Preliminary Analysis of Diet in Post-Metamorphic Northwestern Salamanders (*Ambystoma gracile*)

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Introduction

Understanding amphibian diet across all life stages is critical to understanding amphibians as ecological indicators (Unrine et al. 2005), elucidating their population declines and habitat use patterns (Mahan and Johnson 2007), and determining how habitat loss may affect them at different ecological scales (Rodríguez et al. 2005, Hirschfeld and Rödel 2011). Notably, many terrestrial amphibians appear to contribute to reciprocal subsidies between aquatic and terrestrial habitats because of the patterns of movements of post-metamorphic life stages (Trenham and Shaffer 2005, Whiles et al. 2006, Olson et al. 2007, Greene et al. 2008, O'Donnell and Richart 2012).

As part of a preliminary effort to better understand diets in Pacific Northwest terrestrial amphibians, we examined a series of post-metamorphic Northwestern Salamanders, *Ambystoma gracile* (Baird 1857). A large collection of this species from the mid-1980s Old-growth Study (Aubry and Hall, 1991) was available for examination at the University of Washington Burke Museum of Natural History and Culture. Though the species is common and widespread throughout the Pacific Northwest (Nussbaum et al. 1983, Hoffman et al. 2003), the handful of dietary studies on *A. gracile* (Henderson, 1973, Licht, 1975, Taylor 1984, Efford and Tsumura 1973) have examined exclusively larvae. In this study, our objectives were to: (1) identify the composition of *A. gracile* prey, and (2) determine whether body size or gender influence diet.

Methods

***Specimen selection*.—**We selected 50 *A. gracile* from the large pool (n = 340) of post-metamorphic specimens collected during the Old-growth Study along the west slope of the Cascade Mountains in Washington State (Aubry and Hall 1991). These animals were obtained by pitfall trapping between 10 October and 5 November 1984 and 7 October and 2 November 1985. Intervals between trap checks varied from three to seven days (K. Aubry, pers. comm.). Prior to selection, we measured body size (as snout-vent lengths [SVL]) and assigned each individual a random number. We then selected animals randomly, stratifying selection by 5-mm size class over the available size range (37.0-101.8 mm SVL). This resulted in four animals in each 5-mm size class except that we had only one animal >100 mm SVL, two individuals <40 mm and three in the 55-60 mm size class.

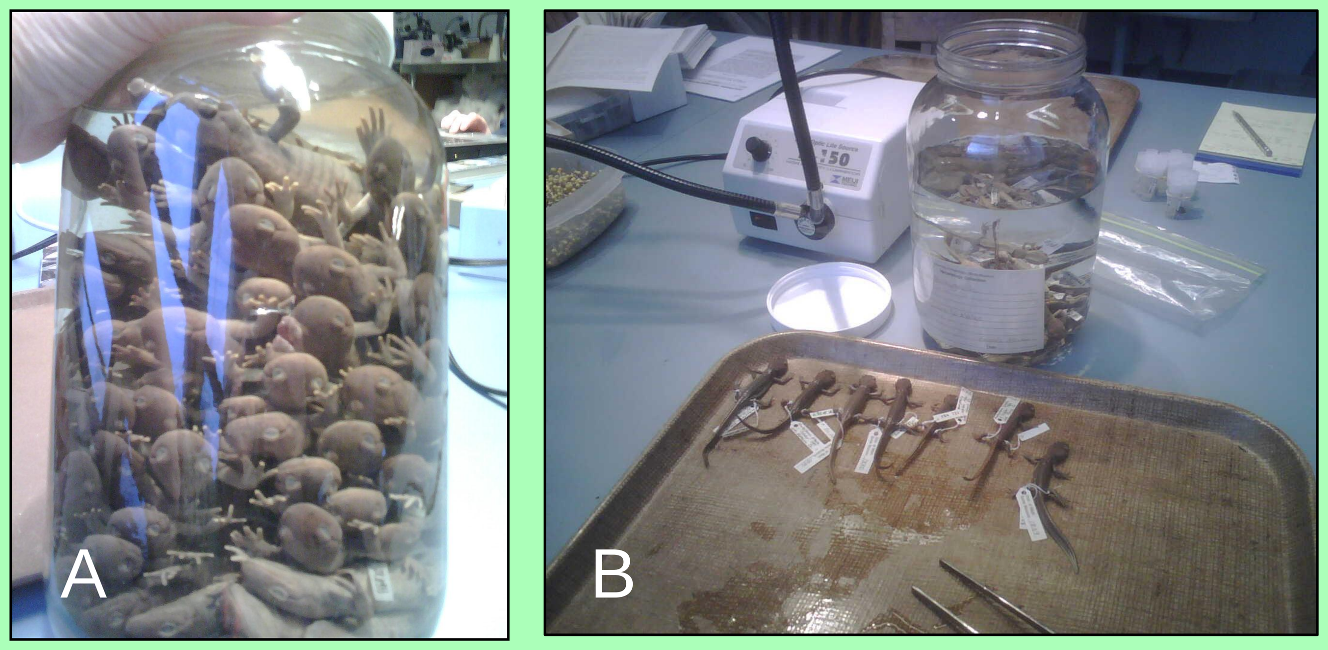

Figure 1. A) One of eight 3.5-L jars containing post-metamorphic A. gracile from which animals were selected for this study; B) Laboratory layout on which post-metamorphic A. gracile were dissected.

***Specimen dissection*.—**We dissected the entire gastrointestinal (GI) tract from each animal, keeping remaining internal organs intact, and removed all contained material. We then sorted the contents into taxon groups and identified taxa to the lowest level of classification practical.

***Analyses*.—**We compared four (≤55, 56-70, 71-85, and 86-100 mm SVL) body size classes in species composition, evenness, dominance, and prey diversity. We collapsed selection size classes to increase sample sizes. We used a Jaccard (1901) coefficient of similarity (J) to compare species composition, a Shannon-Weaver diversity index (H; Shannon and Weaver 1949) to calculate diversity, Pielou's index (E) to evaluate evenness (Brower et al. 1998), and Simpson's index (D_s) to assess domiance (Simpson 1949). A Spearman correlation coefficient (r_s) was used to assess the relationship between body size and each of number of prey and prey numbers by higher-level taxon. We used a Mann-Whitey U to compare numbers of prey by gender (Mann and Whitney 1947). Non-normally distributed data led to our use of the latter methods. We recognize the imperfect nature of comparisons among taxa given unevenness and lack of comprehensiveness of existing systematics, but it allows understandable comparison.

Results

***Dietary Analysis*.—**Of the 50 animals sampled, we recorded a total of 303 individual prey items representing at least 15 different higher-level taxa (order or higher) from 46 individuals (**Table 1**). The remaining four animals (two males and two females all ≤55 mm SVL) had nothing in their GI tracts. Thirty-seven prey were only identified to class (3 Stylommatophora and 34 Arachnida) and were removed from all analyses except for the prey quantity. We found a mean of 6.6 prey items per animal. The three most frequently represented higher-level taxa (collectively 65.3% of all prey) were beetles (Coleoptera), terrestrial pulmonate gastropods (Stylommatophora), and arachnids (Arachnida) with n = 120 (39.6%), 44 (14.5%), and 34 (11.2%), respectively. Larval (n = 70) versus adult (n = 50) beetles were approximately equivalent in abundance. They were found in all size classes except for the two smallest animals examined.

Phylum	Class	Order	Family	Genus	Species	n =
Arthropoda	Arachida	Acari				4
		Araneae	Antrodiaetidae	*Antrodiaetus*	unknown	1
			Linyphiidae	cf *Frederickus*	cf *covei*	1
				Pityohyphantes	cf *tacoma*	1
			Telmidae	*Usofila*	*pacifica*	1
		Phalangida (Opiliones)	Sabaconidae	*Sabacon*	*occidentalis*	2
				Taracus	cf. *pallipes*	17
			unknown			10
		Pseudoscorpionida				6
		Unknown				34
	Chilopoda					6
	Diplopoda					20
	Insecta	Coleoptera (larvae)				70
		Coleoptera (adult)				50
		Collembola				9
		Dipteran (larval)				9
		Hemiptera				8
		Hymenoptera				2
		Lepidoptera (larval)				2
		Orthoptera				8
Mollusca	Pulmonata	Stylommatophora	Arionidae	*Hemphillia*	*dromedarius*	4
					cf. *glandulosa*	1
					unknown	1
				Prophysaon	*foliolatum*	6
					vannattae	3
				Ancotrema	cf. *sportella*	1
					unknown	3
			Haplotrematidae	*Haplotrema*	*vancouverense*	5
				cf. *Haplotrema*		1
				unknown		6
			cf. Haplotrematidae			12

Table 1. Prey numbers and taxa to the lowest level identified for all prey**.** Taxa are listed alphabetically within each taxon levels within each immediately higher taxon.

***Gender and Size Relationships*.—**We found no relationship between gender and body size (Mann-Whitney: U = 1005, U' = 270, P = 0.338, n = 50), gender was unrelated to number of prey (U = 822.5, U' = 259, P = 0.382, n = 50), and prey diversity, evenness, and dominance were similar between genders (females: H' = 1.80, E = 0.683, D_s = 0.257; males: H' = 1.77, E = 0.769, D_s = 0.230). We found a significant positive relationship between body size and the number of millipede (Diplopoda) prey (r_s= 0.563, P = 0.042, n = 14). Comparing diet among the four body size classes, the largest *A. gracile* size class (86-100 mm SVL) had the highest prey diversity (H' = 1.85), the greatest evenness (E = 0.773), and lowest dominance (D_s = 0.230), whereas the smallest size class had the lowest diversity (H' = 1.31) and the highest dominance (D_s = 0.230). Middle size classes (56-70 and 71-85 mm) had intermediate but identical prey diversities (H' = 1.55) and evenness and dominance that were similar to each other.

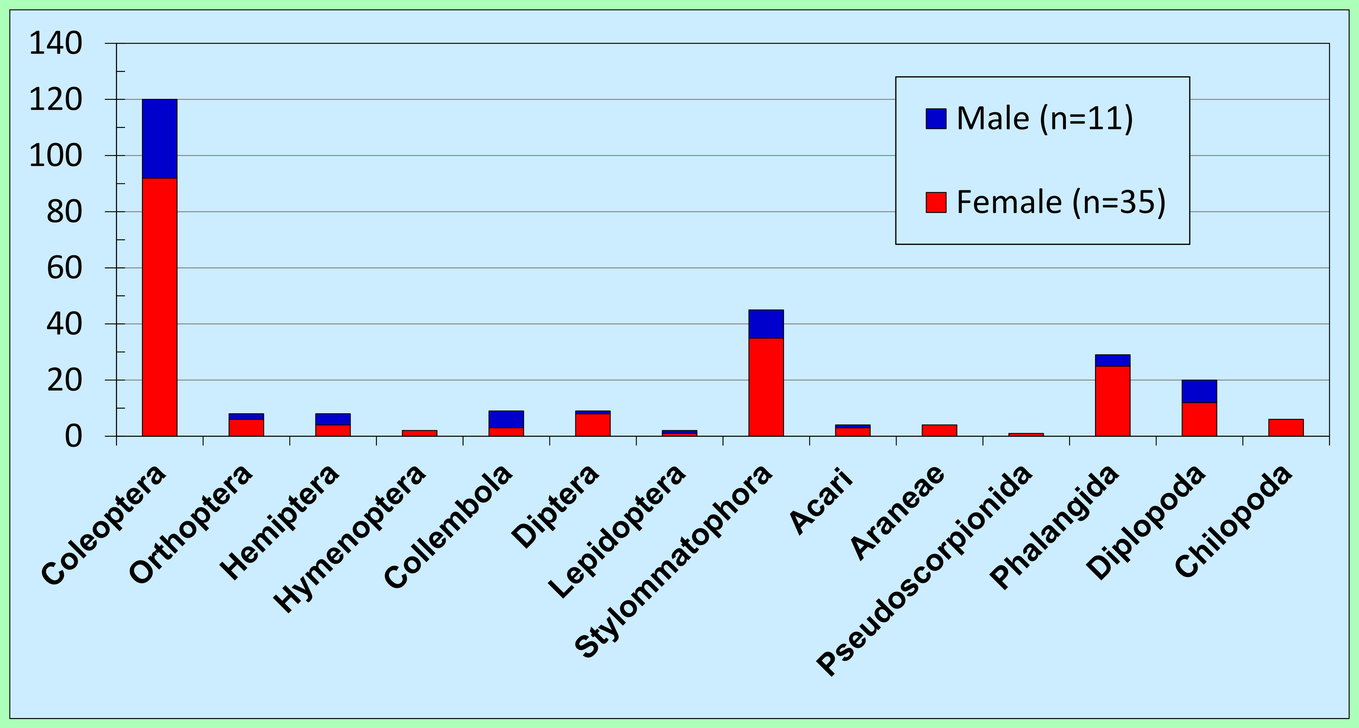

Figure 2. Total prey items by higher-level taxon partitioned by gender.

Discussion

We recorded exclusively invertebrate prey. Dietary studies of *A. gracile* larvae have revealed tadpoles of anurans as prey (Efford and Tsumura 1973, Licht 1975). Lack of vertebrate taxa may reflect unmanageable sizes, effective anti-predator evasion, their availability, or a sample size too small to record the few vertebrates that may be taken. In the case of tadpoles of stilllwater-breeding anurans previously reported, they would generally not be expected in fall samples because of earlier metamorphosis, nor would they be expected in stream-adjacent forested habitats from which these study animals came. However, given that study animals remained in pitfall traps three to seven days prior to preservation, rapid digestibility of tadpoles could also explain their absence.

Given the moderate sample size (46 animals with prey items), our finding of at least 15 higher-level taxa suggests that the prey base is relatively diverse. However, the frequency with which selected lower-level taxa appeared suggests that certain invertebrates were either vulnerable or abundant. For example, individuals of the phalangid (opilionid) genus *Taracus* was moderately frequent; members of this genus exhibit a distinctive freezing behavior when their terrestrial refuge sites are uncovered by human collectors so they may be vulnerable if they exhibit the same behavior when *A. gracile* attempts to prey on them. Insofar as known, the balance of prey except two adult Hymenoptera, which were infrequent, were forest floor forms frequent in the habitat where these post-metamorphic *A. gracile* were collected.

Gender and size analyses revealed little indication of their influence on the diet. Lack of relationship to gender was not surprising since no significant relationship existed between body size and gender. Though we found a positive correlation between body size and number of prey for millipedes, the small sample size hampers understanding the reality of this relationship. Among other salamanders, larger body size is associated with taking larger prey (Brodie and Formanowicz 1983), such a relationship in our sample is implied by the gradient in increased prey diversity and decreased prey dominance between the largest and the smallest *A. gracile* body size classes. One of the analyses we have yet to complete involves taking a direct look at this relationship.

Though this study is the first to examine the diet of terrestrial *A. gracile*, the mode of collection of these animals limits inference about their diet. Low frequency checking of pitfall traps prevents distinguishing prey that may have been trapped and might not be easily accessible outside of the trap environment versus taken prior to the *A. gracile* being trapped. Second, irregular and lengthy trap check intervals create both unwanted variation and bias against more digestible soft-bodied prey. Third, *A. gracile* having been captured in uneven numbers across several disparate study sites also contributes unwanted variation. Lastly, though the *A. gracile* in this study were collected over approximately the same fall interval, collection took place in two different years, so between-year differences in precipitation may further contribute unwanted variation. Nonetheless, this study provides a preliminary analysis of potential prey that can be used as a starting point for further study.

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Acknowledgments

Rod Crawford identified spider taxa.