Lethal and sublethal costs of autotomy and predator presence in damselfly larvae

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Abstract We studied the costs of lamellae autotomy with respect to growth and survival of Lestes sponsa damselfly larvae in field experiments. We manipulated predation risk by Aeshna cyanea dragonfly larvae and lamellae status of L. sponsa larvae in field enclosures and compared differences in numbers, size and mass of survivors among treatments. In the absence of a free-ranging A. cyanea larva, about 29% of the L. sponsa larvae died. This was probably due to cannibalism. The presence of a free-ranging A. cyanea reduced larval survival by 68% compared to treatments in which it was absent or not permitted to forage on L. sponsa damselflies. Across all predator treatments, lamellae autotomy reduced survival by about 20%. The mean head width and mass of survivors was lower in the enclosures with a free-ranging A. cyanea compared to the other two predator treatments. This suggested that larvae grew less in the presence of a free-ranging predator, indicating that increased antipredator behaviours were more important in shaping growth responses than reduced population density. Mass, but not head width, of survivors was also reduced after autotomy. The fitness consequences of these effects for the adults may be pronounced. In general, these field data strongly suggest that lamellae autotomy affects population regulation of damselflies.

Key words Autotomy · Growth · Lestes sponsa · Predation · Survival

Introduction

Autotomy, the amputation of a body part at a breakage plane (McVean 1975), is a well-known escape mechanism both in vertebrates and invertebrates (see references in Althoff and Thompson 1994). Costs of autotomy have largely been inferred from staged encounters with predators (e.g. Dial and Fitzpatrick 1984) and growth experiments (e.g. Althoff and Thompson 1994) under artificial laboratory conditions. Although the costs of autotomy seem obvious, demonstrating their relative importance has been problematic because the underlying mechanisms are often indirect, and compensatory behavioural mechanisms are at work (e.g. Formanowicz et al. 1990). After autotomy, animals may for example shift microhabitat use and reduce their activity levels (e.g. Formanowicz et al. 1990; Martin and Salvador 1992). The form and magnitude of a behavioural response can be modified in the laboratory, distorting our measures of the consequences to components of fitness (Skelly 1992). Moreover, lethal effects of predators in the field may decrease population densities and hence facilitate growth of survivors by reducing density dependence (e.g. Morin 1983). Simple estimates of the costs in laboratory conditions may therefore not reveal true fitness costs in nature. Knowledge of these costs is, however, very important, at both the individual and population level. Theoretical models predict costs when non-lethal injuries are density dependent and when they reduce survival and/or reproduction (Harris 1989). Nevertheless, very little is known about the real costs of autotomy under natural conditions in invertebrates (Smith 1995).

Larval damselflies are characterized by the presence of three leaflike caudal lamellae. These can be autotomized at breaking joints when they are grasped by a predator (Legrand 1974). After autotomy, damselfly larvae try to compensate directly by reducing their activity level (Stoks 1998b) and in the long term by regenerating their lamellae (e.g. Robinson et al. 1991).
Identified costs of lamellae loss in damselflies include increased vulnerability to cannibalism and heterospecific predation, and reduced foraging rates (Robinson et al. 1991; Stoks 1998b). These costs, however, have only been examined in experimental laboratory conditions. In the field, no costs of autotomy under natural conditions have been examined in damselflies. More generally, no lethal costs of autotomy in invertebrates have been found in the field (Smith 1995), and non-lethal costs have not been studied in the field.

In this study, we tested the potential for lethal and non-lethal costs of autotomy by manipulating the lamellae status of the larval damselfly *Lestes sponsa* Hansmann under field conditions. Members of this genus are characterized by their large lamellae (McNeill 1960). Within the genus, lamellae loss is high, with up to 90% of individuals with at least one missing or regenerating lamella and almost 20% missing all three lamellae (Stoks 1998a). *L. sponsa* has a ‘fast’ lifestyle (sensu Johnson 1991). Members of Lestidae can achieve rapid growth due to active searching for prey (Pickup and Thompson 1990; Krishnaraj and Pritchard 1995). Lestid damselfly larvae typically live in small temporary, or otherwise fishless, ponds (e.g. Gower and Kormondy 1963; Lutz 1968; Schneider and Frost 1996). We exposed the damselflies to predation by *Aeshna cyanea* dragonfly larvae because these are important predators within these ponds (e.g. Wellborn et al. 1996; Schneider 1997).

**Materials and methods**

A field experiment to test the lethal and non-lethal impact of predator presence on *L. sponsa* growth was conducted in Fen 34 at the Groot Schietveld, Brasschaat (northern Belgium). This fen is very homogeneous and is dominated by the macrophyte *Juncus bulbosus*. *L. sponsa* is very abundant in these fishless fens.

The experimental design included two levels of lamellae status crossed with three levels of predation risk, each replicated four times. Experimental units were cylindrical enclosures (25 cm diameter, 1 m high). These had a frame of coated iron wire (mesh: 8 x 4 cm) covered with nylon netting (1-mm mesh size). The enclosures were sealed at the bottom with a plastic dish containing approximately 4 cm of sediment. The tops of the enclosures extended 20 cm out of the water and were uncovered. All enclosures were linearly arranged in the fen at the same depth. Inside the enclosures we added the macrophyte *J. bulbosus* from the same fen in natural densities to provide a natural structural heterogeneity. Similar enclosures have been shown to mimic natural conditions for damselfly larvae (McPeek 1990, 1998). Twenty-four enclosures were put in place on 27 May 1998.

Five days later, on 31 May, damselfly larvae were added. This time lag allowed prey items like copepods, branchiopods, midge larvae and small water bugs to colonize the cages. Twenty penultimate instars of *L. sponsa* were placed in each enclosure. Larvae were collected from the very similar ‘Blokvren’, about 200 m south of Fen 34, to avoid disturbance of the aquatic vegetation around the cages. Only larvae with three unregenerated thrice lamellae were used. We randomly assigned larvae to one of the two groups, with or without lamellae. In animals from the latter group, all three lamellae were removed by gently pulling them with two fingers until the animals autotomized these appendages. Animals of the former group were handled similarly, but lamellae were not removed. After lamella removal, larvae were given 30 min to recover before they were placed in the enclosures. One day later on 1 June, we collected dragonfly larvae at Fen 8, about 2 km east of Fen 34, where they were particularly abundant, and added them to the appropriate treatments. Predation risk was manipulated by introducing none, one encaged or one free-ranging penultimate *A. cyanea* dragonfly larva to each enclosure. In the encaged treatment, dragonfly larvae were placed in a cylindrical cage (8 cm diameter, 18 cm long) with a coated iron frame (mesh size 1 cm) covered with nylon netting (mesh size 1 mm). We included two penultimate *Enallagma cyathigerum* damselfly larvae within the cage as food.

After 11 days, the contents of the enclosures were brought to the laboratory and sieved. All dragonfly larvae were recovered alive. All remaining damselfly larvae had moulted towards the ultimate instar. They were immediately frozen. Afterwards, we determined their head width with a dissecting microscope fitted with an ocular micrometer (to the nearest 0.05 mm). Head width is a general measure of size in damselfly larvae (e.g. Dixon and Baker 1988). Dry weight was measured after drying the larvae in a 60°C drying oven for 24 h.

Because the three responses measured at the end of the experiment (number, head width and mass of survivors) may be correlated, we used multivariate analysis of variance (MANOVA) to detect overall effects of predation treatments on response vectors. The number of survivors was not transformed, while mass was log transformed and head width square root transformed to meet assumptions of homoscedasticity of variances and normality of errors. We used the means of individual responses for each enclosure as a measure of the response of that experimental unit, to avoid the lack of independence of individual measures and thus pseudo-replication (Hurlbert 1984). We used univariate analysis of variance (ANOVA) to determine which specific responses accounted for overall differences among treatments in the MANOVA. Because any negative effect of the predator on final mass can be the result of *A. cyanea* preferentially eating large larvae, we also tested for a predator effect on mass by standardizing to a given head width with an ANCOVA (head width as covariate) (Johnson et al. 1984; Pierce et al. 1985; McPeek 1990, 1998). First, we tested for differences in slopes among treatments (the parallelism assumption of ANCOVA). Only if no significant differences are found among the slopes are tests for differences in midpoints of the relationships justified (Sokal and Rohlf 1995).

**Results**

The MANOVA showed that both predation risk and autotomy had significant additive effects on larval *L. sponsa* (Table 1). Below we interpret the results using the univariate tests.

**Number of surviving larvae**

There was no difference in numbers of larvae surviving in the no *A. cyanea* and the encaged *A. cyanea* treatment (ANOVA, contrast testing: $F_{1,18} = 3.07, P = 0.097$; Fig. 1).

| Table 1 MANOVA on the response vector including the numbers, size and mass of larval *Lestes sponsa* that survived the field experiment |
|---|---|---|---|
| Source | df | Wilks’ lambda | $P$ |
| Predation | 6,32 | 0.107 | $<0.001$ |
| Autotomy | 3,16 | 0.523 | 0.014 |
| Predation × Autotomy | 6,32 | 0.738 | 0.52 |