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PREDATORY BEHAVIOR AND PHYSIOLOGICAL RESPONSE OF CHINESE MANTIDS TO TOXIC AND NON-TOXIC LEPIDOPTERAN PREY

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PREDATORY BEHAVIOR AND PHYSIOLOGICAL RESPONSE OF CHINESE MANTIDS TO TOXIC AND NON-TOXIC LEPIDOPTERAN PREY

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ABSTRACT

Prey have evolved a number of defenses against predation, and predators have developed means of countering these protective measures. Monarch caterpillars, *Danaus plexippus*, for example, feed on milkweed plants in the genus *Asclepias* and sequester cardenolides as an anti-predator defense. However, some predators are able to consume this otherwise unpalatable prey. The observation of a Chinese mantid, *Tenodera sinensis*, consuming the body tissue of a monarch caterpillar while 'gutting' the prey (i.e., removing the gut and associated internal organs) without any apparent ill-effects prompted this research. In a series of behavioral trials we explored how adult *T. sinensis* handle and consume toxic (*D. plexippus*) and non-toxic (*Ostrinia nubilalis* and *Galleria mellonella*) caterpillars. In addition, we analyzed differences in the carbon to nitrogen (C:N) ratio and cardenolide content of monarch tissue consumed or discarded by mantids. We found that mantids gutted monarchs while wholly consuming non-toxic species. As expected, monarch gut tissue had a higher C:N ratio than non-gut tissue, confirming the presence of plant material. Although there were more cardenolide peaks in monarch body versus gut tissue, total cardenolide concentration and polarity index did not differ. Although *T. sinensis* treated toxic prey differently than non-toxic prey, gutting did not decrease the mantid's total cardenolide intake. Since other predators consume monarch caterpillars whole, this behavior may be rooted in species-specific vulnerability to particular cardenolides or simply reflect a preference for high-N tissues.

To further investigate the gutting behavior of the mantid, we conducted a second series of behavioral trials in which mantids were offered cardenolide-

containing and cardenolide-free *D. plexippus* caterpillars and butterflies. In addition, we fed mantids starved and unstarved *D. plexippus* caterpillars from each cardenolide treatment and non-toxic *Ostrinia nubilalis* caterpillars. These trials were coupled with elemental analysis of the C:N ratios in gut and body tissues of both *D. plexippus* caterpillars and corn borers. We found that cardenolides did not affect mantid behavior: mantids gutted both cardenolide-containing and cardenolide-free caterpillars. In contrast, mantids consumed both *O. nubilalis* and starved *D. plexippus* caterpillars entirely. *Danaus plexippus* body tissue has a lower C:N ratio than their gut contents, while *O. nubilalis* have similar ratios. It is possible that the gutting behavior is in response to non-cardenolide secondary plant compound and/or an ability to regulate nutrient uptake. The results of this second experiment suggest that while cardenolides are not driving the post-capture prey processing by mantids, it is likely driven by a sophisticated assessment of resource quality.

From our first two experiments, it is clear that the Chinese mantid is able to consume cardenolide-containing monarch caterpillars without immediate adverse effects. Despite discarding the caterpillars' gut contents, mantids still ingest cardenolides sequestered in monarch body tissue. Although mantids do not exhibit immediate adverse reactions when consuming monarch biomass, it is possible that there are long-term fitness costs associated with cardenolide consumption. We tested the hypothesis that monarch caterpillar consumption negatively affects mantid growth and reproductive condition. We assigned lab-reared mantids to one of four toxicity groups that differed in the number of monarch caterpillars offered to adult mantids over a 15-day period. Monarch consumption did not reduce mantid fecundity; all

treatment groups produced similar numbers of eggs. However, mantids in the high-toxicity group produced eggs that were 42% longer on average and devoted 75% more of their biomass toward egg production than those in the control group. This increase in reproductive condition is probably driven by other factors such as mantid size, prey nutritional value and/or diet mixing. Despite consuming similar amounts of prey biomass during the experiment, mantids in the high-toxicity group gained more biomass and were larger than mantids in the other groups. These results, combined with our previous research suggest that the Chinese mantid is able to incorporate monarch prey into its diet without acute or chronic ill-effects.

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DEDICATION

To my brother Robert Price whose blunt remarks about working retail instead of going to school at a family outing one evening during the summer of 2007 reminded me of my passion for learning and inspired me to return to school to complete my B.S and then continue on to graduate school for my PhD.

PREFACE

This thesis is presented in manuscript format. Chapter one has been published in *The Journal of Ecological Entomology* in 2013. Chapters two and three were in review in *The Journal of Environmental Entomology* and *The Journal of Chemical Ecology* at the time this thesis was submitted to the University of Rhode Island.

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CHAPTER ONE

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Chinese mantids gut toxic monarch caterpillars: avoidance of prey defense?

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ABSTRACT

1. Monarch caterpillars, *Danaus plexippus*, feed on milkweed plants in the genus *Asclepias* and sequester cardenolides as an anti-predator defense. However, some predators are able to consume this otherwise unpalatable prey.

2. We observed Chinese mantids, *Tenodera sinensis*, consuming monarch caterpillars by 'gutting' them (i.e., removing the gut and associated internal organs). They then feed on the body of this herbivore without any apparent ill effects.

3. We explored how adult *T. sinensis* handle and consume toxic (*D. plexippus*) and non-toxic (*Ostrinia nubilalis* and *Galleria mellonella*) caterpillars. We analyzed differences in the C:N ratio and cardenolide content of monarch tissue consumed or discarded by mantids.

4. Mantids gutted monarchs while wholly consuming non-toxic species. Monarch gut tissue had a higher C:N ratio than non-gut tissue, confirming the presence of plant material. Although there were more cardenolide peaks in monarch body versus gut tissue, total cardenolide concentration and polarity index did not differ.

5. Although *T. sinensis* treated toxic prey differently than non-toxic prey, gutting did not decrease the mantid's total cardenolide intake. Since other predators consume monarch caterpillars whole, this behavior may be rooted in species-specific vulnerability to particular cardenolides or simply reflect a preference for high-N tissues.

KEYWORDS: *Danaus plexippus, Tenodera sinensis, Ostrinia nubilalis,* prey handling, aposematism, chemical defense, cardenolides

INTRODUCTION

Prey respond to predation risk with a variety of anti-predator defenses including altered life history strategies, morphological defenses, and behavioral changes in foraging behavior and microhabitat use (Lima, 1998; Preisser & Bolnick, 2008). Prey without inducible strategies often compensate with constitutive defenses, such as the production or sequestration of toxic substances, and frequently advertise their defense via aposematism (Duffey, 1980; Nishida, 2002; Ruxton et al., 2004). The stability of aposematic signals make it easier for predators to learn unpalatable prey (Gittleman & Harvey, 1980), thus allowing predators to consistently detect and avoid defended species.

One well-known example of invertebrate aposematism involves the monarch butterfly, *Danaus plexippus*. This species' black and yellow caterpillars feed on host plants in the genus *Asclepias* (Apocynaceae) that contain toxic cardenolides and sequester these toxins in their bodies (Agrawal et al., 2012). These substances have an emetic effect in birds (Brower et al., 1967). While aposematism provides an effective defense against some predators, other predators and parasitoids are able to prey upon *D. plexippus*. Birds such as Orioles (*Icterus* spp.) and Grosbeaks (*Pheucticus* spp.) learn to avoid the toxin-rich cuticle of adults and may develop a physiological insensitivity to the insect's sequestered toxins (Nishida, 2002). Predators such as ants (*Formica montana*) and ladybird beetles (*Harmonia axyridis*) also prey on eggs and early-instar larvae (Koch et al., 2003; Prysby, 2004). In contrast, few predators attempt to consume late-instar caterpillars, although assassin bugs (Hemiptera: Reduviidae) can feed on them (Zalucki & Kitching, 1982) and predatory wasps, *Polistes*

dominulus, will attack and consume monarch caterpillars when more favorable prey types are unavailable (Rayor, 2004).

While carrying out an unrelated field experiment, we observed late-instar Chinese mantids (*Tenodera sinensis),* a generalist predator, consuming *D. plexippus* caterpillars by 'gutting' them and only eating their integument (Fig. 1). *Tenodera sinensis* reacts negatively to chemically defended insects such as *Diabrotica* beetles and milkweed bugs (*Oncopeltus fasciatus*), especially when these herbivores are raised on toxin-containing diets (Ferguson & Metcalf, 1985). They are also able to learn to avoid such encounters: naïve third-instar mantids presented with two toxic *O. fasciatus* in succession take less time to sample and reject the second prey item (Paradise & Stamp, 1991), and it takes fewer than six encounters for late-instar mantids to refuse to attack *O. fasciatus* (Berenbaum & Miliczky, 1984).

We present research exploring predator-prey interactions between *T. sinensis*, *D. plexippus*, and two other species of non-toxic lepidopteran larvae. We observed the behavior and consumption rates of field-collected adult *T. sinensis* when fed *D. plexippus*, non-toxic European corn borers (*Ostrinia nubilalis*), and wax moth larvae (*Galleria mellonella*). We analyzed differences in C:N ratios and cardenolide content between caterpillar tissues consumed or discarded by mantids. We hypothesized that the gutting behavior we observed for monarch caterpillars would not be employed for the two non-toxic prey, and that cardenolide levels would be higher in the monarch gut than in the rest of the body.

METHODS

Insect collection and maintenance: Adult praying mantids were collected in July 2011 from an abandoned agricultural field at East Farm (Kingston, RI), a research facility run by the University of Rhode Island (URI). They were returned to the lab and maintained at 25 ^{o}C in 50 x 25 x 30 cm plexiglass aquariums with plant material as perches. Each aquarium housed two mantids, separated from each other by a piece of cardboard. The mantids were fed house crickets, *Acheta domesticus*, and wax worms, *G. mellonella,* ad libitum until three days before the experiment (see below for details).

Monarch (*D. plexippus*) caterpillars and eggs were collected in August and September 2011 from milkweed plants (*Asclepias syriaca*) growing in a URI-managed farm. Caterpillars were removed from the leaf on which they were feeding; when eggs were found, the entire leaf was collected. Eggs and caterpillars were returned to the lab and kept in a 40 x 40 x 76 cm cage where they were fed cut milkweed. We reared a total of 21 caterpillars (all > 0.5 g).

European corn borers (*O. nubilalis*) were collected in September 2011 from organically-grown flint corn (*Zea mays*) growing in a URI-managed farm. They were kept in the lab and fed ears of corn until the experiment. We reared a total of 15 caterpillars (all > 0.3 g).

Wax worms (the larval phase of *G. mellonella*) were purchased from a local pet store and stored in the refrigerator at 10 ˚C prior to the experiment. At the start of the experiment, they had not consumed any food for 1-3 days.

D. plexippus-only observation trials: We followed standard experimental protocols (Reitze & Nentwig 2011) and starved all praying mantids (n = 11) for three days prior to running the experiment. At the start of each trial, an individual mantid was placed in an 18 x 12.5 x 6 cm clear plastic container, and given five minutes to acclimate. We then placed a pre-weighed *D. plexippus* individual (n = 10) in the container. The interaction was video-recorded from the time the mantid attacked the prey until it was completely consumed or the mantid ceased feeding. We noted whether the mantid engaged in gutting behavior, defined as the predator-induced expulsion of prey organs without any subsequent attempt at consumption.

Trials observing all three prey species: In order to determine if mantids exhibited gutting behavior only when handling *D. plexippus* larvae, we conducted a series of no-choice trials in which we offered mantids non-toxic prey *O. nubilalis* (n = 15) and *G. mellonella* (n = 8) in addition to *D. plexippus* (n = 11). These followed the procedures described above but with the following modifications. First, the plastic container in which the trial was conducted was weighed prior to the start of each trial and after the trial was completed because we found that when the integument of larger caterpillars (i.e., *D. plexippus* and *O. nubilalis*) was punctured, hemolymph often dripped from the cadaver; we did not classify this incidental loss as gutting. We used this final mass to determine the amount of prey biomass discarded.

To determine why mantids gut *D. plexippus*, we disturbed mantids during these trials. As the mantid fed on *D. plexippus*, the gut content expelled from each larva was collected into a pre-weighed 1.5 ml eppendorf tube in order to determine the weight of the expelled material. After each *D. plexippus* larva was gutted, the remaining cadaver

(i.e., the portion of the larva eaten by the mantid in the *D. plexippus*-only trials) was forcibly removed from the mantid and placed in a second pre-weighed eppendorf tube. This tube was re-weighed to determine the weight of the remaining cadaver; both tubes were then frozen at -13 ˚C until their contents could be analyzed. These data were used to determine the larval mass discarded by each mantid.

We analyzed these videos for the following information. First we recorded whether or not the mantid engaged in the gutting behavior. We also recorded the amount of time the mantid spent actively feeding in order to determine predator consumption rate (g/m) of all prey. A total of eight mantids were tested in this experiment (one mantid refused to eat anything, while another mantid escaped, consumed a smaller mantid, and refused to eat thereafter).

CHN and HPLC analysis*:* Each gut and non-gut sample of *D. plexippus* was transferred into an individual 2 ml pre-weighed screw-cap tube and dried in a 45 ^{o}C drying oven for five days. After drying was complete, 1.0-2.2 mg of dried material was removed from each sample for CHN analysis. This material was sent to an analytic chemistry lab at URI (Narragansett RI) for analysis. The remaining dry material from each sample was used for cardenolide analysis.

Cardenolide concentrations were assessed by HPLC, following Bingham and Agrawal (2010). Briefly, oven-dried (40 ^{o}C) tissue from each sample was ground to a fine powder and extracted with 1.8 ml methanol (MeOH). Sample mass ranged from 10-43 mg for gut tissue and 80-159 mg for body tissue. Each extract was spiked with 20 µg digitoxin as an internal standard and sonicated for 20 minutes at 55 °C in a water bath. After centrifugation, the supernatant was collected, dried, resuspended in

300 µl MeOH, and filtered through a 0.45 µm syringe-driven filter unit. 15 µl of extract was then injected into an Agilent 1100 series HPLC and compounds were separated on a Gemini C18 reversed phase column (3 µ m, 150 x 4.6 mm, Phenomenex, Torrance, CA). Cardenolides were eluted on a constant flow of 0.7 ml/min with an acetronile-0.25% phosphoric acid in water gradient as follows: 0-5 min 20% acetonitrile, 20 min 70% acetonitrile; 20-25 min 70% acetonitrile, 30 min 95% acetonitrile, 30-35 min 95% acetonitrile. UV absorbance spectra were recorded from 200 to 400 nm by diode array detector. Peaks with absorption maxima between 217 and 222 nm were recorded as cardenolides and quantified at 218 nm. Concentrations were calculated based on dry mass and standardized by peak areas of the known digitoxin concentration. In addition to total cardenolides, we report cardenolide peak diversity (number of distinct cardenolide peaks per sample) and an index of cardenolide polarity (index $P = \text{sum}[P_i RT_i]$), where RT_i is the retention time of the i^{th} peak, weighted by each peak's relative concentration P_i (Rasmann & Agrawal, 2011).

Statistical analysis: Data on mantid gutting behavior (yes/no) for the three prey species were analyzed using contingency analysis. Because some mantids consumed more than one individual of a given prey species, the effect of prey species on consumption rate (g/min) and percent biomass discarded was tested using a mixed model for repeated measures analysis (Littell et al., 1996). This analytic method is suitable for use in cases where a portion of time series data is missing; in contrast, standard repeated measures ANOVAs excludes all subjects missing any time*treatment data (von Ende, 2001). A two-factor (treatment and time) repeated

measures design was used, and 'mantid' was added as a random factor nested under 'treatment'. Because this design is unbalanced, i.e., not every mantid was fed two individuals from each prey species, the test statistics did not follow an exact *F* distribution. Following recommended procedure, we calculated *P* values using the Satterthwaite method to generate an approximate *F* value with fractional degrees of freedom (West et al., 2006). Although the data on prey handling time was normally distributed, the data on percent biomass discarded was not even when transformed. Because using a non-parametric analysis would have prevented us from incorporating random effects (i.e., 'mantid' nested within 'treatment'), we chose to proceed with a parametric approach. We justify this decision by noting that ANOVAs are robust to departures from normality when per-treatment sample sizes are large (Underwood, 1997), a criterion that our 34-observation data set meets. We performed means separation tests, where appropriate, using Tukey's HSD at $\alpha = 0.05$. Data on C:N ratio, total cardenolide content, number of cardenolide peaks, and polarity index were analyzed using a paired-sample t-test on gut and body tissue from each tested prey individual. We used the same approach to analyze data on individual cardenolides; because of the large number of comparisons, we present both the unadjusted *P*-value as well as the *P*-value corrected for multiple comparisons at $\alpha = 0.05$ using step-up FDR, a sequential Bonferroni-type procedure. Statistical analyses were performed using JMP 9.0.0 (SAS, 2010).

RESULTS

We observed 44 predator-prey encounters between *T. sinensis* and the three prey species. Upon detecting their prey, the mantids would orient on it, grasp the prey

with their forelegs, and begin consuming it. Mantids encountering *G. mellonella* or *O. nubilalis* caterpillars (n = 8 and 15, respectively) ate these prey in their entirety (excluding any hemolymph that fell from *O. nubilalis* prey). In contrast, mantids encountering *D. plexippus* would allow the gut content to fall from the cadaver while feeding, and would not attempt to consume it even after finishing the rest of the cadaver (Fig. 1). Mantids encountering *D. plexippus* larvae gutted 18 of 21 (86%) caterpillars (Fig. 2A; $X^2 = 42.3$, $P < 0.001$). Two of the three individuals that were not gutted were each parasitized by a single late-instar larvae of a tachinid fly; the remaining larvae was extensively infected with a fungal pathogen (likely *Beauveria bassiana*).

The mantid's gutting behavior led to large differences in the mean percent of prey mass discarded (i.e., unconsumed at the end of the feeding bout) by the mantids (Fig. 2B; $F_{2,26.8} = 16.3$, $P < 0.001$). While mantids discarded 41 $\pm$ 3.1% (mean (SE); n = 11) of *D. plexippus* larval mass, they only discarded 14 $\pm$ 4.6% of *O. nubilalis* larval mass and 0% of *G. mellonella* larval mass (Fig. 2B; $P < 0.05$). Mantids that consumed multiple caterpillars of a given prey species did not differ over time in the proportion discarded ($F_{1,11.3} = 0.44$, $P = 0.52$), and there was no time*prey species interaction ($F_{2,11.2} = 0.13$, $P = 0.88$). While the mass discarded from *D. plexippus* caterpillars consisted primarily of its gut, the discarded mass from *O. nubilalis* consisted entirely of hemolymph; mantids never discarded any tissue from either *O. nubilalis* or *G. mellonella* caterpillars. Despite the species-specific differences in gutting behavior, mantids consumed the 'edible' portion of all three prey species at an equal rate (Fig. 2C; $F_{2,21.8} = 0.36$, $P = 0.70$). Again, mantids that consumed multiple prey items of a

given species did not differ in their prey consumption rate over time ($F_{1,8.94} = 0.09$, $P = 0.77$), and there was no time*prey species interaction ($F_{2,8.90} = 0.04$, $P = 0.96$). Gut tissue from *D. plexippus* had a marginally lower concentration of C (38.1 $\pm$ 1.30 (SE) µmol mg^{-1}) than did non-gut tissue (46.2 $\pm$ 3.94 µmol mg^{-1}; $t_8 = 2.18$, $P = 0.061$). However, there was 58% less N in gut (3.1 $\pm$ 0.34 µmol mg^{-1}) versus non-gut tissue (7.5 $\pm$ 0.33 µmol mg^{-1}; $t_8 = 14.97$, $P < 0.001$). As a result, gut tissue had a higher C:N ratio (13.2 $\pm$ 1.22) than non-gut tissue (6.15 $\pm$ 0.44; $t_8 = 4.77$, $P = 0.001$). This suggests that the mantid-discarded *D. plexippus* material consisted mainly of macerated plant tissue, which was low in nutritive value.

Despite the large amount of plant material in the *D. plexippus* gut, there were no differences in total cardenolide content (body: 1.90 $\pm$ 0.77 (SE) µg cardenolides mg^{-1}; gut: 1.74 $\pm$ 0.88) or polarity index (body: 19.2 $\pm$ 2.66; gut: 18.9 $\pm$ 3.46) between mantid-consumed and mantid-discarded herbivore tissue (both $P > 0.10$). There were nearly three times as many cardenolide peaks in *D. plexippus* body versus gut tissue ($t_8 = 11.8$, $P < 0.001$), probably reflecting the breakdown of plant-derived cardenolides into differentially sequestered compounds. For example, the large cardenolide peak in the gut tissue at 10.8 min is twice as concentrated as in the body tissue; the three subsequent peaks, however, are absent from the gut and only in the body tissue, potentially suggesting transformation during sequestration (Fig 3).

DISCUSSION

We found that adult *T. sinensis* can capture and consume late-instar *D. plexippus* caterpillars with no apparent ill effects. The fact that *T. sinensis* handled *D. plexippus* caterpillars differently than *O. nubilalis* and *G. mellonella* larvae appears to

be a behavioral mechanism to reduce exposure to prey toxicity. This interpretation is supported by the fact that all of the tested mantids treated *D. plexippus* caterpillars very similarly (Fig. 2A): chewing open the integument and letting the gut fall out while consuming the remains (Fig. 1). Given that gutted caterpillars were consumed in their entirety, the discarding of ~40% of prey biomass (Fig. 2B) cannot be attributed to mantid satiation. In contrast, *T. sinensis* never gutted either *O. nubilalis* or *G. mellonella* larvae and consumed all non-hemolymph biomass (Figs. 2A, B). While it is possible that larger prey are easier to gut, mantids consumed a substantial amount of *O. nubilalis* gut material that could easily have been avoided by the mantid. In addition, large *O.nubilalis* larvae (e.g., one weighing 0.73 g) were not gutted, whereas smaller *D. plexippus* larvae (e.g., one weighing 0.63 g) were gutted. Once the mantids had gutted the *D. plexippus* caterpillars, they consumed all three prey types at a similar rate (Fig. 2C). This suggests that the mantid considered all three prey types equally palatable.

The gutting behavior we documented in *T. sinensis* is similar to that observed in other predators, many of which are capable of identifying and selectively consuming the least noxious body parts of chemically-defended prey (reviewed in Glendinning, 2007). The European paper wasp *Polistes dominulus*, for instance, will gut *Pieris napi* caterpillars reared on toxic host plants, but not those that were reared on non-toxic plants (Rayor et al., 2007). Conversely, Tanagers (*Pipraeida melanonota*) avoid the toxic integument of ithomiine moths by chewing them until the abdominal content is expelled; they then eat the abdominal contents and discard the rest (Brown & Neto, 1976). Predators that cannot separate the toxic and non-toxic

fractions of unpalatable prey often learn to avoid them entirely. In experiments with cardenolide-containing milkweed bugs, naïve third-instar *T. sinensis* fed a single *O. fasciatus* nymph took much less time to reject a second one (Paradise & Stamp, 1991); similarly, sixth-instar mantids quickly (3-4 exposures) learned to ignore mature *O. fasciatus* (Berenbaum & Miliczky, 1984).

Despite the behavioral data, mantid-consumed and -discarded *D. plexippus* tissue had equal cardenolide concentrations and a similar polarity index. There were more distinct cardenolide peaks in the consumed tissue, likely due the breakdown of plant-derived cardenolides (e.g., at 10.80 nm in Fig. 3) into other forms (e.g., at 12.01, 12.16, and 12.70 nm in Fig. 3). Overall, these results are consistent with previous work showing that cardenolide sequestration occurs in the hemolymph and epidermis of *D. plexippus* (Duffey, 1980) in concentrations equal to or exceeding those of host plant foliage (Agrawal et al., 2012; Malcolm et al., 1989). Our findings appear, however, to reject the hypothesis that mantid gutting of *D. plexippus* caterpillars allows them to avoid cardenolide-rich gut material while consuming the less-defended integument. Below, we discuss potential resolutions to the apparent mismatch between the behavioral data (Fig. 2) and cardenolide analyses.

One explanation for our findings is that the gutting behavior of *T. sinensis* avoids plant-produced cardenolides present in the gut that *D. plexippus* larvae metabolizes into different compounds before sequestering them in their hemolymph and integument. This explanation is consistent with the fact that the three monarch caterpillars that mantids ate whole (individuals containing either tachinid larvae or a fungal pathogen) consumed virtually no *A. syriaca* in captivity. When mantids

punctured the integument and began feeding on these three larvae, no green plant material was present; in contrast, the other 18 monarch guts were green with plant material. Evidence suggests that monarch predators differ in their preference for (or avoidance of) particular body parts. The mouse *Peromyscus melanotis* avidly consumes even high-cardenolide monarch adults, for instance, by opening the abdomen and eating the internal contents while avoiding the integument (Glendinning, 1990); yellowjacket wasps (*Vespula vulgaris*) use similar techniques to prey on adult monarchs (Leong et al., 1990). Although *P. dominulus* wasps prefer more palatable prey over *D. plexippus* larvae (Rayor, 2004), there are reports that they do not gut or otherwise 'selectively process' late-instar monarch caterpillars before eating or feeding them to their offspring (L. Rayor, *unpublished data*, cited in Rayor et al., 2007). Our results may thus be explained by mantids' greater tolerance for monarch-metabolized cardenolides in the integument than for plant-derived chemicals in the gut.

The gutting behavior of *T. sinensis* might also be explained by this obligate carnivore's distaste for partially-digested plant tissue. The digestive system and enzymatic pathways of carnivores are optimized for a heterotrophic diet, and autotrophic biomass differs substantially in a wide range of parameters (reviewed in Price et al., 2011). This explanation is inconsistent with the fact that the mantids consumed similarly-sized *O. nubilalis* larvae in their entirety. Even so, the low nutrient content and likely equal (or greater) toxicity of the gut tissue may help explain the mantid's behavior if the predators can tolerate consumption of the integument but not the material in the gut. Avian insectivores are able to regulate their exposure to

toxins by consuming fewer individuals as prey toxicity increases (Skelhorn & Rowe, 2007); mantids may be similarly able to regulate their toxin loads. Despite being a fairly recent arrival to the east coast of the U.S., *T. sinensis* has rapidly become the dominant invertebrate predator in many old-field ecosystems (reviewed in Snyder & Evans, 2006). The gutting behavior we describe may enable this mantid to utilize otherwise inaccessible prey and thrive in their invaded range. The similar polarity index and total cardenolide content of mantid-consumed versus -discarded tissue also adds an intriguing twist to the monarch-cardenolide-predator interaction first elucidated nearly 50 years ago (Brower et al., 1967). Although total cardenolide content alone may not entirely explain the mantids' behavior, we speculate that the context of the gut's character, largely low nitrogen-containing milkweed tissue, may be critical to the gutting of monarchs.

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AUTHOR CONTRIBUTIONS

JLR and ELP conceived, designed, and performed the experiment. JLR, ELP, and AAA conducted chemical analyses and wrote the manuscript.

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FIGURE LEGENDS

Figure 1: Adult Chinese mantid (*T. sinensis*) gutting a final-instar monarch (*D. plexippus*) caterpillar. For scale, mantid forelegs are ~3 cm in length. Photo credit: Alex Allaux.

Figure 2: A. Percent individuals of each prey type gutted by *T. sinensis*. B. Percent mass of each prey type discarded ± SE. C. Consumption rates for each prey type ± SE.

Figure 3: Concentration of individual cardenolides in *D. plexippus* gut and body (i.e., non-gut) tissue $\pm$ SE. For initial values, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. For adjusted values, § = significant at $\alpha = 0.05$ after step-up FDR Bonferroni-type correction.

Fig. 1

Fig. 2

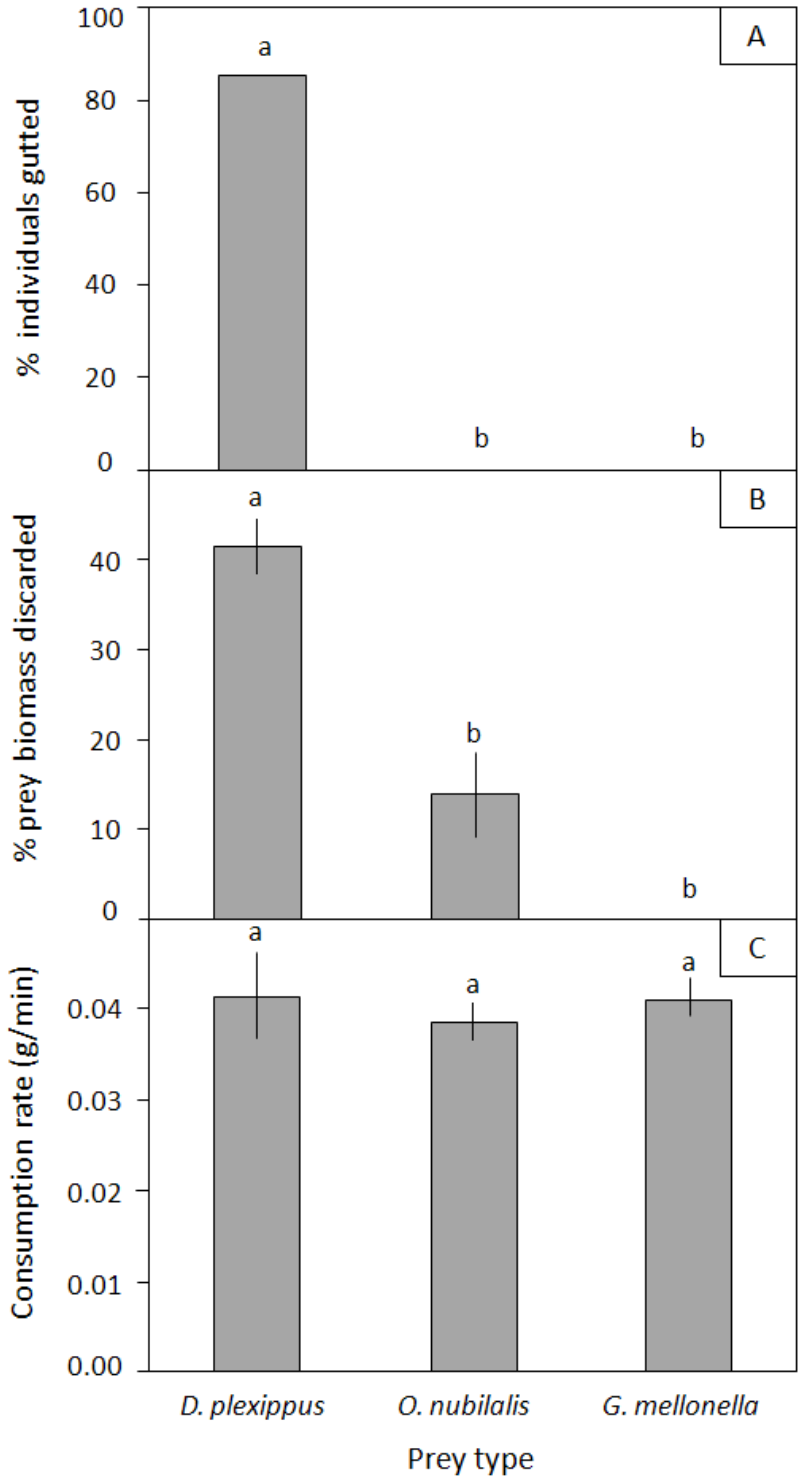
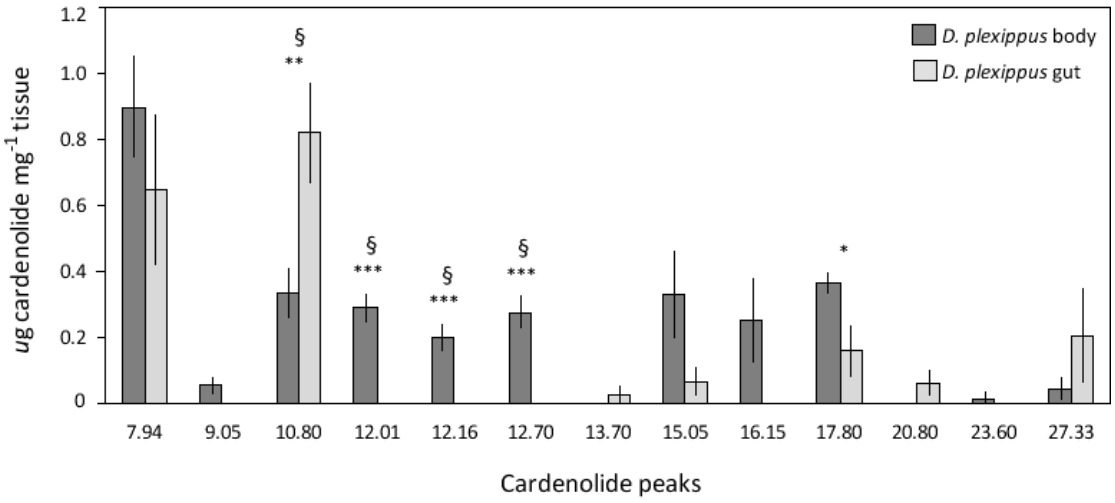

A
B
C
% individuals gutted
% prey biomass discarded
Consumption rate (g/min)
100
80
60
40
20
0
40
30
20
10
0
0.04
0.03
0.02
0.01
0.00
a
b
b
a
b
b
a
a
a
D. plexippus
O. nubilalis
G. mellonella
Prey type

Fig. 3


D. plexippus body
D. plexippus gut
ug cardenolide mg-1 tissue
1.2
1.0
0.8
0.6
0.4
0.2
0
§
**
§
***
§
***
§
***
*
7.94
9.05
10.80
12.01
12.16
12.70
13.70
15.05
16.15
17.80
20.80
23.60
27.33
Cardenolide peaks

CHAPTER TWO

In review in The Journal of Environmental Entomology

Pretty picky for a generalist: impacts of toxicity and nutritional quality on mantid prey processing

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Abstract

Prey have evolved a number of defenses against predation, and predators have developed means of countering these protective measures. Although caterpillars of the monarch butterfly *Danaus plexippus* L. are defended by cardenolides sequestered from their host plants, the Chinese mantid *Tenodera sinensis* Saussure guts the caterpillar before consuming the rest of the body. We hypothesized that this gutting behavior might be driven by the heterogeneous quality of prey tissue with respect to toxicity and/or nutrients. We conducted behavioral trials in which mantids were offered cardenolide-containing and cardenolide-free *D. plexippus* caterpillars and butterflies. In addition, we fed mantids starved and unstarved *D. plexippus* caterpillars from each cardenolide treatment and non-toxic *Ostrinia nubilalis* Hubner caterpillars. These trials were coupled with elemental analysis of the gut and body tissues of both *D. plexippus* caterpillars and corn borers. Cardenolides did not affect mantid behavior: mantids gutted both cardenolide-containing and cardenolide-free caterpillars. In contrast, mantids consumed both *O. nubilalis* and starved *D. plexippus* caterpillars entirely. *Danaus plexippus* body tissue has a lower C:N ratio than their gut contents, while *O. nubilalis* have similar ratios; gutting may reflect the mantid's ability to regulate nutrient uptake. Our results suggest that post-capture prey processing by mantids is likely driven by a sophisticated assessment of resource quality.

KEY WORDS *Danaus plexippus, Ostrinia nubilalis, Tenodera sinensis,* cardenolide, prey processing

Introduction

Prey utilize an array of defenses against predation. Organisms can, for instance, avoid detection via crypsis or disruptive coloration that makes it difficult for predators to identify the boundaries of the prey's body. Prey can also employ behavioral measures to decrease their likelihood of attracting a predator: veeries, *Catharus fuscescens* Stephens, respond to predation risk by decreasing the rate and length of their songs (Schmidt and Belinsky 2013). Once detected, prey can employ secondary defenses such as aggressive or escape behaviors as well as morphological and/or chemical defenses (Ruxton et al. 2004). The presence of trout, for example, can cause macroinvertebrates to alter their drift rates and foraging activity (Simon and Townsend 2003, Eby et al. 2006), as well as their microhabitat use (Lima 1998). Morphological changes are also possible: *Daphnia pulex* Leydig respond to predator cues by producing fewer, but larger, offspring with prominent neck spines (Luening 1994) that make the prey more difficult for predators to attack.

Organisms that lack behavioral and/or morphological defenses may instead deter predation via the production or sequestration of noxious chemical compounds. Prey that adopt this strategy typically possess aposematic coloration that advertises their toxicity (Duffey 1980, Nishida 2002, Ruxton et al. 2004). The nudibranch *Cratena peregrina* Gmelin, for example, uses bright coloration to display its unpalatability to fish predators (Aguado and Marin 2007). In insects, chemical defense and aposematism occurs in multiple orders, including the Hemiptera, Lepidoptera, Coleoptera, and Hymenoptera. Hemipteran milkweed bugs, *Oncopeltus fasciatus* Dallas, feed on cardenolide-rich host plants and sequester these toxins in their bodies;

their contrasting orange-and-black coloration alerts predators to their toxicity (Scudder et al. 1986). Another insect that feeds on milkweed, the Oleander aphid *Aphis nerii* Boyer de Fonscolombe, also sequesters cardenolides and are brightly yellow-and-black colored (Malcolm 1990).

Although chemically-based antipredator defenses are often highly effective, predators have developed a variety of techniques for overcoming them. Floodplain death adders, *Acanthophis praelongus* Ramsay, prey on toxic frogs by biting the prey, injecting it with toxins, and then releasing it. The adder's toxins kill the frog, whose own defensive toxins degrade after it has died; the snake can then eat the formerly-toxic frog without any ill effects (Phillips and Shine 2007). Loggerhead shrikes, *Lanius ludovicianus* Mearnsi, employ a similar strategy for feeding on chemically defended lubber grasshoppers, *Romalea guttata* Beauvois. Grasshoppers captured by the birds are impaled on thorns or barbed wire; the shrikes only return to feed on them once the grasshoppers' defensive toxins have been degraded and their aposematic coloration fades (Yosef 1992). Other predators process prey to feed selectively on the most palatable portion of the prey (Glendinning 2007) or regulate their toxicity burden (Skelhorn and Rowe 2007).

The monarch butterfly, *Danaus plexippus* L., is chemically defended and aposematically colored in both the black-and-yellow larval and black-and-orange adult stage. Their caterpillars sequester toxins when feeding on cardenolide-containing host plants in the genus *Asclepias* (Apocynaceae) (Agrawal et al. 2012). Despite this generally effective chemical defense, *D. plexippus* is susceptible to predation across all life stages. Its invertebrate predators include ants, *Formica montana* Wheeler,

ladybird beetles, *Harmonia axyridis* Pallas (Koch et al. 2003, Prysby 2004), and predatory *Polistes* (Rayor 2004), and *Vespula* wasps (Leong et al. 1990). Birds such as Orioles, *Icterus* spp., Grosbeaks, *Pheucticus* spp., (Nishida 2002) and other vertebrate predators such as *Peromyscus* mice also feed on *D. plexippus* (Glendinning 1990). *Danaus plexippus* caterpillars are also preyed upon by an invasive generalist predator, the Chinese mantid, *Tenodera sinensis* Saussure. We have previously found (Rafter et al. 2013) that mantids consuming toxic *D. plexippus* caterpillars actively reject the gut material, allowing it to fall from the body. However, they consume non-toxic lepidopterans such as European corn borers, *Ostrinia nubilalis* Hubner, and wax worms, *Galleria mellonella* L., in their entirety. These results suggest that the mantids' gutting behavior may be a behavioral mechanism for avoiding prey toxicity. A follow-up analysis of cardenolide levels, however, found that the mantid-discarded guts and mantid-consumed bodies of *D. plexippus* caterpillars contain similar cardenolide concentrations (although the two portions were composed of different individual cardenolides). We also found that gut material has a higher C:N ratio than body material, making it less nutritious. As a result, the mantids' gutting behavior may reflect either their avoidance of individual cardenolides or their need to feed selectively on the most nitrogen-rich portions of their prey (Rafter et al. 2013). We tested these hypotheses by conducting a series of behavioral trials in which we observed mantid prey handling behavior when presented with *D. plexippus* caterpillars reared on toxic cardenolide-containing and control no-cardenolide host plants. We paired the results of this experiment with other work in which we fed mantids starved and unstarved larval *D. plexippus* reared on the two host plants, adult *D. plexippus*

reared on the two host plants, and non-toxic European corn borers. Our results suggest that post-capture prey processing by mantids is likely driven by a sophisticated assessment of resource quality.

Methods

Mantid rearing and maintenance. We collected a single *Tenodera sinensis* egg mass in early April 2012 from an abandoned agricultural field at East Farm (Kingston, RI). It was returned to the lab and maintained at 25^{o}C in a 50 x 25 x 30 cm Plexiglass aquarium until the eggs began to hatch. One day after hatching, 105 nymphs were each placed in individual 1.9L mason jars; the top of each jar was replaced with mosquito netting for ventilation. A single stick was provided for perching; when mantids reached the fourth instar, the stick was replaced with a mesh strip secured under the lid. Water was provided using a water wick made from capped soufflé cups and braided dental cotton inserted through a hole in the lid. The jars were held in a Percival growth chamber with a 16:8 L:D photoperiod and 60-80% humidity at 25˚C during lighted hours and 23˚C during dark hours. The remaining mantids from the egg mass were communally raised in two 50 x 25 x 30 cm aquaria. Each aquarium had several sticks arranged for perching sites. Mantids in both the jars and the aquaria were fed lab-reared apterous fruit flies, *Drosophila melanogaster* Meigen, for the first four instars; following this, they were fed appropriately-sized crickets (*Acheta domesticus* L.). Because crickets will prey on mantids during the molting process, we tested for satiation by using forceps to offer each mantid a cricket before adding crickets to its jar. If the mantid refused to attack the cricket we assumed it was preparing to molt and did not feed it that day; jars with 'molting' mantids were marked

so that we could track whether non-feeding individuals did in fact molt. Mantids that accepted the cricket were fed two additional crickets; we deterred crickets from attacking the mantids by adding fruit flies to the jars for the crickets to eat. Because early-instar mantids have high mortality rates, we replaced any dead Percival-reared mantids with a communally-raised sibling of similar size and developmental stage; we stopped this replacement once a majority of Percival-reared mantids reached the sixth instar. Once mantids reached adulthood, they were fed three crickets daily and no fruit flies. Jars containing adult mantids were removed from the Percival and kept in the lab at ambient room temperature with a 16:8 L:D photoperiod.

Experiment 1: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant)* D. plexippus *caterpillars differently? This experiment tested whether mantids varied in their behavior towards *D. plexippus* caterpillars raised on toxic (i.e., cardenolide-containing) and non-toxic (no-cardenolide) host plants. It tests the hypothesis that the mantids' gutting behavior is a response to the presence of cardenolides in *D. plexippus* gut tissue. Two hundred *D. plexippus* eggs were purchased from Flutterby Gardens (Bradenton, FL, USA) and reared in 50 x 25 x 30 cm aquaria. Half of the emerging larvae were reared on a cardenolide-containing host plant, the common milkweed *Asclepias syriaca* L.; the other half of the emerging larvae were reared on a zero-cardenolide host plant, the swamp milkweed *A. incarnata* L. *Asclepias syriaca* plants were grown from seed while *A. incarnata* plugs were purchased from Northcreek Nursery (Landenberg, PA, USA).

Twenty lepidopteran-naïve adult mantids were randomly assigned to consume late-instar *D. plexippus* larvae raised on either *A. syriaca* (ten mantids) or *A. incarnata* (ten mantids) host plants. All mantids were starved for three days prior to the trial. At the start of each trial, each mantid was weighed, placed into a pre-weighed 23.3 x 15.5 x 16.5 cm plastic container, and allowed to acclimate for five minutes. After the five-minute acclimation period, a pre-weighed caterpillar was placed into the enclosure. We video-recorded each trial from the moment the prey item was placed in the enclosure until the end of the trial. The mantid was given ten minutes to orient on the prey. If the mantid did not orient within this period, the trial ended. Mantids that oriented were given an additional ten minutes to attack the prey. If the mantid did not attack during this period, the trial ended. If the mantid attacked, we recorded five minutes of video following the attack. At the same time, we recorded whether or not the mantid gutted the prey. Every mantid was tested every day for six days during the experiment. Once an individual mantid had attacked prey in two separate trials, we disturbed the remaining trials in which the mantid attacked so that we could collect mantid-dissected gut and body material for CNH analysis. Gut material was collected in a 2 ml pre-weighed screw-cap tube as it fell from the caterpillar. We then pried the remaining cadaver from the mantid and placed it into a second tube. This material was frozen at -13˚C until analyzed.

Experiment 2: Does the presence of plant material in the caterpillar gut affect how mantids handle 'toxic' (cardenolide-containing host plant) and 'non-toxic' (no-cardenolide host plant)* D. plexippus *caterpillars? This experiment tested whether mantid behavior varied as a function of the presence or absence of plant

material in the gut of *D. plexippus* caterpillars reared on cardenolide-containing and no-cardenolide host plants. It tests the hypothesis that mantid gutting behavior is driven by the presence of plant material *per se* rather than by cardenolide concentrations. This experiment was conducted identically to Experiment One (and used the same mantids), but added an additional experimental factor: the presence ('unstarved') or absence ('starved') of plant material in the caterpillar gut. The ten mantids that had previously been fed cardenolide-containing *D. plexippus* caterpillars were split into two groups of five mantids. Mantids in one of the five-mantid groups were fed starved *D. plexippus* caterpillars whose guts were free of plant material ('starved' treatment); mantids in the other five-mantid group were fed *D. plexippus* caterpillars whose guts were filled with plant material ('unstarved' treatment). This design was replicated for the ten mantids that had previously been fed no-cardenolide *D. plexippus* caterpillars, for a total of four five-mantid treatments: starved toxic caterpillars, unstarved toxic caterpillars, starved non-toxic caterpillars, and unstarved non-toxic caterpillars. As in Experiment One, toxic *D. plexippus* caterpillars were raised on *A. syriaca* and non-toxic *D. plexippus* caterpillars were raised on *A. incarnata*. Starved caterpillars were kept without food for 24 hours in order to clear their guts of any plant material; any mantid-attacked 'starved' caterpillars whose guts still contained trace amounts of plant material (apparent as undigested green material within the gut) were excluded from our analysis. Mantid-*D. plexippus* interaction trials were conducted for six days following the same procedure as in the first experiment. We collected caterpillar biomass for chemical analysis once individual mantids attacked twice.

Experiment 3: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) adult* D. plexippus *differently? This experiment tested whether mantids differed in their handling behavior of adult *D. plexippus* butterflies reared on cardenolide-containing versus no-cardenolide host plants. Adult *D. plexippus* are nectar feeders that no longer consume cardenolides; the experiment tested the hypothesis that this ontogenic shift affected how mantids responded to *D. plexippus* reared on different hosts. Twelve *D. plexippus* caterpillars were reared to adulthood, six on *A. syriaca* and six on *A. incarnata*. Twelve mantids used in experiments one and two (six that were fed *A. syriaca* caterpillars, and six that were fed *A. incarnata* caterpillars) were each fed a single *A. syriaca*-reared adult butterfly or a single *A. incarnata*-reared adult butterfly, respectively. For each trial, we noted if the butterfly was gutted and which body parts were discarded by the mantid; all twelve trials took place on the same day.

Experiment 4: Do mantids handle larval* O. nubilalis *differently than* D. plexippus*? This experiment repeated previously-published work (Rafter et al. 2013) finding that non-toxic *O. nubilalis* larvae were consumed in their entirety by mantids that would gut *A. syriaca*-reared *D. plexippus* caterpillars. The current experiment was designed to confirm the results of the 2011 experiment and provide more precise information on how mantids handle prey that do not sequester toxic compounds from their host plant and that may be of higher nutritional value (i.e., lower C:N ratio). Because of the difficulty in finding sufficient late-instar caterpillars, the experiment was conducted in two stages (=trials). In trial one of this experiment, we presented each of 16 lepidopteran-naïve mantids with one late-instar *O. nubilalis* caterpillar

collected from organically-grown flint corn, *Zea mays* L., growing in an experimental farm. The second trial was essentially identical to the first, but took place two weeks later: when we presented each of 12 naïve mantids with one late-instar *O. nubilalis*. Caterpillars were always collected on the day of the trial; both trials lasted one day. Data collection procedures were as above. If mantids did not gut the caterpillars, we froze whole caterpillars and later dissected the caterpillars to isolate the gut and body portions for chemical analysis.

Chemical analysis: All of the preserved caterpillar biomass was stored in plastic tubes and dried in a 45^{o}C drying oven for five days. After drying was complete, 1.0-2.0 mg of dried material was removed from each sample and sent for CNH analysis to the Analytic Chemistry lab at the University of Rhode Island's Graduate School of Oceanography (Narragansett RI).

Statistical analysis: Since post-attack prey handling behavior by mantids did not vary (see results), statistical analysis was unnecessary. Results from the CHN analysis were used to determine the percent carbon and nitrogen in both gut and body tissues and calculate their carbon/nitrogen (C:N) ratios. We analyzed the *D. plexippus* data using a two-way ANOVA that crossed the main factors toxicity (cardenolide-containing or cardenolide-free caterpillars) and body tissue (gut versus body). We analyzed the *O. nubilalis* data using a one-way ANOVA with the main factor body tissue (gut versus body). Where appropriate, we determined among-treatment differences using Tukey-Kramer HSD. All analyses were performed using JMP 9 (SAS Institute, Inc).

Results

Experiment 1: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) **D. plexippus** ***caterpillars differently?*** We analyzed data for 117 predator-prey interactions; predators attacked the prey in 64/114 cases (three caterpillars infected with a fungal pathogen were excluded from the analysis). Regardless of treatment, mantids gutted all the *D. plexippus* caterpillars they attacked (31/31 non-toxic and 33/33 toxic caterpillars, respectively).

Experiment 2: Does the presence of plant material in the caterpillar gut affect how mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) **D. plexippus** ***caterpillars?*** We analyzed data for 113 predator-prey interactions; mantids actually attacked the prey in 20 of the 113 interactions. Mantids gutted all (12/12) of the unstarved prey but none (0/8) of the starved prey.

Experiment 3: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) adult **D. plexippus** ***butterflies differently?*** We analyzed data for 12 predator-prey interactions (six for each toxicity treatment). Mantids did not gut any of the adult butterflies regardless of the larval host plant. In each case, mantids consumed the body while discarding the wings, antennae, and legs. Some mantids appeared to 'taste' the wings, but stopped and returned to feeding on the body.

Experiment 4: Do mantids handle **O. nubilalis** ***differently than*** **D. plexippus*****?*** We observed a total of 28 predator-prey interactions; mantids attacked the prey in 13 of the 28 interactions. In the first trial, six of seven caterpillars were not

gutted, and in the remaining case the mantid stopped feeding entirely. In the second trial, 6/6 caterpillars were not gutted.

Carbon and nitrogen concentrations: Percent carbon was significantly higher in the mantid-consumed body tissue than in the mantid-discarded gut tissue of *D. plexippus* caterpillars ($F_{1, 53} = 31.3$, $p < 0.001$; Fig. 1A). This did not differ between toxic and non-toxic *D. plexippus* ($F_{1, 53}=1.03$, $p=0.31$), and there was no interaction between these factors ($F_{1, 53}=0.10$, $p=0.75$). Percent nitrogen was also higher in body versus gut tissue, and in non-toxic *D. plexippus* ($F_{1, 53}=94.0$, $p<0.001$ and $F_{1,53}=7.47$, $p<0.001$, respectively; Fig 1B); however, the interaction was not significant ($F_{1,53}=1.64$, $p=0.21$). The resulting C:N ratio for *D. plexippus* was higher in the gut versus body tissue, higher in toxic versus non-toxic caterpillars ($F_{1,53}=57.3$, $p<0.001$ and $F_{1,53}=10.6$, $p=0.002$, respectively; Fig. 1C), and there was a significant interaction ($F_{1,53}=9.27$, $p=0.004$). In contrast, there was no difference in the percent carbon, nitrogen, and C:N ratio in *O. nubilalis* guts and bodies ($F_{1,9}=4.52$, $p=0.066$; $F_{1,9}=0.83$, $p=0.39$; and $F_{1,9}=0.24$, $p=0.64$, respectively, Fig. 1). For *D. plexippus*, the C:N ratio of mantid-consumed body tissue was lower than the C:N ratio of mantid-discarded gut tissue; however, mantids eagerly consumed *O. nubilalis* tissue with C:N ratios equal to or greater than those of the *D. plexippus* gut. In other words, mantids consumed tissues with both a higher and lower C:N ratio than the *D. plexippus* guts they rejected.

Discussion

We found no evidence that *D. plexippus*-sequestered cardenolides affected mantid prey handling behavior. Specifically, *T. sinensis* behaved similarly towards *D. plexippus* larvae (experiments 1-2) and adults (experiment 3) reared on cardenolide-

containing *A. syriaca* versus no-cardenolide *A. incarnata*. Since these mantids were lab-reared, their inability/unwillingness to discriminate between cardenolide-containing versus no-cardenolide *D. plexippus* gut tissue must be innate. The lack of a behavioral response to *D. plexippus* adults seems appropriate given that mantids experienced no apparent ill-effects from consuming the cardenolide-laden bodies (Rafter et al. 2013) of *D. plexippus* caterpillars fed *A. syriaca*.

The addition of a starved/unstarved caterpillar treatment to experiment two revealed that the mantids' gutting behavior reflects the active rejection of partially-digested plant material found within the gut. This suggests that rather than avoiding cardenolides, mantids may instead be avoiding the lower-quality (higher C:N ratio) plant material found in the gut tissue (Fig. 1C). This interpretation is further supported by the third experiment that found mantids did not gut adult *D. plexippus*, nectar feeders whose guts are free of plant material. While our three *D. plexippus* experiments support the 'food quality' hypothesis for the mantids' gutting behavior, the results of our fourth experiment (*O. nubilalis* trials) do not. In this experiment, which was intended to confirm results first reported in Rafter et al (2013), we again found that mantids readily consume *O. nubilalis* gut and body tissue. The results of our first three experiments led us to hypothesize that the gut material of *O. nubilalis* caterpillars would be of higher nutritional quality (as indicated by the C:N ratio) than the mantid-discarded portions of *D. plexippus* caterpillars. While we found that both *O. nubilalis* gut and body tissue was relatively high in C and N (Figs. 1A and 1B, respectively), the C:N ratio of mantid-accepted *O. nubilalis* gut tissue equaled or exceeded those of mantid-rejected *D. plexippus* gut tissue (Fig. 1C). Given the

inconsistency in mantid preference for tissues in relation to their respective C:N ratios, this metric does not appear to explain the gutting behavior.

Although *T. sinensis* appears to be insensitive to the presence of cardenolide in *D. plexippus* caterpillars, it does exhibit an adverse reaction when consuming cardenolide-sequestering milkweed bugs, *Oncopeltus fasciatus*. They quickly learn to reject and will eventually completely avoid this prey after few encounters (Berenbaum and Miliczky 1984, Paradise and Stamp 1991). This suggests that the Chinese mantid is tolerant of, rather than unaffected by, cardenolide consumption. Milkweed bugs uptake cardenolides more efficiently and at substantially higher concentrations than do *D. plexippus* (Scudder et al. 1986, Agrawal et al. 2012); mantids may be intolerant to the higher cardenolide concentrations found in milkweed bugs.

An alternate hypothesis for the mantid's behavior is that they may be responding to the presence of other secondary plant compounds. Adult *D. plexippus* have been shown to feed on plants containing pyrrolizidine alkaloids and sequester these compounds; these compounds may play a role in defending adult *D. plexippus* against both vertebrate and invertebrate predators (Kelley et al. 1987, Stelljes and Seiber 1990). These compounds are sequestered during the adult stage, however, and *D. plexippus* butterflies were fed sugar water in this experiment. To our knowledge, there are no reports of *D. plexippus* caterpillars sequestering toxins other than cardenolides. However, plants often employ a suite of defenses against herbivory and maintain multiple defense strategies with little cost (Koricheva et al. 2004). Thus, there are a number of potential toxins that mantids could be responding to in the plant material found in the caterpillar's gut. Many cardenolide-containing plants in the

Apocynaceae, including genus *Asclepias*, also contain alkaloids (Agrawal et al. 2012). In addition, although *A. incarnata* is cardenolide-free, it is not undefended. Both the roots and aboveground biomass contain pregnane glycosides (Warashina and Noro 2000a, b) that are inducible defenses against herbivory (A. Agrawal, personal communication). If mantids are unable to tolerate compounds found in undigested plant material, they might respond by gutting the caterpillar.

Our results may also be influenced by the fact that *D. plexippus* caterpillars and European corn borers feed on different parts of their respective host plants; *D. plexippus* feed on leaves, while corn borers feed on seeds. Corn has been selectively bred for human consumption and is thus relatively undefended compared to milkweed leaves. This further supports the idea that mantids may be gutting *D. plexippus* because of their intolerance to plant compounds found in the leaves of *Asclepias* plants. A number of other species are able to process food items in response to toxicity. Tanagers, *Pipraeida melanonota* Vieillot, reduce the toxicity of ithomiine moths by chewing on them until the abdominal content is expelled; they then eat the abdominal contents while leaving the rest behind (Brown and Neto 1976). The European paper wasp *Polistes dominula* Christ will gut *Pieris napi* L. caterpillars that were reared on toxic host plants, but not those that were reared on non-toxic plants (Rayor et al. 2007). Herbivores such as the meadow vole will cut branches from conifers and leave them uneaten for several days until tannins and phenolic concentrations are reduced sufficiently for the vegetation to be palatable (Roy and Bergeron 1990). Mantids may be similarly reducing their toxin burden by processing prey.

The results of our work illustrate the unexpectedly complex mechanisms determining how Chinese mantids process lepidopteran prey. This predator is responding to a number of chemical cues as it consumes prey items that are heterogeneous in nutritional value and degree of toxicity. Because mantids did not respond to cardenolides in *D. plexippus*, it seems most likely that their gutting behavior is driven instead by other plant secondary compounds and/or the nutritional quality of prey tissue. Irrespective of mechanism, this mantid's ability to efficiently process toxic and non-toxic prey is likely important in allowing this non-native generalist predator to utilize a wide array of prey taxa.

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Author Contributions

JLR, JFV, LGK, and ELP conceived, designed, and performed the experiment. JLR and ELP wrote the manuscript.

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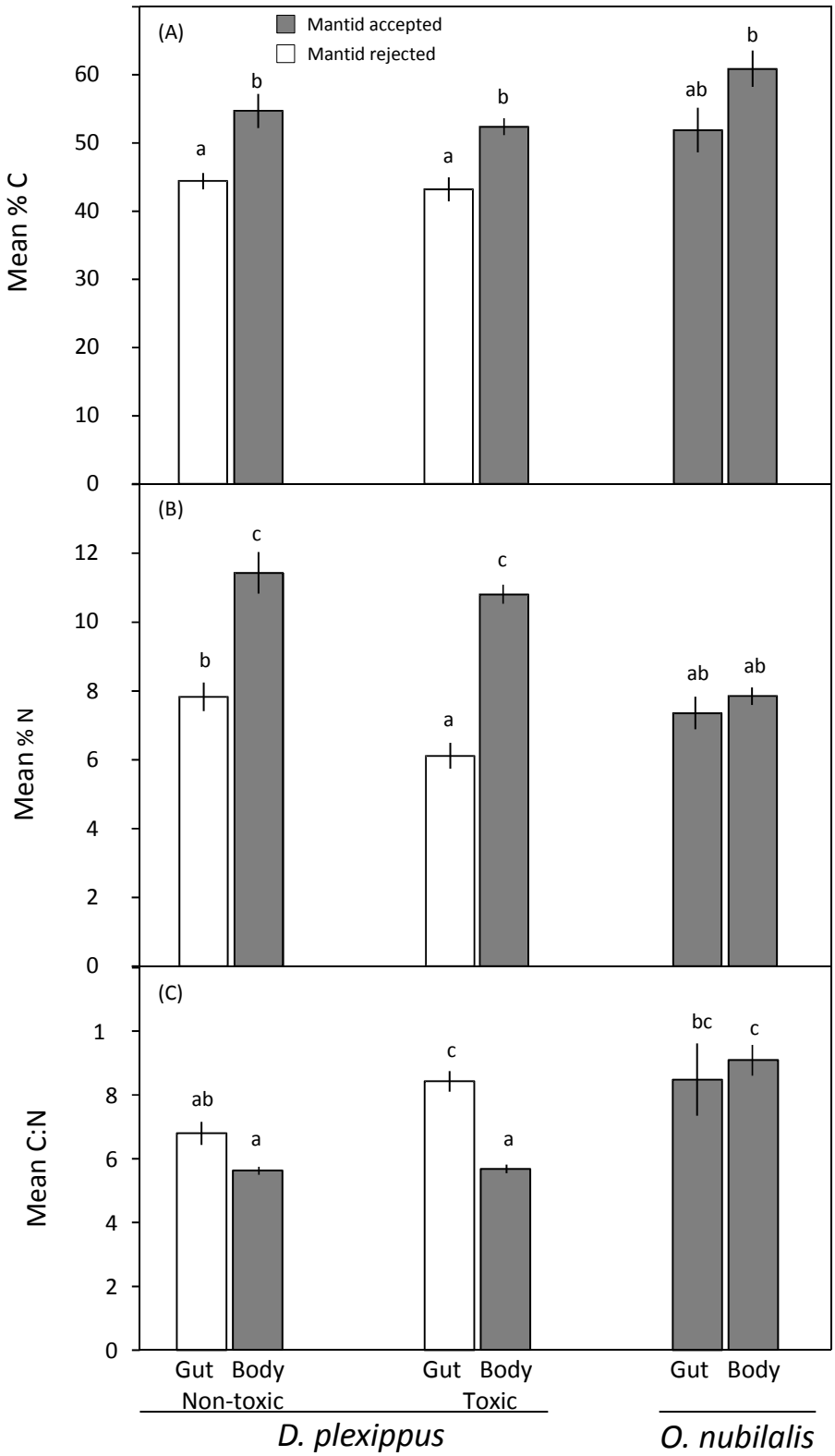
Figure Legend

Figure 1: (a) Mean percent of carbon (C) present in each prey and tissue type ± 1 SE. (b) Mean percent of nitrogen (N) present in each prey and tissue type ± 1 SE. (c) Mean C:N ratio of each prey and tissue type ± 1 SE.

Fig 1


(A)
Mantid accepted
Mantid rejected
Mean % C
(B)
Mean % N
(C)
Mean C:N
Gut
Body
Non-toxic
Toxic
D. plexippus
O. nubilalis

CHAPTER THREE

In review in The Journal of Chemical Ecology

Effects of consumption of toxic monarch caterpillars on Chinese mantid fecundity

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Abstract-Predators that feed on aposematic, chemically-defended prey often experience non-lethal effects after consumption that result in learned avoidance of the prey species. Some predators, however, are able to consume toxic prey without ill-effect. The Chinese mantid, *Tenodera sinensis*, is able to consume cardenolide-containing monarch caterpillars, *Danaus plexippus*, without immediate adverse effects. Although they discard the caterpillars' gut contents, mantids still ingest cardenolides sequestered in monarch body tissue. Although mantids do not exhibit immediate adverse reactions when consuming monarch biomass, it is possible that there are long-term fitness costs associated with cardenolide consumption. We tested the hypothesis that monarch caterpillar consumption negatively affects mantid fecundity and reproductive condition. We assigned lab-reared mantids to one of four toxicity groups that differed in the number of monarch caterpillars offered to adult mantids over a 15-day period. Monarch consumption did not reduce mantid fecundity; all treatment groups produced similar numbers of eggs. However, mantids in the high-toxicity group produced eggs that were 42% longer on average and devoted 75% more of their biomass toward egg production than those in the control group. This increase in reproductive condition is probably driven by other factors such as mantid size, prey nutritional value and/or diet mixing. Despite consuming similar amounts of prey biomass during the experiment, mantids in the high-toxicity group gained more biomass and were larger than mantids in the other groups. Our results suggest that the Chinese mantid is able to incorporate monarch prey into its diet without acute or chronic ill-effects.

Key Words- *Tenodera sinensis*, *Danaus plexippus*, fecundity, monarch, prey toxicity

INTRODUCTION

Chemically-defended species often advertise their protection via aposematism (Duffey 1980; Nishida 2002; Ruxton et al. 2004). These defenses generally involve compounds that are bitter tasting and cause vomiting or other ill effects shortly after consumption. These adverse but non-lethal effects allow predators to learn to avoid consumption of chemically-defended prey (Gittleman and Harvey 1980). These defenses are not always effective, however, and some predators feed on chemically-defended prey without any immediate ill-effects. The ladybird beetle *Harmonia axyridis*, for example, can metabolize toxic alkaloids produced by the coccinellid species on which it feeds (Sloggett and Davis 2010). The harvestman *Mitopus morio* feeds on the larvae of the leaf beetle, *Oreina cacaliae,* and is similarly able to prevent bioactivation and detoxify the toxic pyrrolizidine alkaloids sequestered by the prey (Hartmann et al. 2003). Even predators that lack physiological adaptations can avoid or limit their exposure to prey defenses by processing their prey (Brown and Neto 1976; Glendinning 2007; Rayor et al. 2007) or limiting their consumption (Skelhorn and Rowe 2007).

Even when predators are able to consume toxic prey with seemingly little effect, there may still be fitness costs associated with toxin consumption. When orb web spiders, *Zygiella x-notata*, feed on oleander aphids, *Aphis nerii*, they suffer disorientation and begin to construct webs that are less efficient at prey capture (Malcolm 1989). The two-spotted ladybird beetle, *Adalia bipunctata*, suffers lower fecundity and egg viability when consuming aphids reared on high-glucosinolate plants (Francis 2001).

The Chinese mantid, *Tenodera sinensis*, is a generalist predator that is able to feed on chemically-defended monarch caterpillars, *Danaus plexippus*, with no immediate ill effects. Monarch caterpillars feed on host plants in the genus *Asclepias* (Apocynaceae) that contain cardenolides; the larvae sequester these cardenolides in their bodies, rendering them unpalatable to many predators (Agrawal et al. 2012). We have previously found (Rafter et al. 2013; Rafter et al. unpublished data) that mantids discard the gut tissue from monarch larvae while consuming the rest of the caterpillar. This gutting behavior does not, however, prevent mantids from consuming cardenolides: while the gut and body tissue differ in cardenolide composition, they contain similar cardenolide concentrations (Rafter et al. 2013). Though mantids suffer no immediate ill-effects from consuming monarch larvae, their consumption of this cardenolide-containing tissue may nonetheless have long-term impacts. We tested whether consuming cardenolide-containing monarch caterpillars reduces mantid fecundity.

METHODS AND MATERIALS

Insect Rearing and Maintenance. We collected a *Tenodera sinensis* egg mass in mid-January 2013 from East Farm (Kingston, RI), an abandoned agricultural field. We placed it in a 50 x 25 x 30 cm Plexiglas aquarium that was kept in a Percival growth chamber with a 16:8 L:D photoperiod and 60-80% humidity at 25˚C during lighted hours and 23˚C during dark hours until the eggs began to hatch. After hatching, 105 nymphs were placed in individual 1.9L mason jars, with mosquito netting used in lieu of the tops for ventilation. A mesh strip was secured under the lid to serve as a perching site and water wicks were made using capped soufflé cups with

braided dental cotton inserted through a hole in the lid. These jars were kept in the Percival growth chamber. Mantids in their first four instars were fed lab-reared apterous fruit flies, *Drosophila melanogaster,* purchased from Carolina Biological (Burlington, NC, USA). After mantids reached the fourth instar, they were fed two appropriately-sized crickets daily. Just prior to and during molting, mantids are vulnerable to cricket predation; to prevent this, we tested for satiation by using forceps to offer each mantid a cricket before placing crickets into the jars. If the mantid refused to attack the cricket we assumed it was preparing to molt and did not feed it that day. To help deter crickets from attacking the mantids, we also put fruit flies into the jars for the crickets to eat.

Monarch eggs were purchased from Flutterby Gardens (Bradenton, FL, USA) and reared in the lab on *Asclepias curassavica*, a milkweed species that contains high cardenolide concentrations (Rasmann and Agrawal 2011). Host plants were grown from seed in the University of Rhode Island greenhouse.

Experimental Design. Once mantids reached adulthood, 31 females were randomly assigned to one of four treatments: non-toxic control, low toxicity, medium toxicity, and high toxicity (Table 1). After being assigned to their treatment, all mantids were held for three days without food. As outlined in Table 1, toxicity treatments were defined by the number of late-instar monarchs (0, 1, 5, or 15) offered to a given mantid over a 15-day period (days 4-18). On days during the 15-day treatment period when a mantid was not offered a monarch caterpillar, two crickets (comparable in weight to a single late-instar monarch caterpillar) were offered to the mantid as non-toxic prey. The offering of crickets on non-monarch days was necessary

to prevent mantid starvation in the control (zero caterpillars), low-toxicity (one caterpillar), and medium-toxicity (five caterpillars) treatments. If mantids refused to eat a monarch caterpillar, we continued to offer a caterpillar on subsequent days until the mantid accepted the prey; we did not offer mantids crickets unless they had already accepted the caterpillar. Following the 15-day treatment period, all mantids were fed two crickets daily until day 35. We recorded mantid weight before and after feeding as well as prey weight to determine prey biomass consumed. On day 35 mantids were weighed, anesthetized using a kill jar containing ethyl acetate, and dissected. We removed and weighed the egg mass, counted the eggs, and measured the length of five randomly-chosen eggs from each egg mass. We used the final mantid weight and the weight of the egg mass to determine the percent mantid biomass comprised of eggs. The 35-day length of our experiment ensured that all mantids produced a measurable number of eggs but was too short for them to have laid an egg mass. This allowed us to assess how exposure to monarch-sequestered cardenolides affects egg production and reproductive condition.

Statistical Analyses. Because the data on number of eggs produced, average egg length, and percent mantid biomass comprised of eggs was non-normally distributed, they were analyzed using nonparametric Kruskal-Wallis tests. Among-treatment differences were determined using the post-hoc Steel-Dwass method. Since insect fecundity can vary as a function of prey biomass consumed, we initially attempted to run an ANCOVA using total prey biomass consumed as a covariate. However, our data violated the assumption of homogeneity of regression slopes. Therefore, we used ANOVA to separately analyze data on total prey biomass

consumed (calculated by summing the daily amount of biomass consumed; this was determined using mantid weight before and after feeding) and percent mantid weight gain in each of the four treatments. We determined among treatment differences using post hoc Tukey Kramer HSD tests with $\alpha=0.05$. All data were analyzed using JMP 10 (SAS Institute, Inc.).

RESULTS

Mantids accepted both crickets and monarch caterpillars as prey. Some mantids in the low- and medium-toxicity treatments refused to consume monarch caterpillars on the day offered, but accepted them when offered again in subsequent days. Thus, mantids in the low-toxicity treatment each consumed one monarch caterpillar over the 15-day trial period and mantids in the medium-toxicity treatment consumed an average of 4.7± 0.18 caterpillars over the 15-day trial period. Mantids in the high-toxicity treatment each consumed 15 caterpillars.

Monarch consumption did not affect mantid egg production (Fig. 1a; $\chi^2=5.47$, $p=0.14$). Despite this, both average egg length and percent mantid biomass comprised of eggs differed among treatments (Fig. 1b; $\chi^2=8.56$, $p=0.036$ and Fig. 1c; $\chi^2=12.88$, $p=0.0049$, respectively). Mantids in the high-toxicity group produced 42% longer eggs than those in the control. Mantids in the high-toxicity group also devoted 75% more of their biomass toward egg production than those in the control group. Although mantids in each treatment group consumed similar amounts of prey biomass over the course of the experiment (Fig. 2a; $F_{3,27}=1.97$, $p=0.14$), mantids in the high-toxicity

group gained 18.5, 8.7, and 13.9 percent more biomass than mantids in the medium-toxicity, low-toxicity and control groups, respectively. (Fig. 2b; χ^2=14.10, p=0.0028).

DISCUSSION

We did not observe any acute ill-effects of consuming toxic monarch caterpillars on mantids. Per their typical behavior, mantids that fed on monarchs readily consumed the body tissues and rejected the gut material. This behavior and lack of immediate ill-effect is in agreement with our previous work (Rafter et al. 2013).

Contrary to our expectations, consumption of monarch caterpillars reared on high cardenolide *Asclepias curassavica* did not reduce mantid fecundity. Instead, mantid egg production was unaffected (Fig. 1a) while average egg length and percent mantid biomass comprised of eggs were both greater in the high toxicity group than in the control group (Fig. 1b and 1c, respectively). These data suggest that consumption of monarch prey does not reduce fecundity, but does improve reproductive condition. It is likely, however, that other factors influencing mantid condition are responsible for the observed increase; we discuss these factors below.

The apparent increase in reproductive condition might be explained by differences in the amount of prey biomass consumed by mantids in each treatment group. Prey biomass has been shown to affect insect growth and fecundity, and food-limited adult mantids have lower fecundity (Eisenberg et al. 1981). Because our data violated the assumption of homogeneity of regression slopes we could not run an ANCOVA using 'prey biomass consumed' as a covariate. Instead, we used an ANOVA to determine if there were any among-treatment differences in prey biomass

consumed. This analysis of the total amount of prey biomass consumed revealed no among-treatment differences (Fig. 2a). As a result, mantids in the high-toxicity group put on more biomass than those in all other treatment groups despite consuming similar amounts of prey biomass (Fig. 2b). At the end of the experiment, mantids in the high toxicity group were 28% larger than mantids in the control group ($F_{3,27}$=3.88, p=0.02). Mantids in the high toxicity group could thus exhibit an apparent increase in reproductive condition by virtue of being larger and therefore in better condition for reproduction. Chinese mantids lose an average of 47% of their body mass when they oviposit, with larger mantids producing larger ootheca (Eisenberg et al. 1981). It is possible that, although we could not detect a statistical difference in total prey biomass consumed, biologically significant differences explain the observed results.

While we did not test this, another possible explanation is that the nutrient content of monarchs, although toxic, is higher than that of crickets of comparable biomass. Monarch caterpillars were reared on a suitable host plant, while crickets were fed on a mixed diet of potatoes, apples, and artificial diet. In addition, mantids in toxicity treatments were consuming a mixed diet; crickets and monarchs. Having a mixed diet could improve overall health of the organism and thus explain the observed results. *Agonum dorsale*, a carabid beetle, exhibits the highest fecundity when reared on a mixed diet rather than a pure diet (Bilde and Toft 1994). It is possible then, that although mantids were consuming toxic prey, they were reaping a nutritional benefit through one or both of these mechanisms.

This research, combined with our previous work, suggests that the Chinese mantid is able to incorporate toxic monarch caterpillars into its diet with neither

chronic nor acute ill-effects. The Chinese mantid is a non-native generalist and our use of naïve mantids in this and previous work indicates that mantids are pre-adapted to handle this type of toxic prey. The ability to readily consume toxic prey may in part explain the occurrence of viable and established populations of mantids throughout their introduced range.

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FIGURE LEGENDS

Fig. 1 (a) Mean number of eggs produced ± 1 SE. (b) Mean egg length (mm) ± 1 SE. (c) Mean percent mantid biomass composed of eggs (g) ± 1 SE

Fig. 2 (a) Mean prey biomass consumed over 35 days ± 1 SE. (b) Mean percent change in mantid weight ± 1 SE

TABLE 1 DESCRIPTION OF MANTID TREATMENT GROUPS AND THE NUMBER OF INDIVIDUALS IN EACH GROUP

Treatment Group	**n**	**Treatment description**
Control	9	Offered two crickets daily from day 4 to day 35
Low Toxicity	8	Offered one monarch caterpillar on day 11. Offered two crickets per day all other days until day 35.
Medium Toxicity	7	Offered one monarch on days 6, 9, 12, 15, and 18. Offered two crickets per day all other days.
High Toxicity	7	Offered one monarch caterpillar each day on days 4-19. Subsequently, offered two crickets per day until day 35.

Fig 1

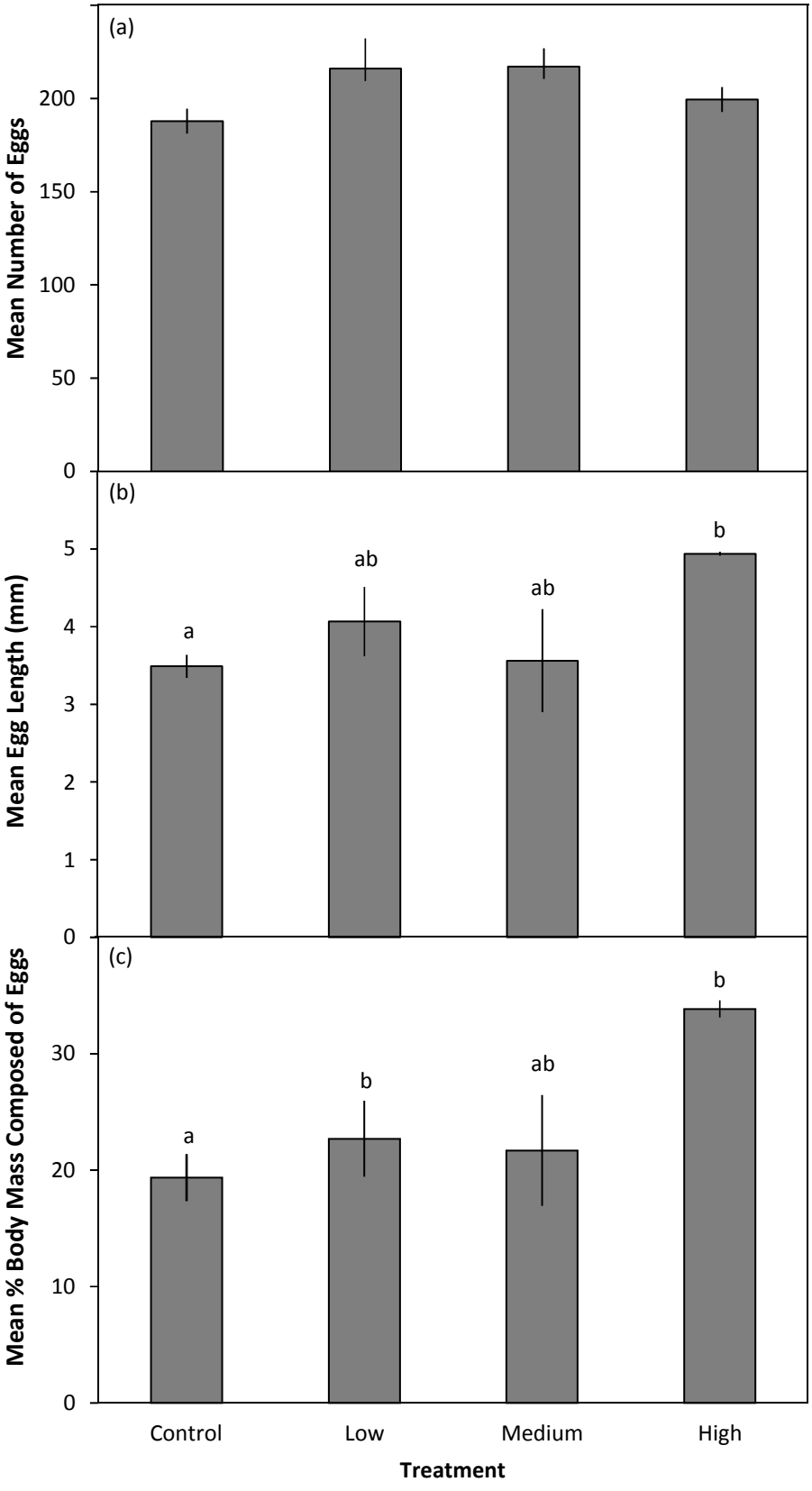
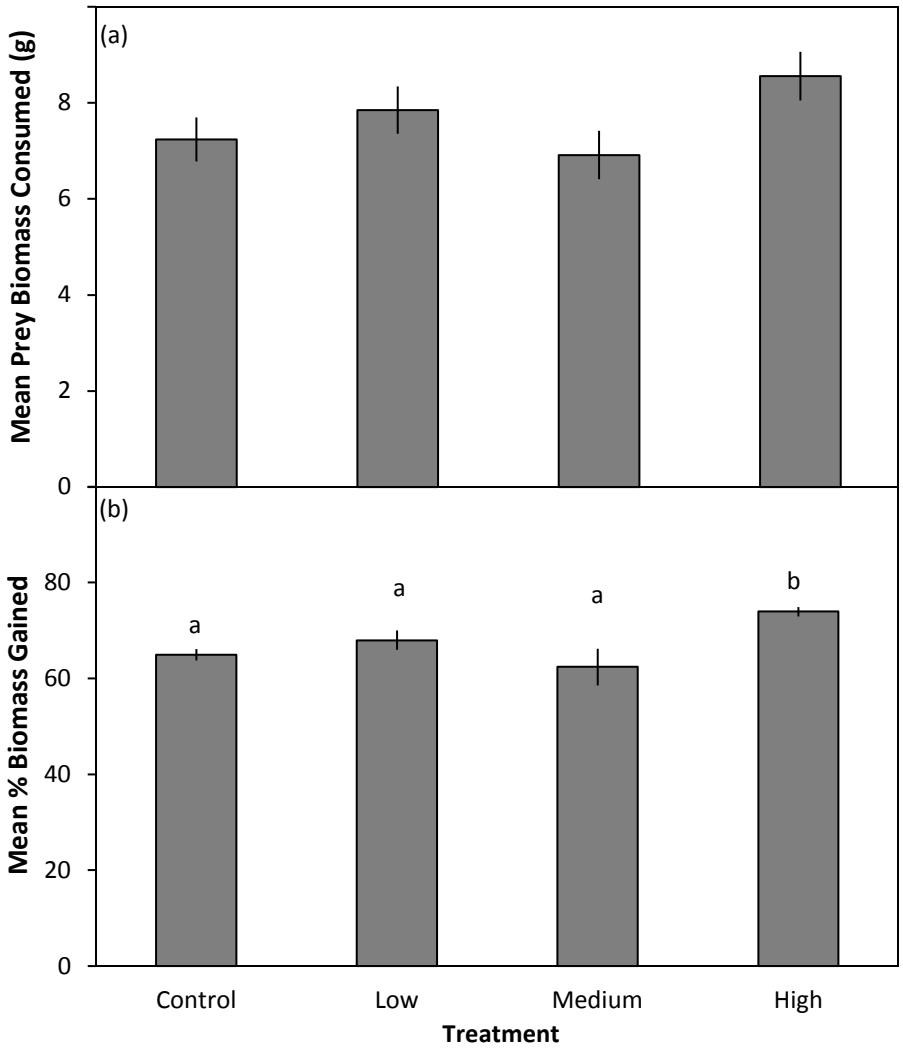

(a)
Mean Number of Eggs
0
50
100
150
200
(b)
Mean Egg Length (mm)
0
1
2
3
4
5
a
ab
ab
b
(c)
Mean % Body Mass Composed of Eggs
0
10
20
30
a
b
ab
b
Control
Low
Medium
High
Treatment

Fig 2


(a)
Mean Prey Biomass Consumed (g)
0
2
4
6
8
(b)
Mean % Biomass Gained
0
20
40
60
80
a
a
a
b
Control
Low
Medium
High
Treatment