

**Rocky Mountains Biological Laboratory
Research Experience for Undergraduates Program (2021)**

**Morph and Sex differences in prey selectivity of tiger salamanders
(*Ambystoma mavortium nebulosum*)**

**Georgenette Sánchez Ruiz
Mentor: Dr. Amanda Klemmer**

Abstract:

The tiger salamander is the top predator of the benthic invertebrate in the ponds at the Mexican Cut Nature Preserve, and hence, one of the main influential factors on the benthic invertebrate community structure. Recent phenomena observed at the MCP, like earlier snowmelt dates of some of the ponds in response to climate change, could potentially result in changes to the community structure by affecting the timeline of encounters between salamanders and their usual prey. To assess changes in predation selectivity of the tiger salamander in response to climate change and how sex and morph alter this feeding relationships, feeding trials were conducted at the MCP. The salamanders representing both morphs and sexes were starved for 24 hours before the trials and placed in 52 cm × 36 ½ cm plastic boxes with different types of their usual prey. The results did not support our hypothesis of shifting diet preferences, fairy shrimp is still the preferred food source for metamorphs, and unexpectedly, also for paedomorphic salamanders. The subsequent preferred prey differentiated between the morphs. An influence of sex on amount of prey consumed was not observed, it is most likely that this is due to the trials being conducted out of mating season. The importance of the study lies in assessing how climate change will affect feeding relationships and community structure of subalpine ponds at the MCP wetland or similar ecosystems.

Introduction

The effects of climate change can be felt in ecosystems across global and local scales. The Mexican Cut Preserve is a wetland ecosystem composed of a complex of 40 subalpine ponds. Usually, the ponds are frozen from late October to early summer, but the effects of climate change have resulted in earlier snowmelt dates for various of the ponds (Wissinger et al. 1999). The tiger salamanders (*Ambystoma mavortium nebulosum*) are the top and only aquatic vertebrate predator at the MCP, and hence, one of the main deciding factors of benthic invertebrate community structure at the subalpine ponds (Whiteman, 1996).

Larval salamanders develop into either an aquatic paedomorph that retains larval traits and stays in its natal pond or a terrestrial metamorph that undergoes metamorphosis (Lackey, 2019). Metamorphs diet is mostly composed of fairy shrimp who are usually found in semi-permanent or vernal ponds, and as a result are not much of an available food source for paedomorphs who tend to be constricted to permanent ponds (Wissinger, 1999). Hatchling larvae tend to feed mostly on copepods and cladocerans, one-to-two-year larvae feed on larger zooplankton, which include fairy shrimps if they manage to survive in semi-permanent ponds. Paedomorphs and larvae over two years old can feed on a variety of size prey, from the largest invertebrates to cannibalize on other salamander larvae (Wissinger, 1999).

During late spring metamorphic salamanders mate and deposit their egg in the permanent and some semi-permanent ponds if they do not freeze completely, where they are able to

survive the winter even when the surface layer of the ponds freeze due to the higher temperatures preserved at the bottom layer that keep it in a liquid state and allows them to survive. The metamorphic salamanders move on to other semi-permanent and vernal ponds where they feed for six to eight weeks before returning to terrestrial habitats (Wissinger, 1999). The metamorph eggs tend to hatch around mid-July, but as a consequence of the pond melting earlier than usual, they have been observed to hatch at earlier dates. It is possible that the metamorph getting into the ponds and their egg hatching at earlier dates could limit encounters with their usual amount and type of nourishment as they may not have hatched by that time. Salamanders feed by foraging their prey and tend to be visual predators, meaning that they will attack the prey they first encounter or notice.

Additionally, because of early ice out paedomorphs were observed to mate twice this year, meaning twice in one summer, a factor that could also affect their feeding relationships with the invertebrates in the ponds. Other phenomena that could potentially influence the feeding selectivity of the salamanders is that fairy shrimp populations have been observed to have reduced in recent years, which is why Elliot Johnston, a PhD student at Amanda Klemmer's Lab, is currently conducting a study on how fairy shrimp populations are affected by salamander predation at the MCP.

The goal of this study is to determine if predation selectivity of the tiger salamander *Ambystoma mavortium nebulosum* has changed in response to changes in the snowmelt dates of some of the ponds caused by climate change. In addition to climate change's effect on feeding behavior, we would like to see if sex and morph of the salamanders alter feeding selectivity. Based on Whiteman et al. 1996, I hypothesized fairy shrimp to be the preferred food source (Whiteman, 1996). However, it was possible that over the past 25 years, because of changes in snowmelt dates of the ponds, salamander mating and fairy shrimp density, they preyed on a wider variety of organisms. It was expected for paedomorphs to not eat as many fairy shrimp, since they are a food source practically unavailable to them. Additionally, I hypothesized females would consume more prey items than males, since they eat more during egg laying and mating season. Due to the observations mentioned above, a new study on the predation selectivity of the tiger salamander will be useful in assessing how the effects of climate change will affect this feeding relationships and as a result the community structure of subalpine ponds at the MCP or similar wetland ecosystems.

Methods

The experiment consisted of feeding trials conducted in 52 cm × 36 ½ cm plastic box mesocosms using salamanders and benthic invertebrate organisms collected from the ponds at the MCP. Two trials with three replications were conducted for each salamander morph and sex type (Fig.1), for a total of twelve plastic boxes. The morph and sex of the salamanders in each mesocosm was randomized.

Salamanders were captured in collaboration with Howard Whiteman's team, whose expertise helped minimize the stress on the salamanders. Metamorphs were collected from pond 8 for the first trial, 8 and 15 for the second trial. Paedomorphs were collected from pond 12 for both trials. For the second trial a female metamorph was missing since there were not many left in the ponds. The salamanders were starved for a period of 24 hours before both trials, but their stomach contents were flushed for the second trial only.

Five prey types were chosen for the experiment; copepods, mosquito larvae, caddisflies, fairy shrimp and chironomids, they were picked from samples collected using a d-frame net from a variety of ponds for both trials. For the second trial no mosquito larvae were found so only four prey types were used. Four individuals of each prey type were placed in each of the plastic boxes with the salamanders. The salamanders and the prey were put together in the plastic boxes and observed for a period of 30 minutes. Data on the bumps, attacks, initial prey item, and number of individuals consumed of the salamanders for each prey type was recorded.

After the trials were over, the plastic box contents were sifted, and organisms left (not consumed) were counted according to their type and subtracted from the original number of individuals to calculate the proportion consumed by the salamander. Photos were taken of the dorsal and ventral side of each salamander to collect data regarding their morph and sex after each of the trials. For statistics, a Two-way Analysis of Variance (ANOVA) test using salamander morph vs. prey selectivity, trial vs. total consumption, morph vs. fairy shrimp consumption, sex vs. total consumption, and total number of encounters vs. morph as predictor were conducted using R programming software (R core Team, 2020).

Results

I found that on average fairy shrimp was the most consumed prey by both morphs during the trials. Both morphs consumed mostly fairy shrimps, but the subsequent preferred prey differentiated between metamorphs and paedomorphs. With mosquitos coming in second for paedomorphs, followed by midges and copepods, and lastly caddisflies. For metamorphs, we found that midges were their second preferred food source, followed by mosquitos and copepods, and lastly caddisflies (Fig.4). However, there was not a significant difference in the amount consumed of each prey by the morphs. There was a significant influence of the variable of prey type ($p = <0.001$) on the types and number of invertebrates consumed. There was not a significant influence of the variable of morph ($p = 0.78$) or the interaction of morph and prey type ($p = 0.44$) (Table 1).

During the first trial metamorphs consumed more fairy shrimps than paedomorphs but there was not a significant difference. However, paedomorphs ended up consuming more fairy shrimps than metamorphs during the second trial, with an actual significant difference (Fig.5). There was a significant influence of the interactions between morph and trial over fairy shrimp consumption ($p = 0.048$) but not from trial ($p = 0.18$) and morph ($p = 0.48$) alone (Table 2). There was not a significant difference in the total amount of invertebrates consumed between females and males (Fig.6). The variable of sex did not have a significant influence over invertebrate consumption, $p = 0.66$ (Table 3). There was an influence of the dates when the trials were conducted on the number of invertebrates consumed, $p = 0.033$ (Table 4, Fig.7). On average, with bumps and attacks, metamorphs had more encounters with the prey. There was a significant influence of trial ($p = 0.053$) on the amount of encounters the salamanders had with the invertebrates (Table 5, Fig.8).

Discussion

I hypothesized that there would be changes in predation selectivity of the tiger salamander due to the early snow melts of some of the ponds caused by climate change, but this was not supported. As predicted, fairy shrimp were the most consumed prey for both morphs, as observed for metamorphs in Whiteman's 1996 paper (Whiteman, 1996) (Fig.4). I observed a significant influence of the prey type variable on consumption of invertebrates, meaning that the type of prey influenced their susceptibility to be eaten by the salamanders. Overall, the salamanders did not seem to have any preference impulse to go directly to a prey, during the trials they foraged and seemed to attack whatever was noticeably in front of them. In various encounters the prey would hang out right in front of their mouths and they did not move to attack. It appeared to take a lot of effort for them to notice the prey at times, which is why one of the possible explanations for fairy shrimps being the preferred food source is that they were one of the biggest, most noticeable, and constantly mobile prey used for the trial.

In the ponds fairy shrimps exist naturally in large groups, compared to mosquito larvae or

midges that tend to be more distributed across the ponds, they are easier to detect, and salamanders can also catch more than one individual per attack. As part of his research, Elliot Johnston collected gut samples from metamorphs and most of the stomach content were composed of fairy shrimps and some copepods. Copepods can also be found in groups in the ponds, but as observed in failed attacks towards both copepods and fairy shrimps, their size makes it easier to escape the salamanders, a reason why they are consumed less than fairy shrimps. The salamanders attempted to attack caddisflies multiple times during the trials but seemed to give up when they found it difficult to get them out of their cases (insert citation). It is largely possible that they prefer prey they can spend the least amount of energy to consume (Wissinger, 2006).

The results both supported and did not support the prediction that paedomorphs would consume less fairy shrimp since they are not usually exposed to that prey type (Wissinger, 1999). Even when metamorphs consumed more fairy shrimps during the first trials, there was not a significant difference in consumption between the morphs. However, for the second trial, paedomorphs consumed more fairy shrimps than metamorphs, with an actual significant difference (Fig.5.). There was a significant influence of the interactions between morph and trial over fairy shrimp consumption, meaning that the date of the trials influenced the fairy shrimp consumption of the morph (Table 2). The observed results are possibly due to the second trial (July 29) being conducted almost two weeks and half after the first trial (July 14), around the time the metamorphs get out of the ponds to get into burrowing holes for the winter and them not feeling as strong as an impulse to forage and feed as they had during the first trial (Wissinger, 1999). During the first trial metamorphs were observed to be more active and aggressive foragers than paedomorphs, as opposed to the behavior they exhibit during the second trial. The result also showed that given the opportunity to encounter fairy shrimp, they would also be a prime food source for paedomorphs.

The effects of this behavioral difference are also reflected in the results of the comparison of total of invertebrates consumed during trial #1 vs. trial #2 (Fig.7.). Overall, there were more invertebrates consumed during the first trial than the second trial, it is possible that the interaction between the dates of the trials and life history traits, like the metamorph preparing to get out of the ponds influence invertebrate consumptions on the salamanders. Even with metamorphs being less active during the second trial, they still had the highest number of encounters with the prey when accounting for both trials (Fig.8) but it was not a significant difference. Even with them being less active foragers during the second trial, they had enough encounters during the first trial to still give them an advantage over the paedomorphs. There was a significant influence of trial on the amount of encounter with the prey, it is possible that the inactivity of the metamorphs during the seconds trial produced a lower number of encounters compared to the first trial (Table 5). Finally, the results did not agree with the prediction that females would consume more prey since they tend to feed more during mating season, since a significant influence of sex on amount of prey consumed was not observed (Table 3, Fig.6). It is most likely that these results are due to the trials being conducted during late summer, out of the mating season that takes place during late spring (Wissinger, 1999). It would be worth to repeat the study in the future earlier in the summer, with a higher

possibility of observing an influence of sex in the trials and also avoiding the metamorphs behavior been affected by their exit from the ponds.

References:

Lackey ACR, Moore MP, Doyle J, Gerlanc N, Hagan A, Geile M, Eden C, Whiteman HH (2019) Lifetime Fitness, Sex-Specific Life History, and the Maintenance of a Polyphenism. *Am Nat* 194:230–245.

Wissinger, S. A., Bohonak, A. J., Whiteman, H. H., & Brown, W. S. (1999). Subalpine wetlands in Colorado: habitat permanence, salamander predation, and invertebrate communities. *Invertebrates in freshwater wetlands of North America: ecology and management*. Wiley, New York, 757-790.

Wissinger, Scott & Whissel, John & Eldermire, Charles & Brown, Wendy. (2006). Predator defense along a permanence gradient: Roles of case structure, behavior, and developmental phenology in caddisflies. *Oecologia*. 147. 667-78. 10.1007/s00442-005-0303-1.

Whiteman, H. H., Wissinger, S. A., & Brown, W. S. (1996). Growth and foraging consequences of facultative paedomorphosis in the tiger salamander, *Ambystoma tigrinum nebulosum*. *Evolutionary Ecology*, 10(4), 433-446.

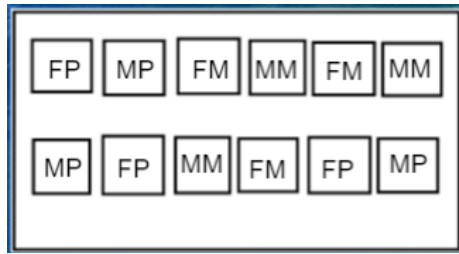
Figures and Tables:



(Fig. 1. Metamorphic *Ambystoma mavortium nebulosum* salamander from a pond at the Mexican Cut Preserve)



(Fig. 2. Paedomorphic *Ambystoma mavortium nebulosum* salamander from a pond at the Mexican Cut Preserve)



(Fig.3. Drawing exemplifying the feeding trials set up. There were three plastic boxes (replications) out of twelve for each morph and sex. Three boxes containing female paedomorphs, three containing female metamorph, three containing a male paedomorph, and three containing a male metamorph. Their positions were randomized.

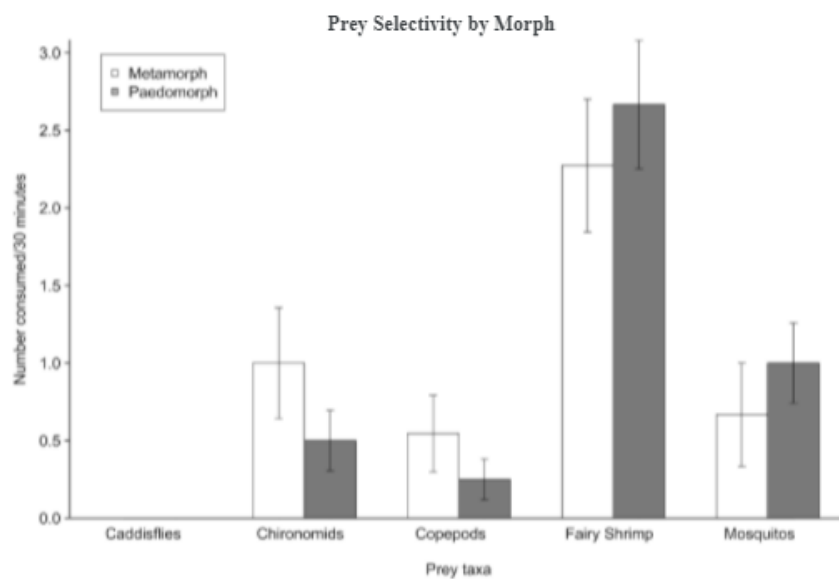


Fig.4. Average number of each prey type consumed by salamander morph during feeding trials conducted at the MCP during July 14 and July 29. Fairy shrimp was the most consumed prey by both morphs.

Table #1: Statistical Values of Two -Way ANOVA for Prey Selectivity by Morph

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Prey Type	4	82.51769788	20.62942447	25.46204107	2.64E-14
Morph	1	0.0655451168	0.0655451168	0.08089961309	0.776707601
	9				

Prey Type:Morph	4	3.094204554	0.7735511384	0.9547620139	0.436170866 9
Residuals	94	76.15909091	0.8102030948	NA	NA

*Significant influence of prey type ($p = <0.001$), not significant influence of morph ($p = 0.78$) and prey type: morph ($p = 0.44$) over prey type consumption of metamorphs vs. paedomorphs.

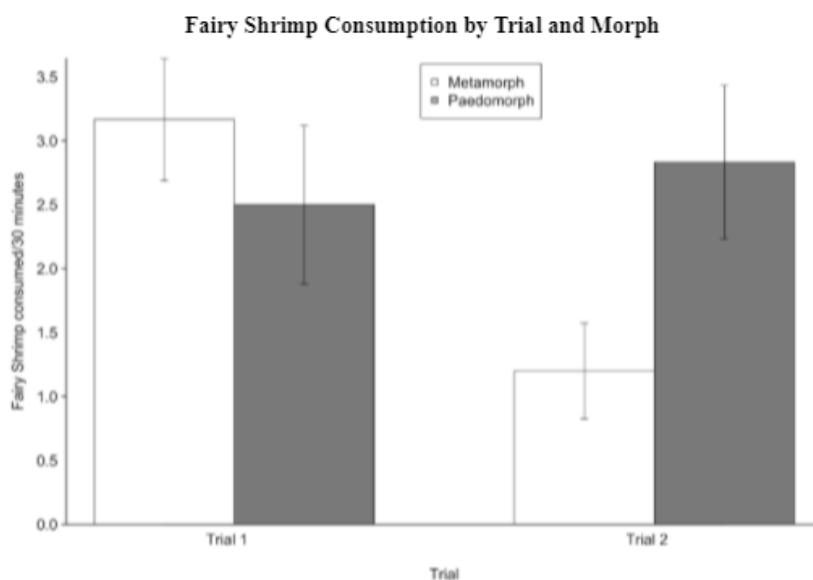


Fig.5. Average fairy shrimp consumed during each trial by the different salamander morphs. During the first trial metamorphs consumed more fairy shrimps than paedomorphs but there was not a significant difference. During the second trial paedomorphs consumed more fairy shrimps than metamorphs with a significant difference.

Table #2: Statistical Values of Two -Way ANOVA for Fairy Shrimp Consumption by Trial and Morph

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Morph	1	0.8906455863	0.8906455863	0.5293722463	0.4757419295
Trial	1	3.324675325	3.324675325	1.976084395	0.1759459746
Morph:Trial	1	7.557142857	7.557142857	4.491732459	0.04745506153

Residuals	19	31.96666667	1.68245614	NA	NA
-----------	----	-------------	------------	----	----

*Significant influence of morph: trial ($p = 0.048$), not significant influence of morph ($p = 0.48$) and trial ($p = 0.18$) over fairy shrimp consumption.



Fig.6. Average invertebrate consumption of females vs. males during feeding trials conducted at the MCP during July 14 and July 29. No significant difference of consumption of invertebrates between the sexes.

Table #3: Statistical Values of Two -Way ANOVA for Total Consumption of Invertebrates of Females vs. Males

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	1	1.048829	1.048829	0.21829	0.64566
Trial	1	22.61642	22.61642	4.707084	0.042928
Sex:Trial	1	0.000799	0.000799	0.000166	0.989846
Residuals	19	91.29048	4.804762	NA	NA

*Significant influence of trial ($p = 0.043$), not significant influence of sex ($p = 0.66$) and sex:trial ($p = 0.99$) over total invertebrate consumption.

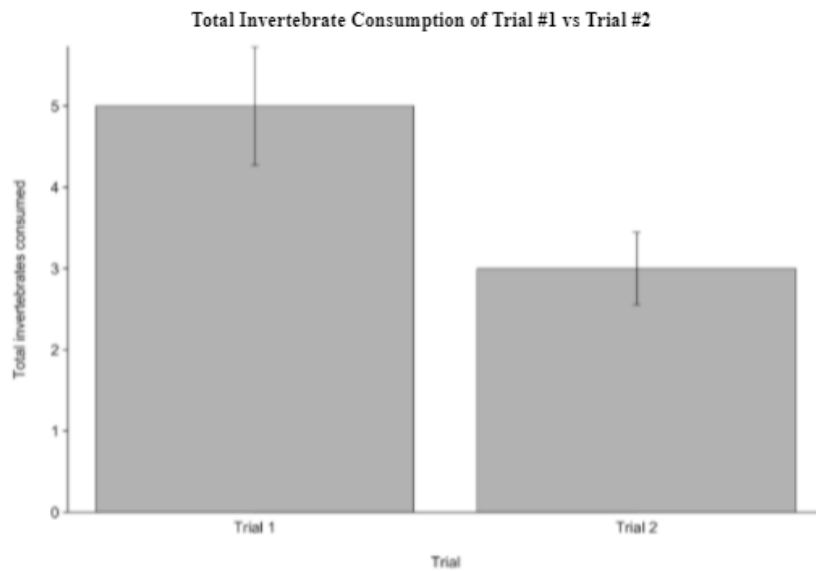


Fig.7. Significant difference in mean total of invertebrates consumed during feeding trial #1 vs. trial #2 conducted at the MCP.

Table #4: Statistical Values of Two -Way ANOVA for Total Invertebrates Consumption of Trial #1 vs. Trial #2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
as.factor(Trial)	1	22.95652174	22.95652174	5.240075614	0.03253446157
Residuals	21	92	4.380952381	NA	NA

*Significant influence of trial ($p = 0.033$) dates on total of invertebrates consumed.

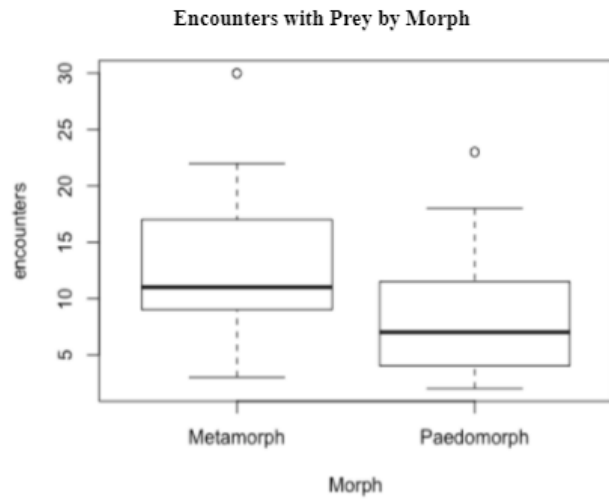


Fig.8. Mean encounters with prey of metamorphs vs. paedomorphs during feeding trials conducted at the MCP during July 14 and July 29. Not significant difference of encounters between the morphs.

Table #5: Statistical Values of Two -Way ANOVA for Encounters with Prey by Morph

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Morph	1	112.722332	112.722332	2.433685438	0.1352552597
Trial	1	197.0133478	197.0133478	4.253536162	0.05311183221
Morph:Trial	1	0.8396825397	0.8396825397	0.01812882268	0.8943099827
Residuals	19	880.0333333	46.31754386	NA	NA

*Significant influence of trial ($p = 0.053$), not significant influence of morph ($p = 0.14$) and morph:trial ($p = 0.89$) on encounters with prey of metamorphs vs. paedomorphs.