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PRIMARY RESEARCH PAPER

The effect of prey density on the developmental time of larvae of an aquatic beetle: *Tropisternus setiger* (Insecta, Coleoptera: Hydrophilidae)

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Abstract Predaceous larvae of the water scavenger beetle *Tropisternus setiger* (Germar) are common inhabitants of variable environments in which prey availability may vary widely. We conducted laboratory experiments to assess the effect of prey density on developmental times and survivorship of the preimaginal stages of *T. setiger*. We also examined the effect of the number of consumed prey on the larval size of instar III. Four different prey densities (one, two, four, and eight preys a day) were tested and both developmental time and survivorship differed significantly among them. Larvae fed one or two preys daily showed a longer developmental time and a lower survivorship than larvae fed four or eight preys a day. Moreover the consumption of four preys a day increased larval developmental success, and to consume one prey a day affected survivorship through the larval period. On the other hand, prey density had no effect on the final larval size.

Keywords *Tropisternus setiger* · Hydrophilidae · Prey density · Developmental time · Survivorship · Larval size

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Introduction

Developmental time is an important factor in the life history of an organism, and in predators it may be strongly influenced by prey density (Peckarsky, 1984). In natural environments the number of prey consumed by a predator may be related, among other factors, to the availability of prey in the habitat. Under laboratory conditions several experiments have shown that prey density becomes an indicator of the quantity of consumed food (Quiroz-Martínez & Badii, 1990; Allan, 1995; Elliot, 2003).

Previous studies suggest that the amount of ingested food strongly affects the duration of larval instars and the survivorship of insects (Chapman, 1998; Amalraj & Sivagnaname, 2005; Mikolajewsky et al., 2005). In some species, it may also affect their morphology (Chapman, 1998; Mikolajewsky et al., 2005). The main

purpose of this study was to analyze, under laboratory conditions, the effect of prey density on the developmental time, survivorship, and larval size of the water scavenger beetle *Tropisternus setiger* (Germar) (Hydrophilidae: Coleoptera). Larvae of *T. setiger*, as those of most hydrophilids, are obligate predators (Oliva et al., 2002). They undergo three larval instars and a short pupal stage before completing their development (Fernández & Domizi, 1983; Archangelsky, 1997). Information on larval morphology of this genus, and of the Hydrophilidae family, can be found in Archangelsky (1997) and Archangelsky et al. (2005). Hydrophilids are important in aquatic and riparian habitats, and in South American urban landscapes *Tropisternus* is one of the most common genera found in permanent ponds and urban rain pools (Fontanarrosa et al., 2004; Fischer et al., 2000a). Furthermore *Tropisternus setiger* has been recorded several times in habitats of fluctuating environmental conditions such as temporary pools (Fischer et al., 2000a; Campos et al., 2004) in which prey availability may vary widely.

Even though some ecological studies of *T. setiger* have been performed (Fernández & Domizi, 1983; Balseiro & Fernández, 1992), there are no studies addressing the effect of variable prey density on the development of this South American species. Hosseinie (1976) studied the effect of the amount of food on a North American species of this genus, *T. lateralis nimbatus* (Say), and Fischer et al. (2000b) studied the developmental time and the survivorship of the South American species *T. lateralis limbatus* (Brullé).

Materials and methods

The specimens used for the study were collected in two localities: Laguna La Zeta (42°53′ S, 71°21′ W) and Aldea Escolar (43°08′ S, 71°32′ W), both in the Futaleufú Department (Chubut province, Argentina). The study was performed during the month of January 2004. Seventy adults of *T. setiger* were collected with a D-frame net, 10 additional egg cases were also gathered at the same sites. Adults were kept in small aquaria and fed on commercial fish food; egg cases were removed daily. Each egg case was placed in a small container (tissue culture plates with 6 cells, one egg case per cell). The adults kept in the laboratory produced 14 egg cases.

A total of 98 larvae were obtained from the egg cases. After hatching larvae were transferred to individual cells due to their cannibalistic habits. First instars were kept in tissue culture plates with 12 cells (diameter = 2.14 cm, high = 1.8 cm), second and third instars in plates with 6 cells (diameter = 3.5 cm, high = 2.2 cm). Each cell was filled with water (about two thirds of its volume), and each larva had a small stick as support. Larvae of the same egg case were assigned to different treatments. Four treatments were used; these consisted of feeding each larva with a predetermined amount of prey all through its development from larval to prepupal stage. The amounts of prey chosen were 1, 2, 4, and 8 preys a day (T_1, T_2, T_4, and T_8). The number of replicates was: T_1 $N = 26$, T_2 $N = 25$, T_4 $N = 24$, y T_8 $N = 23$.

Two different types of prey were used: chironomid larvae (*Chironomus* sp., Diptera: Chironomidae), and freshwater shrimp (*Hyallela* sp., Amphipoda). The type and size of prey offered each day was similar for each larval stage in all treatments (the prey size was adjusted to each of the three larval stages of *T. setiger*). For each larva the number of ingested, dead, and live prey was controlled every day. Day of moulting was also registered. Once larvae reached the prepupal stage they were fixed in boiling water and stored in 75% ethyl alcohol in order to take measures of different sclerotized regions of the body. Ten larvae of each treatment were measured; the measures taken were the following: length of head capsule along midline (in dorsal view), length of antennae, length of metatibiotarsus and length of metafemur.

Larvae were kept in the laboratory at room temperature; therefore the photoperiod and temperature regime was similar for all treatments and all larvae.

Variation of developmental times (in days) for each prey density was analyzed with one-way ANOVA. Tukey's tests (unequal *N*) were used to perform *a posteriori* contrasts when a significant

response was found with ANOVA ($P < 0.05$). The parametric assumption of the homogeneity of variances was checked and met (Levene's Test for homogeneity of Variances; Sokal & Rohlf, 1980). Normality assumption was also tested but in one case even though data were transformed they did not fit to normal distribution (Kolmogorov–Smirnov's Test). Nevertheless we considered that the validity of ANOVA is only slightly affected even by considerable deviations from normality, especially as N increases (Zar, 1996).

Survivorship for each larval stage was calculated using the number of individuals molting to the next stage.

In order to see if the survivorship at the end of the larval development and in each stage was independent of prey density, a χ^2 Test for k independent samples was performed. This same test was used to compare survivorship between first, second, and third larval stages of larvae under the same prey treatment.

On the other hand, since data did not conform to the assumption of homogeneity of variances, we compared morphometric data using Kruskal–Wallis's Test (non-parametric statistics; Siegel, 1994).

All analyses were performed using the statistical package STATISTICA 5.1. ('98) and STATISTIX 1.0 ('96).

Results

Ingested prey

The number of ingested prey was considered equivalent to the amount of food-ingested daily (Table 1).

Developmental time

Larvae fed on 8 preys a day (T_8) completed their development faster than larvae of other treatments. They were followed by larvae fed 4 preys a day (T_4), the latter were followed by larvae fed 2 preys daily (T_2), and the last to finish their development were those larvae fed 1 prey a day (T_1). The information is summarized in Table 2.

Table 1 Mean ± standard deviation of consumed prey by each larval stage of *T. setiger*

Prey density	Instar I	Instar II	Instar III
1	0.98 ± 0.06 (11)	1 ± 0.0 (9)	1 ± 0.0 (1)
2	1.95 ± 0.17 (12)	2 ± 0.0 (10)	2 ± 0.0 (9)
4	3.74 ± 0.34 (19)	3.89 ± 0.20 (16)	4 ± 0.0 (15)
8	6.8 ± 0.96 (15)	7.5 ± 0.62 (13)	7.98 ± 0.03 (11)

The number of larvae present in each instar is written between brackets

Kolmogorov–Smirnov's Test showed that only data from the first larval instar were not normally distributed (Instar I: d = 0.26, $P < 0.01$; Instar II: d = 0.138, P = n.s.; Instar III: d = 0.121, P = n.s.; Total larval development: d = 0.126 P = n.s). Results from Levene's Test were not significant in any case ($P > 0.15$).

One way ANOVA indicated that prey density had a significant effect on the duration of all larval instars and the total developmental time (Instar I: F = 16.8, $P < 0.001$; Instar II: F = 100.1, $P < 0.001$; Instar III: F = 66.3, $P < 0.001$; Total larval Development: F = 135.5, $P < 0.001$).

Tukey comparisons indicated that the mean of the developmental time of first instar larvae fed one prey a day was significantly longer than that of the other treatments ($P < 0.003$). The developmental time of first instar larvae fed two, four, and eight preys daily did not show significant differences among themselves ($P > 0.23$).

Second instar larvae under T_1 did show a significantly longer development than those larvae under T_2 (Tukey's Test, $P < 0.001$). Additionally, the duration of the second instar of larvae fed one and two preys a day was significantly longer than those of larvae fed four and eight preys daily (Tukey's Test, $P < 0.001$). No significant difference between larvae of T_4 and T_8 was observed (Tukey's Test, $P > 0.55$).

Developmental times of third instar larvae were significantly different among T_2, T_4, and T_8 treatments (Tukey's Test, $P < 0.001$). Data of T_1 were not analyzed because only one-third instar larva reached prepupal stage. While carrying out the ANOVA between the developmental times of third instar larvae and total developmental time, T_1 was excluded since we only had a single data.

Table 2 Mean ± standard deviation of developmental time (days) of the three larval instars and total larval development of *T. setiger* for each treatment

Larval instar	1 prey	2 prey	4 prey	8 prey
Instar I	4.3 ± 0.8 (11)	2.7 ± 0.9 (12)	2.6 ± 0.7 (19)	2.1 ± 0.6 (15)
Instar II	8.2 ± 0.8 (9)	5.8 ± 0.9 (10)	3.6 ± 0.6 (16)	3.2 ± 0.6 (13)
Instar III	24 ± 0.0 (1)	17.8 ± 1.9 (9)	13.5 ± 1.9 (15)	8.5 ± 1.4 (11)
Development*	38 ± 0.0 (1)	27.1 ± 1.9 (9)	21.1 ± 1.8 (15)	14.9 ± 1.1 (11)

*Total developmental time. The number of larvae present in each instar is written between brackets

Nevertheless it is important to point out that even though the differences could not be statistically analyzed, this single larva (third instar of T_1) finished its development several days after the other three treatments.

Tukey comparisons indicated that when larvae finished their development, total developmental times were significantly different among all treatments included in the analysis (T_2, T_4, and T_8, $P < 0.001$).

Survivorship

Survivorship rate of *T. setiger* larvae varied among the four treatments (Table 3). The highest survivorship values are those of larvae in T_4, followed by larvae of T_8, T_2, and T_1 respectively. Significant differences in survivorship could be seen between larvae of T_1 and those of the remaining treatments ($\chi^2_{(3)}$, $P < 0.001$); among larvae of T_2, T_4, and T_8 no significant differences were observed.

Within first instar larvae those of T_4 showed a significantly higher survivorship than those larvae of T_1 and T_2 ($\chi^2_{(3)}$, $P < 0.01$); no significant differences were observed between larvae of T_4 and T_8, neither among larvae of T_1, T_2, and T_8 ($\chi^2_{(3)}$, $P > 0.05$).

Survivorship of second instar larvae was high in all treatments, and no significant differences were observed ($\chi^2_{(3)}$, $P > 0.73$).

In third instar, larvae under T_1 showed a very low survivorship, significantly lower than those of the other three treatments ($\chi^2_{(3)}$, $P < 0.001$).

In the same treatment, when comparing the survivorship in each stage, the analysis showed that only the treatments with low prey density (T_1, T_2) had significant differences among larval stages. Larvae of T1 presented low survivorship values for the first and third instars, while those of the second instar were significantly higher ($\chi^2_{(2)}$, $P < 0.01$). For T_2 only the first instar showed a low survivorship rate, while there was no significant difference between second and third instars ($\chi^2_{(2)}$, $P < 0.01$).

Morphometrics

A Kruskal–Wallis analysis for the morphometric measures did not show any significant difference among the four treatments. Mean values of these variables are shown in Table 4.

Discussion

The results of this study clearly show that larvae of *T. setiger* reared at low prey densities have a longer developmental time (Table 2). This is consistent with the results of Hosseinie (1976) when he reared larvae of *Tropisternus lateralis nimbatus*. Hosseinie (1976) observed that lower

Table 3 Survivorship rate of *T. setiger* larvae when reared on different prey densities

Larval instar	1 prey ($N = 26$)	2 prey ($N = 25$)	4 prey ($N = 24$)	8 prey ($N = 23$)
Instar I	0.42 (11)	0.48 (12)	0.79 (19)	0.65 (15)
Instar II	0.82 (9)	0.83 (10)	0.84 (16)	0.86 (13)
Instar III	0.11 (1)	0.9 (9)	0.94 (15)	0.85 (11)
Development *	0.04 (1)	0.36 (9)	0.62 (15)	0.48 (11)

*Total developmental time. The number of larvae present in each instar is written between brackets

Table 4 Measurements of selected morphological structures. The number of measured specimens is written between brackets

	Mean ± standard deviation (mm)
Head capsule	1.15 ± 0.05 $_{(40)}$
Antenna	1.12 ± 0.07 $_{(40)}$
Femur	1.08 ± 0.04 $_{(40)}$
Tibiotarsus	0.80 ± 0.03 $_{(40)}$

amounts of food increased the developmental time of each larval stage, and concluded that larvae need to reach a predetermined physiological state before moulting into pupa. In agreement with Hosseinie's observations (1976), our results also indicate that larvae of *T. setiger* needed to attain a certain physiological state (or size or weight) before moulting into the next larval instar or becoming prepupae. Furthermore, both experiments suggest that larvae reared at low prey densities will require more time to reach this physiological state.

Only one larva of T_1 was able to reach the prepupal stage (survivorship of 4%), suggesting that the amount of nutrients provided by just one prey a day are not enough to complete the life cycle successfully (Table 3). Conversely larvae of treatments T_2, T_4, and T_8 were able to finish their larval development successfully, and no significant differences in survivorship were observed. Nevertheless, the developmental time increased inversely to the amount of food ($T_2 > T_4 > T_8$) (Table 2).

First instar larvae of T_4 had a significantly higher survivorship than those of T_1 and T_2. On the other hand, there is no significant difference between larvae of T_1 and T_2 with those of T_8. This suggests that some other factor is affecting the survivorship of first instar larvae of T_8. This has been reported for other aquatic insects; for example *Aedes aegypti* larvae reared with excess of food in the laboratory showed a lower larval survival (Arrivillaga & Barrera, 2004). In our experience overcrowding could be a possible cause for this lower survival. First instar larvae were placed in smaller containers and since *T. setiger* larvae respond to tactile stimuli, a higher density of prey could prevent larvae of *T. setiger* from feeding undisturbed (larvae already feeding may drop the prey and try to catch another in response to tactile stimuli, personal observations). Nevertheless more detailed studies should be designed in order to investigate the feeding behavior of these larvae under stressful conditions (prey overcrowding). On the other hand, with the available information we cannot rule out the possibility that a higher growth rate could be the cause of this higher mortality as suggested by Nylin & Gotthard (1998) for Lepidoptera larvae.

In contrast with our expectations, the morphometric data of the last larval instar did not show any differences among the four treatments. This means that the amount of ingested food does not affect body size (at least at the level of sclerotized structures). Therefore, the response of *T. setiger* larvae to lower amounts of food seems to be an increment in the developmental time rather than a change in the final larval size. Similar responses have been observed in other insect species (Janz et al., 1994; Nylin & Gotthard, 1998). Conversely, this fact conflicts with the preliminary results of Hosseinie (1976) with *T. lateralis nimbatus*, in which a slight reduction in size was observed.

This study supports the fact that prey density influences the duration of each larval instar, the total developmental time, and the survivorship of *T. setiger* larval stages. In opposition, it does not affect the size of the last larval instar or prepupa. Even though there are other factors that could affect larval development such as temperature and photoperiod (Sweeney, 1984; Thomas, 1993; Nylin & Gotthard, 1998; Zilahi-Balogh et al., 2003; Barahona et al., 2005), as these other factors were equal for all reared specimens we can assume that they were not significant in this study.

These beetles are easy to rear; they also have a short life cycle, which makes them an interesting subject for laboratory studies. The results of this study can be applied to several activities that require laboratory rearing of this species (or of related species) such as behavioral or ecological studies, since the optimal prey density for the development and survivorship of this species seems to be 4 preys a day.

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