

# Survival and growth of the caddisfly *Limnephilus flavastellus* after predation on toxic eggs of the Rough-skinned Newt (*Taricha granulosa*)

B.G. Gall, E.D. Brodie III, and E.D. Brodie, Jr.

**Abstract:** The Rough-skinned Newt (*Taricha granulosa* (Skilton, 1849)) possesses a powerful neurotoxin, tetrodotoxin, in the skin that is secondarily deposited in the ova. Although assumed to serve an antipredator function in the eggs, empirical evidence of the toxin's role in preventing egg predation is lacking. In this study, we characterized the aquatic macroinvertebrate community at a location sympatric with extremely toxic newts and estimated the abundance of caddisflies. We tested aquatic macroinvertebrates sympatric with toxic newts for their capacity to consume the toxic eggs, and examined the propensity of egg predation and its effect on growth of the only known predator of newt eggs, caddisfly larvae. *Limnephilid* caddisfly larvae were the only invertebrate observed to consume substantial quantities of toxic newt eggs. Survival and growth of the caddisfly *Limnephilus flavastellus* Banks, 1918 continued when larvae consumed toxic eggs and did not differ from *L. flavastellus* that also had access to an alternative food source (detritus). *Limnephilus flavastellus* that had access to eggs + detritus consumed a similar number of eggs compared with those provided with eggs only. These results, combined with the abundance of caddisflies, suggest that caddisflies are important predators of eggs of *T. granulosa*.

**Résumé :** Le triton rugueux (*Taricha granulosa* (Skilton, 1849)) possède une puissante neurotoxine, la tétrodoxtine, dans la peau qui se dépose secondairement dans les œufs. Bien qu'on présume que la toxine joue un rôle antiprédateur dans les œufs, il n'existe pas de données empiriques montrant que son rôle est de prévenir la prédation des œufs. Dans notre étude, nous caractérisons la communauté de macroinvertébrés aquatiques dans un site qui contient en sympatrie ce triton extrêmement toxique et nous estimons l'abondance des trichoptères. Nous vérifions chez les macroinvertébrés aquatiques qui cohabitent avec les tritons toxiques leur capacité à consommer des œufs toxiques; nous avons aussi déterminé la tendance à la prédation des œufs et son effet sur la croissance chez les larves de trichoptères, les seuls prédateurs connus des œufs de tritons. Les larves de trichoptères limnéphilidés sont les seuls invertébrés que nous avons observés consommer des quantités importantes d'œufs toxiques de tritons. La survie et la croissance du trichoptère *Limnephilus flavastellus* Banks, 1918 se poursuivent lorsque les larves consomment des œufs toxiques et ne diffèrent pas de celles des *L. flavastellus* qui ont en plus accès à une source de nourriture de rechange (détrit). *Limnephilus flavastellus* qui ont accès à des œufs + détrit consomment un nombre semblable d'œufs, par rapport à ceux qui ne reçoivent que des œufs. Ces résultats, compte tenu de l'abondance des trichoptères, laissent croire que les trichoptères sont d'importants prédateurs des œufs de *T. granulosa*.

[Traduit par la Rédaction]

## Introduction

Toxicity is a common antipredator strategy and empirical evidence indicates that toxic prey often escape predatory encounters uninjured, thus conferring a selective advantage to those individuals (Fisher 1930; Paradise and Stamp 1991; Williams et al. 2003; Gall et al. 2011). Some toxic or distasteful species secondarily provide the same protection to their offspring by depositing the chemical defenses in the ova. These compounds protect the developing embryo until hatching, whereupon behavioral defenses can reduce the risk of predation or the juvenile can sequester its own toxin.

Although embryonic toxins may prevent predation by some predators, it is unlikely that any defense will confer complete protection (Orians and Janzen 1974). For example, Licht (1968, 1969) established that eggs of toads (genus *Bufo* Laurenti, 1768) are unpalatable to a variety of potential predators including leeches (genus *Batrachobdella* Viguiet, 1879), larval Northwestern Salamanders (*Ambystoma gracile* (Baird, 1859)), and fishes (black bullhead, *Ameiurus melas* (Rafinesque, 1820); longear sunfish, *Lepomis megalotis* (Rafinesque, 1820); green sunfish, *Lepomis cyanellus* Rafinesque, 1819; threespine stickleback, *Gasterosteus aculeatus* L., 1758; cutthroat trout, *Oncorhynchus clarkii* (Richardson,

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1836)). However, the Common Garter Snake (*Thamnophis sirtalis* (L., 1758)) frequently eats adult bufonids and is not deterred from consuming toad eggs (Licht 1968). Eggs of the saddled toby (*Canthigaster valentini* (Bleeker, 1853)) are unpalatable to two species of sympatric reef fish, but are consumed by a closely related species (Gladstone 1987). Nonetheless, egg toxicity clearly limits the scope of predators that can consume the eggs and likely confers a fitness advantage to those organisms that supply their embryos with this defense.

One of the most toxic vertebrates, newts of the genus *Taricha* Gray, 1850, deposit large quantities of the neurotoxin tetrodotoxin (TTX) in the ova (Twitty 1937; Mosher et al. 1964; Wakely et al. 1966; Hanifin et al. 2003). Although the toxin is also located in the skin and serves an antipredator function in adult newts (Brodie 1968; Brodie et al. 1974), it is unknown if the toxin confers similar protection to the eggs. Tetrodotoxin is an extremely powerful toxin, blocking the pore of voltage-gated sodium channels thereby stopping the propagation of action potentials and rapidly leading to asphyxiation and death (Kao 1966; Narahashi et al. 1967). Tetrodotoxin quantities range between 672 and 2767 ng in a single egg (Hanifin et al. 2003), enough to kill up to 14 mice (20 g each) in 10 min. Although it is unlikely that many predators consume these eggs, Lehman (2006) observed larval caddisflies (order Trichoptera) consuming newt eggs at some localities. Furthermore, caddisflies are known to consume the eggs of other amphibians (Murphy 1961; Dalrymple 1970; Richter 2000; Romano et al. 2008). If caddisflies exhibit the same susceptibility to TTX as mice, a single egg contains enough TTX to kill 500–3700 caddisflies.

We evaluated the possible interactions between newt eggs and potential predators by first characterizing the macroinvertebrate community at one of our study sites and providing these invertebrates with toxic eggs in the laboratory. Because caddisflies have been observed to consume newt eggs, we specifically examined if consumption of newt eggs prevents larval caddisfly growth and development. Finally, we estimated the abundance of caddisflies to determine their potential impact on the newt population.

## Materials and methods

### Study area and macroinvertebrate sampling

Invertebrate sampling took place at a series of eight man-made ponds (Soap Creek ponds) located in the Central Willamette Valley, near Corvallis, Oregon, USA. Located on the eastern edge of the Coast Range Mountains, the surrounding vegetation consists primarily of coniferous forest. The ponds are arranged in two rows of four, with the upper row 4 m higher than the lower ponds. Each pond is 22.86 m × 91.44 m square and gently slopes to a depth of 3–4 m at the easternmost edge. The ponds within a row are separated by 2 m wide berms.

For the present study, we haphazardly selected four ponds to serve as sampling sites. Invertebrate sampling occurred on 11 March 2010. We used two sampling techniques to document the diversity of the macroinvertebrate community and the abundance of caddisflies. Caddisfly abundance was estimated using a 19 L bucket (0.285 m diameter) with the bottom cut out. The bottomless bucket was rapidly pushed into

the substrate and mesh strainers (aperture = 1 mm<sup>2</sup>) were used to move all the contents into shallow trays. The contents of the trays were sorted and all macroinvertebrates were preserved in formalin – acetic acid – alcohol (FAA). Five within-pond samples were collected from each pond by haphazardly selecting a location at a depth up to 37 cm and proceeding at 2 m intervals across the pond from this location. An estimate of caddisfly abundance was calculated by dividing the area of the pond by the area of the bucket then multiplying by the mean number of caddisflies collected in the five samples collected per pond. Although this technique makes the assumption of even distribution across the pond, it gives a rough estimate of density and allows us to evaluate the potential influence of caddisflies on the newt population. The process of moving to the bucket sampling location may have slightly disturbed the water around the sampling site, resulting in mobile invertebrates being underrepresented in the samples. Therefore, we also sampled macroinvertebrates by dip-netting in each pond for 1 h to further assess the diversity of the macroinvertebrate community. These samples were visually examined and subsamples were transferred to ice-chests and transported to Utah State University for identification and egg consumption experiments.

Gravid female Rough-skinned Newts (*Taricha granulosa* (Skilton, 1849); henceforth newts) were collected by hand from Soap Creek ponds for the collection of eggs (see below) in March 2009 and 2010. Newts were transported to Utah State University where they were housed in 5.7 L containers with 2 L of tap water filtered by reverse osmosis. To initiate egg deposition and ensure eggs were of similar developmental stage, females were injected with 2 µL/g of luteinizing hormone releasing hormone ([des-Gly<sup>10</sup>, D-His(Bzl)<sup>6</sup>]-LH-RH ethylamide; Sigma #L2761). Eggs were collected 24 h after the initiation of deposition and frozen (caddisfly growth experiment) or immediately given to potential predators (egg predation by aquatic invertebrates).

### Consumption of newt eggs by aquatic invertebrates

We tested macroinvertebrates from 5 different orders and 11 families collected from Soap Creek ponds for their propensity to consume recently deposited newt eggs (Table 1). The sample sizes of potential egg predators tested was unequal because of differences in availability. Invertebrates were housed individually in clear plastic cylinders (9 cm diameter × 4 cm tall) with a plastic cap affixed to the bottom. Small holes were punched in the sides to allow aerated water to pass into the cylinder. Invertebrates were randomly assigned to 1 of 10 cylinders placed inside a shallow tray filled with 3 L of filtered tap water and two aerators. Each tray was maintained in an environmental chamber at 6 °C.

Recently deposited newt eggs were collected and combined from five female newts. Five of these eggs were placed in each cylinder and monitored daily for egg consumption for 16 days. The eggs were examined upon termination of the experiment for signs of predation. To ensure that the lack of egg consumption was not the result of aversion to feeding in the test containers, each invertebrate was provided with alternative prey, bloodworms or conditioned detritus (see below), at the conclusion of testing. All potential predators fed on at least one of the alternative diets within the confines of the test tanks.

### Consumption of newt eggs by caddisflies

Because caddisflies appeared to be the only major predator of *Taricha* eggs (see Results), we tested whether caddisflies would feed on eggs when a detritus food source was available. The case-making caddisflies (suborder Integripalpia) construct portable cases out of plant or mineral material that aid in respiration and defense (Wiggins and Currie 2008). As a larva grows, additional material is added to the anterior end of the case and the change in case length over time can be used to estimate growth.

Fifty-four caddisfly *Limnephilus flavastellus* Banks, 1918 were measured to the nearest 0.01 mm (case length) using digital calipers, individually placed in mesh bottom cups, and randomly assigned to one of three 37 L aerated aquaria with 15 L of filtered tap water at 12 °C. Each *L. flavastellus* was then randomly assigned to one of three treatments: detritus only ( $n = 18$ ), newt eggs only ( $n = 18$ ), or eggs + detritus ( $n = 18$ ), with an equal number of each treatment occurring within each aquarium. The median length of *L. flavastellus* in each treatment was not significantly different at the start of the experiment (Kruskal–Wallis one-way ANOVA;  $H_{[2]} = 0.224$ ,  $P = 0.894$ ). Several thin strips of artificial silk leaves were added to each cup to provide *L. flavastellus* with media for case construction.

Each *L. flavastellus* in the egg-only and egg + detritus treatments was given five newt eggs. Eggs were previously collected from 15 female newts, combined, and killed by freezing (–80 °C) to ensure toxicity was similar throughout the experiment and between treatments; TTX concentration may decrease as development proceeds (Twitty 1937). Freezing is a common technique to preserve tissue samples for TTX analysis (Hanifin et al. 1999, 2003). Although not tested during this experiment, *L. flavastellus* also consume live eggs (B.G. Gall, unpublished data). Animals in the detritus and egg + detritus treatments were given three conditioned maple leaves. Conditioned maple leaves were prepared by collecting sugar maple (*Acer grandidentatum* Nutt.) leaves shortly after abscission in October 2009 and maintained dry until further use. In January 2009, leaves were placed in aerated aquaria with filtered tap water and a small amount of pond detritus to facilitate the buildup of beneficial bacteria and fungi (henceforth conditioned detritus). The number of eggs consumed by each *L. flavastellus* was recorded every 24–48 h, upon which the consumed eggs were replaced. All eggs and detritus were replaced every 7 days. Detritus was replaced more frequently when large quantities were consumed. Because of the difficulties in quantifying small pieces of detritus and because our goal was to provide an excess of an alternative food source, we did not attempt to quantify the amount of detritus consumed. All *L. flavastellus* with access to detritus (including *L. flavastellus* in the egg + detritus treatment) consumed this resource; partially consumed leaves were frequently replaced. Eggs and detritus were never reused. Case length was measured at the completion of trials, providing an estimate of developmental rate (Anderson 1974). Trials were terminated after 2 weeks and *L. flavastellus* were not retested.

Two *L. flavastellus* in the egg-only treatment pupated before the completion of trials and were excluded from the analyses. To determine whether an alternative food source affected egg consumption, we compared the number of eggs

eaten between the egg-only and egg + detritus treatments with a Student's  $t$  test. We compared the change in growth (increase in case length) between *L. flavastellus* in the three treatments by subtracting the initial length from the final length and comparing the change in length between the treatments with a randomized complete block design with subsamples. Tank was used as the blocking factor and cups within tank as subsamples. The Tukey–Kramer multiple comparisons method was used to identify differences between treatments. Because of the reduced power from blocking by tank and to account for the conservative nature and limited ability to detect small differences between means of Tukey–Kramer post hoc comparisons, we used an adjusted  $\alpha$  value (determined a priori) for post hoc comparisons of 0.10 (Day and Quinn 1989). Analysis was performed with GLIMMIX in SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) with the nobound option. Finally, we examined the role that the size of *L. flavastellus* had on the number of eggs a larvae consumed by combining the egg-only and egg + detritus treatments and comparing the number of eggs consumed to initial length using linear regression. All data met assumptions for parametric statistics.

## Results

### Macroinvertebrate sampling

Invertebrates from 18 genera and 13 families were collected in samples from the three ponds (Table 1). No invertebrates were present in the bucket samples that were not found in the dip-net samples. A total of 494 invertebrates were collected during bucket sampling from all four ponds. The most abundant invertebrate in the bucket samples were caddisflies, comprising 95.7% of the invertebrates collected. Four species of caddisfly were present, with *L. flavastellus* comprising the majority (Table 2). In total, as many as 774 000 caddisflies (Table 2) may populate each 2 090 m<sup>2</sup> pond.

### Egg predation by macroinvertebrates

Caddisflies were the only invertebrate to consume newt eggs, except for a single planorbid snail that consumed one egg (Table 1). Each caddisfly species tested consumed newt eggs (Table 1).

### Egg predation by *L. flavastellus*

All *L. flavastellus* offered newt eggs consumed at least one egg over the 14-day trial. *Limnephilus flavastellus* in all three treatments increased in size during the trials; however, change in growth was significantly different between the treatments ( $F_{[2,4]} = 6.19$ ,  $P = 0.059$ ; Fig. 1). Consumption of eggs did not hinder growth but actually facilitated it; *L. flavastellus* in the egg-only and egg + detritus treatments grew more than *L. flavastellus* in the detritus-only treatment (egg only:  $t_{[4]} = -2.87$ ,  $P = 0.095$ ; egg + detritus:  $t_{[4]} = -3.16$ ,  $P = 0.072$ ; Fig. 1). There was no significant difference in the growth between *L. flavastellus* provisioned with eggs only or eggs + detritus ( $t_{[4]} = -0.14$ ,  $P = 0.99$ ). In addition, *L. flavastellus* with access to an alternative food source (detritus) consumed a similar number of eggs (eggs consumed =  $14.72 \pm 2.02$  (mean  $\pm$  SE)) compared with *L. flavastellus* that had access to eggs only (eggs consumed =  $12.94 \pm 2.12$ ) ( $t_{[32]} = -0.61$ ,  $P = 0.55$ ; Fig. 2). Larger *L. flavastellus*



**Table 1.** Macroinvertebrates collected at Soap Creek ponds and tested for their propensity to consume toxic eggs of Rough-skinned Newts (*Taricha granulosa*).

Class, order, family	Species	No. of invertebrates offered eggs	Eggs consumed
Insecta			
Odonata			
	Libellulidae	11	No
	Aeshnidae	1	No
	Coenagrionidae	6	No
Hemiptera			
	Belostomatidae	1	No
	Notonecta	2	No
	Nepidae	NA	Not tested
Coleoptera			
	Noteridae	3	No
	Dytiscidae	1	No
Trichoptera			
	Limnephilidae		
	<i>Limnephilus flavastellus</i>	3	Yes
	<i>Limnephilus concolor</i> Banks, 1899	3	Yes
	<i>Grammotaulius betteni</i> Hill-Griffin, 1912	2	Yes
	<i>Limnephilus occidentalis</i> Banks, 1908	15	Yes
Gastropoda			
	Lymnophila		
	Physidae	4	No
	Lymnaeidae	2	No
	Planorbidae	4	Yes

**Note:** NA, not available. “No” indicates that eggs were neither damaged nor consumed, whereas “yes” indicates that eggs were consumed.

**Table 2.** Species and estimated population sizes of caddisflies (Trichoptera) sympatric with a population of toxic Rough-skinned Newts (*Taricha granulosa*).

Species	Total collected	Mean number per sample	Estimated population size per pond
<i>Limnephilus flavastellus</i>	430	21.5	704 484
<i>Limnephilus concolor</i>	29	1.5	47 512
<i>Limnephilus occidentalis</i>	10	0.5	16 383
<i>Grammotaulius betteni</i>	4	0.2	6 553

**Note:** All species consumed living and freeze-killed newt eggs in the laboratory.

consumed more eggs than smaller *L. flavastellus* ( $F_{[1,32]} = 17.94$ ,  $R^2 = 0.359$ ,  $P < 0.001$ ; Fig. 3).

**Discussion**

The presence of TTX in the eggs of newts has been postulated to serve as a defense against egg predators (Twitty 1966; Daly et al. 1987). The eggs from the population that we sampled contain the highest levels of TTX in an egg known in the animal kingdom (1.53 µg TTX/egg; Hanifin et al. 2003), yet a single invertebrate, sympatric larval caddisflies (a single planorbid snail also consumed one egg), were able to consume these toxic eggs. Moreover, the presence of an alternative food resource (detritus) did not affect the number of eggs eaten by *L. flavastellus*, indicating egg predation may be common in an ecological context. *Limnephilus flavastellus* given a diet of eggs only or eggs + detritus grew equally well, and outgrew *L. flavastellus* that had access to detritus only. These data indicate that ingestion of TTX does not kill *L. flavastellus* predators, but these individuals con-

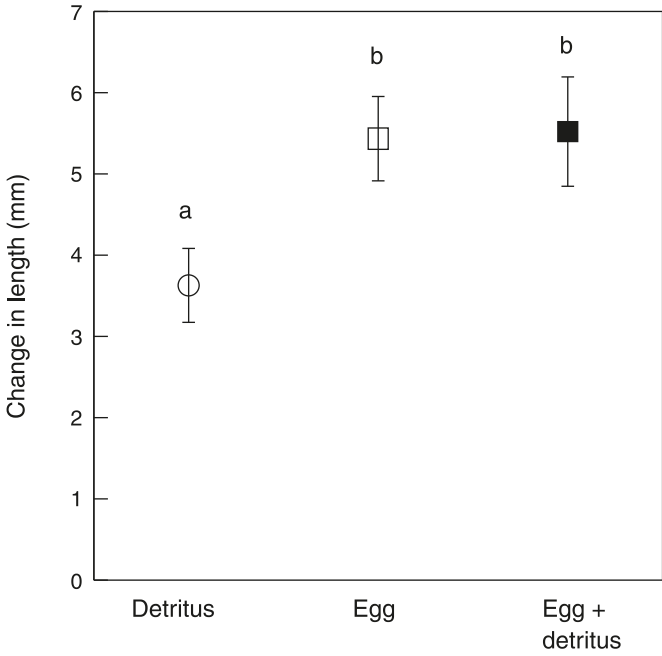
tinue to grow and develop (this study) and may eventually pupate and eclose as functional adults (B.G. Gall, personal observation).

Ingestion of toxic foods is often assumed to have physiological costs that negatively affect growth of an organism (Freeland and Janzen 1974; Behmer et al. 2002). Detoxifying dangerous compounds requires energy and nutrients that could otherwise be used for growth or reproduction (Sorensen et al. 2005). Consuming toxic eggs may entail a cost relative to some other animal-based resource, yet consuming this immobile and plentiful resource is beneficial relative to a detritus-only diet. *Limnephilus flavastellus* continued to grow when provided with toxic eggs, suggesting individuals from this locality are resistant to TTX. At present it is unknown what metabolic pathway is utilized to excrete, detoxify, or sequester TTX.

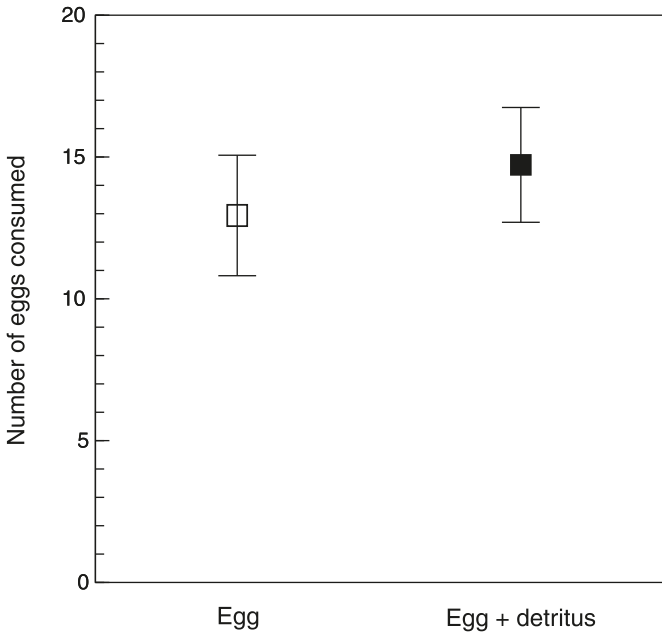
Although limnephilid caddisflies are primarily detritivores, their food is limited in resources important for growth (e.g., lipid, protein, carbohydrate; Suberkropp et al. 1976; Cummins and Klug 1979), and field and laboratory observations

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**Fig. 1.** Growth (mm) of the caddisfly *Limnephilus flavastellus* observed in larvae fed detritus only, eggs of Rough-skinned Newts (*Taricha granulosa*) only, or eggs + detritus. Growth was measured as the change in case length. Different letters indicate significant differences ( $P \leq 0.1$ ) between treatments. Values are means  $\pm$  SE.

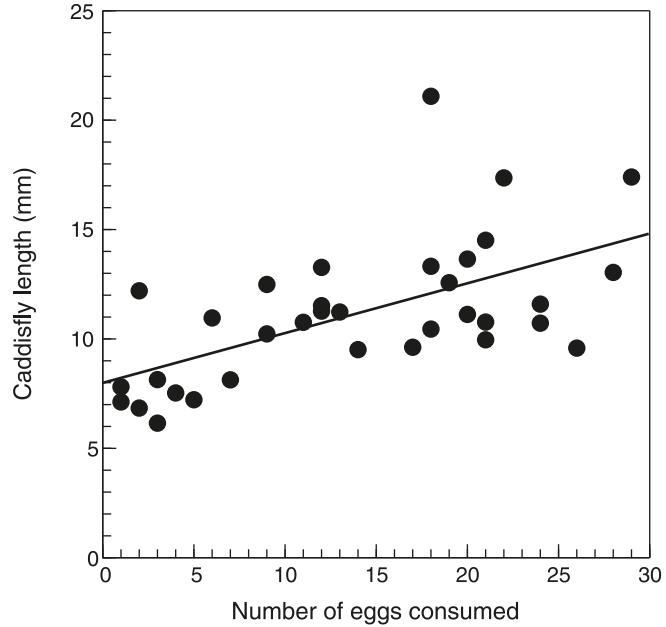


**Fig. 2.** The caddisfly *Limnephilus flavastellus* with access to an alternative food source (detritus) consumed a similar number of eggs of Rough-skinned Newts (*Taricha granulosa*) (eggs consumed =  $14.72 \pm 2.02$ ) as *L. flavastellus* that had access to eggs only (eggs consumed =  $12.94 \pm 2.12$ ) (Student's  $t$  test,  $t = -0.61$ ,  $P = 0.55$ ). Values are means  $\pm$  SE.



indicate that they are opportunistic scavengers and predators of animal matter (Brusven and Scoggan 1969; Gallepp 1974; Wissinger et al. 1996). Adult caddisflies reared exclusively on a diet of detritus are often smaller and have more wing

**Fig. 3.** Regression of the number of eggs of Rough-skinned Newts (*Taricha granulosa*) consumed by individual caddisfly *Limnephilus flavastellus* versus the initial length of *L. flavastellus* in the egg-only and egg + detritus treatments (treatments were combined because no significant difference in number of eggs consumed was detected). Larger *L. flavastellus* consumed more eggs over the course of the experiment than smaller *L. flavastellus* ( $F_{[1,32]} = 17.94$ ,  $R^2 = 0.359$ ,  $P < 0.001$ ). Values are means  $\pm$  SE.



abnormalities than field-caught specimens (Anderson 1976), and experimental evidence indicates consumption of animal matter is important for normal growth and development. Anderson (1976) conducted laboratory experiments on the growth of caddisfly larvae fed diets of detritus and detritus supplemented with animal matter. He found that supplementing animal matter reduced developmental time and yielded 50% larger larvae compared with caddisflies fed detritus only. Majecki and Majecka (1996) demonstrated that growth of the larval caddisfly *Oligotricha striata* (L., 1758) was accelerated when they consume eggs of European Frogs (*Rana temporaria* L., 1758) compared with *O. striata* fed detritus only from their natural habitat. This increase in growth and decrease in developmental time was attributed to additional protein or lipid resources in the supplemented food, and alternative foods containing these compounds are likely important to caddisfly growth in natural environments (Anderson 1976; Cummins and Klug 1979).

Both lipids and protein are major components of amphibian eggs (Duellman and Trueb 1994), and caddisfly larvae have been observed eating the eggs of several amphibian species (Murphy 1961; Dalrymple 1970; Rowe et al. 1994; Majecki and Majecka 1998; Richter 2000). At our study site, newt populations can reach local densities of 6000 individuals per pond (Isaac and Bond 1963; Smith 1967), and a single female may deposit 400–650 eggs (Hanifin et al. 2003). Although sex ratios of *T. granulosa* from our study site are unknown, Janzen and Brodie (1989) documented a male:female sex ratio of 3.8:1 at a pond in Lane County, Oregon, with many more females present on land that had not yet entered

the pond. At our study site, if 2000 females lay, on average, 500 eggs each, then one million eggs would be deposited in a single year in a single pond. This massive reproductive output by newts is temporally limited, typically occurring from March through July (Nussbaum et al. 1983; B.G. Gall, personal observation). The final stage of rapid caddisfly growth (when larvae are large and consume the greatest number of eggs) coincides with newt oviposition, and ingestion of protein-rich foods during this phase is particularly important for proper development (Anderson 1976; Anderson and Cummins 1979; Cummins and Klug 1979). Moreover, caddisflies are very dense at this locality, possibly as many as three quarters of a million animals populating each pond. If each animal consumes, on average, 10 eggs, then as many as 30 times the reproductive output of the newt population could be consumed in a single season. Newt eggs are clearly not available to caddisflies in the same manner used in our experiments; newts attach their eggs to vegetation and may fold leaves around them (Miaud 1994). Nevertheless, caddisflies are likely major predators of newt eggs.

Although functioning in the last stage of a predation event, toxicity has the potential to reduce the number of predators capable of consuming a particular prey species. However, antipredator defenses are unlikely to deter all potential predators and even extremely toxic *Taricha* eggs are vulnerable to predation. The abundance of caddisflies, apparent importance of protein-based food resources on growth, and the continued growth and development after consuming tetrodotoxin indicate that caddisflies are major predators on the toxic egg stage of *T. granulosa*.

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