance penalties when planthoppers were raised on nutrient deficient plants. For example, the survival of *P. dolus* was significantly enhanced when it was raised on plants with higher nitrogen content (10N versus 0N treatment) (Figure 1.4A) (main effect of N on survival;  $F_{1,99} = 8.31$ ;  $P = 0.005$ ). Its survival, however, was not significantly affected by phosphorus fertilization (main effect of P on survival;  $F_{1,99} = 0.71$ ;  $P = 0.5$ ). For *P. marginata*, raising it on plants with increased nitrogen or phosphorus content did translate into improved survival (main effect of N on survival,  $F_{1,90} = 15.77$ ;  $P = 0.0001$ ) (main effect of P on survival;  $F_{3,90} =$

7.4;  $P = 0.002$ ) (Figure 1.4B), and there was a significant interaction as the effect of P on survival was non-linear ( $N \times P$ ;  $F_{3,90} = 5.92$ ;  $P < 0.0001$ ).

In general, enhancing *Spartina* quality via nitrogen and phosphorus fertilization also had significant positive effects on the body size of female planthoppers, although *P. marginata* benefited relatively more. For instance, *P. dolus* exhibited a significant increase in body size (tibia length) when raised on N-fertilized *Spartina* (main effect of N on tibia length  $F_{1,75} = 9.37$ ;  $P = 0.003$ ), but showed no response to P-subsidized plants (main effect of P on tibia length;  $F_{3,75} = 1.11$ ;  $P = 0.3$ ) (Figure 1.5A). In contrast, fertilization of *Spartina* with both N (main effect of N on tibia length;  $F_{1,61} = 38.82$ ;  $P < 0.0001$ ) and P (main effect of P on tibia length;  $F_{3,61} = 2.5$ ;  $P = 0.06$ ) resulted in a significant increase in the body size of *P. marginata* (Figure 1.5B). There was no effect of wing form on body size nor was there any interactive effect with either N or P fertilization ( $P > 0.05$ ).

Feeding on nitrogen-rich *Spartina* (10N) decreased the development time of *P. dolus* females by 4 days compared to when they were reared on 0N treatment plants ( $F_{1,75} = 10.5$ ;  $P = 0.002$ ), but there was no significant change in development time on plants fertilized with increasing amounts of phosphorus ( $F_{3,75} = 0.19$ ;  $P = 0.8$ ) (Figure 1.6A). As with survival and body size, both N and P fertilization of *Spartina* promoted faster development in *P. marginata* (Figure 1.6B). Adding N fertilizer to *Spartina* resulted in a significant 8 day reduction in the development time (main effect of N on development time;  $F_{1,61} = 37.44$ ;  $P < 0.0001$ ). With increasing P fertilization of *Spartina*, there was a non-linear change in development rate; the most rapid development occurred on plants in the 6P treatment compared to the remaining treatments (main effect of P on development

time;  $F_{3,61} = 3.64$ ;  $P = 0.02$ ). There was no effect of wing form on development time nor was there any interactive effect with either N or P fertilization ( $P > 0.05$ ).

### Field Experiment

#### Effect of Nutrient Subsidies on *Spartina* Biomass and N and P Content

Both N and P fertilization significantly increased the nitrogen content (%N) of *Spartina* in the field (Figures 1.7 and 1.8). Overall, nitrogen subsidy increased the N content of *Spartina* from  $1.86 \pm 0.03$  % to  $2.86 \pm 0.30$  % (main effect of N on %N;  $F_{1,124} = 472.20$ ;  $P < 0.0001$ ). Phosphorus fertilization enhanced nitrogen uptake and resulted in a more modest increase in the nitrogen content (%) of *Spartina* from  $2.27 \pm 0.03$  % to  $2.45 \pm 0.03$  % (main effect of P on %N;  $F_{1,124} = 14.27$ ;  $P = 0.0002$ ).

The habitat where the N fertilization treatment was applied also significantly influenced the nitrogen content of *Spartina*. On average, the nitrogen content of *Spartina* growing in the meadow habitat ( $2.5 \pm 0.03$  %, Figure 1.7A) was significantly greater than the nitrogen content of *Spartina* growing in the tidal creek habitat ( $2.25 \pm 0.03$  %;  $F_{1,124} = 23.68$ ;  $P < 0.0001$ ) (Figure 1.7B).

On the date planthoppers were sampled (July 22), the nitrogen content of *Spartina* was significantly higher in plots receiving the nitrogen subsidy ( $3.1 \pm 0.08$  %) than in plots that did not ( $2.1 \pm 0.08$  %;  $F_{1,23} = 65.97$ ;  $P < 0.0001$ ). Likewise, the N-content of *Spartina* was significantly greater in plots fertilized with phosphorus ( $2.7 \pm 0.08$  %) compared to controls ( $2.4 \pm 0.08$  %;  $F_{1,23} = 9.63$ ;  $P = 0.005$ ) (Figure 1.7C). On this date there was no difference in the nitrogen content of plants between the meadow and tidal creek habitats (main effect of Habitat;  $F_{1,23} = 0.37$ ;  $P = 0.5$ ).

There was a significant interactive effect of fertilization treatment, date, and habitat on the phosphorus content (%) of *Spartina* ( $N*P*Date*Habitat$ ;  $F_{4,94.3} = 2.65$ ;  $P = 0.03$ ; Figures 1.8A-B). In control plots (0N0P) and in nitrogen subsidized plots (+N), the P content of *Spartina* declined from May to July in both habitats, and there was no difference in the P content of *Spartina* in these two treatments. By contrast, in the meadow habitat, there was a significant increase in the P content of *Spartina* in plots receiving a phosphorus subsidy (0N+P) and a synergistic seasonal increase in the P content of *Spartina* in plots receiving both N and P subsidies (+N+P) compared to controls (Figure 1.8A). In the tidal creek habitat, although phosphorus fertilization resulted in an increase in the P content of *Spartina*, the seasonal increase in P content in response to the +N+P treatment seen in meadow *Spartina* (Figure 1.8A) was not as evident in tidal creek plants (Figure 1.8B). The difference in the P content of *Spartina* between plants in the meadow and tidal creek habitats that received both N and P subsidies was particularly evident on the date planthopper were sampled (Figure 1.8C). On this date, meadow plants contained twice as much foliar phosphorus as those in the tidal creek habitat ( $N*P*Habitat$ ,  $F_{1,24} = 16.8$ ;  $P = 0.0004$ ).

There was a significant interactive effect of the fertilization treatments and habitat on the aboveground biomass of *Spartina* ( $N*P*Habitat$ ;  $F_{1,17.3} = 5.94$ ;  $P = 0.03$ ; Figure 1.9). Nitrogen fertilization resulted in a significant increase in *Spartina* biomass in the meadow habitat, a response that did not occur in plants growing in the tidal creek. Neither phosphorus fertilization nor the application of both N and P significantly altered *Spartina* biomass in the meadow habitats relative to controls. However, the biomass of

plants receiving the phosphorus subsidy was significantly less than those that were fertilized with only nitrogen.

#### Mismatch in N and P content between *Spartina* and *Prokelisia*

The mismatch in nitrogen (%) and phosphorus (%) content between *Spartina* and both planthopper species was even more extreme than that observed in the laboratory data (compare Figure 1.10 with 1.3). The nitrogen content of *Spartina* growing in the field was 5 times less than that for either planthopper species ( $F_{2,75.4} = 1334$ ;  $P < 0.0001$ ) (Figure 1.10A), and more than three times less in phosphorus content than either *Prokelisia* species ( $F_{2,77.2} = 221$ ,  $P < 0.0001$ ) (Figure 1.10B).

#### Effect of Nutrient Subsidies on Planthopper Density

Although both *Prokelisia* species responded with population increases on nitrogen-subsidized *Spartina*, *P. marginata* exhibited a relatively stronger response than *P. dolus* (Figure 1.11). Nitrogen fertilization of *Spartina* promoted a significant increase in the density of *P. dolus* (main effect of N;  $F_{1,55} = 30.47$ ;  $P < 0.0001$ ), and there was a greater increase in the density of macropters than flightless brachypters on plots that received a nitrogen subsidy compared to those that did not (N\*Wing form;  $F_{1,54.9} = 6.49$ ;  $P = 0.01$ ) (Figures 1.11A-B). The density of *P. dolus* was not affected by phosphorus fertilization (main effect of P;  $F_{1,55} = 0.08$ ;  $P = 0.8$ ). Overall, *P. dolus* was found in greater densities in the meadow habitat than on tidal creek *Spartina* (main effect of Habitat;  $F_{1,55} = 5.46$ ;  $P = 0.02$ ) (Figures 1.11A-B).



For *P. marginata*, macropters selectively colonized N-subsidized *Spartina* a density response not observed for flightless brachypters (N\*Wing form;  $F_{1,96} = 20.06$ ;  $P < 0.0001$ ), and the increase in macropter density on nitrogen fertilized *Spartina* was greater in the tidal creek habitat than the meadow habitat (N\*Wing form\*Habitat;  $F_{1,96} = 4.11$ ;  $P = 0.046$ ) (Figures 1.11C-D). There was no density response of *P. marginata* to P-subsidized *Spartina* in either habitat (main effect of P;  $F_{1,96} = 0.19$ ;  $P = 0.7$ ). For both planthopper species, there was no difference in the response of males and females to fertilized plants, and plant biomass was not a significant covariate ( $P > 0.05$ ).

## Discussion

### *Nutrient-limitation in Spartina alterniflora*

*Spartina* responded vigorously to nitrogen fertilization in both laboratory and field experiments. In the laboratory, high rates of nitrogen subsidy (30N and 60N) resulted in a remarkably high nitrogen content of *Spartina* (~7 %N) (Figure 1.1A), but also promoted severe mortality (Figure 1.2B), suggesting that nitrogen fertilization has a toxic effect at high application rates. Furthermore, the greatest increase in plant biomass occurred at a moderate level of nitrogen addition (10N) and not at the highest fertilization levels (Figure 1.2A), further suggesting the adverse effects of excess nitrogen input.

In the field, nitrogen fertilization resulted in increased *Spartina* biomass in the meadow habitat, but not in the tidal creek habitat (Figure 1.9). Also, the nitrogen content of field plants, particularly those in the meadow habitat, was further enhanced by the

application of both N and P (Figure 1.7), suggesting that nitrogen uptake is influenced by ambient levels of phosphorus.

Phosphorus fertilization in the laboratory did not result in dramatic changes in the elemental content or biomass of *Spartina* compared to nitrogen fertilization. Although the P content of *Spartina* increased with P fertilization, a greater response occurred when both N and P were added to growing *Spartina* (Figure 1.1B), suggesting that nitrogen facilitates phosphorus uptake. Plant biomass did increase with P addition, but the increase occurred only when a comparison was made to plants deprived of P altogether (Figure 1.2A). *Spartina* mortality was unaffected by phosphorus subsidy in the laboratory (Figure 1.2C). Phosphorus fertilization had only minor effects on *Spartina* plants in the field as well. Phosphorus fertilization alone resulted in a slight increase in the P content of *Spartina* (Figure 1.8) and no increase in plant biomass (Figure 1.9). Only when phosphorus was applied along with nitrogen did the P content of *Spartina* increase, a response that was more evident in meadow *Spartina*. Notably, phosphorus fertilization did not affect the biomass of *Spartina* in either habitat.

Overall, there is extensive data suggesting that *Spartina* is strongly nitrogen limited and that evidence for phosphorus limitation is weak, suggesting that *Spartina* has a greater physiological demand for nitrogen than phosphorus. Previous research on other Atlantic coastal marshes also shows greater N-limitation than P-limitation in *Spartina* (Patrick and Delaune 1976, Broome et al. 1983, Osgood and Zieman 1993). That the greatest effects of phosphorus subsidy on the P content of *Spartina* were seen when both nitrogen and phosphorus were applied compared to when phosphorus was applied alone,

suggesting that nitrogen demands are more crucial and must be met first (Patrick and Delaune 1976).

In the field, *Spartina* growing in meadow habitats exhibited a greater growth response to nitrogen fertilization compared to plants growing in the tidal creek habitat (Figure 1.9), a response that has been documented previously (Broome et al. 1975, Mendelssohn 1979, Broome et al. 1983, Webb 1983). This result is consistent with reports in the literature that *Spartina* is more nitrogen limited in high marsh habitats that incur less tidal subsidy of nutrients and face problems of nutrient uptake under salt stressed conditions (Webb 1983, Gallagher et al. 1988, Ornes and Kaplan 1989, House et al. 1998, Osgood and Zieman 1998).

#### *Nutrient-limitation and its consequences for Prokelisia planthoppers*

The nitrogen and phosphorus content of *Spartina alterniflora* is remarkably low compared to the body nitrogen content of both *Prokelisia* species (Figure 1.3 and 1.10), suggesting that both N and P may limit their performance and adversely influence their density. When *P. dolus* and *P. marginata* were raised on plants with an enriched nitrogen signature, they survived better, grew to a larger size, and developed more rapidly than when they developed on nitrogen-deficient plants (Figures 1.4, 1.5 and 1.6). Moreover, both species achieved significantly higher densities on nitrogen-subsidized plants in the field (Figure 1.11). Although both planthopper species responded positively to nitrogen-fertilized *Spartina*, *P. marginata* experienced greater fitness consequences than *P. dolus* under nitrogen-deficient plants. For *P. marginata*, its survival declined by 50%, its body size was reduced 12%, and its development time to adult was extended by 8 days when

reared on nitrogen-deficient compared to nitrogen-enriched *Spartina*. In contrast, *P. dolus* suffered only 25% reduction in survival, a 6% decrease in body size, and a 4-day developmental delay when raised on the same nitrogen-poor plants.

Phosphorus limitation was not as severe as nitrogen limitation for *P. marginata*, and was not detected at all in *P. dolus* even though there was a significant mismatch between the phosphorus content of *Spartina* and that of both *Prokelisia* species. Nonetheless, *P. marginata* did show a significant increase in survival and body size on phosphorus-enhanced plants compared to controls, but like *P. dolus* failed to show a positive density response to phosphorus-fertilized *Spartina* in the field (Figures 1.4, 1.5 and 1.6).

The tempered response of *P. dolus* to nitrogen- and phosphorus-deficient *Spartina* compared to *P. marginata* may be due to a greater investment in feeding musculature and hence its ability to compensate for nutrient deficiencies by increasing its ingestion rate of phloem (Denno et al. 2000, Chapter 3). Planthoppers and leafhoppers feed by inserting their stylets into phloem and xylem tissues respectively (Backus 1985, Cook and Denno 1994). Then using a cibarial pump (modified esophagus), cell sap is ingested. The cibarial pump is driven by a series of dilator muscles that insert on the interior of the face, and face size is positively related to the cross-sectional mass and thus the power of the cibarial muscles to ingest cell sap (Backus 1985) For *Prokelisia* planthoppers, *P. dolus* has a much broader face and likely commitment to subtending cibarial musculature than *P. marginata* (Denno et al. 1987, Denno et al. 2000) Chapter 3). *Prokelisia dolus* is likely more capable than *P. marginata* of increasing food uptake in response to any reductions in plant nitrogen, and thus its reduced sensitivity to host plant nutrition. Data

suggest that species-level differences in cibarial pump investment and subsequent feeding ability dictate planthopper responses to N and P limitation for both species, as wing forms within a species performed equally.

Compensatory feeding and the ability to maintain adequate performance on nutrient limited host plants has been documented in several species of insect herbivores, particularly chewing insects (Raubenheimer and Simpson 1993, Kingsolver and Woods 1998, Lavoie and Oberhauser 2004). Also, a xylem-feeding leafhopper has also been shown to increase consumption in relation to low nitrogen availability (Brodbeck et al. 1999).

Phosphorus limitation in *Spartina* had only minor adverse effects on the performance of both *Prokelisia* species. Given the discrepancy in phosphorus content between *Spartina* and *Prokelisia*, a mismatch that was as large as that for nitrogen content, I fully expected phosphorus limitation to have very adverse effects on planthopper performance growth, as has been observed in other organisms (Urabe and Watanabe 1992, DeMott et al. 1998, Sterner and Schulz 1998, Elser et al. 2001, Makino et al. 2002, Urabe et al. 2002, Xenopoulos et al. 2002). However, the unusually high N content of both species (approximately 11%), which is approximately the same as that for predators and significantly greater than the average for phytophagous herbivores (9.65%N) (Fagan et al. 2002b), may impose unusual nitrogen demands and underlie the relatively more important role of nitrogen than phosphorus in the nutritional ecology of *Prokelisia*. Considering that predators feed at a trophic level with a more comparable N content (difference between 11%N and 9%N) than herbivores feeding on plants (difference

between 9%N and 1.5%N), the extremely high N content of *Prokelisia* planthoppers is remarkable.

The two *Prokelisia* species have evolved very different strategies for coping with nutrient deficiencies in their host plant. Data suggests that *P. dolus* uses compensatory feeding (Chapter 3), whereas *P. marginata* has evolved a dispersal strategy that allows it to effectively track spatial changes in host plant quality (Denno 1983, Roderick 1987, Denno et al. 2002, Denno et al. 2003, Figure 1.11). In fact, all data points to a phenotypic trade-off between the two strategies with a greater commitment to flight musculature in *P. marginata* and a greater investment in the musculature associated with enhanced ingestion in *P. dolus*. When its option of dispersal is eliminated, and *P. marginata* is confined on nutrient-deficient host plants, its performance is drastically affected, far more so than that of *P. dolus*. Thus, despite similar body nitrogen and phosphorus content, and extremely analogous mismatch in N and P composition compared to *Spartina*, the two *Prokelisia* species incur the penalties of depleted host-plant nutrition quite differently. This research underscores the importance of interfacing an understanding of herbivore life-history strategies with ecological stoichiometry in order to interpret the consequences of macronutrient limitation on herbivore performance and population dynamics.

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## Figure Descriptions

Figure 1.1. Effect of 4 levels of nitrogen fertilization (0N, 10N, 30N, and 60N) and 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) on the (A) nitrogen content (%) and (B) phosphorus content (%) of *Spartina alterniflora* plants grown under laboratory conditions. Note that the nitrogen content of *Spartina* increased significantly with the addition of N and P fertilizer. *Spartina* also exhibited a significantly greater increase in phosphorus content with the addition of both N and P fertilizer treatments. Means ( $\pm$  SE) are reported.

Figure 1.2. (A) Effect of 4 levels of nitrogen fertilization (0N, 10N, 30N, and 60N) and 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) on the aboveground biomass ( $\text{g/m}^2$ ) of *Spartina alterniflora* plants grown under laboratory conditions. Note that maximum biomass was achieved in the 10N treatment, and that any level of phosphorus subsidy above 0P resulted in increased biomass. (B) Effect of 4 levels of nitrogen fertilization (0N, 10N, 30N, and 60N) and (C) 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) on the mortality (%) of *Spartina alterniflora* plants. Plant mortality was highest on the 60N treatment plants and the phosphorus treatments had no effect on mortality. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 1.3. The (A) nitrogen content (%) and (B) phosphorus content (%) of *Spartina alterniflora*, and the planthoppers *Prokelisia dolus* and *Prokelisia marginata*

raised on *S. alterniflora* plants grown under laboratory conditions. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 1.4. Effect of 2 levels of nitrogen fertilization (0N and 10N) and 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) applied to *Spartina alterniflora* on the survival (%) of (A) *Prokelisia dolus* and (B) *Prokelisia marginata* raised on the 8 treatment combinations of plants in the laboratory. Nitrogen fertilization of *Spartina* resulted in an increase in survival of both *Prokelisia* species. Means ( $\pm$  SE) are reported.

Figure 1.5. Effect of 2 levels of nitrogen fertilization (0N and 10N) and 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) applied to *Spartina alterniflora* on the body size (tibia length in mm) of female (A) *Prokelisia dolus* and (B) *Prokelisia marginata* raised on the 8 treatment combinations of plants in the laboratory. Nitrogen fertilization of *Spartina* resulted in an increase in the body size of both *Prokelisia* species. Means ( $\pm$  SE) are reported.

Figure 1.6. Effect of 2 levels of nitrogen fertilization (0N and 10N) and 4 levels of phosphorus fertilization (0P, 2P, 6P, and 12P) applied to *Spartina alterniflora* on the development time (days to adult molt) of female (A) *Prokelisia dolus* and (B) *Prokelisia marginata* raised on the 8 treatment combinations of plants in the laboratory. Nitrogen fertilization of *Spartina* promoted rapid development in both *Prokelisia* species. Means ( $\pm$  SE) are reported.

Figure 1.7. Effect of nitrogen and phosphorus fertilization treatments (0N0P, N, P, and both N and P) on the nitrogen content (%) of *Spartina alterniflora* growing in (A) a high-marsh meadow habitat and (B) a low-marsh tidal creek habitat at Tuckerton, New Jersey. (C) Effect of the same fertilization treatments on the nitrogen content of *Spartina* growing in meadow and tidal creek habitats on 22 July 2002, the date on which planthoppers were sampled. Nitrogen subsidy enhanced the nitrogen content of *Spartina* in both habitats. Means ( $\pm$  SE) are reported.

Figure 1.8. Effect of nitrogen and phosphorus fertilization treatments (0N0P, N, P, and both N and P) on the phosphorus content (%) of *Spartina alterniflora* growing in (A) a high-marsh meadow habitat and (B) a low-marsh tidal creek habitat at Tuckerton, New Jersey. (C) Effect of the same fertilization treatments on the nitrogen content of *Spartina* growing in meadow and tidal creek habitats on 22 July 2002, the date on which planthoppers were sampled. The combination of nitrogen and phosphorus fertilization resulted in the highest phosphorus content of *Spartina*, particularly in the meadow habitat. Means ( $\pm$  SE) are reported.

Figure 1.9. Effect of nitrogen and phosphorus fertilization treatments (0N0P, N, P, and both N and P) on the biomass (g dry mass) of *Spartina alterniflora* growing in a high-marsh meadow and a low-marsh tidal creek habitat at Tuckerton, New

Jersey. Nitrogen subsidy resulted in a significant increase in biomass only in the meadow habitat. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 1.10. The (A) nitrogen content (%) and (B) phosphorus content (%) of *Spartina alterniflora*, and the planthoppers *Prokelisia dolus* and *Prokelisia marginata* sampled on *S. alterniflora* plants in the field on a salt marsh at Tuckerton, New Jersey. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 1.11. Population density [ $\log(N+1)/m^2$ ] of the wing forms (macropters and brachypters) of *Prokelisia dolus* in (A) meadow and (B) tidal creek habitats in plots of *Spartina* fertilized with nitrogen (+N) or in plots not receiving a nitrogen subsidy on a salt marsh at Tuckerton, New Jersey. Density responses of the wing forms of *Prokelisia marginata* to the same nitrogen treatments are shown in (C) meadow habitat and (D) tidal creek habitats. Macropters of *P. dolus* were more abundant on fertilized *Spartina* compared to non-fertilized control plots only in the meadow habitat, and flightless brachypters showed no density response to fertilized *Spartina* in either habitat. Macropters of *P. marginata* were more abundant on fertilized *Spartina* compared to controls in both habitats, a response that was particularly strong in the tidal creek. Flightless brachypters of this planthopper showed no density response to fertilized *Spartina* in either habitat. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 1.1.

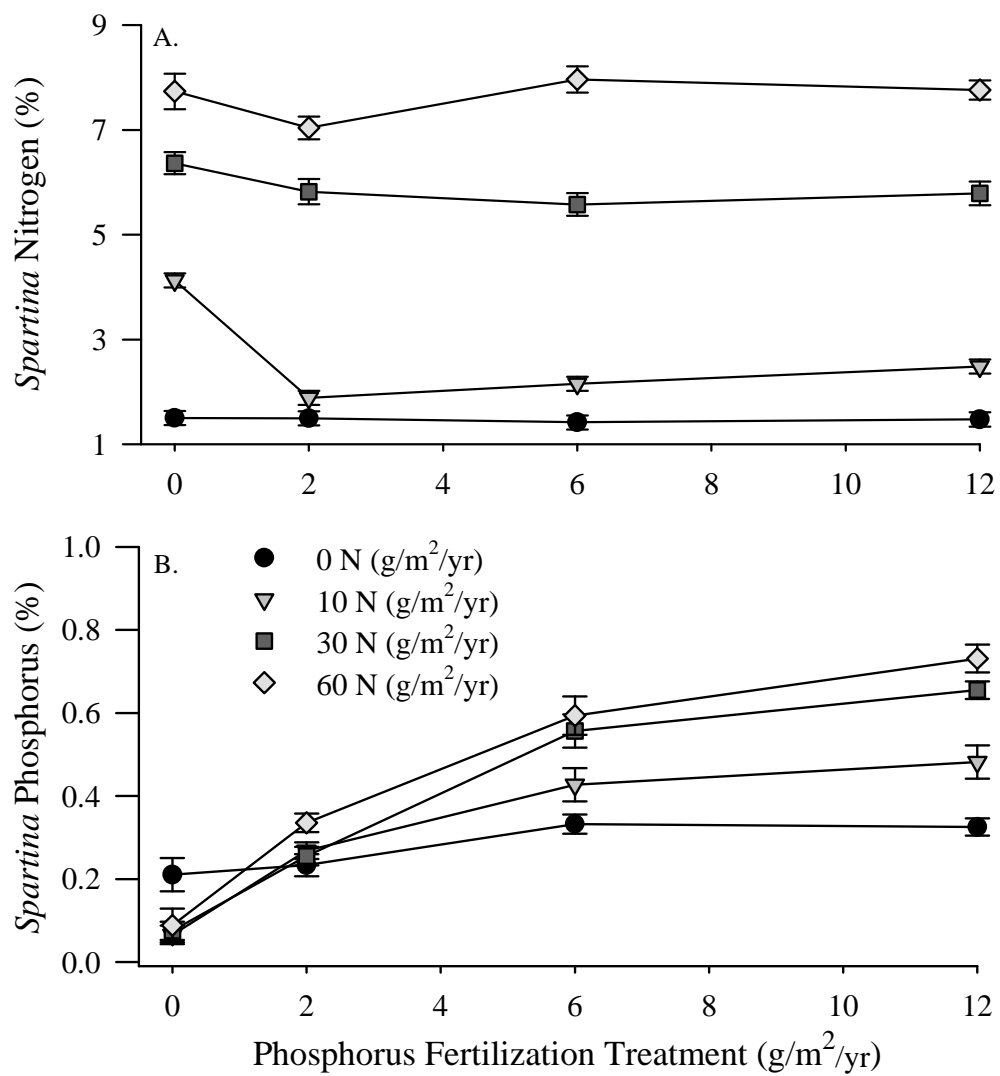


Figure 1.2.

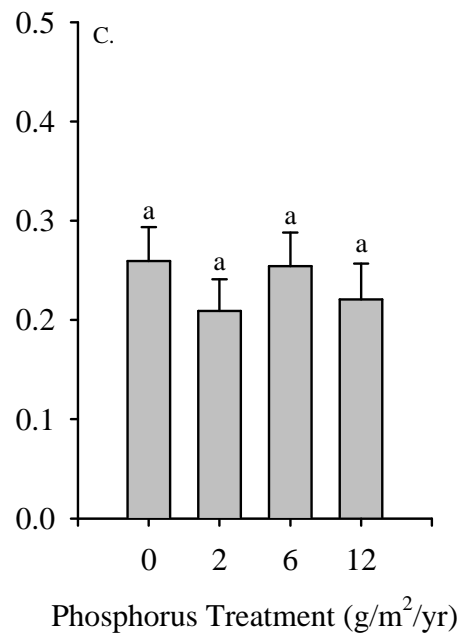
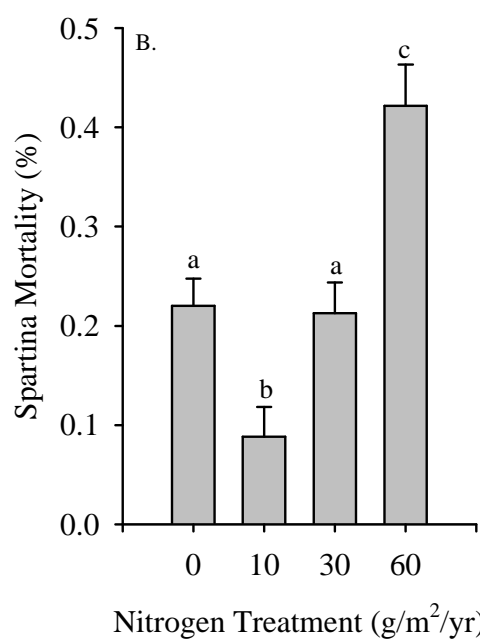
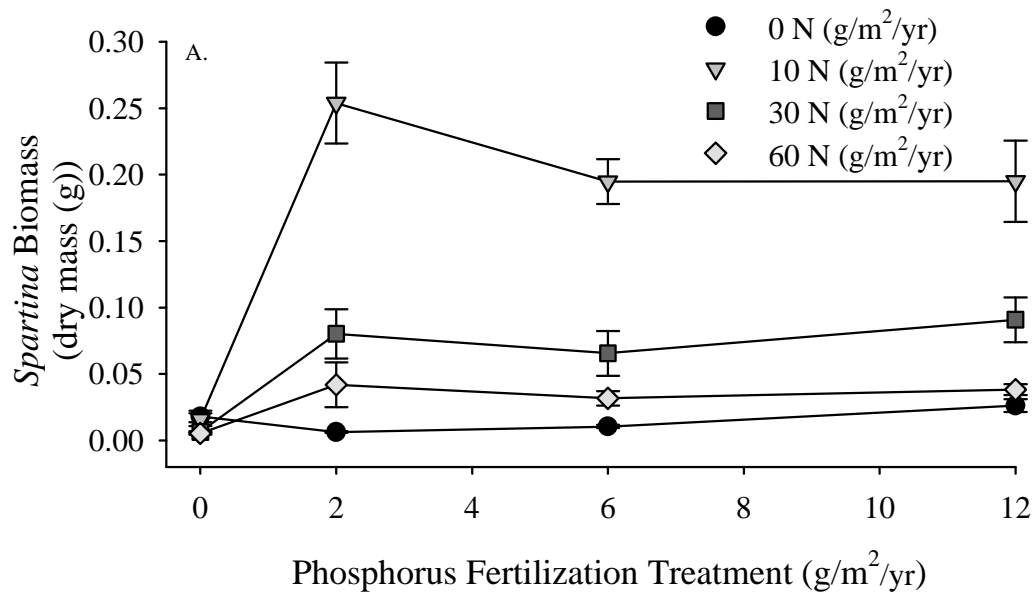




Figure 1.3.

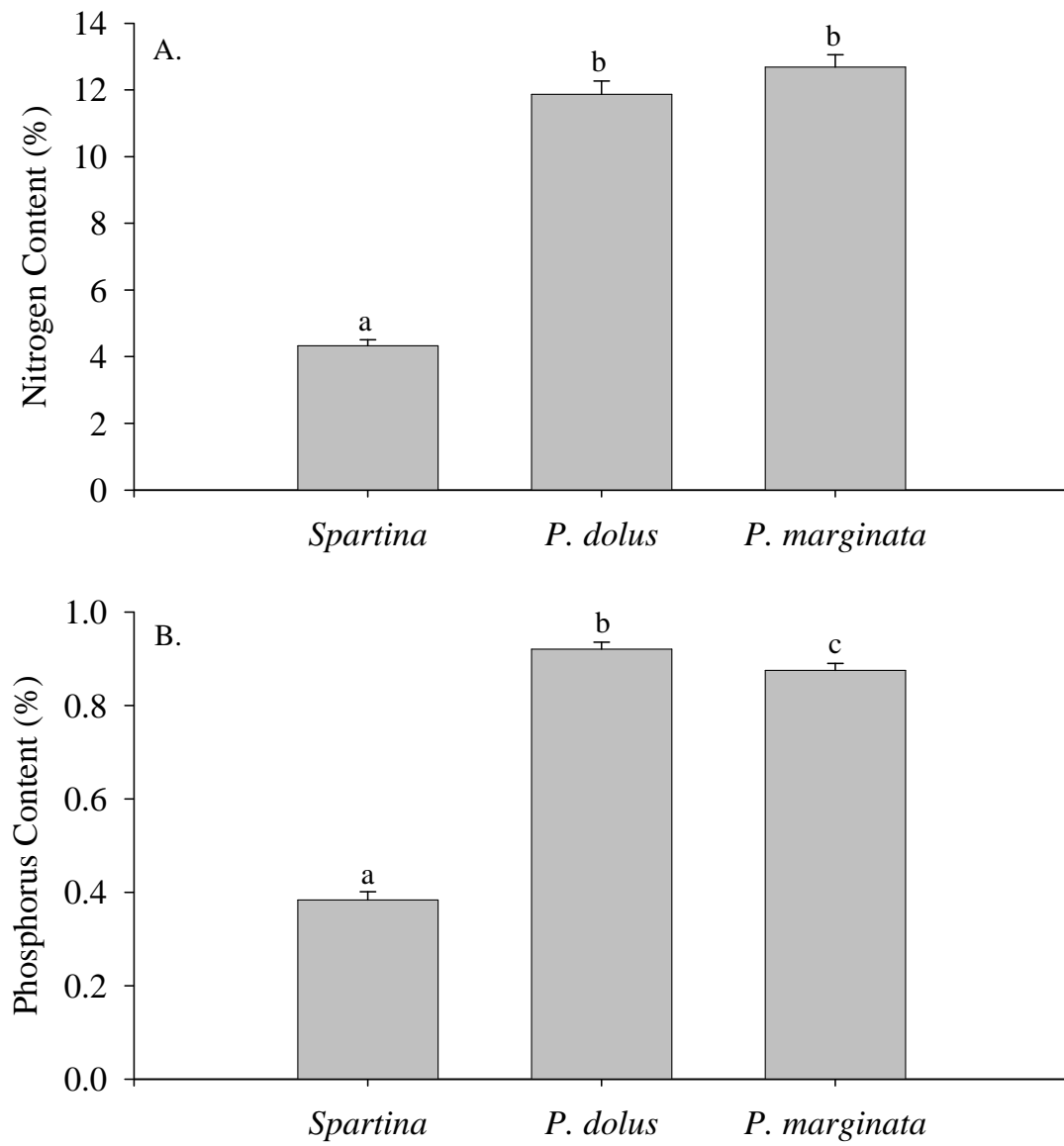


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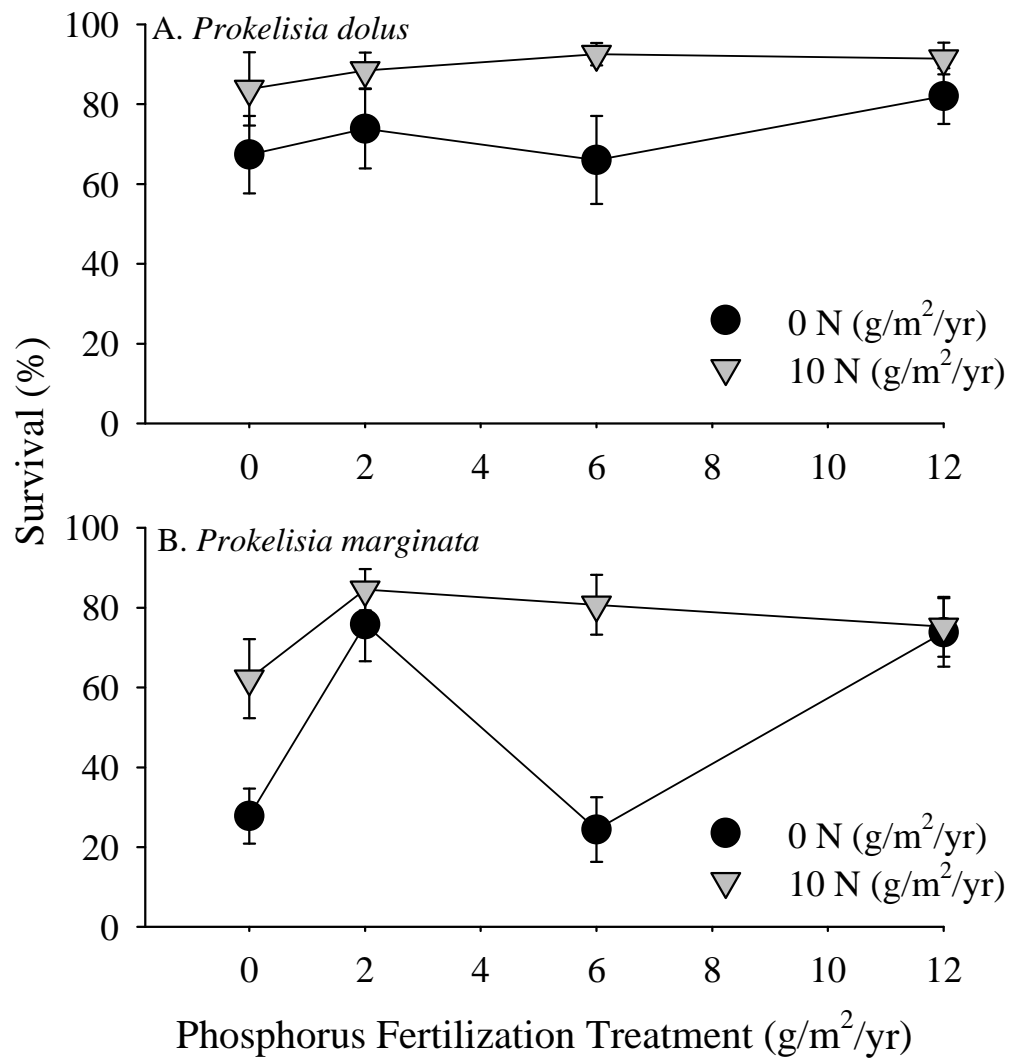


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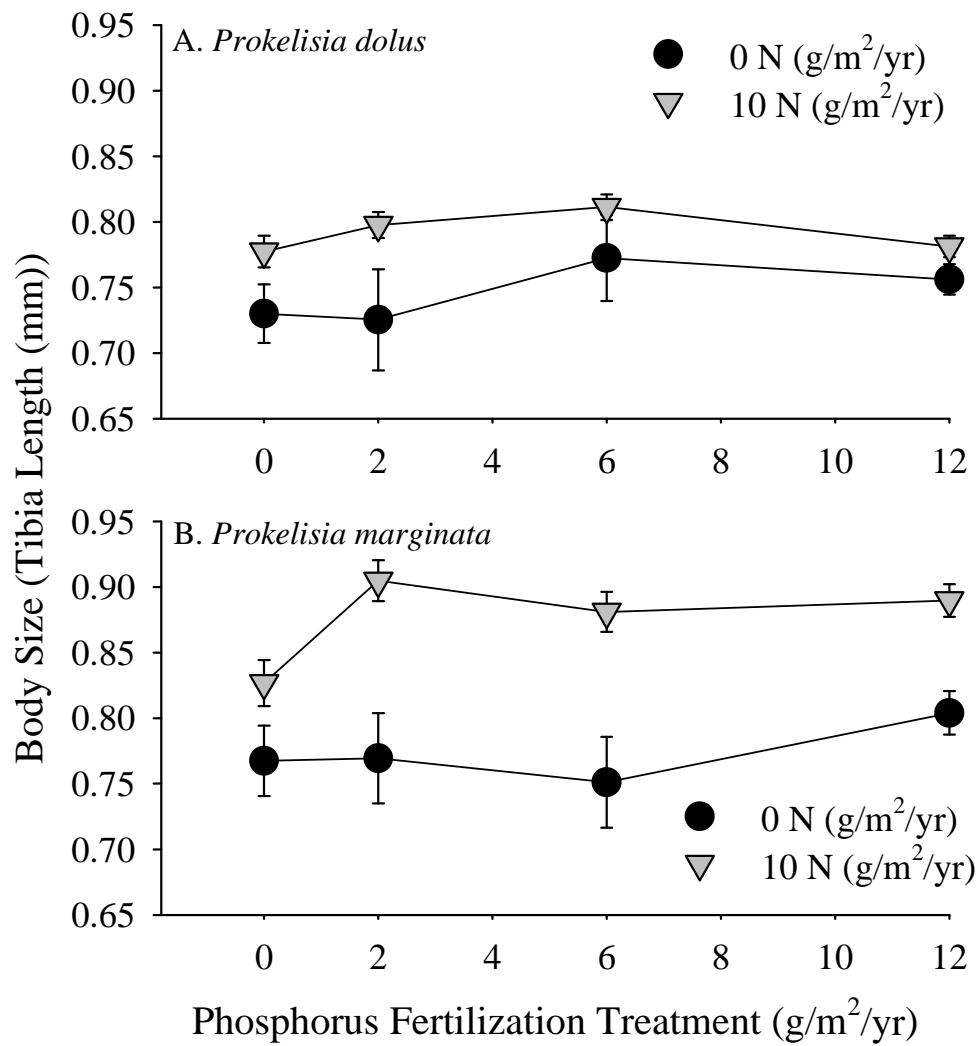


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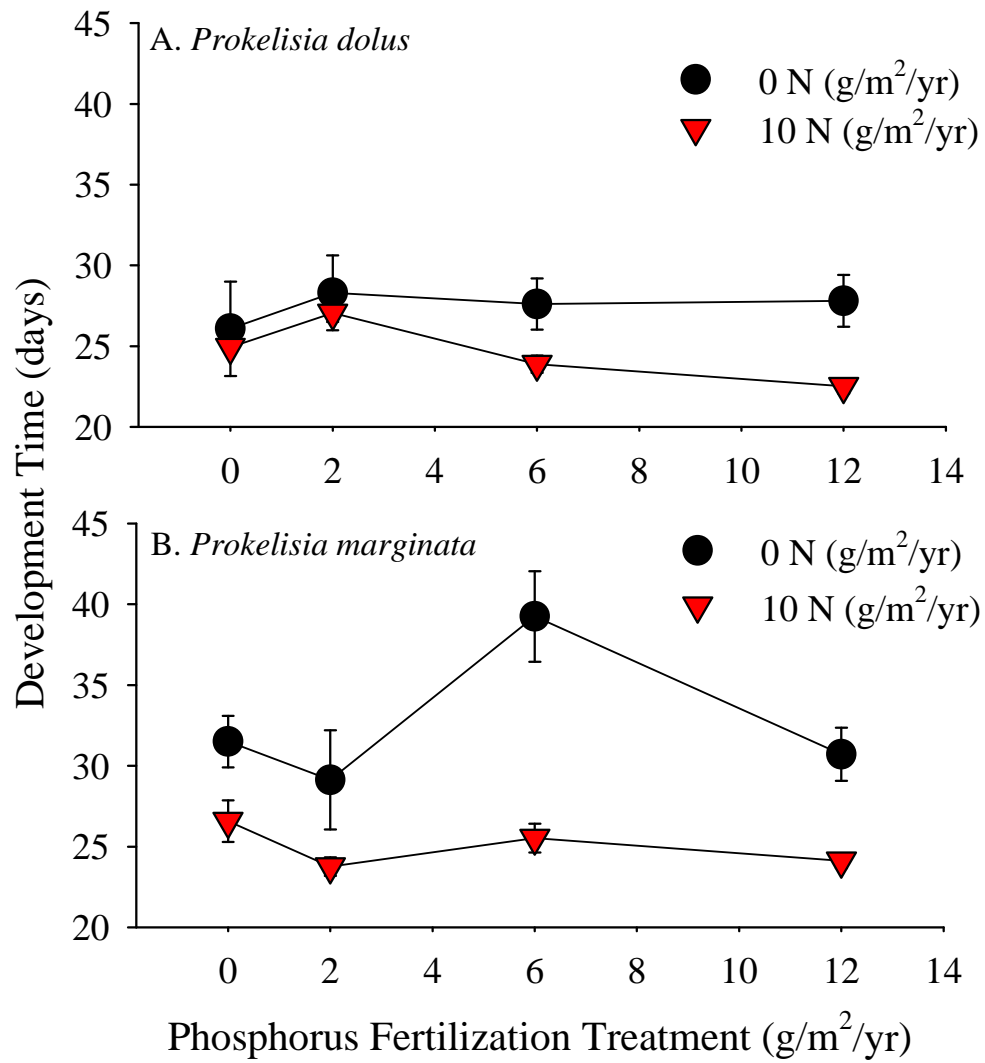


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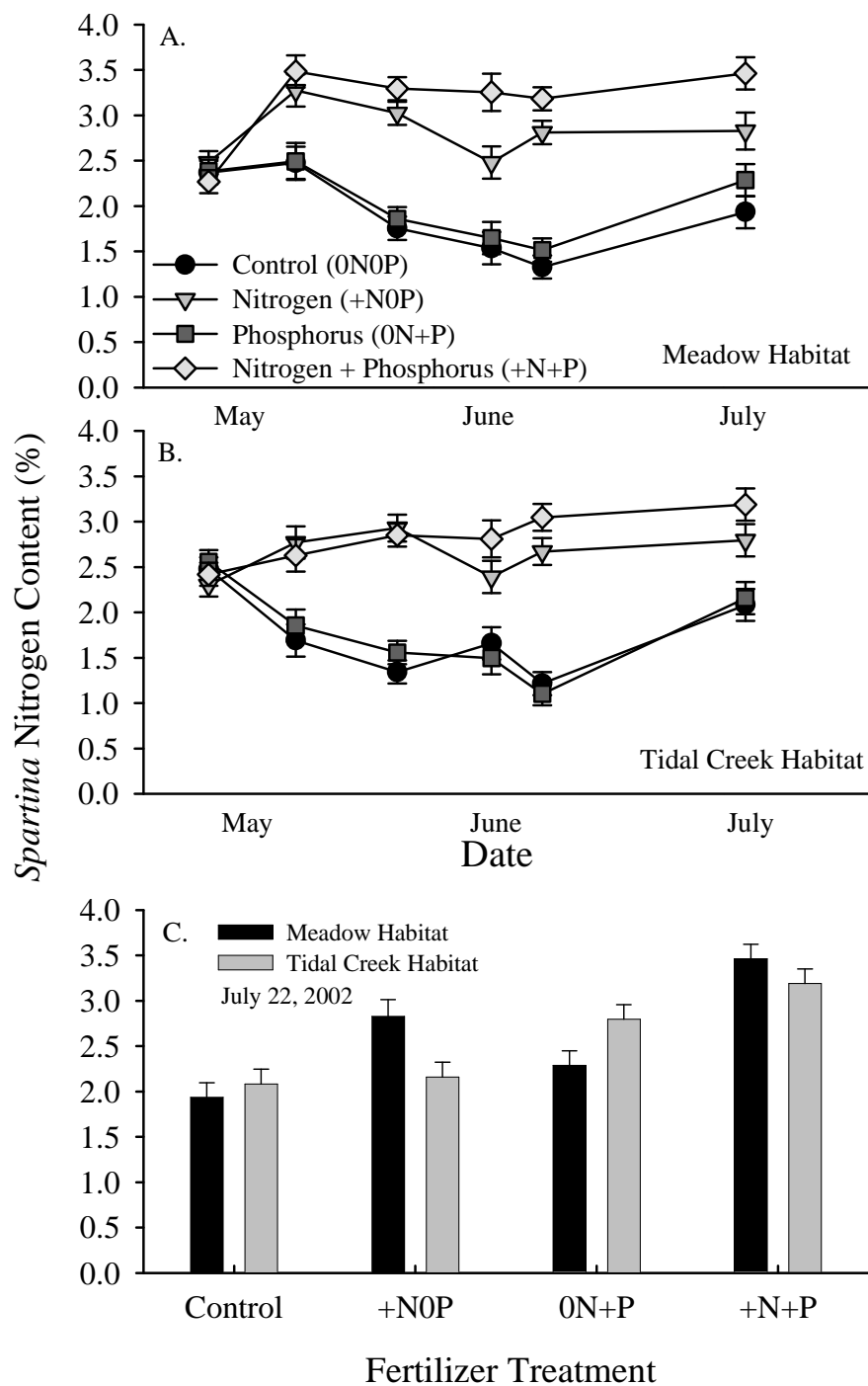


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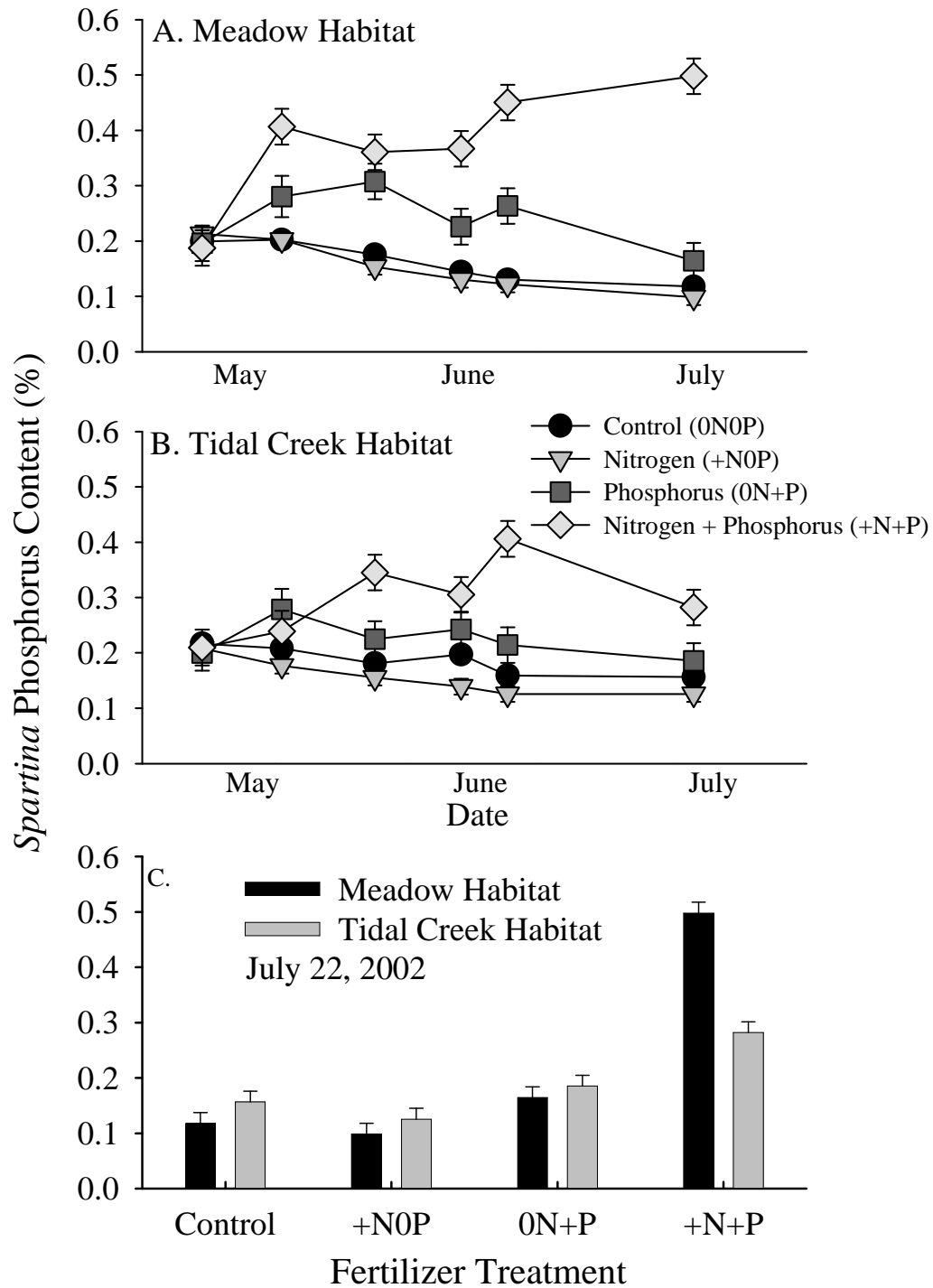


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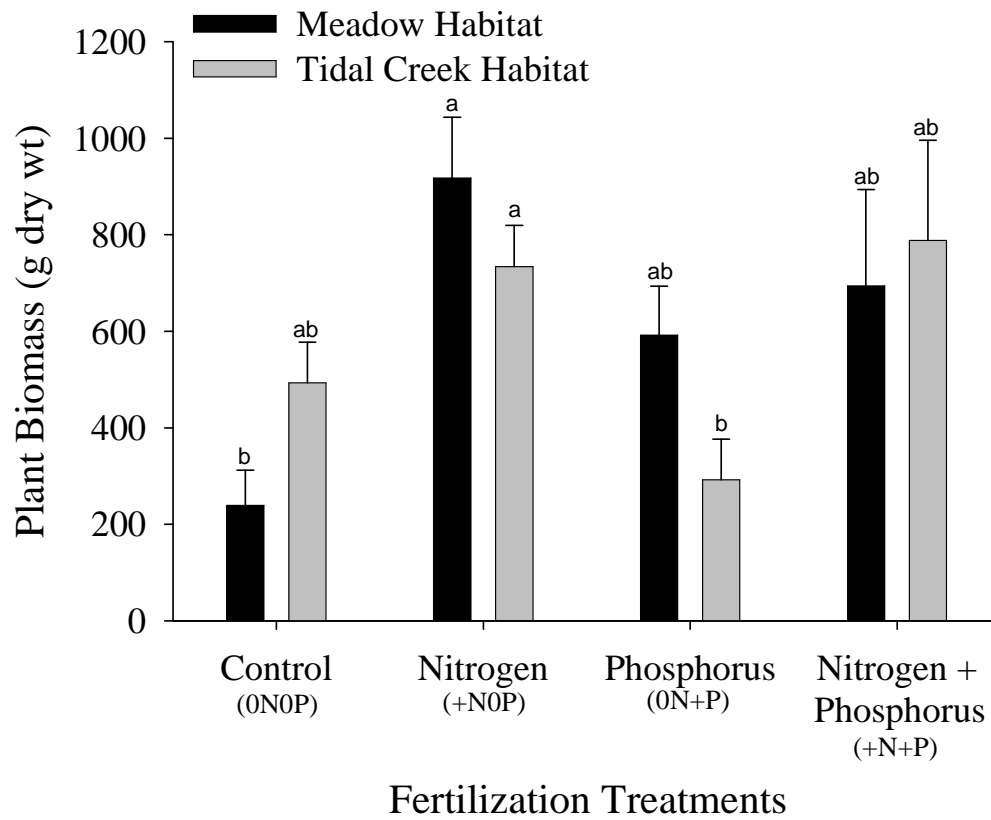


Figure 1.10.

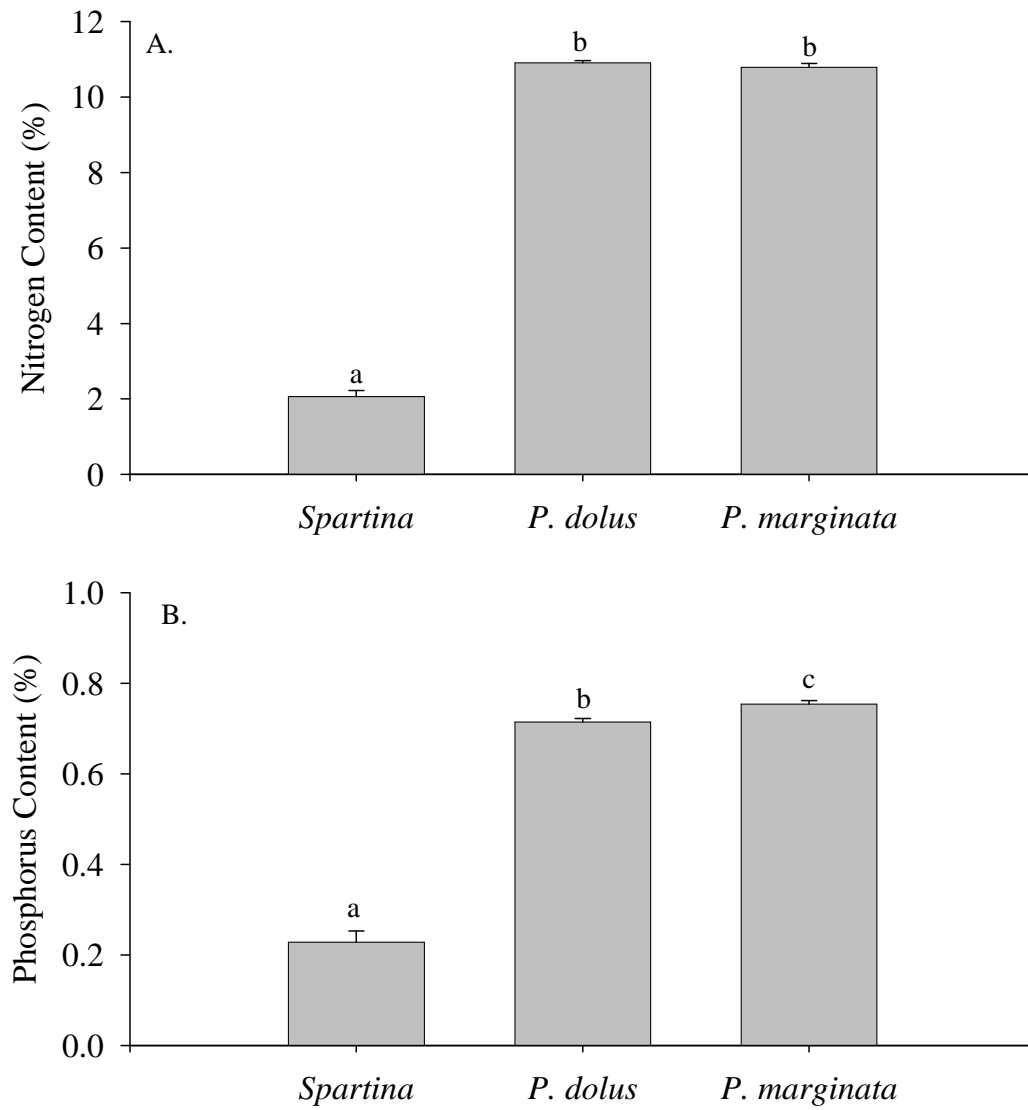
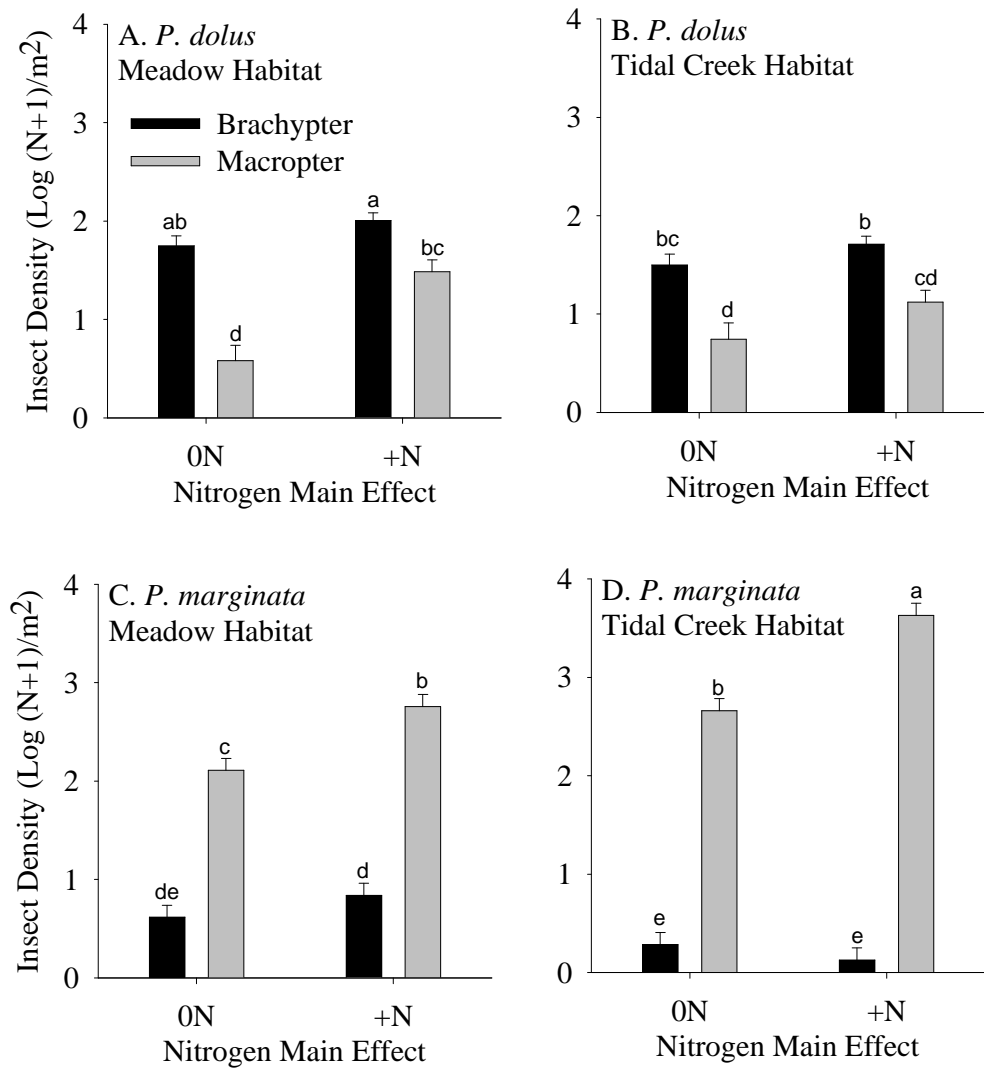




Figure 1.11.



## Chapter 2: Homeostatic regulation of body elemental composition and its consequences for growth in two phytophagous insects

### Introduction

Ecological stoichiometry provides an integrative approach for analyzing community and food web structure because all organisms are composed of the same major elements [carbon (C), nitrogen (N), and phosphorus (P)], whose balance affects species interactions, production, nutrient cycling, and food-web dynamics (Elser et al. 1996, Huxel 1999, Fagan et al. 2002b, Sterner and Elser 2002, Denno and Fagan 2003). Of these three major elements, nitrogen in particular has been cited as the essential nutrient that is most limiting to phytophagous insects (McNeill and Southwood 1978, Mattson 1980, Strong et al. 1984, White 1993). Phosphorus, however, has been largely overlooked as a potentially important nutrient for phytophagous insects, but recent studies suggest that P may be far more important in terrestrial systems than previously thought (Ayers et al. 2000, Elser et al. 2000a, Schade et al. 2003). Notably, carbon occurs in surplus in terrestrial plants (Elser et al. 2000a), and eliminating excess carbon may prove costly to insect herbivores (Raven 1983, White 1993).

Food webs, particularly those in terrestrial systems, are built on an extremely nutrient-poor but carbon-rich autotroph base (high C:N and C:P ratios) (Elser et al. 2000a). By contrast, terrestrial consumers have significantly lower C:N and C:P ratios, indicating a dramatic elemental mismatch in body elemental composition relative to their food resources. Thus, phytophagous insects face two problems; not only must these

herbivores obtain nutrients from nutrient-limited food resources, but they must also process excess amounts of carbon to acquire limited nutrients (Raven 1983).

Despite the elemental inequalities between phytophagous insects and their host plants, herbivores are able to maintain their elemental body composition via homeostasis. Homeostasis is the ability to regulate elemental body composition despite feeding on stoichiometrically mismatched food resources, and is a general tenet of ecological stoichiometry (Sternern and Elser 2002). Homeostatic regulation in phytophagous insects can occur through mechanisms such as the differential disposal (excretion or respiration) of excess elements (Slansky and Feeny 1977, Elser et al. 1996, DeMott et al. 1998, Darchambeau et al. 2003, Trier and Mattson 2003). The physiological regulation of elemental body content in invertebrates was originally thought to be strict, such that elemental composition was maintained despite wide variation in the elemental composition of food resources (Sternern and Elser 2002). Recent studies, however, indicate that individuals may alter their body elemental content when they are exposed to food resources that deviate significantly from their own elemental composition (Plath and Boersma 2001, Cross et al. 2003, Elser et al. 2003). Thus, organisms may struggle to regulate their optimal elemental composition when faced with nutrient-poor and carbon-rich food resources. The result of homeostatic regulation, especially when organisms feed on nutrient-deficient food, is a cost that is often imposed on performance, namely decreased growth rate (Elser et al. 1996, Plath and Boersma 2001, Sternern and Elser 2002, Elser et al. 2003, Raubenheimer and Simpson 2004). Growth rate penalties occur because organisms use limited nutrients for maintenance, which are then unavailable for maximizing performance (Sternern and Elser 2002).

Not surprisingly, insect herbivores have evolved a variety of life-history strategies and physiological mechanisms that allow them to increase their intake of limiting nutrients (McNeill and Southwood 1978, White 1993). One mechanism for coping with deteriorating plant nutrition is to disperse to better quality host plants in other habitats (Denno et al. 2002). Alternatively, remaining on poor quality plants and waiting for nutritional conditions to improve may be an option if herbivores are able to compensate, either by storing nutrients as a buffer against times of poor food quality (Woods et al. 2002), by increasing the volume of food they ingest (feeding compensation) (Lavoie and Oberhauser 2004, Raubenheimer and Simpson 2004) or by increasing their nutrient assimilation efficiency (Prestidge 1982b, Brodbeck et al. 1996, Lavoie and Oberhauser 2004, Raubenheimer and Simpson 2004).

This study is the first to employ a stoichiometric framework to the homeostatic regulation of elemental body composition (C, N and P) and its consequences for the growth of terrestrial insect herbivores. Using two phytophagous planthoppers (*Prokelisia dolus* and *P. marginata*; Hemiptera: Delphacidae) with divergent life-history strategies, this research identifies a mismatch in elemental composition between these herbivores and their host plant, examines life-history related differences in the homeostatic regulation of elemental body composition, and identifies any costs imposed by homeostasis. The life-history trait that differs so strikingly between these two planthoppers is their dispersal ability; *P. marginata* is a migratory species whereas *P. dolus* is comparatively sedentary (Denno et al. 1996, Denno and Peterson 2000). Both planthopper species are wing-dimorphic with both flight-capable adults (macropters that can disperse long distances) and flightless adults (brachypters) present in the same

population (Denno et al. 1989, Denno et al. 1996, Peterson and Denno 1997, Zera and Denno 1997). An approximate assessment of dispersal ability can be obtained by comparing the fraction of macropters in populations, which is high for *P. marginata* (>90%) and low for *P. dolus* (<20%) (Denno et al. 1996).

Species-related differences in dispersal ability may indicate that these two planthoppers use different mechanisms to cope with deteriorating host-plant nutrition (Denno et al. 2002). *Prokelisia marginata* is known to disperse and colonize nitrogen-enriched host-plant patches in the field whereas *P. dolus* is a poor colonizer of such patches (Denno 1983, Roderick 1987, Denno et al. 2002). When confined to nutrient-deficient host plants, *P. marginata* exhibits reduced performance (Chapter 1), suggesting that dispersal may be the primary mechanism used by this species to escape nutrient limitation. Although the performance of both *Prokelisia* species is adversely affected when they develop on low-quality plants, *P. dolus* has the ability to survive on and tolerate nitrogen- and phosphorus-deficient plants better than does *P. marginata* (Denno et al. 2002, Chapter 1). Thus, inversely associated with dispersal capability in these two species is their ability to tolerate poor plant nutrition, probably via differences in feeding compensation (Denno et al. 2000, Chapter 3). This relationship potentially derives from a potential trade-off between flight musculature and the cibarial musculature associated with the ingestion of phloem sap and thus their ability to enhance food intake on nutrient-deficient host plants (Chapter 3). Apparently, these two species cope with deteriorating host-plant quality in two fundamentally different ways, *P. marginata* by migrating to better quality host plants in other habitats and *P. dolus* by tolerating periods of low plant

quality until nutrient conditions improve (Cook and Denno 1994, Denno et al. 2000, Chapter 3).

Using a blend of laboratory and field experiments, the objectives of this study were three: (1) to establish any mismatch in the elemental composition of macronutrients (C, N, and P) between planthoppers and their host plant, (2) to investigate homeostatic differences between the *Prokelisia* species in their ability to maintain their body elemental composition when fed a range of plants differing in elemental stoichiometries, and (3) to determine any species-specific growth costs associated with homeostasis. The elemental currencies used for this study were the ratios of C:N, C:P, and N:P and how they differed between host plants and the *Prokelisia* species. Carbon:nutrient ratios examine the potential consequences of limited N or P in the context of excess C, whereas N:P can be used to examine the relative importance of N or P in homeostatic regulation (Sternner and Elser 2002).

I hypothesize that both *Prokelisia* species will maintain their body elemental composition (e.g., C:N ratio) when fed a range of plants that vary in their elemental stoichiometry (e.g., C:N ratios). However, the cost of homeostasis (decreased growth) will be greater for *P. marginata* than *P. dolus* and for macropters compared to brachypters. This prediction derives from the enhanced ability of *P. dolus* and brachypters of both species to compensate for nutrient deficiencies by increasing their ingestion rate (Chapter 3). Moreover, dispersive species may have greater nutrient demands than sedentary species due to investments in flight fuel (Cook and Denno 1994, Denno 1994, Dudley 2000). Thus, a greater investment in dispersal should restrict the compensatory feeding ability of *P. marginata*, as well as the macropters of both species,

and result in decreased growth rate on nutrient-deficient plants. Overall, the divergent strategies should favor *P. dolus* and penalize *P. marginata* when confined to and challenged by nutrient-poor host plants. This study will be among the first to examine the interaction between life-history strategy, homeostatic ability, and the consequences for growth of stoichiometric imbalances in macronutrients between insect herbivores and their host plants.

## **Study Site and System**

### Study Site

The relationship in elemental composition (C, N, and P) between *Prokelisia* planthoppers and their cordgrass host plant *Spartina alterniflora* were studied in the laboratory and in the field on an expansive intertidal marsh in the Great Bay-Mullica River estuarine system at the end of Great Bay Boulevard and just north of the Rutgers University Marine Station, Tuckerton, Ocean County, New Jersey (39° 30.8' N, 74° 19.0' W). The vegetation of this marsh and other mid-Atlantic marshes is dominated by the perennial cordgrass *Spartina alterniflora*, where it grows in extensive pure stands within the intertidal zone (Denno 1983, Gallagher et al. 1988, Mendelssohn and Morris 2000).

### Macronutrient variability in *Spartina alterniflora*

Within the intertidal zone, the structure and nutritional content of *Spartina alterniflora* varies dramatically with elevation due to differences in nutrient subsidy by tides/groundwater and salinity that alters nutrient uptake (Denno 1983, Gallagher et al.

1988, House et al. 1998). Moving up the elevational gradient from low-marsh habitats (tidal creek banks) to high-marsh plateaus (meadows), *Spartina* plants generally decrease in nutrient content (nitrogen and phosphorus) and height (Denno 1983, Ornes and Kaplan 1989). In particular, nitrogen uptake can be limited on the high marsh under conditions of elevated salinity that arise from evaporation (Mendelssohn 1979, Webb 1983). Thus, natural variation in the nitrogen and phosphorus content of *Spartina* occurs in the field presenting potential local problems to plant-feeding insects that must meet their nutrient demands.

*Responses to host-plant nutrition and population dynamics of Prokelisia planthoppers*

*Prokelisia marginata* and *P. dolus* are the most abundant herbivores on Atlantic coastal marshes where they feed exclusively on *Spartina alterniflora* (Denno 1977, Denno et al. 2002). Responses of the two *Prokelisia* species to the nitrogen content of *Spartina* suggest that both species are nitrogen limited, but that *P. marginata* is more sensitive to fluctuating plant nitrogen than its congener (Cook and Denno 1994, Denno et al. 2002, 2003, Chapter 1). For instance, although both species select the most nitrogen-rich plants or plant parts on which to feed and oviposit, *P. marginata* colonizes nitrogen-enriched plants at higher densities than *P. dolus* (Denno 1983, 1985, Roderick 1987, Denno et al. 2002). Moreover, what little data exists suggests that both species exhibit enhanced survival and performance on nitrogen-fertilized *Spartina*, but that *P. marginata* shows a stronger response (Denno 1985, Denno and McCloud 1985, Denno et al. 1996, Olmstead et al. 1997). *Prokelisia dolus* also has the ability to withstand nitrogen-deficient plants better than *P. marginata* (Denno et al. 2000, Chapter 1). Existing data



suggests that nitrogen is limiting for both *Prokelisia* species, that phosphorus is less limiting than nitrogen, and that the performance of *P. marginata* is more adversely affected by N- and P-deficient host plants than its congener (Chapter 1).

There is a dramatic difference in population dynamics between the two *Prokelisia* species. *Prokelisia dolus* is a relatively sedentary species (most adults are flightless) that is restricted primarily to high-marsh meadows of *Spartina* (Denno et al. 1996). By contrast, *P. marginata* is a highly mobile species (most adults are macropterous) along the Atlantic coast and undergoes annual inter-habitat migrations between overwintering sites on the high-marsh and more favorable low-marsh habitats where development occurs (Denno et al. 1996). Outbreaks of both planthopper species are associated with nitrogen-rich host plants, but most striking are the outbreaks of *P. marginata* that occur frequently on low-marsh *Spartina* (Denno and Peterson 2000)

## Methods

Experiments were conducted in the laboratory and field to: (1) determine the mismatch in elemental composition (C, N, and P) between *Prokelisia* planthoppers and their *Spartina* host plant, (2) evaluate the ability of both *Prokelisia* species to maintain their elemental body composition when fed a range of host plants varying in elemental stoichiometry, and (3) discover any growth penalties that arise from homeostasis on nutritionally-deficient host plants.

### Laboratory Assessment

A 4x4 factorial fertilization regime was initiated in May 2000 to create a range of host plant elemental compositions (C:N, C:P, and N:P ratios) on which the stoichiometry, homeostatic ability, and growth of *Prokelisia* planthoppers was assessed. Potted *Spartina* plants (3-5 plants per pot grown in sand substrate) were grown in flats (80 pots per flat in each of two flats) in the greenhouse and exposed to four levels of nitrogen subsidy (0,10,30,60g/m<sup>2</sup>) and four levels of phosphorus addition (0,2,6,12g/m<sup>2</sup>), all crossed. To achieve the desired treatments, plants in each treatment combination were fertilized every three weeks from May until July. Nitrogen was applied as ammonium nitrate (0, 5g, 16g, 32g / flat / application) and phosphorus was supplied as triple super phosphate (0g, 0.8g, 2g, 5g / flat / application). The macronutrient treatments are hereafter referred to as 0N, 10N, 30N and 60N for nitrogen and 0P, 2P, 6P and 12P for phosphorus. The range of N and P fertilization rates was chosen to bracket those used in previous laboratory experiments and to include the spectrum of *Spartina* nutrient contents that occurs naturally in the field (Denno et al. 2000). Plants for this experiment were grown from seed obtained from Environmental Concern, Inc. St. Michaels, MD. Most plants in the 30N and 60N treatments eventually died creating imbalance in the number of plants available for use in experiments (Chapter 1).

#### Elemental Composition (C:N:P) of *Spartina*

The mean elemental composition of *Spartina* (C:N:P) due to the fertilization treatments was determined for 6 randomly chosen pots containing planthopper-free plants harvested on 26 July. Foliar nutrient content was used as an index of the phloem

nutrients available to planthoppers because the two plant variables are related (Youssefi et al. 2000). Harvested plant samples (sample = aboveground leaf material in each pot (no culms)) were oven dried for 48 hours at 60°C and ground in a Wiley Mill. The leaf powder was then analyzed for N and C content using a Perkin-Elmer 2400 CHN analyzer, and for P content using persulfate digestion (Clesceri et al. 1998). All elemental ratios used in analyses (e.g., C:N) are atomic ratios. The mean elemental composition (C:N, C:P, N:P) for *Spartina* for each fertilization treatment was used as the dependent variable for the homeostasis and growth rate analyses below. A grand mean for *Spartina* elemental composition (C:N, C:P, N:P) was also determined as stated below to compare plant and herbivore stoichiometries.

#### Elemental Composition and Growth Rate of *Prokelisia* Species

To determine the effects of nutrient treatment on the growth rate of planthoppers, cohorts of 3 to 5 first instar nymphs of each *Prokelisia* species were placed separately onto caged treatment plants (20 initial replicate cohorts per treatment) on 17 July (see Denno et al. 2000 for cage design). The original design was for each *Prokelisia* species to be raised on 16 different treatment combinations (4 levels of N and 4 levels of P fertilization) of *Spartina*. However, severe plant death in the 30N and 60N treatments, and sporadic plant death in the remaining treatments resulted in uneven replication such that 3-12 replicates remained per treatment. Emerging adult planthoppers were sorted to species, sex, and wing form prior to determining their development time to adult (days), body size (indexed as tibia length in mm) and C, N, and P content. Nymphs for the

experiment were obtained from lab cultures initiated by ovipositing adults collected from the Tuckerton field site.

Growth rate was assessed as tibia length gained per day and was calculated as final tibia length (mm)-initial tibia length (mm)/development time (days). For this calculation, initial tibia length was determined independently for ten 1<sup>st</sup>-instar nymphs for both species (1<sup>st</sup> instars are less than 0.1mm in body length). The average growth rate for each replicate cohort was determined for use in forthcoming analyses. Following the measurement of tibia length, adult planthoppers were pooled by replicate (2-5 individuals) and oven dried prior to analysis for C, N and P content as above (Clesceri et al. 1998). Only female planthoppers were used in these determinations because too few adult males emerged during the experiment. Thus, growth rate and elemental composition was determined for females (both wing forms) of both *Prokelisia* species.

#### Mismatched Elemental Stoichiometry between *Spartina* and the *Prokelisia* Species

To establish that the elemental composition of *Spartina* differed from that of the *Prokelisia* planthoppers, the overall average C:N, C:P, and N:P of *Spartina* was compared to the overall average C:N, C:P, and N:P of *Prokelisia dolus* and *P. marginata* using a mixed-model analysis of variance with organism type ( *P. dolus*, *P. marginata* or *Spartina*) as the independent variable. The grand means of all experimental treatment plants and planthoppers raised on those plants were used to generate the elemental ratios.

### Homeostatic Regulation of Body Elemental Composition in *Prokelisia*

To determine if planthoppers were able to maintain their elemental composition when reared on plants of different elemental compositions, I calculated H, which measures the strength of homeostasis and varies from 1 to infinity (Sterner and Elser 2002). Homeostasis is calculated as:  $H = 1/[\text{absolute value of slope of the relationship between log (insect elemental composition) versus log (plant elemental composition)}]$ . If the slope of the relationship = 1, then  $H=1$ , and there is a direct positive relationship between the elemental composition of the herbivore and that of its host plant (you are what you eat), and there is no evidence for homeostasis. With an  $H > 1$ , variation in elemental body composition of the herbivore is small compared to variation in host plant composition and homeostasis exists. To determine the slope of the relationship between insect elemental composition and plant elemental composition, a weighted regression approach was used. Mean plant elemental composition (*Spartina* C:N, C:P, or N:P) for each fertilizer treatment combination was log-transformed and used as the continuous independent variable. The dependent variable for each analysis was the log-transformed planthopper body elemental composition C:N, C:P, or N:P of females. Each planthopper species and wing form combination was analyzed separately. The slopes from these analyses were then used to calculate  $H_{C:N}$ ,  $H_{C:P}$ , and  $H_{N:P}$  for each planthopper species and wing form combination.

However, there are degrees of homeostatic regulation that are determined by the significance of the slope of the relationships articulated above. Homeostasis is considered strictly regulated if the slope is not significantly different from zero, and only strongly regulated if the slope, although not 1, is significantly different from zero. Thus,

the significance of the slope of the relationships was examined to ascertain the strength of homeostasis. As such, a significant slope indicates the failure of planthoppers to maintain a constant elemental body composition against a changing background of host-plant stoichiometry. Contrasts were used to test for differences in slope (homeostasis regulation) between wing forms within a species.

#### Growth Cost Associated with Homeostasis

To evaluate the cost of maintaining a constant elemental body composition for each *Prokelisia* species (females only) and wing form, growth rate (tibial length gained/day) was regressed against plant elemental composition (separate analyses for C:N, C:P, N:P; weighted regression). A significant negative slope indicates that growth is reduced with a decrease in plant quality (e.g., with an increase in C:N). Contrasts were used to test for differences in slope (growth penalty) between species or wing forms.

#### Field Assessment

A 2x2x2 factorial fertilization experiment was initiated in May 2000 at Tuckerton to create a range of host plant elemental compositions (C:N, C:P, and N:P ratios) on which the relationship between *Spartina* elemental composition and *Prokelisia* planthopper elemental composition was assessed. Two levels of nitrogen fertilizer (0, 80g/m<sup>2</sup>) and phosphorus fertilizer (0, 80g/m<sup>2</sup>) were applied to small plots (2m<sup>2</sup>) in each of two habitats (meadow and tidal creek). The design was a randomized double-block design with two sites (marsh areas) each containing 2 habitat types and each of these containing 2 blocks of the 4 treatment combinations. Thus, each nutrient treatment

combination was replicated 8 times for a total of 32 plots. Fertilizer treatments (0N0P, 0N+P, +N0P, +N+P) were randomly assigned to a single plot (2x2m<sup>2</sup>) within each block. Nitrogen was applied as ammonium nitrate (0 or 236g / plot / application) and phosphorus was added as triple super phosphate (0g or 176g / plot / application) on four dates (May 9 and 21, June 4 and 17). This range of N and P fertilization rates was selected because it includes rates used by others to achieve *Spartina* nutrient contents that occur naturally in the field (Patrick and Delaune 1976, Denno et al. 2000). Treatments were established in both habitat types to ensure the presence of both *Prokelisia* species; *P. marginata* occurs abundantly in low-marsh *Spartina* whereas *P. dolus* predominates in *Spartina* meadows (Denno et al. 1996).

#### Elemental Content (C, N, and P) of *Spartina*

The elemental composition of *Spartina* plants (C:N, C:P, N:P) in each plot was determined on 24 June, the date planthoppers were sampled. The elemental content of *Spartina* was determined by taking 5 snippets (leaves from the upper third of different 5 *Spartina* stems) per plot. Snippet samples were oven dried at 60°C for 48 hours, ground in a Wiley Mill, and the powder was analyzed for C, N and P content as above (Clesceri et al. 1998). The mean C:N, C:P, and N:P content of *Spartina* was determined for each plot and used as the dependent variable in the regression analysis below. The grand mean of *Spartina* C:N, C:P, and N:P was determined and used to evaluate the trophic disparity in elemental composition between *Spartina* and the *Prokelisia* species as described below.

### Elemental Content (C, N, and P) *Prokelisia* Planthoppers

Planthopper adults were collected once in each plot on June 24, 2002. A D-vac suction sampler was used to remove all available adults from the center of each plot by suctioning for approximately 3 minutes in each plot. Care was taken not to collect within 0.3 meters of the edge of each plot to decrease the probability of new immigrants from being sampled. Planthopper samples were kept on ice until reaching the laboratory where they were stored at -20°C. Subsequently planthoppers were sorted to species, wing form, and sex and then dried. Up to twenty individuals of each species, wing form, and sex were taken from each plot and analyzed for their C, N and P content as above (Clesceri et al. 1998).

### Mismatch in Elemental Stoichiometry between *Spartina* and the *Prokelisia* Species

To establish that the elemental composition of *Spartina* differed from that of the *Prokelisia* planthoppers (females), the overall average C:N, C:P, and N:P of *Spartina* was compared to the overall average C:N, C:P, and N:P of *Prokelisia dolus* and *P. marginata* using a mixed-model analysis of variance with organism type (*P. dolus*, *P. marginata* or *Spartina*) as the independent variable. The grand means of all experimental treatment plants and planthoppers raised on those plants were used to generate the elemental ratios.

### Homeostatic Regulation of Body Elemental Composition in *Prokelisia*

To determine if female planthoppers were able to maintain their elemental composition when developing on plants of different elemental compositions in the field, weighted regression analyses (using log-transformed means of plant and insect elemental



compositions from the 32 plots) were performed to determine the relationship between plant elemental composition and planthopper body elemental composition (C:N, C:P, and N:P analyzed separately) for both wing forms of both *Prokelisia* species. A significant slope indicates the failure of planthoppers to maintain a constant elemental body composition against a changing background of host-plant stoichiometry.

### Statistical analyses

Prior to analysis, data (residuals) were assessed for normality and homogeneity of variances (SAS 2002). If residual variances were heterogeneous, variance partitioning was conducted and the best model was chosen using Bayesian Information Criterion. The degrees of freedom were calculated using the Kenward-Roger method. All means comparisons were conducted using a Tukey adjustment to account for inflated comparison-wise error rates.

## **Results**

### Laboratory Assessment

#### Mismatch in Elemental Stoichiometry between *Spartina* and the *Prokelisia* Species

There was a remarkable difference in elemental stoichiometry between both *Prokelisia* species and that of their *Spartina* host plant (Figure 2.1). Both *Prokelisia* species had a significantly lower C:N ( $F_{2,130} = 116.81$ ;  $P < 0.0001$ ; Figure 2.1A) and C:P ratio ( $F_{2,124} = 44.46$ ;  $P > 0.0001$ ; Figure 2.1B) than their host plant, suggesting that *Spartina* is a nutrient-deficient food source relative to the inherent nitrogen and

phosphorus demands of *Prokelisia*. Also, the N:P ratio of *Spartina* was significantly higher than that for either *Prokelisia* species and *P. marginata* had a slightly greater N:P ratio than *P. dolus* ( $F_{2,114} = 7.86$ ;  $P = 0.0006$ ; Figure 2.1C), suggesting that N occurs in excess of P in *Spartina* relative to planthopper stoichiometry.

### Homeostatic Regulation of Body Elemental Composition in *Prokelisia*

All evidence suggests that *P. dolus* was able to maintain its body C:N and C:P composition despite having been raised on *Spartina* plants with highly variable elemental composition (Figure 2.2). All H values were greater than one suggesting some level of homeostasis ( $H_{C:N} = 500$ ,  $H_{C:P} = 25$ ,  $H_{N:P} = 33$ ). Moreover, no slope of the relationship between the C:N or C:P elemental ratio of *P. dolus* and that of *Spartina* differed significantly from 0 indicating strict homeostasis ( $\beta_{C:N} = 0.002$ ,  $P = 0.9$ , Figure 2A;  $\beta_{C:P} = 0.04$ ,  $P = 0.3$ , Figure 2.2B). Also, there was no significant difference in the homeostatic regulation of C:N or C:P body content between the brachypterous and macropterous wing forms of *P. dolus* ( $P > 0.05$ ). There was evidence for strong but not strict homeostatic regulation of N:P ( $\beta_{N:P} = 0.03$ ,  $P = 0.01$ ), a result that was evident for both wing forms ( $H_{N:P, Macropter} = 11$ ,  $\beta_{N:P, Macropter} = 0.09$ ,  $P = 0.05$ ;  $H_{N:P, Brachypter} = 12$ ,  $\beta_{N:P, Brachypter} = 0.08$ ,  $P = 0.06$ ; Figure 2.2C).

For *P. marginata* at the species level (wing forms pooled), there was evidence for strong but not strict homeostasis (Figure 2.3). Although H values were all greater than one ( $H_{C:N} = 9$ ,  $H_{C:P} = 14$ ,  $H_{N:P} = 12$ ) indicating some homeostatic regulation of elemental composition, slopes of the relationship between the C:N, C:P and N:P contents of planthoppers and that of *Spartina* differed significantly from zero or were marginally

significant ( $\beta_{C:N} = 0.11$ ,  $P = 0.06$ ;  $\beta_{C:P} = -0.07$ ,  $P = 0.02$ ,  $\beta_{N:P} = 0.08$ ,  $P = 0.06$ ) suggesting that homeostasis was not strict (Figure 2.3 A, B and C). A closer examination revealed that the significant species level responses of *P. marginata* were largely attributable to differences in the homeostatic ability of each wing form. Although brachypters were able to maintain strict homeostatic regulation of their C:N and C:P body stoichiometry ( $H_{C:N} = 50$ ,  $\beta_{C:N} = -0.02$ ,  $P = 0.9$ , Figure 2.3A;  $H_{C:P} = 14$ ,  $\beta_{C:P} = -0.07$ ,  $P = 0.2$ , Figure 2.3B), macropters were not ( $H_{C:N} = 4$ ,  $\beta_{C:N} = 0.24$ ,  $P = 0.002$ , Figure 2.3A;  $H_{C:P} = 14$ ,  $\beta_{C:P} = -0.07$ ,  $P = 0.06$ , Figure 2.3B). There was weak evidence that macropters ( $H_{N:P} = 14$ ,  $\beta_{N:P} = 0.07$ ,  $P = 0.2$ ) but not brachypters ( $H_{N:P} = 4$ ,  $\beta_{N:P} = 0.23$ ,  $P = 0.04$ ) regulated their N:P stoichiometry (Figure 2.3C). However, the slope of the relationship for brachypters is driven by one point, and coupled with the lack of this wing form emerging on the high N:P plant treatments, this significance of the relationship must be interpreted with caution. Overall, evidence suggests that *P. dolus* is better able to regulate its elemental body composition than *P. marginata*, and that the brachypters of *P. marginata* are better capable of homeostatic regulation than are the macropters of this species.

#### Growth Costs Associated with Homeostasis

The growth rate of both *P. dolus* and *P. marginata* declined as the C:N and C:P content of *Spartina* increased ( $\beta_{C:N, P. dolus} = -0.0002$ ,  $P < 0.0001$ ;  $\beta_{C:N, P. marginata} = -0.00036$ ,  $P < 0.0001$ ;  $\beta_{C:P, P. dolus} = -1.7 \times 10^{-6}$ ,  $P < 0.0001$ ;  $\beta_{C:P, P. marginata} = -4.3 \times 10^{-6}$ ,  $P = 0.008$ ; Figures 2.4 and 2.5 respectively). However, the slopes of the relationships were significantly different between the two *Prokelisia* species, and significantly steeper in both cases for *P. marginata* than *P. dolus* (species contrasts; C:N,  $P = 0.001$ ; C:P,  $P =$

0.07). Thus, as the nutritional quality of *Spartina* decreased with regard to either C:N or C:P, the growth of *P. marginata* was protracted relatively more than that for *P. dolus* raised on the same plant treatments, suggesting a greater cost associated with the maintenance of body stoichiometry. There was no significant relationship between growth rate and the N:P content of *Spartina* for either *Prokelisia* species ( $\beta_{N:P, P. dolus} = -0.00001$ ,  $P = 0.09$ ;  $\beta_{N:P, P. marginata} = 5.2 \times 10^{-6}$ ,  $P = 0.7$ ; Figure 2.6). Wing form did not affect the relationship for either species ( $P > 0.05$ ).

### Field Assessment

#### Mismatch in Elemental Stoichiometry between *Spartina* and the *Prokelisia* Species

When evaluated in the field, there was also a striking difference in elemental stoichiometry between both *Prokelisia* species and that of their *Spartina* host plant (Figure 2.7). Both *Prokelisia* species had a significantly lower C:N ( $F_{2,65.8} = 56.29$ ;  $P < 0.0001$ ; Figure 2.7A) and C:P ratio ( $F_{2,67.7} = 38.36$ ;  $P < 0.0001$ ; Figure 2.7B) than their host plant, suggesting that *Spartina* is a nutrient-deficient food source relative to the inherent nitrogen and phosphorus demands of *Prokelisia*. Unlike results from the laboratory experiment, the N:P ratio of *Spartina* was significantly lower than that for either *Prokelisia* species and *P. marginata* had a slightly lower N:P ratio than *P. dolus* ( $F_{2,69.3} = 12.43$ ;  $P < 0.0001$ ; Figure 2.7C), suggesting that P occurs in excess of N in *Spartina* relative to planthopper elemental composition.

## Homeostatic Regulation of Body Elemental Composition in *Prokelisia*

There was no relationship between the C:N content of brachypters and that of their *Spartina* host plant for either *P. dolus* or *P. marginata* suggesting strict homeostasis ( $H_{C:N, P. dolus} = 100$ ,  $\beta_{C:N, P. dolus} = -0.01$ ,  $P = 0.8$ ;  $H_{C:N, P. marginata} = 500$ ,  $\beta_{C:N, P. marginata} = -0.002$ ,  $P = 0.9$ ; Figures 2.8A and 2.9A). In contrast, there was a significant negative relationship between the C:N content of the macropters of both species and the C:N content of *Spartina* ( $H_{C:N, P. dolus} = 11$ ,  $\beta_{C:N, P. dolus} = -0.09$ ,  $P = 0.01$ ;  $H_{C:N, P. marginata} = 12$ ,  $\beta_{C:N, P. marginata} = -0.08$ ,  $P = 0.02$ ; Figures 2.8A and 2.9A). These results suggest that plots with the highest quality *Spartina* plants in the field (those with the lowest C:N content) contained macropters that on average were characterized by a higher C:N content.

With regard to C:P stoichiometry, there was no relationship between the C:P body content of either *P. dolus* or *P. marginata* and that of their *Spartina* host plant ( $H_{C:P, P. dolus} = 16$ ,  $\beta_{C:P, P. dolus} = 0.06$ ,  $P = 0.09$ ;  $H_{C:P, P. marginata} = 200$ ,  $\beta_{C:P, P. marginata} = -0.005$ ,  $P = 0.9$ ; Figures 2.8B and 2.9B). Brachypterous individuals of both species exhibited a positive relationship between body N:P content and that of *Spartina* ( $H_{C:N, P. dolus} = 7$ ,  $\beta_{N:P, P. dolus} = 0.13$ ,  $P = 0.0005$ ;  $H_{C:N, P. marginata} = 5$ ,  $\beta_{N:P, P. marginata} = 0.19$ ,  $P = 0.02$ ; Figures 2.8C and 2.9C respectively). Macropters did not exhibit any association between body N:P content and *Spartina* N:P content ( $H_{C:N, P. dolus} = 33$ ,  $\beta_{N:P, P. dolus} = 0.03$ ,  $P = 0.4$ ;  $H_{C:N, P. marginata} = 25$ ,  $\beta_{N:P, P. marginata} = 0.4$ ,  $P = 0.2$ ; Figure 2.8C and Figure 2.9C).

## **Discussion**

The C:N and C:P elemental ratios in *Spartina* are vastly higher than those for either *Prokelisia* species, underscoring the relatively poor nutrient quality of their host plant resource (Figures 2.1 and 2.7). This macronutrient mismatch between phytophagous insects and their host plant resources is widely documented in the literature, especially with regard to nitrogen (McNeill and Southwood 1978, Mattson 1980, Waring and Cobb 1992, White 1993, Awmack and Leather 2002, Huberty and Denno 2004). Moreover, the C:N ratio of *Spartina* used in the laboratory experiment (C:N ~ 22) was higher than that for plants in the field (C:N ~ 26), a result probably attributable to the higher rates of nitrogen application used in the laboratory. However, C:P ratios were similar (C:P ~ 550) between lab and field plants. The elevated N content of fertilized *Spartina* in the lab relative to P resulted in a higher N:P ratio for lab plants (N:P ~ 45) than field plants (N:P ~ 25). This difference translated into a higher N:P ratio for lab plants than either *Prokelisia* species (N:P ~ 30), whereas the reverse was the case when the N:P ratio of field plants is compared. In general, published N:P ratios for phytophagous insects are similar (mean = 26.4) than those recorded for their host plants (mean = 28.0) (Elser et al. 2000a, Perkins et al. 2004).

Despite extreme variation in the elemental stoichiometry (C, N, and P composition) of their *Spartina* host plant, both *Prokelisia* species maintained a relatively constant elemental balance of macronutrients in their bodies, thereby exhibiting some level of homeostasis. However, there was a clear difference in the strength of homeostatic ability between the two *Prokelisia* species, as evidenced from the laboratory experiment where planthoppers were confined on plants with particular elemental compositions. There was evidence for strict homeostasis in *P. dolus* (both wing forms)

with regard to C:N and C:P regulation (Figure 2.2), whereas *P. marginata* exhibited only strong homeostasis (Figure 2.3). This species-level difference was attributable to the macropters of *P. marginata* that, unlike brachypters of this species, were unable to maintain a constant C:N and C:P body composition when reared across a range of plants with variable elemental compositions (Figure 2.3A and B). For less than strict homeostasis, the expectation is for a positive relationship, however weak, between the elemental composition of the herbivore and that of the plant, as was the case for the macropters of *P. marginata* with regard to their C:N composition (Figure 2.3A). The negative relationship between the C:P of macropters and that of their host plant (Figure 2.3B), although it provides general support for imperfect homeostasis (Sternern and Elser 2002), was unexpected. This negative C:P relationship may derive from macropters accumulating P as a result of increasing ingestion rate to meet nitrogen demands on treatment plants also very deficient in N. Notably, nitrogen is more limiting than phosphorus for *P. marginata* (Chapter 1) and this planthopper is unable to completely meet its nitrogen requirements on nitrogen-poor plants as evidenced by an elevated C:N ratio (Figure 2.3A). Thus, the low C:P ratio of macropters on P-deficient plants may have resulted from phosphorus accumulation and storage, a phenomenon known to occur in other insect herbivores (Woods et al. 2002). As in *P. marginata*, recent studies have also shown that strict homeostatic regulation may not be as universal as stoichiometric dogma once predicted (Plath and Boersma 2001, Cross et al. 2003, Elser et al. 2003).

Homeostatic regulation of macronutrient composition in invertebrates including insect herbivores is achieved by a combination of mechanisms. Increased consumption rate and/or assimilation rate of scarce nutrients (often N and P), coupled with the

selective excretion of excess nutrients (often carbon), can provide an essential balance (Prestidge 1982b, Raubenheimer and Simpson 2004). Gut modifications, such as the filter chamber present in planthoppers and aphids, facilitate the elimination of excess carbon (sugars) and the assimilation of amino nitrogen (Raven 1983, Abisgold et al. 1994, Rhoades et al. 1997). Surplus carbon can be eliminated as well by increasing respiration rate when feeding on carbon-rich food resources (Zanotto et al. 1993, Zanotto et al. 1997). Such diet induced thermogenesis is a well-recognized mechanism of carbon elimination in the nutritional ecology of vertebrates, may also be a mechanism employed by invertebrates (Trier and Mattson 2003). There are physiological limits, however, to achieving homeostatic regulation, especially for herbivorous insects faced with eliminating copious amounts of carbon in order to meet their nitrogen (and perhaps also P) demands. For example, gut capacity and throughput time may limit the degree to which increased ingestion can compensate for eating nutrient-poor food (Denno and Fagan 2003). Moreover, compensatory feeding on low-quality food can lead to increased levels of dietary toxins, toxins that can have negative consequences for fitness, especially for insect herbivores (Slanksy and Wheeler 1992). In general, metabolic and growth costs attributed to homeostasis can be severe (Plath and Boersma 2001). Although the presence of nitrogen-synthesizing endosymbionts, like those that occur in planthoppers, can partially diminish the costs of feeding on nutrient-deficient plants (Hongoh and Ishikawa 2000, Wilkinson and Ishikawa 2001), they clearly do not eliminate them altogether. As evidenced by this study, there are clear growth penalties for both *Prokelisia* species associated with homeostasis and feeding on nutrient-deficient food, but the consequences are particularly severe for *P. marginata* (Figures 2.4 and 2.5).



One can ask why *P. dolus* is better able to regulate its body C:N and C:P composition and suffers fewer developmental costs in terms of reduced growth on nutrient-deficient plants than *P. marginata*. The answer may lie with a major difference in their life history strategies that underlies the way they cope with landscape-level changes in host-plant quality. Data suggests that *P. dolus* employs compensatory feeding when plant quality deteriorates (Denno et al. 2000, Chapter 3), whereas *P. marginata* has evolved a dispersal strategy that allows it to exploit temporary habitats and effectively track spatial changes in host plant quality, particularly nitrogen (Denno 1983, Roderick 1987, Denno et al. 2002, Denno et al. 2003). In fact, most data points to a phenotypic trade-off between the two strategies with a greater commitment to flight musculature in *P. marginata* and a greater investment in the musculature associated with enhanced ingestion (cibarial musculature) in *P. dolus* (Chapter 3). When the option of dispersal is eliminated, and *P. marginata* is confined on nutrient-deficient host plants, both homeostatic capability and growth are more adversely affected than for *P. dolus*. Not surprisingly, macropters, the wing form with a greater investment in flight musculature than cibarial musculature (Denno et al. 1987, Denno et al. 1989, Denno and Fox unpublished data, Chapter 3), exhibit poorer homeostatic capability than the brachypters of this species (Figure 2.3A). Thus, despite very similar body nitrogen and phosphorus contents, and a very similar mismatch in N and P stoichiometry compared to *Spartina*, the two *Prokelisia* species differ considerably in the ways they cope with deteriorating host plant nutrition (dispersal versus compensatory feeding), a difference that I argue affects their ability to regulate body stoichiometry and buffer potential associated growth penalties.

There was one evident inconsistency in particular between laboratory and field results. In the lab, there was no relationship (slope not different from 0) between the C:N of *P. dolus* (both macropters and brachypters) and that of *Spartina*, providing clear evidence for strict homeostasis (Figure 2.2A). Likewise, the brachypters, but not macropters, of *P. marginata* exhibited strict homeostatic regulation of body C:N (Figure 2.3A). Notably, the slopes of C:N the relationship for macropters of both *Prokelisia* species were positive in the lab with *P. marginata* showing a slope that was significantly greater than zero. By contrast, field-sampled macropters of both *Prokelisia* species exhibited significant negative slopes of the relationship between body C:N and plant C:N, a relationship that occurred because macropters with a high body C:N were collected from low C:N treatment plants (Figures 2.8A and 2.9A).

Unlike the laboratory experiment where planthoppers were confined to specific nutrient treatments, there was no clear way to determine if field-collected planthoppers developed on the treatment plants from which elemental composition was determined. This is particularly true for macropters of *Prokelisia* that consistently emigrate from nitrogen-deficient plants to colonize better quality plants elsewhere (Cook and Denno 1994, Denno et al. 2002). Brachypters are much more likely to have developed as residents as they are far less mobile and can move only a few meters during their adult lifetime (Denno 1983, 1994, Denno et al. 1996). Notably, the brachypters of both species showed strict homeostasis of C:N and C:P body composition both in the lab and the field. Why then the discrepancy for macropters between lab and field results? In general, macropters selectively seek out nitrogen-rich hosts to colonize (Cook and Denno 1994, Denno et al. 2002). Moreover, macropterous adults, especially those of *P. marginata*, are

likely to reflect the C:N composition of the plants on which they developed (Figure 2.3A). Thus, macropters with a high C:N ratio are more likely to have emerged from nitrogen-poor plants with a high C:N content. I argue that such nitrogen-deficient individuals will have higher nitrogen demands than macropters emerging from better quality plants. The only way that they can recover penalties imposed during development (slow growth and small body size, Figure 2.4) is to colonize nitrogen-rich plants where survival and fecundity can be vastly improved (Cook and Denno 1994). This argument is particularly true for females, the sex employed to determine all stoichiometric relationships in this study, because they have nitrogen demands for reproduction that far exceed those of males (Cook and Denno 1994, Denno 1994). Thus, in the field where dispersal dynamics occur, I contend that the negative relationship between the C:N content of macropters and that of *Spartina* is the result of selective colonization of N-rich plants by N-poor dispersers.

Alternatively, theory posits that stoichiometric demands of consumers can be best met by feeding of food with an identical elemental composition (Sterner and Elser 2002). Thus, high C:N macropters should colonize high C:N plants whereas low C:N dispersers should do the reverse. However, this paradigm may not prevail if the absolute amount of nitrogen in food is very low, as can be the case for *Prokelisia* planthoppers on very nitrogen-deficient *Spartina* (<1.5% N)(Cook and Denno 1994, Chapter 1). Under such carbon-rich conditions fitness penalties can be severe (Cook and Denno 1994, Chapter 1), and the ingestion/assimilation costs of obtaining sufficient nitrogen may be extreme given that phloem feeders are known to regulate their carbon intake (sucrose) by reducing ingestion rate (see Abisgold et al. 1994).

Another unlikely explanation for the occurrence of high C:N macropters on low C:N plants in the field stems from possible ontogenetic differences in C:N stoichiometry and the demands they impose that are known to occur in other invertebrates (Villar-Argaiz et al. 2002). For aquatic crustaceans, both C:N and C:P ratios decrease during development from immatures to adults, suggesting that the nutritional demands of adults differ from those of early developmental stages (Villar-Argaiz et al. 2002). For instance dispersing adults (macropters), including those of delphacid planthoppers, use carbon-rich flight fuels (glycogen and lipids) during migration ((Kisimoto and Rosenberg 1994, Dudley 2000). Thus, one might expect adults, especially dispersing macropters, to have high-energy (carbon) demands that are satisfied to some extent by colonizing carbon-rich host plants. Using this line of reasoning, macropters, especially those characterized by a high C:N content, should selectively colonize high C:N plants to meet high carbon demands, when in fact they do the reverse (Figures 2.8A and 2.9A). All arguments considered, the most likely explanation for the colonization of low C:N plants by high C:N macropters lies in meeting their nitrogen demands.

There was widespread evidence from both the lab and the field that females of both *Prokelisia* species, especially brachypterous ones, showed a significant positive relationship between body N:P and *Spartina* N:P content, suggesting strong but not strict homeostasis (Figures 2.2C, 2.3C, 2.8C and 2.9C). The N:P content of an organisms can be used to examine the relative importance of N or P in homeostatic regulation (Sterner and Elser 2002). That the N:P content of *Prokelisia* planthoppers was elevated above homeostatic expectations on high N:P plants likely reflects the relatively greater demands for nitrogen than phosphorus. Thus, nitrogen may be selectively ingested or assimilated

to meet high demands. Because females have increased nitrogen requirements during reproduction, and because brachypters have a higher reproductive rate than macropters (Denno et al. 1989, Cook and Denno 1994, Denno 1994), they may selectively assimilate more nitrogen than macropters as was evidenced by the steeper slopes for brachypters of the relationship between body N:P and plant N:P. Overall, N:P relationships observed in this study are consistent with the finding that nitrogen is more limiting than phosphorus for both *Prokelisia* species and that its scarcity in *Spartina* has relatively greater fitness consequences (Chapter 1).

This research is among the first studies to determine homeostatic regulation of macronutrient content and its consequences for growth in terrestrial insect herbivores. Both *Prokelisia* species were somewhat able to regulate their macronutrient body composition despite that of their host plant, but *P. dolus* was better able to do so and suffered fewer associated growth penalties than *P. marginata*. The disparity in homeostatic regulation between the two planthoppers is clearly associated with a life-history difference, namely dispersal ability. Moreover, dispersal ability appears to constrain ingestion rate and vice versa. Specifically, there appears to be an allocational trade-off between flight musculature and thus dispersal (overdeveloped in *P. marginata*) and the cibarial musculature required to extract phloem sap (overdeveloped in *P. dolus*), especially under conditions of plant stress (Chapter 1, Huberty and Denno 2004). Thus, the two closely related planthoppers employ two somewhat mutually exclusive strategies for meeting their nitrogen requirements, especially when nutrients are limiting. *Prokelisia marginata* disperses to nitrogen-rich patches elsewhere, whereas *P. dolus* employs feeding compensation that allows it to increase its feeding rate on nutrient

deficient plants and pre-adapts it for homeostatic regulation and reduced growth costs. This research emphasizes the importance of considering the life history strategies of consumers when examining their homeostatic abilities and associated consequences for growth. Because the *Prokelisia* species play keystone roles in their community (Denno et al. 2002), and because they differ so dramatically in their growth and elemental composition in response to host-plant stoichiometry, the consequences of plant stoichiometry could cascade differentially throughout the food web to affect interactions with herbivores, risk of predation, intraguild predation, and food-web dynamics at large (Denno et al. 2000, Denno and Fagan 2003, Fagan and Denno 2004, Matsumura et al. 2004).

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## Figure Descriptions

Figure 2.1. The (A) C:N content, (B) C:P content, and (C) N:P content of *Spartina alterniflora*, and the planthoppers *Prokelisia dolus* and *Prokelisia marginata* raised on *Spartina* plants grown under laboratory conditions. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 2.2. Relationship between (A) the C:N content, (B) C:P content, and (C) N:P content of the macropters and brachypters of *P. dolus* and the respective elemental content of the *Spartina* host plant on which they were raised in the laboratory. The lack of a significant relationship for both wing forms in A and B indicates strict homeostasis. C. Species-level relationship (both wing forms pooled) between N:P content of *P. dolus* and *Spartina* was significantly positive [*P. dolus* N:P<sub>species</sub> = 1.36 + 0.07 (*Spartina* N:P);  $R^2 = 0.17$ ;  $P = 0.01$ ], as was the relationship between N:P content of brachypters [*P. dolus* N:P<sub>brachypters</sub> = 1.35 + 0.08 (*Spartina* N:P);  $R^2 = 0.14$ ;  $P = 0.06$ ] and macropters [*P. dolus* N:P<sub>macropters</sub> = 1.31 + 0.09 (*Spartina* N:P);  $R^2 = 0.40$ ;  $P = 0.05$ ] and their *Spartina* host plant. The positive relationship between the N:P content of brachypters and that of *Spartina* is driven by a single observation. Regression line shown is the species-level relationship.

Figure 2.3. Relationship between (A) the C:N content, (B) C:P content, and (C) N:P content of the macropters and brachypters of *P. marginata* and the respective elemental content of the *Spartina* host plant on which they were raised in the



laboratory. The lack of a significant relationship for brachypters in A and B and for macropters in C indicates strict homeostasis. The slopes of the elemental relationships for macropters in A and B differed from 0 indicating strong but not strict homeostasis. The positive relationship between the N:P content of brachypters (C) and that of *Spartina* is driven by a single observation. Regression lines shown are for the significant macropter relationship in A [*P. marginata*  $C:N_{macropters} = 0.41 + 0.24 (Spartina\ C:N)$ ,  $R^2 = 0.35$ ;  $P = 0.002$ ] and B [*P. marginata*  $C:P_{macropters} = 2.38 + -0.08 (Spartina\ C:P)$ ,  $R^2 = 0.14$ ;  $P = 0.06$ ] and the significant brachypter relationship in C [*P. marginata*  $N:P_{brachypters} = 1.22 + 0.23 (Spartina\ N:P)$ ,  $R^2 = 0.24$ ;  $P = 0.05$ ].

Figure 2.4. Relationship between the growth rate of (A) *P. dolus* and (B) *P. marginata* and the C:N content of the *Spartina* plants on which they were raised in the laboratory. Although both species exhibited a negative relationship (*P. dolus*  $Growth\ Rate_{C:N} = 0.32 + -0.00020 (Spartina\ C:N)$ ,  $R^2 = 0.15$ ,  $P < 0.0001$ ; *P. marginata*  $Growth\ Rate_{C:N} = 0.36 + -0.00036 (Spartina\ C:N)$ ,  $R^2 = 0.29$ ,  $P < 0.0001$ ), the slope was significantly steeper for *P. marginata* than *P. dolus* ( $P = 0.001$ ) suggesting that its growth was reduced relatively more as plant quality decreased (increase in C:N). Wing forms (macropters and brachypters) did not differ in their growth response.

Figure 2.5. Relationship between the growth rate of (A) *P. dolus* and (B) *P. marginata* and the C:P content of the *Spartina* plants on which they were raised in the

laboratory. Although both species exhibited a negative relationship [*P. dolus* Growth Rate<sub>C:P</sub> = 0.029 + -1.7 x 10<sup>-6</sup> (*Spartina* C:P), R<sup>2</sup> = 0.03, *P* < 0.0001; *P. marginata* Growth Rate<sub>C:P</sub> = 0.031 + -4.33 x 10<sup>-6</sup> (*Spartina* C:P), R<sup>2</sup> = 0.05, *P* < 0.0008], the slope was marginally steeper for *P. marginata* than *P. dolus* (*P* = 0.07) suggesting that its growth was reduced relatively more as plant quality decreased (increase in C:P). Wing forms (macropters and brachypters) did not differ in their growth response.

Figure 2.6. Relationship between the growth rate of (A) *P. dolus* and (B) *P. marginata* and the N:P content of the *Spartina* plants on which they were raised in the laboratory. The relationship was not significant for either species, and wing forms (macropters and brachypters) did not differ in their growth response.

Figure 2.7. The (A) C:N content, (B) C:P content, and (C) N:P content of *Spartina alterniflora*, and the planthoppers *Prokelisia dolus* and *Prokelisia marginata* occurring on *Spartina* plants growing in the field on a salt marsh at Tuckerton, New Jersey. Means (± SE) with the same letter are not significantly different.

Figure 2.8. Relationship between (A) the C:N content, (B) C:P content, and (C) N:P content of the macropters and brachypters of *P. dolus* and the respective elemental content of the *Spartina* host plant on which they occurred on a salt marsh at Tuckerton, New Jersey. The lack of a significant relationship for the brachypters in A and B and the macropters in B and C indicates strict

homeostasis. Slopes of the relationship differed from 0 for macropters in A [*P. dolus* C:N<sub>macropters</sub> = 0.89 + - 0.09 (*Spartina* C:N), R<sup>2</sup> = 0.19, *P* = 0.01] and for brachypters in C [*P. dolus* N:P<sub>brachypters</sub> = 1.32 + 0.13 (*Spartina* N:P), R<sup>2</sup> = 0.34, *P* = 0.0005] suggesting strong but not strict homeostasis. Regression lines shown are for (A) macropters and (C) brachypters.

Figure 2.9. Relationship between (A) the C:N content, (B) C:P content, and (C) N:P content of the macropters and brachypters of *P. marginata* and the respective elemental content of the *Spartina* host plant on which they occurred on a salt marsh at Tuckerton, New Jersey. The lack of a significant relationship for the brachypters in A and B and the macropters in B and C indicates strict homeostasis. Slopes of the relationship differed from 0 for macropters in A [*P. marginata* C:N<sub>macropters</sub> = 0.90 + - 0.08 (*Spartina* C:N), R<sup>2</sup> = 0.16, *P* = 0.02] and for brachypters in C [*P. marginata* N:P<sub>brachypters</sub> = 1.27 + 0.19 (*Spartina* N:P), R<sup>2</sup> = 0.24, *P* = 0.02] suggesting strong but not strict homeostasis. Regression lines shown are for (A) macropters and for (C) brachypters.

Figure 2.1.

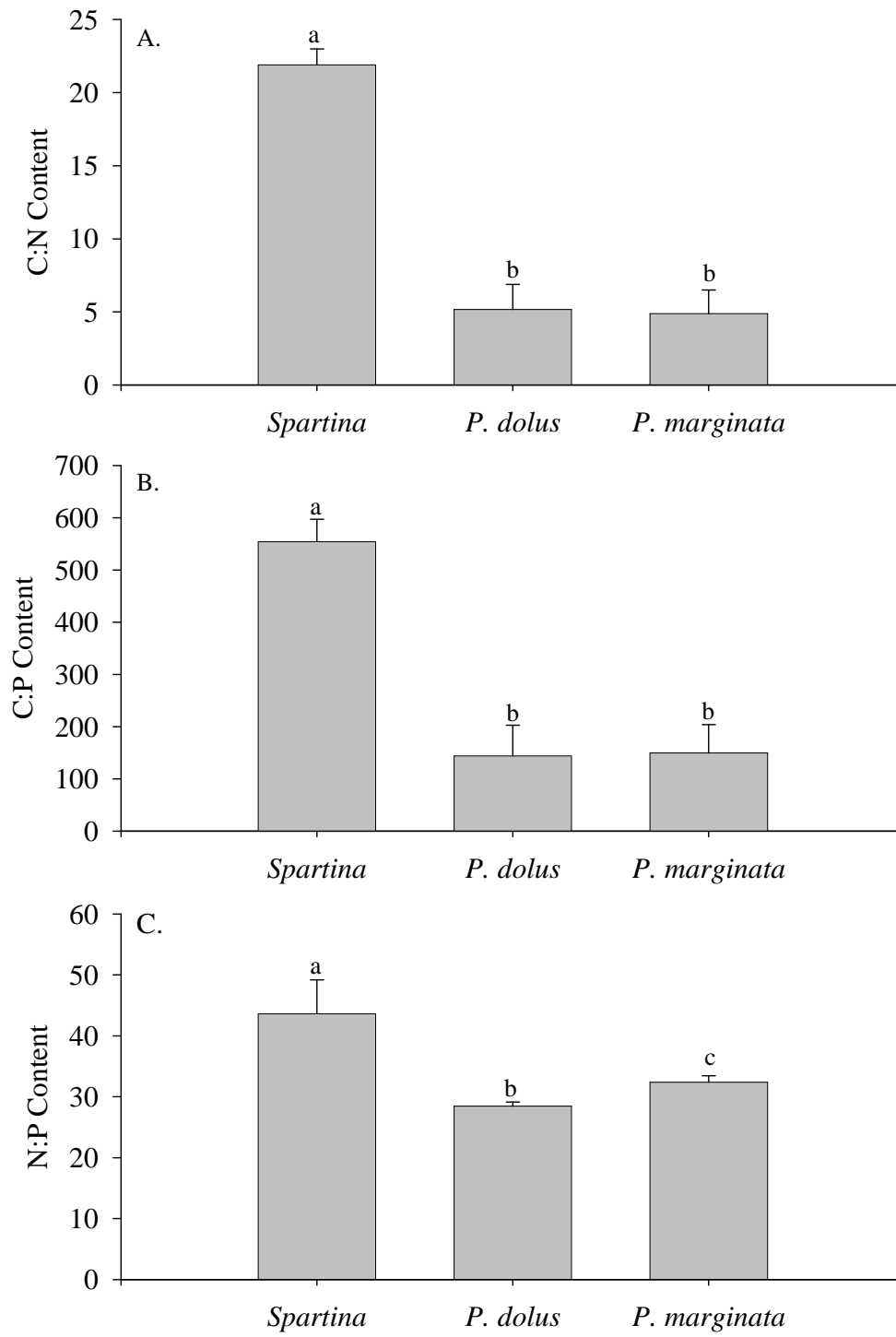


Figure 2.2.

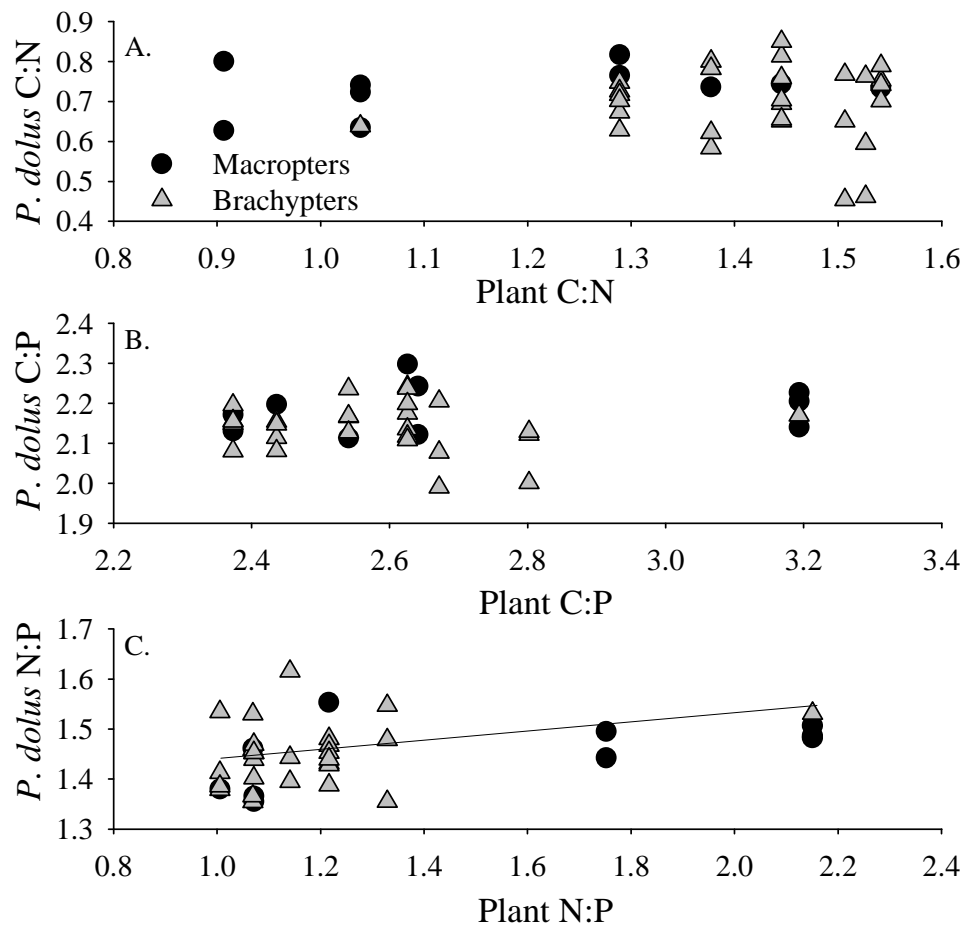


Figure 1 consists of three scatter plots (A, B, and C) showing the relationship between plant nutrient ratios and *P. marginata* nutrient ratios for two forms: Macropter (black circles) and Brachypter (grey triangles). Each plot includes a linear regression line.

- Plot A:** *P. marginata* C:N (Y-axis, 0.4 to 1.0) vs. Plant C:N (X-axis, 0.8 to 1.6). The regression line shows a positive correlation.
- Plot B:** *P. marginata* C:P (Y-axis, 2.0 to 2.4) vs. Plant C:P (X-axis, 2.0 to 3.4). The regression line shows a negative correlation.
- Plot C:** *P. marginata* N:P (Y-axis, 1.2 to 1.8) vs. Plant N:P (X-axis, 0.8 to 2.4). The regression line shows a positive correlation.

Figure 2.4.

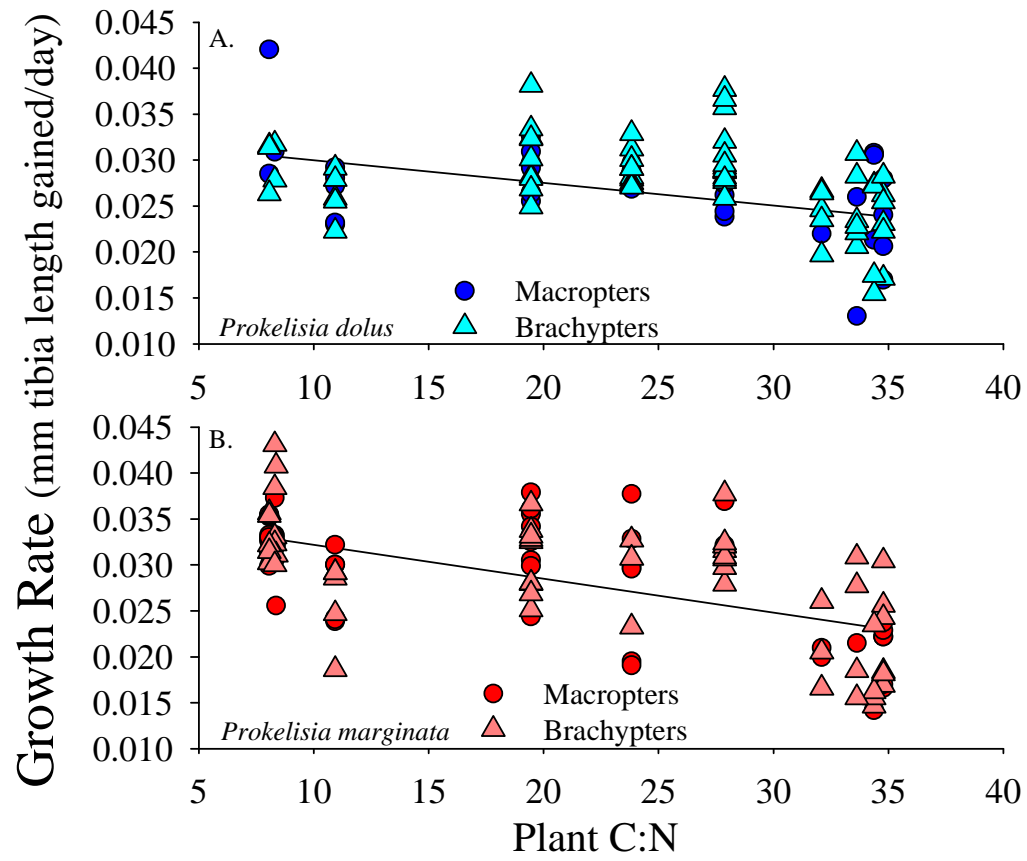


Figure 2.5

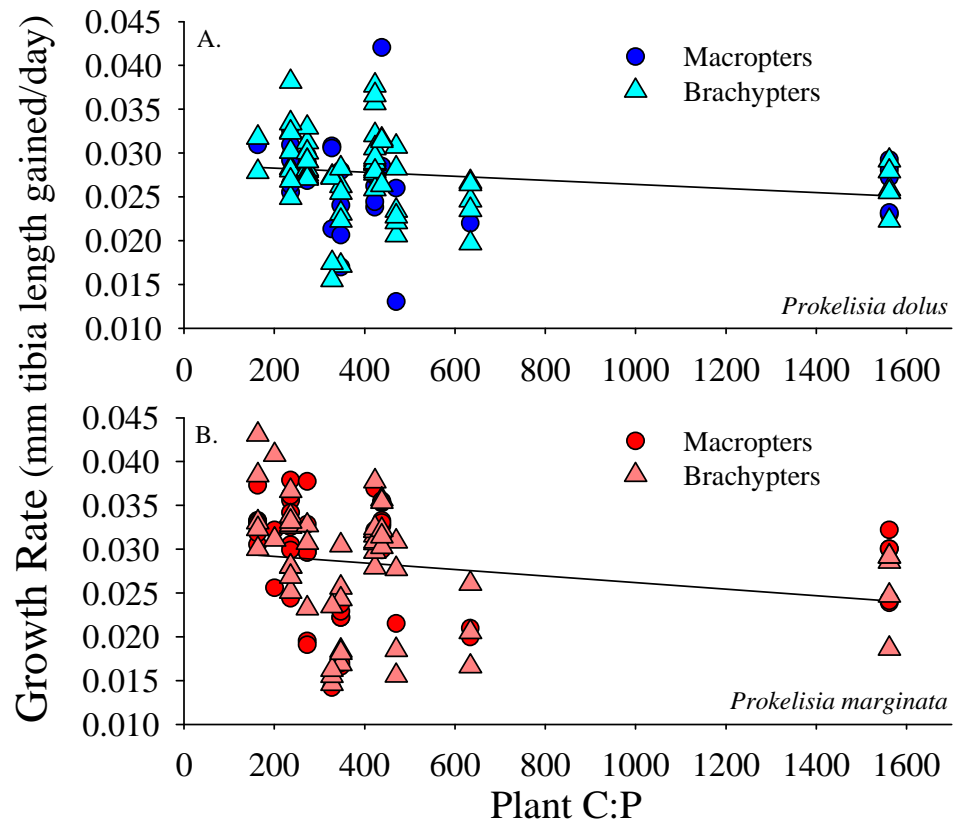




Figure 2.6.

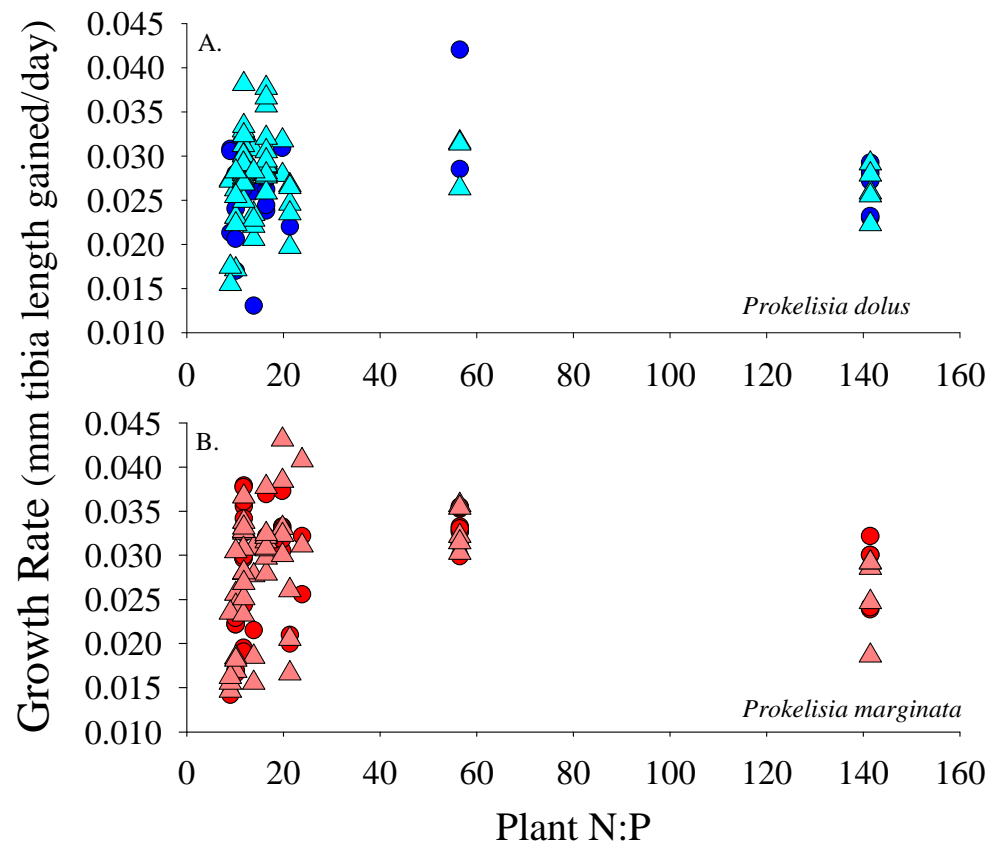


Figure 2.7.

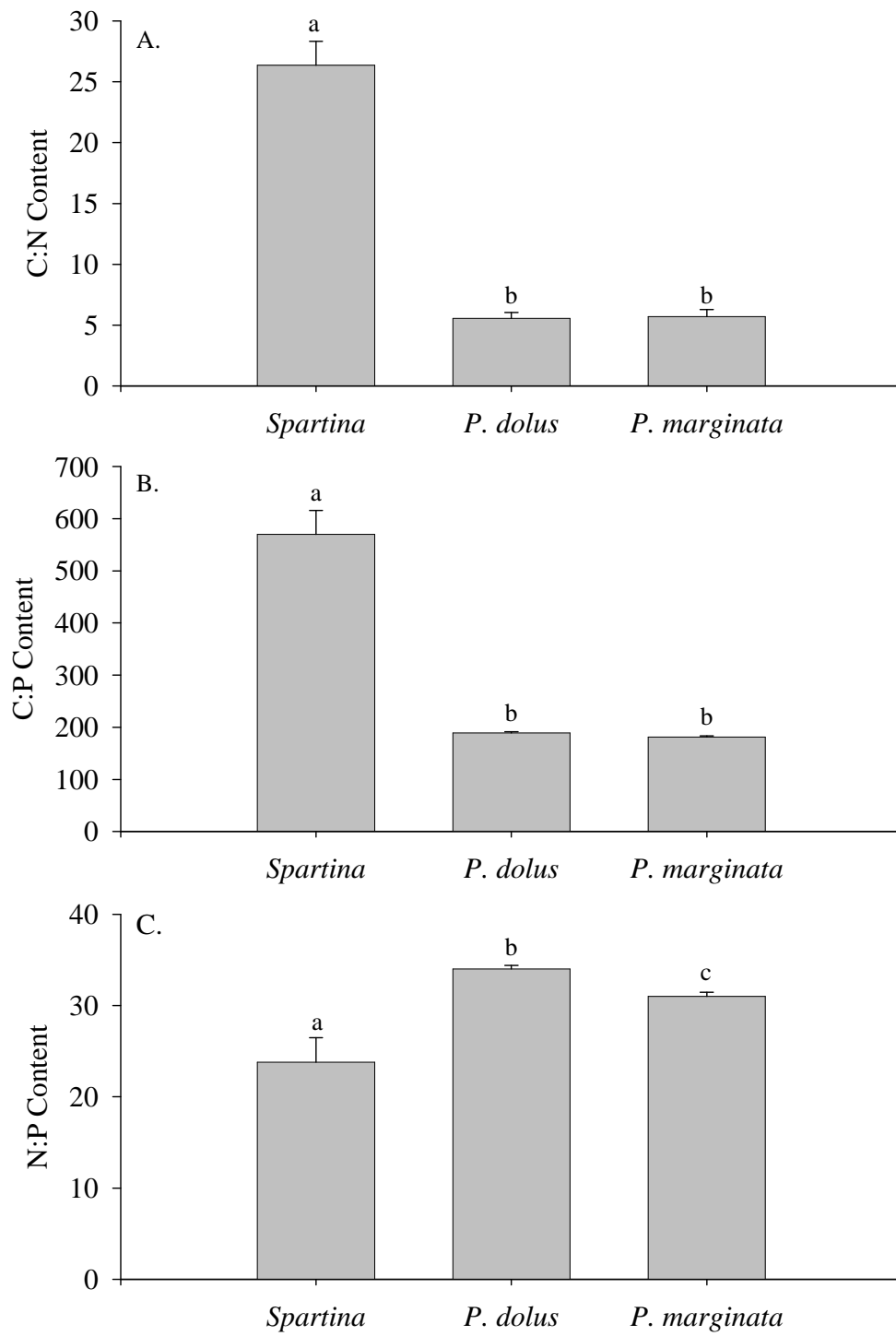


Figure 2.8.

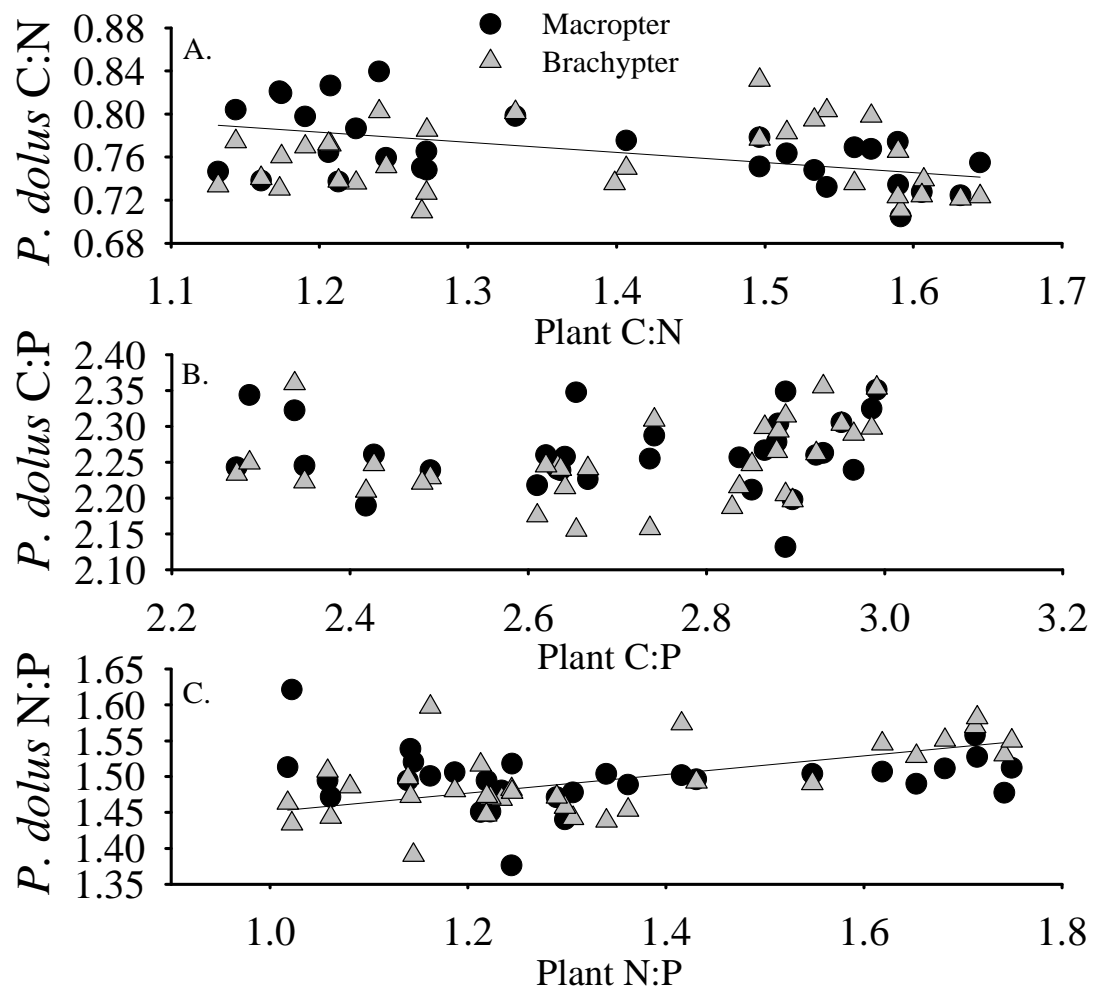
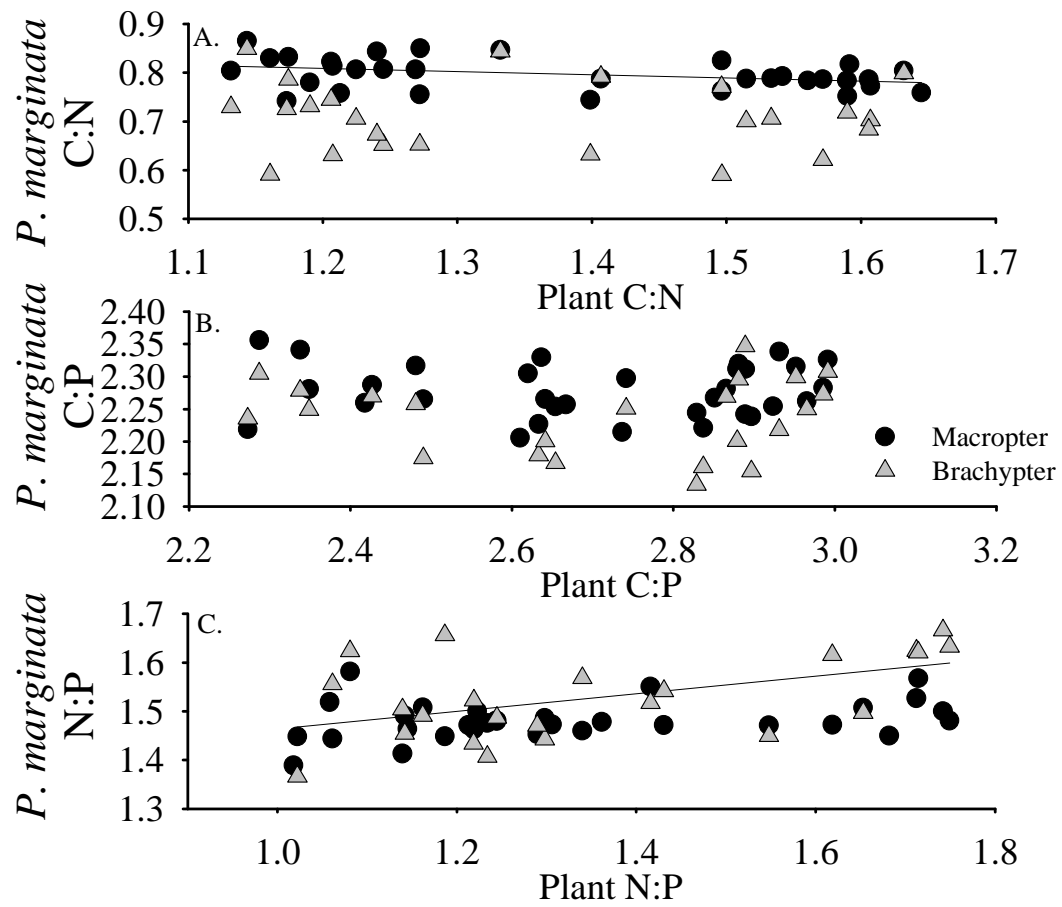


Figure 2.9.



## Chapter 3: Trade-off between dispersal and feeding morphology in two phytophagous insects

### Introduction

Dispersal by flight provides organisms the opportunity to synchronize reproduction with favorable resources across spatially diverse landscapes, and thus plays a key role in the evolution of insect life history strategies (Southwood 1977, Roff 1990, Roff and Fairbairn 1991, Denno 1994, Zera and Denno 1997). For example, dispersal is thought to be essential for the success of species that exploit ephemeral habitats or must negotiate complex-structured, three-dimensional space (Denno et al. 1991, Denno et al. 1996, Denno et al. 2001). Specifically, dispersal allows for the effective tracking of changing resources (e.g., nutrient-rich host plants, optimal oviposition sites, and favorable over-wintering habitats). In addition to its pivotal role in life-history theory, dispersal also acts as a stabilizing force in metapopulation dynamics (den Boer 1981, Hanski 1999), influences species interactions (Denno et al. 2000), and directly affects gene flow and the genetic structure of populations (Peterson and Denno 1997, 1998, Mun et al. 1999). Moreover, a high incidence of dispersal is characteristic of many of our severe agricultural and forest insect pests (Berryman 1988, Pedgely 1993, Kisimoto and Rosenberg 1994). Thus, dispersal has wide-spread consequences for both population ecology and pest management (Rabb and Kennedy 1979, Cappuccino and Price 1995, Denno et al. 2001).

Dispersal, however, does not occur without costs, costs that are often imposed on

life-history traits associated with reproduction (Roff 1986, Roff and Fairbairn 1991, Zera and Denno 1997). Moreover, wing-dimorphic insects such as planthoppers, aphids, crickets, and true bugs have proved invaluable for investigating trade-offs between dispersal and other life history traits because flight-capable and flightless forms are so easily recognized (Solbreck 1978, Denno et al. 1991, Roff and Fairbairn 1991, Tanaka 1993, Dixon 1998, Zera and Brink 2000). Populations of most wing-dimorphic insects contain both flightless adults (brachypters with reduced wings or wingless apterae) and flight-capable adults (macropters or alates) that possess fully-developed wings and can disperse long distances, distances over 1000 km in some cases (Denno 1994, Kisimoto and Rosenberg 1994, Dixon 1998). Traditionally, dispersal costs have been evaluated by comparing reproductive traits between brachypters and macropters or between apterae and alates with the expectation of reduced reproductive effort in the flight-capable morph (Zera and Denno 1997). Indeed, for females of wing-dimorphic insects, there is widespread evidence that macropters have reduced fecundity, extended age to first reproduction, or reduced offspring size compared to their flightless counterparts (Solbreck 1978, Roff 1986, Denno et al. 1989, Roff and Fairbairn 1991, Denno 1994, Zera and Denno 1997). More recently, similar trade-offs between dispersal and siring capability have been found in the males of wing-dimorphic insects (Langellotto et al. 2000, Langellotto and Denno 2001). Underlying antagonistic trade-offs between dispersal and reproduction are differences in resource allocation between macropters and brachypters, given a limited energy and/or nutrient budget. For example, in macropters, relatively more resources are allocated to flight muscles and the biochemistry associated with muscle maintenance and flight, whereas in brachypters assimilated and synthesized

nutrients are allocated more to reproductive effort (Zera et al. 1998, Zera and Brink 2000, Zhao and Zera 2002).

Historically, phenotypic trade-offs involving dispersal have focused almost exclusively on differences in allocation between flight and reproduction (Zera and Denno 1997). However, resource investments in dispersal may also result in reduced resource allocation to other so-called “third-party traits” (e.g., compensatory feeding) that are not directly associated with reproduction (Zera et al. 1998). Such traits, however, remain largely uninvestigated for any insect despite their potential importance for performance and survival. In this chapter I provide evidence that investment in the musculature associated with ingestion, and thus the ability to compensate for nutrient-poor food resources, trades off with dispersal capability in two wing-dimorphic planthoppers (*Prokelisia marginata*, *P. dolus*: Hemiptera, Delphacidae). Clues to identifying a potential trade-off between dispersal and compensatory feeding lie in differences in life history strategy, response to nutrient limitation, competitive ability, and population dynamics between these two phloem-feeding insects. For example, *P. marginata* invests far more in dispersal ability than *P. dolus* (Denno et al. 1991). An approximate assessment of dispersal ability can be obtained by comparing the fraction of macropters in populations, which is high for *P. marginata* (>90%) and low for *P. dolus* (Denno et al. 1991, Denno et al. 1996). Moreover, there is evidence for a greater investment in flight apparatus per individual in species that are characterized by a high fraction of macropterous adults (e.g., *P. marginata*) compared to species that are primarily brachypterous (e.g., *P. dolus*) (Fairbairn and Desranleau 1987, Fairbairn 1994). Despite its greater investment in flight capability, *P. marginata* performs and survives far poorer

on nutrient-deficient host plants low in nitrogen and phosphorus content, and is less able to regulate its own macronutrient composition (nitrogen to carbon and phosphorus to carbon ratio) than *P. dolus* (Chapters 1 and 2). Moreover, *P. marginata* is a poor interspecific competitor compared to *P. dolus*, an ability that is mediated by tolerance to feeding induced reductions in plant quality (Denno et al. 2000). The common denominator underlying these differences in performance and competitive ability between the *Prokelisia* species may be the ability to increase ingestion rate as food quality decreases and thus compensate for nutrient reductions (Slansky and Feeny 1977, Raubenheimer and Simpson 1993, Cook and Denno 1994, Lavoie and Oberhauser 2004). Compensatory feeding ability may be particularly critical given that phytophagous insects in general face a food resource that is extremely nutrient poor (e.g., low nitrogen and phosphorus contents) compared to their own body composition (e.g., high nitrogen and phosphorus content), a nutrient mismatch that imposes tremendous nutrient demands on consumers with potentially drastic consequences for growth (McNeill and Southwood 1978, Mattson 1980, White 1993, Cook and Denno 1994, Ayers et al. 2000, Elser et al. 2000a, Schade et al. 2003, Huberty and Denno 2004).

For planthoppers, compensatory feeding occurs when ingestion rate is increased as the concentration of macronutrients (e.g., amino nitrogen) in the host plant decreases (Waloff 1980, McNeill and Prestidge 1982, Prestidge 1982a, Backus 1985, Cook and Denno 1994). Perhaps this is made possible by a large commitment to the musculature associated with ingestion. Planthoppers feed by inserting their stylets into phloem tissues (Backus 1985, Cook and Denno 1994). Then using a cibarial pump (modified esophagus), cell sap is ingested. The cibarial pump is driven by a series of dilator



muscles that insert on the interior of the face (clypeus), and face size is positively related to the cross-sectional mass and thus the power of the cibarial muscles to ingest cell sap (Backus 1985). For *Prokelisia* planthoppers, *P. dolus* has a much broader face than *P. marginata* (Denno et al. 1987), suggesting a greater investment in subtending cibarial musculature. Preliminary data suggests that *P. dolus* invests more in cibarial musculature than *P. marginata* at the species level with a trend toward greater cibarial pump musculature in brachypters compared to macropters for both species (Denno and Fox, unpublished data).

Although dispersal and compensatory feeding have been discussed as alternative strategies for coping with deteriorating plant nutrition and meeting nutrient demands (McNeill and Southwood 1978, Cook and Denno 1994), they have never been linked by virtue of a phenotypic trade-off. If a trade-off indeed exists, then dispersal ability should constrain compensatory feeding to some extent and vice versa. In this context, the objectives for this study were to: (1) compare differences in investment in the flight and feeding morphology between *P. dolus*, *P. marginata*, and between the brachypters and macropters of both species, and (2) compare differences in feeding compensation (ingestion rate) between the two species and their wing forms. Differences in flight and feeding musculature were measured indirectly by comparing head metrics (face widths and areas, head weights) and thoracic metrics (width, length, area, weight) between the two planthopper species and wing forms. Differences in feeding compensation (ingestion) between the species and wing forms were assessed by measuring honeydew production (liquid excretory product) on host plants differing in nutritional quality (% nitrogen, % phosphorus, and C:N and C:P ratios). In phloem-feeding insects such as

planthoppers, ingestion and excretion rates are positively related (Prestidge 1982a, Brodbeck et al. 1993, Brodbeck et al. 1995, 1996); thus, honeydew production was used as a surrogate for ingestion rate.

If a trade-off between dispersal and compensatory feeding is in place at the species level, I expect *P. marginata* will show a relatively greater investment in flight (larger and heavier thorax) than cibarial apparatus (reduced face area and head weight), and that *P. dolus* will show the reverse. Likewise, with a presumed greater investment in cibarial musculature and thus enhanced compensatory feeding, I predict that *P. dolus* will exhibit greater honeydew production than *P. marginata*, especially on nitrogen-deficient host plants. I also predict that macropters compared to brachypters will exhibit a greater investment in flight than feeding morphology (cibarial musculature) and will produce less honeydew. By examining patterns of investment in dispersal and feeding apparatus between the species and wing forms of *Prokelisia* planthoppers, I aim to identify for the first time a “third party trait,” namely compensatory feeding, that is negatively associated with flight capability. Identifying such a trade-off between dispersal and a non-reproductive trait should mandate a broader view of life history theory and the selective pressures affecting the evolution of particular life-history strategies.

## Study System

*Prokelisia* planthoppers were used to test hypotheses concerning differential investment in flight and feeding as it affects compensatory feeding as a mechanism to cope with nutrient limitation. *Prokelisia marginata* and *P. dolus* are the most abundant

herbivores on Atlantic coastal marshes where they feed exclusively on *Spartina alterniflora* (Denno et al. 2002, Denno et al. 2003). Both species are wing dimorphic with flight-capable macropters (adults with fully-developed wings) and flightless brachypters (adults with vestigial hind wings) present in the same population (Denno et al. 1991, Denno et al. 1996). However, most adults of *P. marginata* are macropterous (>90%) whereas those of *P. dolus* are primarily brachypterous (>80%), a difference that reflects a marked difference in population dynamics between the two species. *Prokelisia dolus* is a relatively sedentary species that is restricted primarily to high-marsh meadows of *Spartina* (Denno et al. 1996). By contrast, *P. marginata* is a highly mobile species along the Atlantic coast and undergoes annual inter-habitat migrations between overwintering sites on the high-marsh and more favorable low-marsh habitats where development occurs (Denno et al. 1996). Outbreaks of both planthopper species are associated with nitrogen-rich host plants, but most striking are the outbreaks of *P. marginata* that occur frequently on low-marsh *Spartina* (Denno and Peterson 2000).

Although both *P. marginata* and *P. dolus* exhibit population increases on nitrogen-enriched *Spartina*, all evidence suggests that the two species cope with nutrient-deficient *Spartina* differently (Cook and Denno 1994, Denno et al. 2002, Denno et al. 2003, Chapters 1 and 2). For instance, both species select the most nitrogen-rich plants or plant parts on which to feed and oviposit but the macropters of *P. marginata* colonize nitrogen-rich plants at tremendously high densities compared to *P. dolus* (Denno 1983, 1985, Denno et al. 2002). Moreover, both species exhibit enhanced survival and performance on nitrogen-fertilized *Spartina*, (Cook and Denno 1994, Olmstead et al. 1997) but *P. marginata* shows a much stronger response (Chapter 1). *Prokelisia dolus*

also has the ability to withstand nitrogen-deficient plants better than *P. marginata* (Denno et al. 2000, Chapters 1 and 2). Thus, existing data suggests that nitrogen is limiting for both *Prokelisia* species, but that *P. marginata* copes with spatial changes in host-plant quality by dispersal to more favorable habitats, whereas *P. dolus* is able to remain on site and wait out periods of inadequate plant nutrition (Cook and Denno 1994, Denno et al. 2000).

## Methods

### *Investment in flight versus feeding morphology in Prokelisia planthoppers*

#### Head and thorax metrics

Patterns of investment to flight and feeding morphology (head and thorax metrics) in the *Prokelisia* species (both wing forms) and were determined from laboratory-reared planthoppers. Upon emergence, adults were sorted to species and wing form and were stored in ethyl alcohol (70%) prior to measurement of head and thorax features using an optical micrometer. Following measurement, adult bodies were separated into heads and thoraces (wings removed) that were dried at 60°C for 48 hours and then weighed individually. In all, measurements and weights were made on 15 adult females of *P. dolus* (5 macropters and 10 brachypters) and 20 females of *P. marginata* (10 macropters and 10 brachypters).

Investment in feeding musculature was assessed indirectly by measuring several head metrics: face (clypeus) width (mm) and face area (width x length in mm) as indexes of the surface available for cibarial muscle attachment, and head weight (mg) as a surrogate for cibarial muscle mass. Investment in flight morphology was determined from several thorax metrics: width of mesothorax (mm), width of metathorax (mm), average thorax width (mesothorax width + metathorax width  $\div$  2), length of mesothorax + metathorax (mm), and length (mm) and weight (mg) of entire thorax (prothorax + mesothorax + metathorax). The area of the entire thorax (mm<sup>2</sup>) was calculated in three ways: entire length (prothorax to metathorax) x mesothorax width, entire length x metathorax width, and entire thorax length x average thorax width. The prothorax was included in the determination of thorax length (despite lacking wings) because it is small compared to the other thoracic segments and yet allows a more precise estimate of thorax length. Individual tibia lengths (mm) were also measured and used to control for differences in body size between individuals.

Area (face and thorax) and weight (head and thorax) measurements were also used to calculate two indexes of relative investment in flight versus feeding capability: face area/thorax area (entire thorax length x average thorax width) and head weight/thorax weight. Higher ratios suggest a greater investment in feeding relative to flight morphology.

### Statistical analysis

The effects of species, wing form, and their interaction on head metrics, thorax metrics, and investment indexes were determined using ANCOVA with tibia length as

the covariate (SAS 2002). Prior to analysis, data (residuals) were assessed for normality and homogeneity of variances (SAS 2002). If residual variances were heterogeneous, variance partitioning was conducted and the best model was chosen using Bayesian Information Criterion. The degrees of freedom were calculated using the Kenward-Roger method. All means comparisons were conducted using a Tukey adjustment to account for inflated comparison-wise error rates. If data were transformed for statistical analysis, means and standard errors are presented as untransformed data.

#### *Spartina* and planthopper culture

Planthoppers for this experiment were reared on fertilized plants to control for any possible nutrient-deficiency effects on muscle allocation. Potted *Spartina* plants (3-5 plants per pot grown in sand substrate) were greenhouse grown from seed in flats (80 pots per flat in each of eight flats) exposed to a fertilization regime of nitrogen and phosphorus that supports optimal *Spartina* and *Prokelisia* growth (Chapter 1). Nitrogen subsidy was  $10\text{gN/m}^2$  (supplied as 5g ammonium nitrate / flat / application) and phosphorus subsidy was  $12\text{gP/m}^2$  (supplied as 5g triple super phosphate / flat / application). To achieve the desired treatments, plants in each treatment combination were fertilized every three weeks from April 2003 until June 2003. Plants for this experiment were grown from seed obtained from Environmental Concern, Inc. St. Michaels, MD.

*Prokelisia* nymphs were reared to adult by placing 3 to 5 first instar nymphs of each *Prokelisia* species onto caged plants on 1 July 2003 (see Denno et al. 2000 for cage design). Approximately 500 cohorts of each species were started in order to assure the

emergence of the rarer wing forms of each species (macropterous *P. dolus* and brachypterous *P. marginata*, respectively). Nymphs were obtained from lab cultures initiated from ovipositing adults collected from the Tuckerton field site.

### *Honeydew production in planthoppers as an index of ingestion and feeding compensation*

#### Honeydew production in planthoppers on plants of variable nutrient quality

Feeding compensation is the ability to increase ingestion rate as host plant quality decreases, which can be measured indirectly as excretion rate (honeydew production) (Prestidge 1982a, Brodbeck et al. 1993, Brodbeck et al. 1995, 1996). Honeydew production was determined for both *Prokelisia* species by feeding them *Spartina* grown under different nutrient regimes. Potted *Spartina* plants (3-5 plants per pot) were greenhouse grown from seed in flats (80 pots per flat, two flats per treatment) and exposed to one of three fertilization treatments to create a range of plant qualities: 10g/m<sup>2</sup> of nitrogen only, 12g/m<sup>2</sup> of phosphorus only, or both. To achieve the desired treatments, plants in each treatment combination were fertilized every three weeks from April until June 2003. Nitrogen was applied as 5g ammonium nitrate / flat / application and phosphorus was supplied as 5g triple super phosphate / flat / application. Plants for this experiment were grown from seed obtained from Environmental Concern, Inc. St. Michaels, MD. The macronutrient treatments are hereafter referred to as +P (only phosphorus fertilization), +N (only nitrogen fertilization), and +N+P (both phosphorus and nitrogen fertilization).

Honeydew production was determined from planthoppers confined in clip cages on treatment plants. After adult emergence from cultures (see above), cohorts consisting of 10 females of a single species and wing form combination were placed into small cylindrical clip cages (1.5 cm in diameter x 6cm in length and made of clear cellulose butyrate plastic) that contained a cup-shaped piece of pre-weighed filter paper. Cohorts were then randomly assigned to plants in one of 3 fertilized *Spartina* treatments (+P, +N, +N+P). Because few brachypterous females of *P. marginata* emerged during the rearing process, only macropters of this species were available for testing. Both wing forms of *P. dolus* were sufficiently abundant for assessment of honeydew production.

Leaves were positioned in the tops of clip cages to ensure that honeydew fell down and collected on filter paper below. After 48 hours of feeding, cohorts were removed and the filter paper was re-weighed. Honeydew production (mg/48 hrs) was measured as the difference between pre- and post- filter paper weight. Thirteen replicate cohorts were established for each wing form by plant nutrition-treatment combination for *P. dolus* as well as 14 replicates of each plant-treatment combination for the macropters of *P. marginata*. Eleven planthopper-free “control cages” were also placed onto plants in each of the 3 treatment categories for 48 hours and filter paper was re-weighed ensure that any change in filter paper weight was due to the presence of planthoppers.

#### Effect of fertilization treatments on plant nutrient content

To determine the effect of the fertilization treatments on the nutrient content (% nitrogen, % phosphorus, % carbon) of *Spartina*, six replicates per treatment of planthopper-free *Spartina* leaves (all aboveground living biomass in each pot) were



harvested just prior the start of the honeydew production experiment (15 August 2003). Foliar nutrient content was used as an index of the phloem nutrients available (Youssefi et al. 2000). Leaves were oven dried for 48 hours at 60°C, ground in a Wiley Mill and subsequently analyzed for %C and %N using a Perkin-Elmer 2400 CHN analyzer, and for %P by persulfate digestion (Clesceri et al. 1998). All elemental ratios used for analyses are atomic ratios.

### Statistical analysis

The effect of the plant-nutrition treatments on honeydew production (mg/48 hrs) was analyzed separately for the two *Prokelisia* species and the wing forms of *P. dolus* using ANOVA (SAS 2002). To confirm that changes in filter paper weight were due to honeydew production, filter paper weight in planthopper-free controls (averaged across plant fertilization treatments) was compared to that in the planthopper-containing treatment (averaged across species, wing forms and plant fertilization treatments) using ANOVA. For the control treatment, change in filter paper weight over the 48 hour experimental period (initial versus final weight) was compared using a t-test. The effect of the plant-nutrition treatments on the nutrient content of *Spartina* (%N, %P, C:N, C:P) was assessed using ANOVA. Prior to analysis, residuals were assessed for normality and homogeneity of variances, variance partitioning was conducted and the best model was chosen using Bayesian Information Criterion, degrees of freedom were calculated using the Kenward-Roger method and means were compared using a Tukey adjustment to account for inflated comparison-wise error rates (SAS 2002).

## Results

### *Investment in flight versus feeding morphology in Prokelisia planthoppers*

Brachypters of *P. dolus* had wider faces (clypeus) and a larger facial area than macropters of this species and both the brachypters and macropters of *P. marginata*, which did not differ from each other [significant Species\*Wing form interaction for both face width ( $F_{1,31} = 6.8$ ,  $P = 0.01$ ) and face area ( $F_{1,30} = 10.7$ ,  $P = 0.003$ )] (Figure 3.1A and B). Thus, there is a greater surface for the attachment of cibarial dilators in the brachypters of *P. dolus*, suggesting a greater potential for increasing ingestion rate when need be. Because brachypters are the predominant wing form in populations of *P. dolus* whereas macropters dominate populations of *P. marginata*, *P. dolus* may have a generally greater potential for compensatory feeding than *P. marginata*.

In general, thoracic metrics (widths and areas of mesothorax, metathorax, and entire thorax) indicated little difference in thorax size between the wing forms of *P. dolus*, whereas in *P. marginata* macropters had consistently larger thoraces than brachypters (Figure 3.2A, C and D; Figure 3.3 A, B, and C). Significant Species\*Wing form interactive effects on thoracic metrics document this pattern: width of mesothorax ( $F_{1,30} = 7.8$ ,  $P = 0.009$ ), length of mesothorax + metathorax ( $F_{1,30} = 8.07$ ,  $P = 0.008$ ), length of entire thorax ( $F_{1,30} = 6.9$ ,  $P = 0.01$ ), and area of the thorax calculated as length x mesothoracic width ( $F_{1,30} = 13.47$ ,  $P = 0.001$ ), length x metathoracic width ( $F_{1,30} = 6.56$ ,  $P = 0.02$ ), and length by average thoracic width ( $F_{1,30} = 9.8$ ,  $P = 0.004$ ). The only exception to this general pattern was the absence of a species effect ( $F_{1,30} = 0.09$ ,  $P = 0.8$ ), wing

form effect ( $F_{1,30} = 1.6$ ,  $P = 0.2$ ) and their interactive effect on the width of the metathorax ( $F_{1,30} = 2.65$ ,  $P = 0.1$ )(Figure 3.2B).

Brachypters of *P. dolus* had heavier heads than macropters of this species, a difference in head weight that was not evident between the wing forms of *P. marginata* (significant Species\*Wing form interaction;  $F_{1,27} = 7.96$ ,  $P = 0.009$ ; Figure 3.4A).

*Prokelisia marginata* had a heavier thorax ( $0.1 \pm 0.004$  mg) than *P. dolus* ( $0.09 \pm 0.005$  mg) when averaged across wing forms ( $F_{1,30} = 3.95$ ,  $P = 0.05$ ; Figure 4B). Also, macropters of both species had heavier thoraces than brachypters ( $F_{1,30} = 8.43$ ,  $P = 0.007$ ), and there was no interactive effect of species and wing form on thorax weight ( $F_{1,30} = 0.8$ ,  $P = 0.4$ ) (Figure 3.4B).

Indices of investment suggest differential allocation to flight and feeding morphology in the two *Prokelisia* species. As evidenced by the face-area-to-thorax-area index, *P. dolus* as a species exhibited a significantly greater investment in feeding ( $1.6 \pm 0.004$ ) than *P. marginata* ( $1.4 \pm 0.003$ ), which allocated more to flight ( $F_{1,31} = 14.89$ ,  $P = 0.0005$ ; Figure 3.5A). This index also showed that brachypters allocated more to feeding morphology ( $0.17 \pm 0.003$ ) than macropters ( $0.14 \pm 0.004$ ) which invested more in flight ( $F_{1,31} = 28.81$ ,  $P < 0.0001$ ), a pattern that occurred for both *Prokelisia* species (no significant interactive effect of species and wing form;  $F_{1,31} = 1.87$ ,  $P = 0.2$ ). The head-weight-to-thorax-weight index indicated that brachypters of *P. dolus* allocated more to feeding than macropters of this species, whereas the wing forms of *P. marginata* showed no difference in allocation (significant Species\*Wing form interaction,  $F_{1,26} = 4.42$ ;  $P = 0.05$ ; Figure 3.5B).

Altogether, head and thorax metrics as well as investment indices indicated that: (1) *P. dolus* invests more in feeding morphology than *P. marginata*, which allocates more to flight and (2) brachypters in general invest more in feeding and less in flight than macropters. Moreover, discrepancies in investment in feeding and flight between the wing forms were exaggerated differently in the *Prokelisia* species: (3) brachypters invested more in feeding than macropters, a difference that was relatively greater in *P. dolus* than *P. marginata*, and (4) macropters allocated more to flight than brachypters, a difference that was enhanced in *P. marginata* compared to *P. dolus*.

#### *Honeydew production in planthoppers as an index of ingestion and feeding compensation*

Fertilization produced a range of plants with different nutrient contents on which planthopper honeydew production could be assessed (Figure 3.6). There was a significant treatment effect on the nitrogen content of *Spartina*, which varied from 2.3%N for plants that received only phosphorus to 3.5%N for plants subsidized only with nitrogen ( $F_{2,15} = 30.54$ ,  $P < 0.001$ ; Figure 3.6A). In general, these values are high compared to those for *Spartina* plants in the field that usually average less than 1.5% nitrogen content (Denno 1983, Ornes and Kaplan 1989, Denno et al. 2002, Chapter 1). Phosphorus content also differed significantly among treatments with values ranging from 0.1% (N only treatment) to 0.3% (N+P treatment) ( $F_{2,13} = 78.87$ ,  $P < 0.0001$ ; Figure 3.6B), values that are far more representative of those for field plants (Ornes and Kaplan 1989, Chapter 1). The fertilization treatments also resulted in significant effects on the C:N and C:P contents of *Spartina* ( $F_{C:N,2,11.6} = 36.23$ ,  $P < 0.001$ ;  $F_{C:P,2,13} = 118.76$ ,  $P < 0.001$ ) (Figures 3.6C and D, respectively). Thus, because nitrogen is far more limiting

than phosphorus for *Prokelisia* planthoppers (Chapter 1), the poorest quality plants (lowest %N and highest C:N ratio) were those in the +P treatment followed by plants in the +N+P treatment; plants in the +N treatment were of the highest quality.

Honeydew production by *P. dolus* did not differ across plant treatments, despite variation in plant quality ( $F_{2,20} = 0.35$ ,  $P = 0.7$ ; Figure 3.7A). Moreover, neither wing form nor its interactive effect with fertilization treatment affected honeydew production (Wing form,  $F_{1,20} = 0.24$ ,  $P = 0.6$ ; Wing form\*Treatment,  $F_{2,20} = 0.22$ ,  $P = 0.8$ ) (Figure 3.7A). Thus, there was no evidence that *P. dolus* altered its ingestion/excretion rate in response to variable plant quality.

Species-level comparisons of honeydew production were also made using macropters. Even though there was no effect of the plant-nutrition treatments on honeydew production for either species ( $F_{2,14.9} = 0.28$ ,  $P = 0.7$ ), the excretion rate of *P. dolus* ( $0.56 \pm 0.1$  mg/48 hours) was more than twice that for *P. marginata* ( $0.22 \pm 0.06$  mg/48 hours) ( $F_{1,14.8} = 8.02$ ,  $P = 0.01$ ; Figure 3.7B). Although there was no interactive effect of species and plant treatment on honeydew production ( $F_{2,14.9} = 0.44$ ,  $P = 0.6$ ), there was a non-significant trend for *P. marginata* to excrete more honeydew on less nutritious plants (+P compared to +N+P treatment), a trend that was not evident in *P. dolus* (Figure 3.7B).

Two pieces of information suggest that honeydew excretion was accurately assessed. First, there was a significant increase in filter paper weight (the substrate on which honeydew collected) over the time course of the experiment in cages containing planthoppers ( $F_{2,35} = 15.7$ ,  $P < 0.0001$ ). Second, there was no change in filter paper weight in planthopper-free control cages ( $t_{1,35} = 0.6$ ,  $P = 0.6$ ).

## Discussion

Using wing-dimorphic *Prokelisia* planthoppers, the aim of this study was to establish the existence of a trade off between dispersal ability and a “third party trait,” namely compensatory feeding that allows an organism to cope with deteriorating plant quality. The essence of the trade off is a predicted allocational difference in thoracic muscle mass that allows for long-distance flight verses the cibarial musculature that governs the ability to ingest phloem sap. Thus, the expectation was that investment in dispersal would impose costs on compensatory feeding and vice versa, and that individuals could not maintain both a high commitment to dispersal and an efficient compensatory feeding ability. Accordingly, my approach was to compare head and thorax morphology between two planthopper species, *P. dolus* a sedentary species (most adults are brachypterous) and *P. marginata* a migratory species (most adults are macropterous), and to assess their abilities to compensate for poor plant quality by increasing their ingestion rate. I extended my investigation to include an intraspecific assessment of the head and thorax morphology of the flightless and volant wing forms of both species and to assess their respective abilities for compensatory feeding.

Overall, my results provided robust evidence for a species-level trade-off between the morphology associated with flight and that related to feeding. The head and thorax metrics I used as surrogates for cibarial and flight musculature indicated that *P. dolus* invests more in feeding than flight morphology, whereas *P. marginata* exhibits the reverse allocation pattern. Moreover, the same metrics suggest that investments in flight

by macropters (heavy thoraces) occur at the expense of feeding morphology (light heads) and that the reverse pattern occurs in brachypters with relatively light thoraces and heavy heads compared to macropters (Figure 3.4). However, the allocation pattern is complex in that the discrepancy in head and thorax morphology between the wing forms is exaggerated differently between the two *Prokelisia* species. Although brachypters generally invested more in feeding morphology (larger faces and heavier heads) than macropters, this difference was relatively greater in *P. dolus* than in *P. marginata* (Figure 3.5). Likewise, macropters allocated more to flight (larger and heavier thoraces) than brachypters, but this difference was generally greater in *P. marginata* than *P. dolus* (Figures 3.2, 3.3, 3.4B and 3.5). Thus, wing form differences in head morphology, which are presumably associated with feeding capability, are far greater in the sedentary species which must contend with on site fluctuations in host plant quality. Similarly, wing form differences in thorax morphology are exaggerated in the migratory species which copes with deteriorating plant nutrition via dispersal (see Denno 1994, Denno et al. 2000). These latter data are consistent with the argument that selection for dispersal favors a positive correlation between the proportion of flight-capable adults in the population and the flight capability (investment in flight morphology) of the macropterous morph (Fairbairn and Desranleau 1987, Fairbairn and Butler 1990, Roff and Fairbairn 1991, Fairbairn 1994). Thus, macropters from predominantly macropterous species are better fliers than macropters from species that are largely brachypterous (Fairbairn and Desranleau 1987, Fairbairn and Butler 1990). Likewise, selection may favor a greater investment in the head morphology associated with feeding in the brachypterous morph of sedentary species, as my data suggests (Figure 3.5).

Thus, my data support the contention that species-level differences in life-history strategy influence patterns of investment between feeding and flight. Variation in investment between macropters and brachypters within a species occurs largely in traits that reflect the major life style of the species. For example, the wing forms of *P. dolus*, the largely immobile species, showed variation in the head morphology associated with compensatory feeding, but minimal differences in allocation to the thoracic morphology linked with flight. Similarly, for the migratory *P. marginata*, there were great differences in thoracic morphology between the wing forms, but only negligible differences in the head metrics associated with feeding capability. Collectively, these data support the view that selection for a particular trait (e.g., dispersal) affects a suite of other associated physiological traits and patterns of allocation (Fairbairn and Desranleau 1987, Roff and Fairbairn 1991, Fairbairn 1994).

One could argue that the head metrics I used as surrogates for cibarial musculature are in fact associated with other functions. The most obvious of these alternatives might be vision and associated eyes and optic lobes of the brain. Thus, the larger head of *P. dolus*, especially the brachypterous morph (Figure 3.5A) may result from selective pressures associated with sight rather than feeding. All evidence points to the contrary, because large eyes and visual acuity in flies and bumble bees are associated with males that are extremely mobile and rely on dispersal to locate mates (Menzel et al. 1991, Hornstein et al. 2000). Accordingly, it should be the macropters of *P. marginata* with the largest heads, which in fact is not the case. Moreover, the cross sectional area of the cibarial muscles of *P. dolus* is larger than that for *P. marginata* (Denno and Fox unpublished data). Regarding my use of thorax metrics as indicators of flight



musculature, there is a positive association between thorax size and flight in insects (Chai and Srygley 1990, Hill et al. 1999, Fric and Konvicka 2002). Thus, I am confident that the surrogates I used to assess flight and feeding musculature were accurate indicators.

Based on its greater investment in cibarial musculature (Figures 3.4 and 3.5), the expectation was for *P. dolus* to exhibit a higher ingestion rate and compensatory feeding ability than *P. marginata*. Likewise, brachypters were expected to have a greater ingestion capacity and thus compensatory feeding ability than macropters. Because ingestion and excretion rates are highly correlated in phloem-feeding insects such as planthoppers (Prestidge 1982a, Brodbeck et al. 1993, Brodbeck et al. 1995, 1996), I used honeydew production as an index of ingestion rate. Overall, there was little support regarding predictions for ingestion and feeding compensation between species and wing forms.

*Prokelisidolus* produced more than twice the amount of honeydew as *P. marginata* (Figure 3.7), suggesting a much higher ingestion capacity. At the level of species, the greater ingestion capacity of *P. dolus* compared to *P. marginata* is likely related to its increased ability to survive, perform, and regulate its macronutrient composition on nutrient deficient host plants (Chapters 1 and 2). However, the species-specific difference in ingestion rate (honeydew production) is not clearly attributable to allocational differences in cibarial musculature. For instance, despite the dramatic discrepancy in honeydew production between the macropters of *P. dolus* and *P. marginata*, there was no morphological evidence suggesting a greater allocation to feeding musculature in the macropters of *P. dolus* (Figures 3.1, 3.4 and 3.5).

Similarly, within-species predictions regarding ingestion rate and feeding morphology were not well supported. For example, there was no difference in honeydew production between the wing forms of *P. dolus* (Figure 3.7), even though brachypters apparently invest much more in feeding morphology than macropters (Figure 3.5B). Moreover, because honeydew production did not increase on the nutrient-deficient treatment plants, there was no evidence for compensatory feeding in *P. dolus* (Figure 3.7). Admittedly, the nitrogen content in all treatment plants was high ( $> 2\%$ ) which may not have challenged *P. dolus* to increase its ingestion rate to meet nutrient demands. However, additional evidence suggests that despite extreme variation in host plant quality (%N, %P, and C:N), both wing forms of *P. dolus* regulate their body composition of macronutrients equally well (Chapter 2). Alternatively, nutrient demands might be met on poor-quality host plants by increasing the assimilation efficiency of nitrogen rather than enhancing ingestion rate (Abisgold et al. 1994). Thus, the negative association between feeding and flight morphology between the wing forms of *P. dolus* does not apparently extend to affect actual differences in consumption capacity.

There is evidence suggesting a trade-off between flight and reproduction in *P. dolus*, with flightless brachypters exhibiting a higher reproductive potential than flight-capable macropters (Denno et al. 1989), a pattern commonly observed in many wing-dimorphic insects (Zera and Denno 1997). However, to document the existence of a physiological trade-off, it is necessary to verify that morph-related differences in trait investment are the result of differences in resource allocation and not due to the differential consumption of nutrients (Zera and Denno 1997, Zera and Harshman 2001). Indeed, the similar ingestion rates (honeydew production) of the wing forms of *P. dolus*

suggest that the trade-off between reproduction and flight evident in this species does not result from differences in nutrient consumption. Thus, this study provides robust support for the existence of an actual trade-off between flight and reproduction in *P. dolus*.

The physiology underlying life history trade-offs in wing dimorphic insects has received much attention in the recent years (Zera et al. 1998, Zera and Brink 2000, Zhao and Zera 2002). In the context of this study, life histories bear heavily on the ability of phytophagous insects to cope with the fundamental stoichiometric mismatch that exists between their nutrient composition and that of their host plants (Elser et al. 2000a, Fagan et al. 2002a). Differences in dispersal ability may influence a species' response to nutrient limitation by virtue of constraints placed on "third party traits" such as compensatory feeding. My results reveal that although investments in flight likely constrain feeding morphology that such constraints do not clearly translate into differences in ingestion rate or compensatory feeding. Resource allocation differences that underlie the strategies used by phytophagous insects to cope with nutrient limitation have rarely been identified. Toward this end, this study presents the first attempt to link dispersal and feeding investments as antagonistic traits within and between species.

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## Figure Descriptions

Figure 3.1. (A) Face width (mm) and (B) face area (mm<sup>2</sup>) of the female wing forms (brachypters and macropters) of *Prokelisia dolus* and *P. marginata*. A larger face indicates a greater surface of attachment for the cibarial muscles that control ingestion, and thus a greater potential investment in the musculature regulating ingestion rate and capacity. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.2. Thorax size of the female wing forms (brachypters and macropters) of *Prokelisia dolus* and *P. marginata* as indexed by the (A) width of mesothorax (mm), (B) width of the metathorax, (C) length of the mesothorax + metathorax (mm), and (D) length of the entire thorax (prothorax + mesothorax + metathorax (mm)). A larger thorax suggests a greater volume for housing flight muscles, and thus a greater potential investment in dispersal capability. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.3. Thorax area (mm<sup>2</sup>) of the female wing forms (brachypters and macropters) of *Prokelisia dolus* and *P. marginata* as indexed calculated by (A) length (prothorax to metathorax) x mesothorax width, (B) length x meta-thorax width, and (C) thorax length x average thorax width. A larger thorax suggests a greater volume for housing flight muscles, and thus a greater potential investment in dispersal capability. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.4. (A) Head weight (mg) and (B) thorax weight (mg) of the female wing forms (brachypters and macropters) of *Prokelisia dolus* and *P. marginata*. A heavier head indicates a greater potential mass of cibarial muscles that control ingestion and thus a greater investment in the musculature regulating ingestion rate and capacity. A heavier thorax suggests a greater potential mass of flight muscles and

thus a greater investment in dispersal capability. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.5. Indices of investment in feeding versus flight musculature for the female wing forms (brachypters and macropters) of *Prokelisia dolus* and *P. marginata*. Indices were calculated as (A) face area ( $\text{mm}^2$ )/thorax area ( $\text{mm}^2$ ) and (B) head weight (mg)/thorax weight (mg). A high value indicates a greater potential investment in cibarial musculature compared to flight musculature, whereas a low value suggests the reverse. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.6. Effect of fertilization treatment [phosphorus only (+P), nitrogen only (+N), and both (+N+P)] on the nutrient content of *Spartina*: (A) nitrogen (%), (B) phosphorus (%), (C) C:N, and (D) C:P. Means ( $\pm$  SE) with the same letter are not significantly different.

Figure 3.7. Effect of disparity in *Spartina* nutrition, achieved by differential fertilization [phosphorus only (+P), nitrogen only (+N), and both (+N+P)], on the honeydew production (mg/48 hours) of (A) the female brachypters and macropters of *Prokelisia dolus*, and (B) the female macropters of *P. dolus* and *P. marginata*. There was no effect of the plant nutrient treatments on the honeydew production of either species, but *P. dolus* excreted more than twice as much honeydew as *P. marginata* (B) suggesting a much higher ingestion rate. Control clip-cage results are shown for illustrative purposes. Means ( $\pm$  SE) are reported.

Figure 3.1.

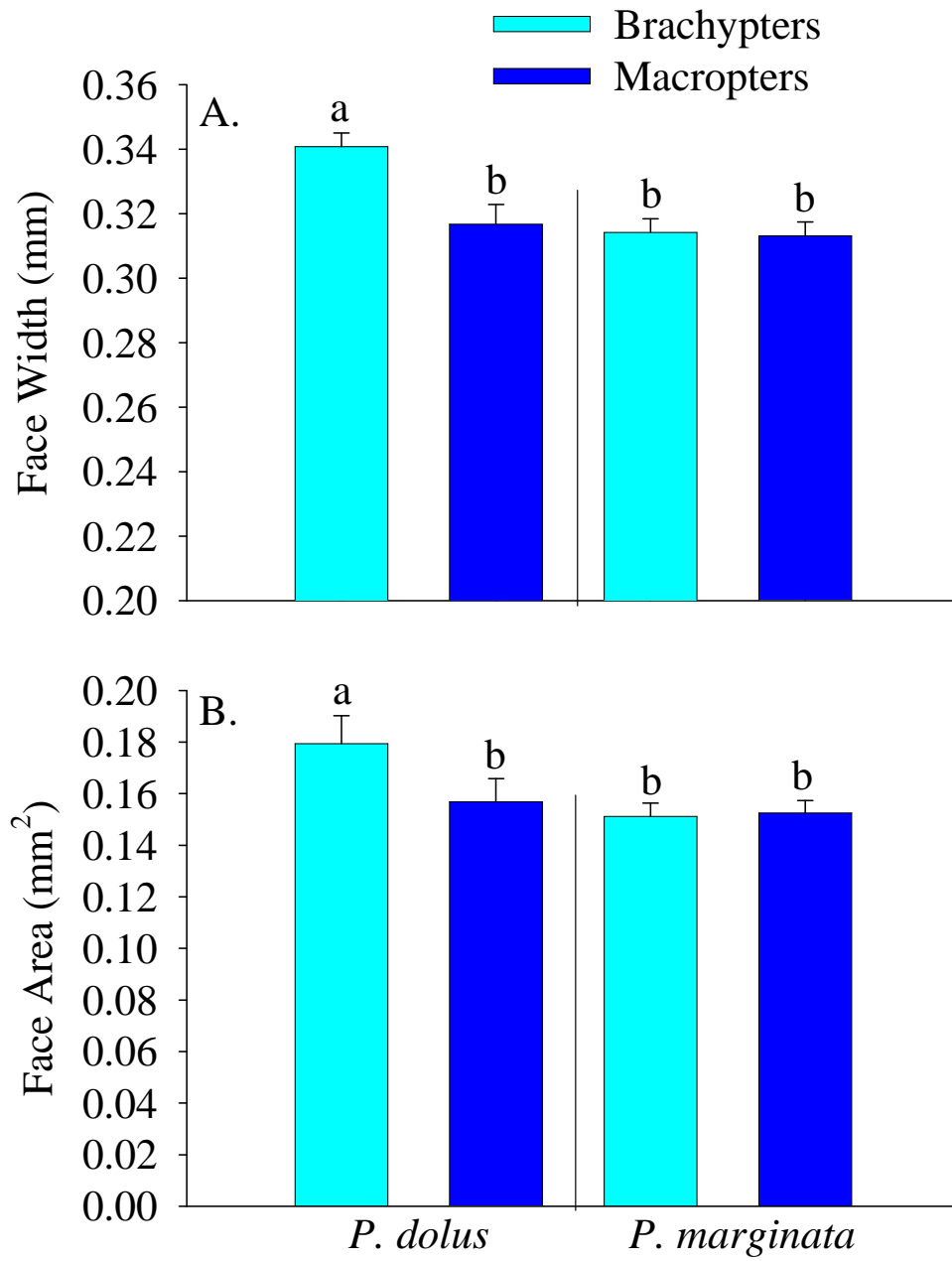


Figure 3.2.

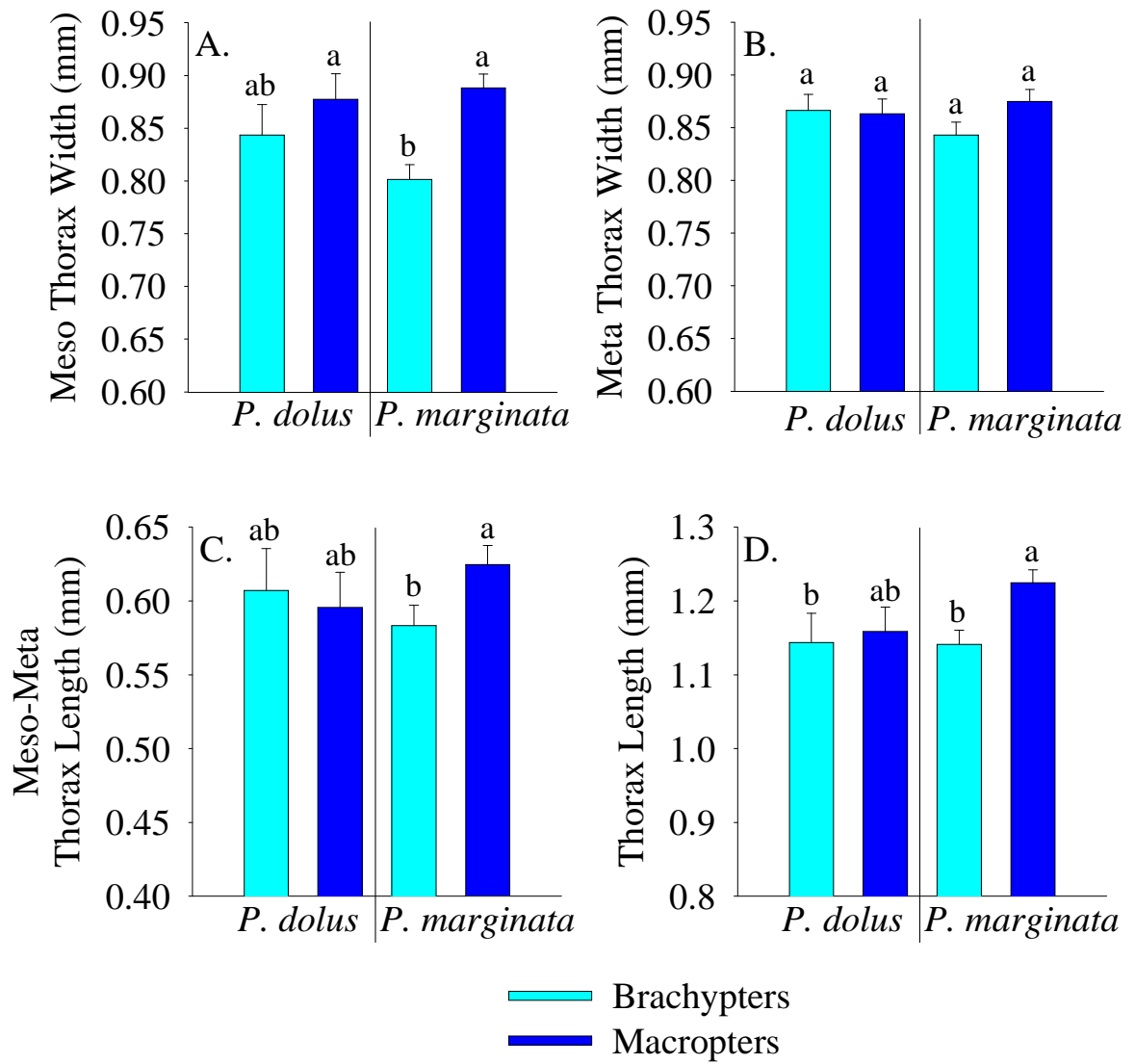




Figure 3.3.

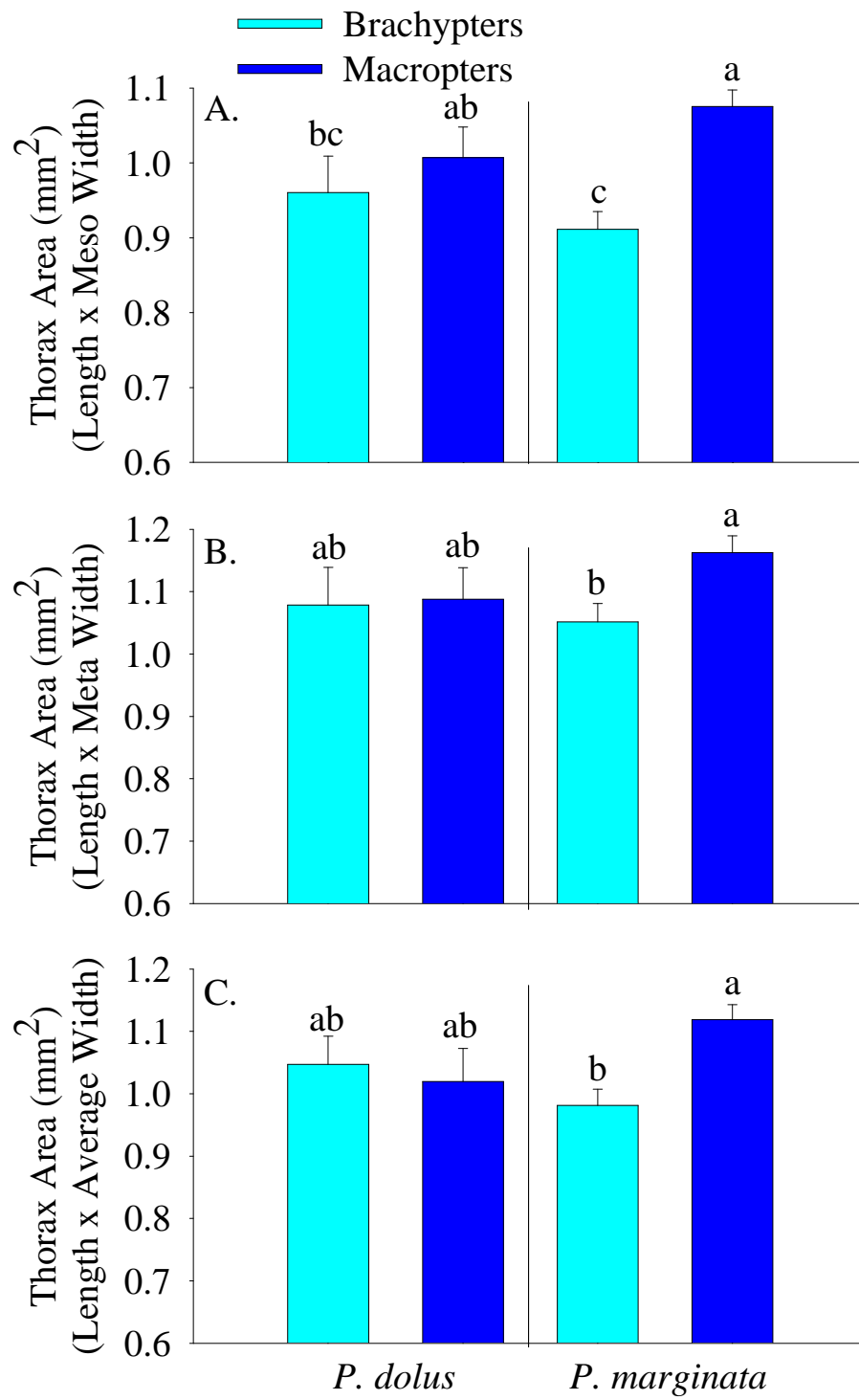


Figure 3.4

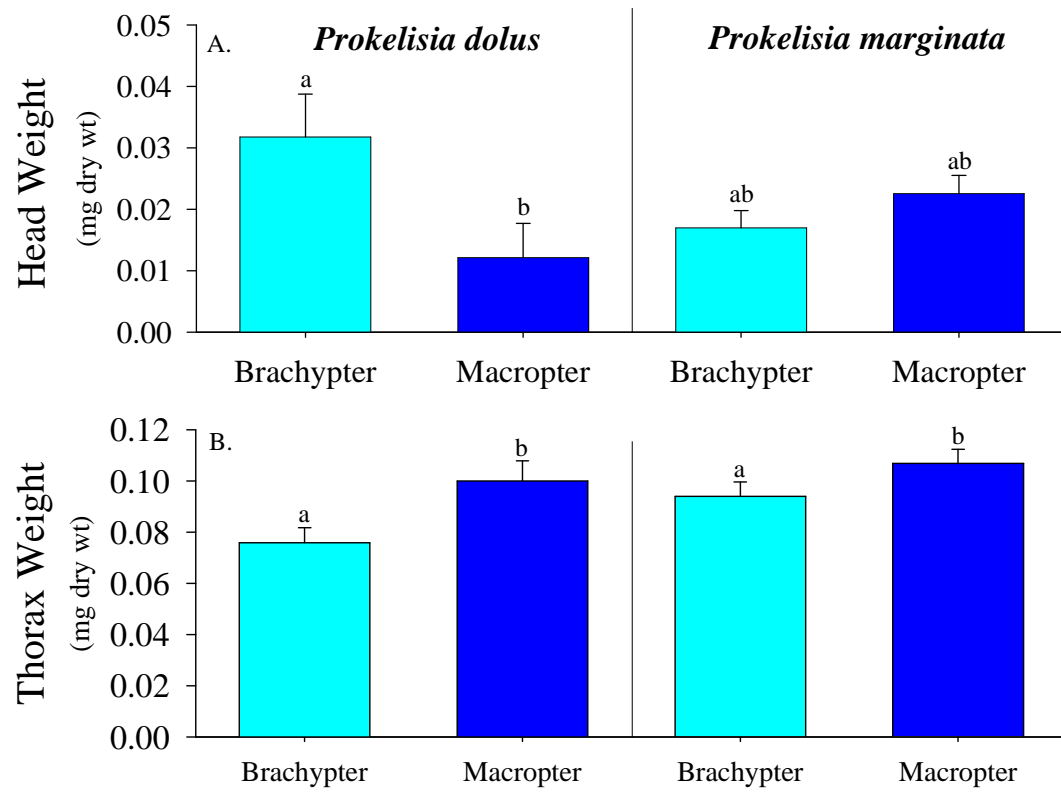


Figure 3.5

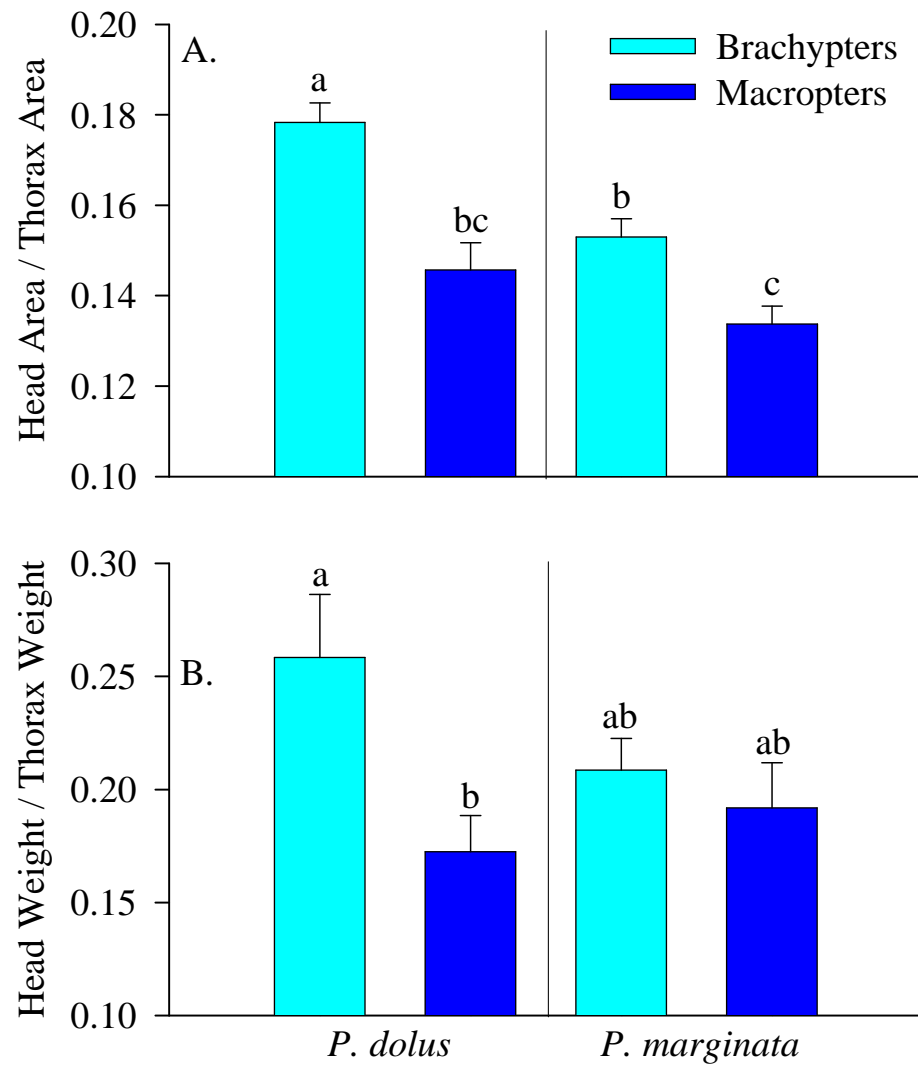


Figure 3.6

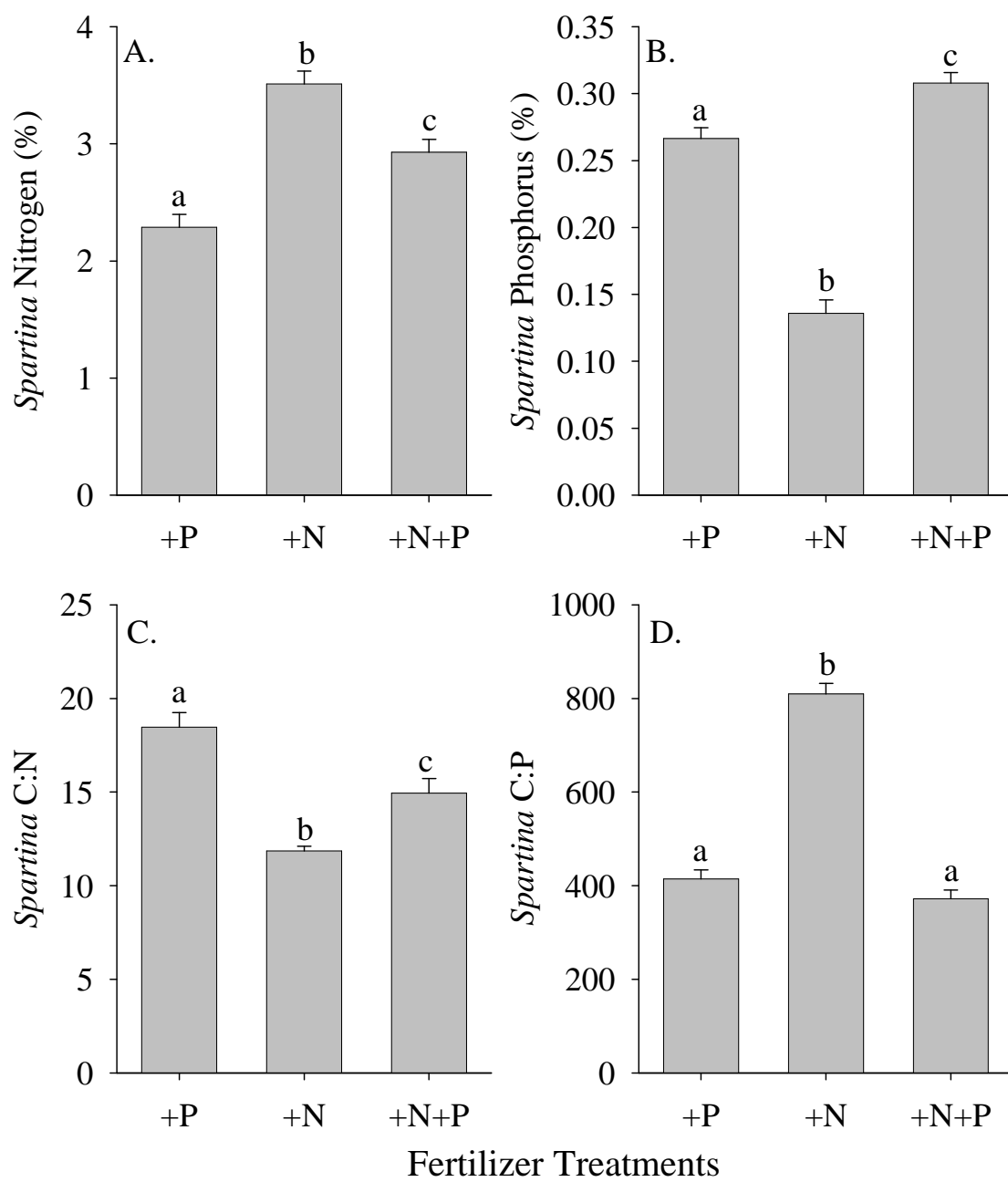
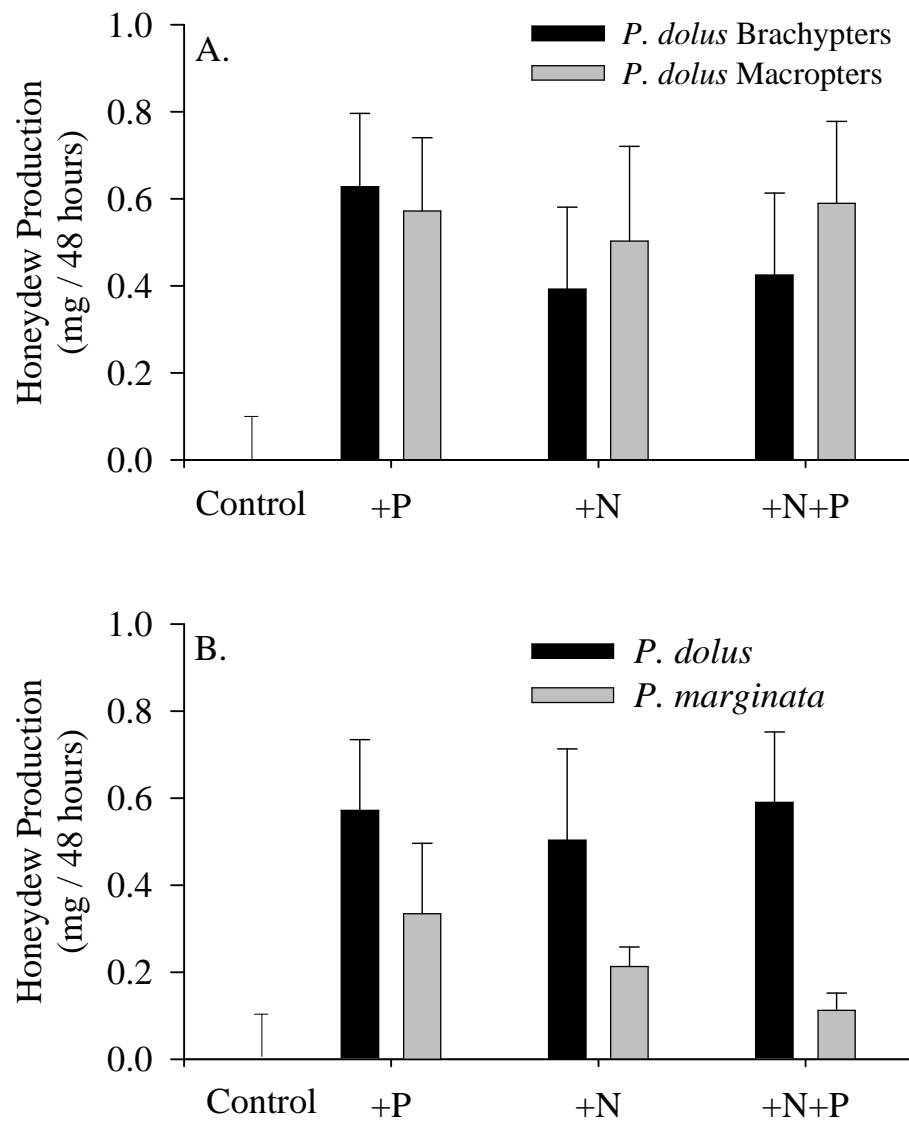


Figure 3.7



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