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LIFE HISTORY PLASTICITY IN A DAMSELFLY: EFFECTS OF COMBINED TIME AND BIOTIC CONSTRAINTS

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Abstract. Optimal values for life history traits are expected to depend upon environmental conditions during development and the period within which development is constrained (e.g., biotic factors and time constraints, respectively). Theory predicts that life history responses to both biotic factors and time constraints may be both direct and behaviorally mediated. Few experimental studies of life histories have considered the joint effects of biotic factors and time constraints, and fewer still have been able to disentangle direct from behaviorally mediated effects. We studied such interactions by manipulating the perceived time to the onset of winter, predation risk, and food resources level in larvae of the damselfly *Lestes sponsa.* In the first experiment (predation × time constraint), the presence of a predator caused an overall reduction in foraging activity, development rate, and mass at emergence. However, larvae that had less time available before the end of the season, increased foraging activity and development rate, while mass at emergence decreased. These results suggest that the observed changes in life history characters were behaviorally mediated in the presence of predators. In contrast, life history responses of time-constrained larvae occurred independently of the behavioral changes and, therefore, were direct. In the second experiment (food level × time constraint) larvae under high food levels had a higher foraging activity, increased development rate, and higher growth rates, compared to low food-level treatments. Time-constrained larvae accelerated development and had a smaller mass at emergence at high food levels than larvae that were not time constrained. In contrast, and opposite to predictions, time-constrained larvae at low food levels had the slowest development rate and the largest mass at emergence. We suggest that larvae in the latter group were aiming to delay emergence to the next season (cohort splitting). Our results suggest that both behaviorally mediated and direct responses to biotic factors and time constraints are a feature of the life history of this damselfly.

Key words: damselflies; development; food resource; growth rate; Lestes sponsa; *life history; mass at metamorphosis; phenotypic plasticity; predation risk; time constraints; trade-offs.*

INTRODUCTION

The age and size at maturity of most organisms is plastic and highly variable. Given the importance of age and size to fitness, understanding this variation has become a principal focus of life history studies (Roff 1992, Stearns 1992, Charlesworth 1994). Most explanations rely on variation in the balance between the advantages of conflicting goals of reaching maturity at an early age, and achieving large size. Typically, early maturation will reduce cumulative prereproductive mortality, but comes at the cost of reduced fecundity associated with a smaller size. Clearly, the optimal balance between these conflicting demands will depend on the ambient levels of resources and predation risk. A great deal of theory and tests address the role of these two variables in determining optimal age and size at maturity (reviews in Werner and Gilliam 1984, Stearns and Koella 1986, Kozlowski and Wiegert 1987, Berrigan and Charnov 1994, Abrams and Rowe 1996).

It has also become apparent that the seasonal environments in which most organisms live, impose a time constraint on life histories (e.g., Forrest 1987, Nylin et al. 1989, Rowe and Ludwig 1991, Wiklund et al. 1991, Rowe et al. 1994, Abrams et al. 1996). Examples include those amphibians that must metamorphose prior to pond drying, and insects that must reproduce or deposit fat prior to the onset of winter. In general, maturity or metamorphosis early in a season is favored. Empirical studies of amphibians (Semlitsch and Wilbur 1988, Newman 1992, Reques and Tejedo 1997, Laurila and Kujasalo 1999) and insects (Nylin et al. 1989, Leimar 1996, Blanckenhorn 1998, Gotthard 1998, Nylin and Gotthard 1998, Johansson and Rowe 1999, Plaistow and Siva-Jothy 1999), typically demonstrate that organisms do indeed respond to an approaching time horizon by accelerating development rate. Such accelerated development usually comes at a cost to size.

Life history theory has made significant steps toward

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predicting how life history transitions will be jointly affected by both time constraints and biotic factors (Ludwig and Rowe 1990, Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams and Rowe 1996, Abrams et al. 1996). There are numerous studies showing that manipulations of resources and predators, do affect these life histories (Solbreck et al. 1989, Skelly and Werner 1990, Peckarsky et al. 1993, Ball and Baker 1996, Weeks and Meffe 1996, Hechtel and Juliano 1997, Anholt and Werner 1998). Studies that have included time constraints and at least one biotic factor are, however, very few (Reques and Tejedo 1997, Blanckenhorn 1998, Laurila and Kujosalo 1999, Plaistow and Siva-Jothy 1999). Therefore, a series of experiments testing current theory are called for.

A second significant step in life history theory is the inclusion of adaptively flexible growth rates, in the formulation of optimal strategies (e.g., Rowe and Ludwig 1991, Houston et al. 1993, Werner and Anholt 1993, Abrams and Rowe 1996, Abrams et al. 1996). The logic behind this is that life history responses are not only determined directly by environmental factors, but also indirectly through behaviorally mediated shifts, whereby animals adaptively vary their growth rates. These analyses often show that the inclusion of flexible growth rates often changes the predicted effects of various factors (e.g., predation risk) on life history traits. At the least, both behavioral and life history traits are expected to respond independently to these factors and disentangling their effects is often instructive. An often-observed example is the indirect, behaviorally-mediated decrease in size at maturity in the presence of predation risk (Skelly and Werner 1990, Peckarsky et al. 1993, Ball and Baker 1996). Here, the entire effect of predators can be attributed to the reduced growth of prey, resulting from reduced foraging at high predation risk. To understand and disentangle direct and behaviorally mediated responses to time constraints and biotic factors, experiments that include behavioral assays are needed. Little is also known about how a combination of time constraints and a biotic factor affects behavioral traits, and hence potentially mediates indirect life history effects. So far, only Laurila and Kujasalo (1999) have studied behavioral responses under combined conditions and have shown that the effects of time constraints and biotic factors on behavior of tadpole larvae are not additive. Their study did not, however, include direct estimates of foraging effort, and did not consider time constraints in terms of light regimes, which are much more common cues in nature than the pond-drying cue they used.

In our opinion, theory is out ahead of the existing data, and therefore, a series of experiments testing current theory is called for. Most authors have looked at life history responses to time constraints or biotic factors independent of one another. Fewer still have included measurements of behavior. A daunting task for empirical ecologists is to experimentally disentangle the various factors affecting the life history, and to search for some generality. In the current study, we conduct a pair of experiments to determine the effects of time constraints, predation risk, and food level on the life history of a damselfly. Moreover, we conduct frequent detailed behavioral assays of foraging and activity, so that we can distinguish behaviorally mediated from direct responses to the various manipulated factors. Our general a priori predictions are: (1) Time constraints should lead to increased foraging effort and increased development. The accelerated development comes at a cost of reduced size at maturity. (2) Increased predation risk could, according to theory, take two forms (Abrams and Rowe 1996). Individuals could increase foraging effort to escape predation in the larval stage. In that case we would expect accelerated growth and development, and probably a smaller size at maturity. This would be expected if the cost of increased foraging effort is low. If the cost of increased foraging effort is high we would expect reduced foraging effort and reduced size at maturity. (3) Increased resource availability should result in a higher foraging effort (McNamara and Houston 1994), which in turn should result in a higher growth and development rate.

METHODS

We conducted two independent experiments to assess the behavioral and life history responses to predation risk, food level, and an approaching time horizon. In the first, conducted in Belgium, we jointly manipulated predation risk and light regime (photoperiod). In the second, conducted in Sweden, we jointly manipulated food level and light. Considerable effort was made to ensure that these two experiments were conducted in a similar manner, however, some differences remained. Therefore, we treat the results of each experiment independently.

In both experiments, the damselfly *Lestes sponsa* (Hansemann) was the test organism. This species diapauses over winter in the egg stage, has a brief larval period in spring, then emerges and reproduces in the summer (Corbet 1999). Lestids respond to photoperiod with changes in life cycle length (Lutz 1968). Therefore, lestids that have an obligate univoltine life cycle, like *L. sponsa*, are ideal organisms to study the effects of time constraints. Moreover, field studies suggest that populations of *L. sponsa* are under intense predation pressure from fish (Macan 1966), and may be food limited (Pickup et al. 1984).

For both experiments, eggs of *L. sponsa* were collected from a small pond (18 m^2) in Belgium, by sampling stems of *Juncus* sp. in which females had oviposited. The eggs were brought to the laboratory where they were hatched by immersing the stems in water, at either ''late season'' or ''early season'' light regimes (see *Experiment* 1, below, for details on the light regimes). Hatching began two to four days after immersing, and larvae for each experiment were collected at day 10. For each experiment, day 10 was set to day 0 of the experiment. Food levels for appropriate development without mortality effects were based on pilot experiments and prior studies (Lawton et al. 1980, Baker 1982).

Experiment 1: time constraint and predation risk

In the first experiment, hereafter referred to as the ''predation experiment,'' we determined the effect of a time constraint (early- and late-hatching larvae) and predation risk (nonlethal predator absent and present) on behavioral and life history variables in a 2 × 2 factorial design. Eggs were collected on 29 March. In the laboratory, they were haphazardly divided into two groups, with each group allocated to six plastic containers (32 × 15 × 15 cm) filled with 1.5 L of aged tap water. Each of the two groups of six hatching containers were transferred into one of two walk-in climate rooms, which differed in light regime. Temperature was 20°C ± 1°C. Light regimes in the two rooms were set to simulate those that would be experienced by larvae hatching early and late in the growing season at the site of egg collection in Belgium. The starting photoperiod for the early treatment was set at 15 March (lights on at 0658 and off at 1847) and the late treatment at 1 June (0435–2047). Photoperiods were thereafter adjusted weekly in the climate rooms to simulate the natural progress of the light cycle. To minimize potential confounding physical differences between the two climate rooms, all larvae and their respective light condition were rotated between climate rooms on a weekly basis.

Eggs began hatching after 2 d. After 10 d, a total of 50 larvae were collected haphazardly from the six hatching containers in each light regime. These were individually transferred to transparent plastic cups (15 × 10 × 11 cm height) containing 150 mL of water. Larvae were held and observed in the plastic cups until emergence. We further grouped cups into tanks that could hold our predators. Groups of five cups were kept floated with styrofoam within a larger plastic tank (80 × 40 × 25 cm) filled with 64 L of aged tap water. Therefore, at each light regime, there were 10 tanks. Of these 10 tanks, five were randomly assigned to each of the predator treatments. Water in all tanks was replaced every two weeks and all tanks were permanently aerated with air stones. To facilitate emergence, a piece of plastic netting was placed in each cup a few days prior to emergence. Hence, of the 50 larvae in each light treatment, 25 were reared at each predator level, and therefore, each cell in this 2 × 2 factorial design was replicated 25 times.

We used perch (*Perca fluviatilis*) as a predator. Odonate larvae are part of the natural prey of perch (Rask 1986), and *L. sponsa* often co-occurs with it (F. Johansson, *unpublished manuscript*). In each light regime, the five predator tanks contained two perch (mean ± 1 SE, 10.5 ± 1.0 cm; $n = 10$). Perch were fed chironomids three times a week and larval *L. sponsa* once weekly. A square piece of plastic netting 2 cm on a side replaced part of the bottom of each cup. Hence, in the predator treatment, larvae received visual and chemical cues of feeding perch (through the plastic netting). The remaining five tanks lacked any cues indicating presence of perch.

All larvae were fed laboratory-reared (newly hatched) brine shrimp, each Monday, Wednesday and Friday. Mean number of shrimp were 199.3 (± 9.9 shrimp per ration), based on 10 randomly chosen samples. From day 80 of the experiment, larvae were instead fed a ration of 20 field-collected *Daphnia* sp. (2.37 ± 0.12 mm; $n = 10$) 5 d/wk until emergence. We increased food ration because larger larvae require more food. Increasing brine shrimp densities with the growth of the larvae would be ineffective, because brine shrimp survive only for a couple of hours in fresh water, and *Lestes* larvae could not consume enough brine shrimp during this short survival period to maintain growth and development.

Experiment 2: time constraint and resource level

In this experiment, hereafter referred to as the ''resource experiment,'' we determined the effect of a time constraint (early and late hatching larvae) and food levels (low and high) on behavioral and life history variables in a 2 × 2 factorial design. Eggs were collected on 20 March. They were brought into the laboratory where they were haphazardly divided into two groups of five hatching containers (height 13 cm, diameter 32 cm) containing 4 L of water. Each group of five was thereafter transferred to one of two walk-in climate rooms, which differed in light level. Temperature was 20° ± 1°C. Light regimes in the two rooms were set exactly as those in the predation experiment.

Fifty larvae in each light regime were individually transferred to plastic containers (height 16 cm, diameter 18 cm) containing 250 mL of water. The water in these containers was exchanged every two weeks. Larvae were fed laboratory-reared (newly hatched) brine shrimp until day 80 of the experiment, and thereafter they were fed field-collected *Daphnia pulex* (2.10 ± 0.05 mm; $n = 20$) until emergence. High-food larvae were fed brine shrimp every day, and low-food larvae were fed shrimp every second day. A mean of 127 ± 7.4 shrimp were given per ration, based on 10 randomly chosen samples. High-food larvae were fed with 40 *Daphnia*/d, and low-food larvae with 20 *Daphnia*/d, respectively, until emergence. Mean mass of one brine shrimp and one *Daphnia* was 9.7 μg and 130 μg, respectively. Of the 50 larvae in each light regime, 25 received low food levels and 25 received high food levels. Hence, each cell in the 2 × 2 factorial design was replicated 25 times. As in the predation experiment, photoperiod was adjusted weekly, and treatments and organisms were rotated between climate rooms each week.

TABLE 1. Factor loadings of the behavioral traits of *Lestes sponsa* on the first two and three principal components for the two experiments: time constraint–predation and time constraint–resource.

	Loadings (predation)		Loadings (resource)		
Behavior	PC1	PC2	PC1	PC2	PC3
Orient	**0.912**	0.286	**0.960**	0.111	0.063
Advance	**0.751**	−0.364	**0.877**	0.039	0.038
Strike	**0.928**	0.253	**0.952**	0.172	0.126
Capture	**0.940**	0.268	**0.963**	0.154	0.052
Success rate	**0.725**	0.295	**0.586**	0.009	−0.447
Walk	···	···	0.054	−0.481	**−0.823**
Distance	**0.713**	**−0.618**	0.484	**−0.810**	0.036
Swim	0.381	**−0.652**	0.050	**−0.791**	0.507

Notes: No walks were performed in the predation experiment; hence this variable was excluded from the analyses. Variables that loaded high are boldface.

Response variables

For each experiment, we measured behavior of larval damselflies at two scales. On a ''fine-grained scale,'' we recorded the frequency of eight behavioral variables over a 15-min observation period. We hereafter refer to these eight variables as the behavioral variables. Observations were conducted on days 25, 50, and 75 of the experiment. The eight behavioral variables were: orient = orientation of the head toward a prey but without moving legs; advance = movement towards a prey by moving one or more legs; strike = strike with the labium towards a prey; capture = successful capture of a prey; success rate = captures divided by strikes; walks = movement not directed towards a prey; distance = total distance (in centimeters) a larvae moved during the observation period; swim = swimming in the water column free of the substrate. Behavioral observations were conducted beginning 15 min after larvae had received their brine shrimp ration. Ten randomly chosen replicate individuals were observed in the predation experiment and eight in the resource experiment.

On a ''coarse-grained scale,'' we estimated activity rate (hereafter referred to as activity) on days 40, 60, and 80. We placed a 2 × 2 cm grid pattern on the bottom (predation experiment) or the top (resource experiment) of the experimental arena, and we noted the position of each larva at intervals of 15 min over a 4-h period (0830–1230). Activity rate was scored as the number of times a larva had moved during the 4-h period (minimum = 0; maximum = 16), and was replicated on each date 15 times in the predation experiment, and 10 or 11 times in the resource experiment. Estimates of activity of predation-experiment larvae and high-food larvae were conducted just prior to daily feeding. For low-food larvae, activity was estimated on days in which no ration was given.

Development time (age at emergence) of larvae was the number of days from the beginning of the experiment until emergence. When larvae emerged, the adults were sexed and weighed. Mass was determined by drying individuals for 48 h at 60°C and then weighing them. Growth rate was calculated as ln (mass at emergence) divided by the number of days until emergence.

Analysis

To reduce the number of variables and the problems associated with their covariance in the behavioral experiment, we analyzed data with Principal Component Analysis (PCA) followed by Analysis of Variance (ANOVA). All behavioral observations were $\ln(x + 1)$ transformed to homogenize variances and were thereafter entered into a single matrix. The PCA was run on the correlation structure among the variables. We tried several rotation methods but chose to use nonrotated axes, as this allowed the most straightforward interpretation. Since 85% of the variance was explained by the first three (for the resource experiment) and first two (for the predation experiment) principal components, we used the scores from these three or two components for further analysis in repeated-measures ANOVAs, with sampling day as the repeated measure. Activity rate (x^2 transformed for the resource experiment) was analyzed with repeated measures (sampling day) ANOVAs. Age and mass (size) at emergence, total number of moults at emergence, and growth rate were analyzed with ANOVAs.

For the predation experiment, larvae were initially blocked for analysis by the larger tanks they were in. However, we have dropped this blocking factor in our presentation of the results, since it was consistently nonsignificant. Initially, we also analyzed both experiments with sex as a factor; however, we did not include it in the final models as it too was consistently nonsignificant. All statistical analyses were performed in SYSTAT (1998).

RESULTS

Predation experiment

Over 90% of the variation in the behavioral variables was accounted for by the first two PC axes (Table 1). The behaviors orient, advance, strike, capture, and success rate were all correlated with PC1, which accounted for 61.7% of the variation. Swim was correlated with

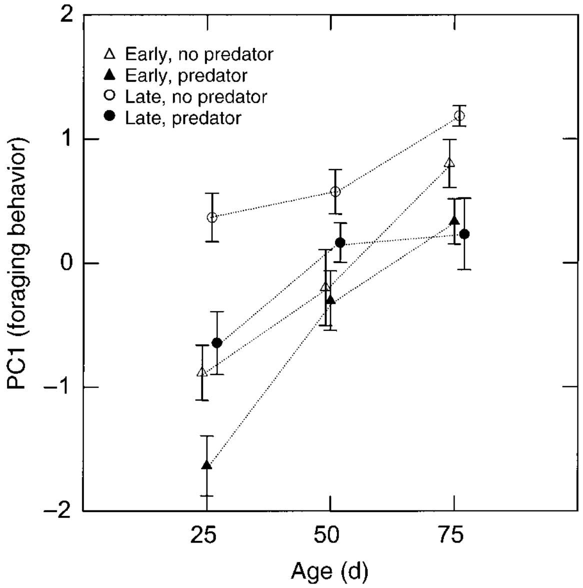

FIG. 1. Mean scores for the foraging behavior PC1 (± 1 SE) against development day for *Lestes sponsa* larvae in the predation experiment (see Table 2).

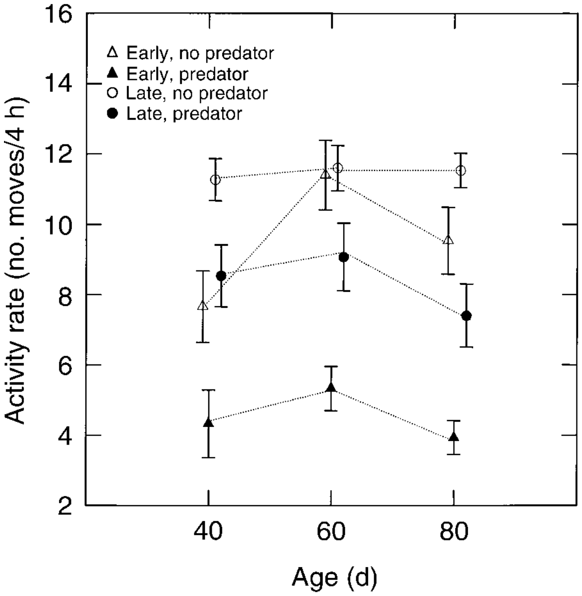

FIG. 2. Mean activity (± 1 SE), estimated as number of moves by *Lestes sponsa* larvae in the predation experiment (see Table 3).

PC2, which accounted for 19.7% of the variation. Distance was correlated with PC1 and 2. No walks were observed. Larvae displayed the variables associated with PC1 more frequently in the late treatment and in the absence of the predator, with no significant interaction between these effects (Fig. 1, Table 2). These behaviors also increased in frequency as larval development proceeded (Fig. 1, Table 2). A significant interaction between day and time constraint suggests that behaviors did not increase with age to a similar degree in early and late larvae. There were no significant treatment effects on PC2 scores.

In keeping with the behavioral data, larvae were more active in the late treatment and in the absence of predators (Fig. 2, Table 3). A significant within-subjects effect of day resulted from a peak of activity on day 60 (Fig. 2, Table 3).

The life history variables of growth, age, and mass at emergence, were all significantly affected by the time constraint and predation risk (Fig. 3, Table 4). Larvae

TABLE 2. *F* values and significance levels from repeated-measures ANOVAs on the scores from the first three and two principal components, respectively, on the behavioral variables of *Lestes sponsa* larvae.

	Predation experiment			Resource experiment			
Treatment	df	PC1	PC2	df	PC1	PC2	PC3
Between subjects							
Time constraint (T)	1	**19.177*****	0.754	1	0.009	1.371	0.721
Predation (P)	1	**18.472*****	2.454				
Resource (R)				1	**7.105***	0.010	3.409
$T \times P$	1	1.489	0.222				
$T \times R$				1	0.061	0.100	0.008
Error	36			28			
Within subject							
Day (D)	2	**43.310*****	1.267	2	**13.387*****	1.136	0.335
$D \times T$	2	**5.794***	1.807	2	0.829	0.300	1.473
$D \times P$	2	2.512	1.714				
$D \times R$				2	1.468	1.428	0.129
$D \times P \times T$	2	0.085	1.029				
$D \times R \times T$				2	1.987	1.531	1.668
Error	72			56			

Notes: For the predation experiment, treatments were time constraint (early, late) and predation risk (no predator, predator). For the resource experiment treatments were time constraint (early, late) and food level (high, low). Day of measurement (25, 50, and 75) was used as the repeated measure.

* $P < 0.05$; *** $P < 0.001$.

TABLE 3. Results from two-way repeated-measures ANOVA on activity by *Lestes sponsa* larvae.

	Predation experiment			Resource experiment		
Treatment	df	*F*	*P*	df	*F*	*P*
Between subjects						
Time constraint (*T*)	1	25.727	<0.001	1	1.858	0.181
Predation (*P*)	1	51.774	<0.001			
Resource (*R*)				1	1.536	0.223
$T \times P$	1	2.727	0.104			
$T \times R$				1	0.908	0.347
Error	56			37		
Within subjects						
Day (*D*)	2	4.544	0.012	2	0.470	0.627
$D \times T$	2	1.822	0.166	2	0.209	0.812
$D \times P$	2	1.694	0.188			
$D \times R$				2	0.666	0.576
$D \times T \times P$	2	1.092	0.339			
$D \times T \times R$				2	0.287	0.751
Error	112			74		

Notes: Treatments in the predation experiment were time constraint (early, late) and predation risk (no predator, predator). Treatments in the resource experiment were time constraint (early, late) and food level (high, low). Day of measurement (40, 60, and 80) was used as the repeated measure.

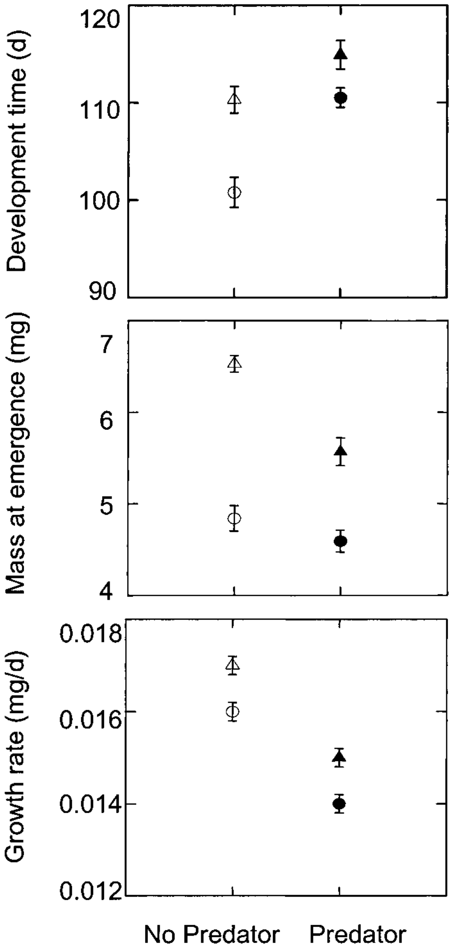

FIG. 3. The effect of time constraint and predation risk on life history variables in *Lestes sponsa* (see Table 4). Triangles denote early larvae (no time constraint), and circles denote late larvae (time constraint). Error bars represent ± 1 SE.

in the predator treatment had relatively low growth rates, as would be predicted by their reduced foraging and activity rates. Reduced growth was accompanied by an increased development time, as may also be expected. However, these effects did not balance, as mass at emergence was significantly reduced in the predator treatment. In contrast, late larvae grew at a lower rate, despite their increased foraging and activity rate. Development rate was accelerated, and mass at emergence dramatically decreased in the late treatment. It is quite possible that the slightly lower mean growth rates that we observed in the late treatments resulted from a decreased duration of exposure to the higher food ration of *Daphnia* initiated at day 80 (a by-product of their accelerated development). The only significant interaction between main effects on these life history traits resulted from a particularly strong effect of the late treatment on mass at emergence in the no-predator treatment. There were 8.0 ± 0.2 instars before emergence (mean ± 1 SE), and the treatments had no effect on instar number (Table 4).

In summary, the predation experiment suggests that perceived predation risk slowed the life history of this damselfly. Reductions occurred in foraging behavior, activity rate, development rate, and mass at emergence. The effect of an approaching time constraint (the late treatment) was to speed up the life history. The late treatment caused increases in foraging behavior, activity rate, and development rate, while growth rate and mass decreased.

Resource experiment

The eight behavioral variables were readily interpreted in terms of three of the principal component axes, which together explained 85.6% of the variation (Table 1). Orient, advance, strike, capture, and success rate were highly correlated with PC1, which explained

TABLE 4. Results from ANOVAs on *Lestes sponsa* development time, mass at emergence, growth rate, and instar at emergence.

	Predation experiment			Resource experiment		
Treatment	df	*F*	*P*	df	*F*	*P*
Development time						
Time constraint (*T*)	1	25.31	<0.001	1	4.14	0.045
Predation (*P*)	1	26.88	<0.001			
Resource (*R*)				1	303.88	<0.001
T × *P*	1	3.387	0.070			
T × *R*				1	21.20	<0.001
Error	71			70		
Mass at emergence						
Time constraint (*T*)	1	109.33	<0.001	1	15.63	0.002
Predation (*P*)	1	22.45	<0.001			
Resource (*R*)				1	4.32	0.041
T × *P*	1	7.72	0.007			
T × *R*				1	25.95	<0.001
Error	71			70		
Growth rate						
Time constraint (*T*)	1	21.76	<0.001	1	21.76	0.356
Predation (*P*)	1	52.30	<0.001			
Resource (*R*)				1	52.30	0.131
T × *P*	1	0.219	0.641			
T × *R*				1	0.219	0.385
Error	71			70		
Instar at emergence						
Time constraint (*T*)	1	0.139	0.710	1	0.38	0.540
Predation (*P*)	1	2.494	0.119			
Resource (*R*)				1	9.58	0.003
T × *P*	1	0.493	0.485			
T × *R*				1	0.093	0.762
Error	71			70		

Notes: Treatments in the predation experiment were time constraint (early, late) and predation risk (no predator, predator). Treatments in the resource experiment were time constraint (early, late) and food level (high, low).

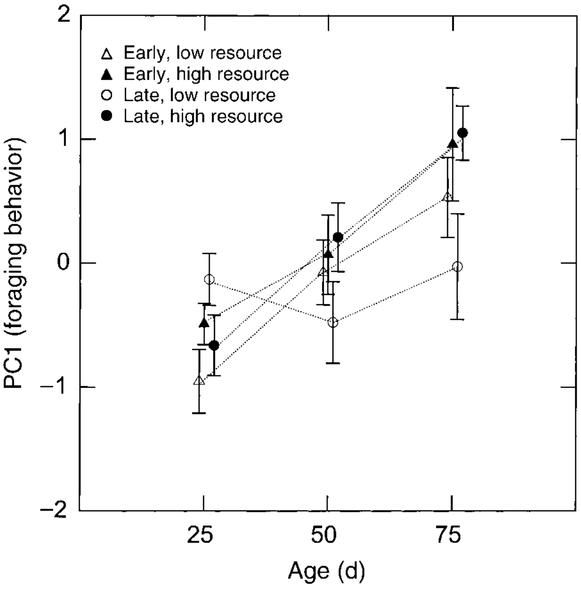

FIG. 4. Mean scores for the foraging behavior PC1 (± 1 SE) plotted against development day for *Lestes sponsa* larvae in the resource experiment (see Table 2).

51.3% of the variance. Distance and swim were correlated with PC2, which explained 19.8% of the variance. Finally, walk was correlated with PC3, which explained 14.5% of the variance. Only those behavioral variables correlated with PC1 were significantly affected by the treatments (Fig. 4, Table 2). Larvae displayed these behaviors more frequently at the high food level, and as development progressed.

No clear patterns in activity differences were found between treatments (Fig. 5), and as such, there were no significant effects in the repeated measures ANOVA (Table 3). Note that most data are very close to the maximum attainable in our design (16 movements in the 4-h period). The only exception is the mean for those individuals in the early, low-food treatment.

The response of life history traits in the resource experiment differed from those in the predation experiment, in that shared main effects did not show the same patterns of significance, and interaction effects were strong (Fig. 6, Table 4). At high food, larvae reared in the late-season light treatment had an accelerated development and a smaller mass at emergence, as was the case in the predation experiment. However, at low food, the opposite was true. Moreover, high food resulted in larger mass at emergence in larvae reared in the early treatment, but smaller mass in larvae reared

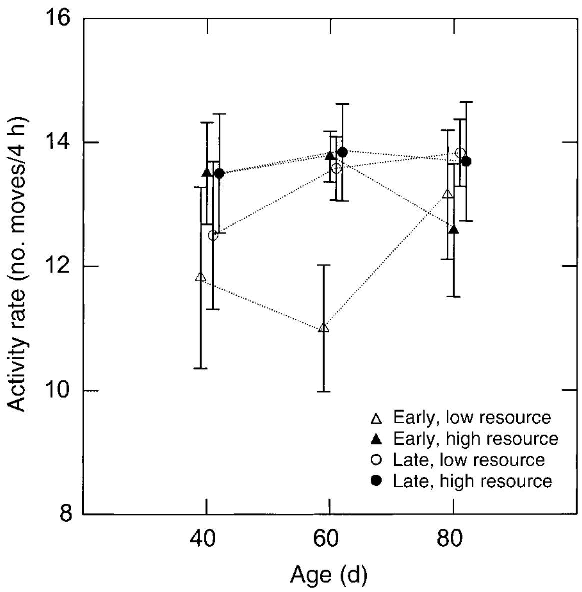

FIG. 5. Mean activity (± 1 SE), estimated as number of moves and plotted against development day for *Lestes sponsa* larvae in the resource experiment (see Table 3).

in the late treatment. Both of these interaction effects resulted from the unexpected slow development and large mass achieved by larvae reared in the late treatment in the low resource treatment. In short, larvae reared in the late treatment delayed emergence beyond that of any other treatment, and thereby achieved a mass greater than any other treatment. We had expected the opposite. Mean number of instars before emergence was significantly higher for larvae reared with low food (9.0 ± 0.2 instars) compared to high food (8.4 ± 0.2 instars). No other significant instar effects were found (Table 4).

DISCUSSION

Our study is one of the few to consider the joint effects of predation risk and food level with the sort of time constraints that are so common to seasonal life histories (Blanckenhorn 1998, Laurila and Kujasalo 1999, Plaistow and Siva-Jothy 1999). We have demonstrated that behavioral and life history traits of *Lestes sponsa* larvae were all affected in interesting ways by both time constraints and biotic factors, and that these effects were often additive. In some cases, life history responses corresponded with expected direct effects; in others, they could be attributed to behaviorally mediated responses (indirect effects). For example, data from both experiments suggest that time constraints shaped life history responses directly. Larvae approaching a time horizon, if anything, increased foraging behavior and activity rates, usually accelerated development, and paid for this with a reduced growth and by emerging at a smaller mass. In contrast, the biotic factors (predation risk/food level) seemed to affect life history traits indirectly, through behavioral alterations. For example, predators caused a reduction in foraging and activity levels, and as a result, reduced development rates and reduced mass at emergence.

Time constraints and predation risk

The effects of time constraints and predators in our experiments are in accord with theory, and with the observations of previous experiments. In the predation experiment, late larvae increased the frequency of their foraging behavior and their activity rates. This result corresponds to predictions from theory (e.g., Ludwig and Rowe 1990, Houston et al. 1993, Werner and Anholt 1993, Abrams et al. 1996). Despite the associated mortality costs of increasing foraging effort (Anholt and Werner 1998), larvae are expected to increase risky foraging in an attempt to partially compensate for the reduced time available for growth and development. We know of only two other studies that explored the effects of time constraints, operating at the life history

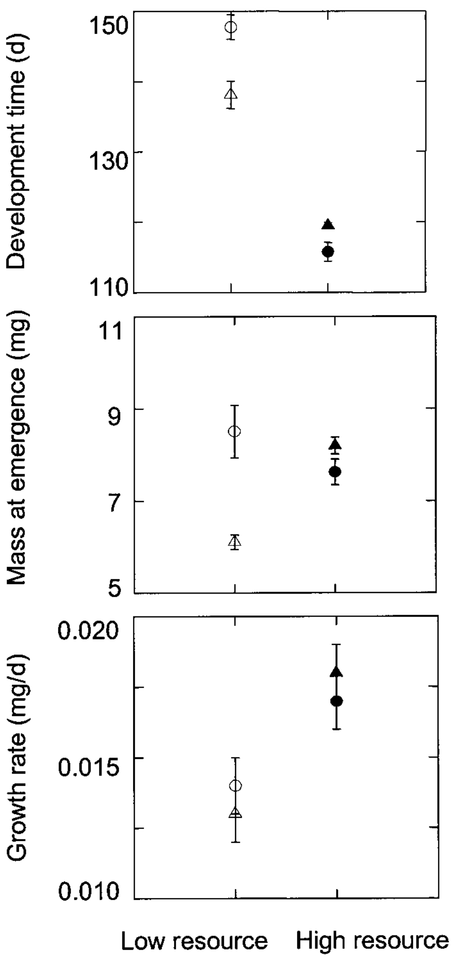

FIG. 6. The effect of time constraint and resource level on life history variables in *Lestes sponsa* (see Table 4). Triangles denote early larvae (no time constraint), and circles denote late larvae (time constraint). Error bars are 1 SE.

scale, on larval behavior. Johansson and Rowe (1999) reported that late larvae of a related damselfly species increased their activity rate, and like this study, did so very early in the larval period. In a study of frog tadpoles, Laurila and Kujasalo (1999) found just the opposite response in activity rate, and explained that result as an effect of decreasing water level.

As has been reported in several other experiments, time-constrained larvae accelerated development (Newman 1988*a, b,* Nylin et al. 1989, Reques and Tejedo 1997, Blanckenhorn 1998, Johansson and Rowe 1999, Laurila and Kujasalo 1999). Such acceleration has usually been accompanied by a decrease in size at the subsequent life history transition. The combination of accelerated development and reduced size under a time constraint is predicted both by models that include flexible growth (Abrams and Rowe 1996, Abrams et al. 1996) and those that do not allow for flexible growth (Rowe and Ludwig 1991). Therefore, accelerated development and reduced size is expected, independent of any associated behaviorally mediated changes in growth rate. Most relevant experimental studies have not been able to disentangle behaviorally mediated from direct life history responses. Johansson and Rowe (1999) were able to demonstrate that an observed accelerated development under time constraints was independent of growth rate. We suggest that results of our predation experiment support this view, because late larvae accelerated development without increasing growth rate. We wish to stress that there still might have been some differences in growth rate between late and early larvae. Since the former had access to the *Daphnia* ration for a shorter time (a by-product of their accelerated development) they may have grown at a slightly faster rate during this period.

Predators tended to slow the behavior and life history of these damselflies. Activity level and frequency of foraging behavior were reduced throughout the larval stage. Many experiments have shown similar effects, although usually over a smaller portion of the life history. These include studies on our species (Stoks 1998) and many other taxa (reviewed in Lima and Dill 1990, Lima 1998). Our results are predicted under some conditions in some relevant theory (Werner and Anholt 1993, Abrams and Rowe 1996, Abrams et al. 1996). These include cases where the predation mortality cost of activity is high.

In the presence of predators, damselflies reduced development rate and emerged at a smaller mass. Theory often, though not always, predicts that individuals should accelerate development when juvenile predation rate is increased (e.g., Ludwig and Rowe 1990, Abrams and Rowe 1996, Abrams et al. 1996). An explanation for this discrepancy may be found in the activity and growth data. Under high predation risk, larvae dramatically reduced their activity levels and suffered a corresponding decrease in growth rate. Reduced growth rate typically leads to delayed development (reviewed in Berrigan and Charnov 1994), and such an effect was demonstrated in our resource experiment. We suggest that it was this behaviorally mediated reduction in growth that resulted in a delay of development. This view is supported in Fig. 7, where it can be seen that age at maturity in the predation experiment was simply a function of growth. This sort of behavioral or growth-mediated life history response to predation risk has been reported several times before (e.g., Skelly and Werner 1990, Peckarsky et al. 1993, Ball and Baker 1996). Although a direct response is predicted by many theoretical treatments (reviewed in Abrams and Rowe 1996), very few studies have actually demonstrated such a response (but see Stibor and Machácěk 1998). Interestingly, there was an interaction between the time constraint and predation risk on mass at emergence, with late individuals emerging at about the same mass regardless whether predators were present or absent (Fig. 3). This may reflect the presence of a critical size required for emergence. There is some evidence for such a critical size for emergence in *L. sponsa* (Stoks 1999, 2000).

Time constraints and resources

The results of the resource experiment contrast in many ways with those of the predation experiment. In the resource experiment, there was little variation among treatments in behaviors, and the observed life history responses included strong interactions among treatments. Foraging behavior did increase at the higher food level, as may be expected, because the net energy gain of increased effort is higher (Sih 1984, Abrams 1992, McNamara and Houston 1994). We did not see any effect of time constraint on behavior or activity, as is predicted by theory (Houston et al. 1993, Werner and Anholt 1993, Abrams et al. 1996). We suspect that our assessment of foraging behaviors and activity levels may have been flawed in this experiment. First, activity level of individuals in all treatments was very close to the maximum (16) allowed in our design (compare Figs. 2 and 5). A second reason to suspect both the foraging behavior and activity level results in this experiment is that they do not match those of the first experiment or prior experiments (Johansson and Rowe 1999).

The strong interactive effects of time constraints and food levels, that we observed on life history variables, is intriguing (Fig. 6). At high food levels, development was accelerated, as would be expected from high growth rates (reviewed in Berrigan and Charnov 1994). Late individuals further accelerated development, as was observed in the predation experiment, our prior experiment on a related damselfly (Johansson and Rowe 1999), and as is predicted by theory (e.g., Ludwig and Rowe 1990, Rowe and Ludwig 1991, Abrams et al. 1996). Finally, mass at emergence was lower in late individuals when at high food (despite a lower growth rate; Fig. 6). Again, these results match those of our first experiment and our prior work. However,

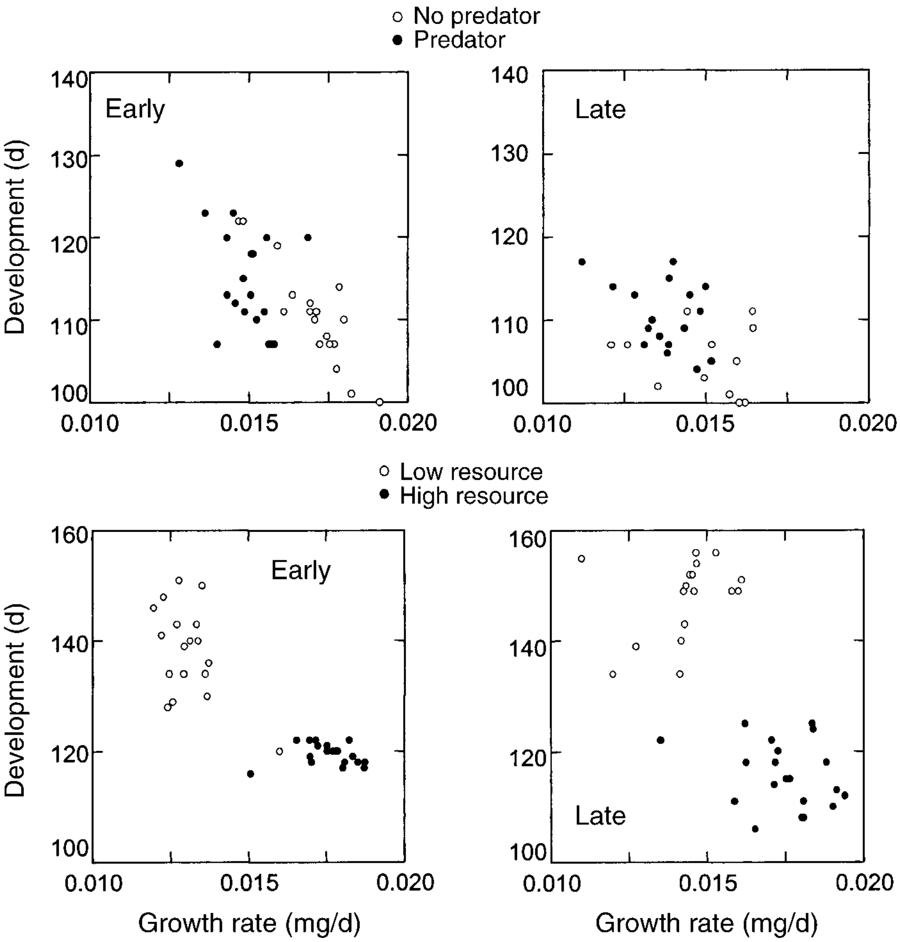

FIG. 7. Development time plotted against growth rate for individual *Lestes sponsa* larvae from the predation and resource experiment.

there were two surprising results in the low-food treatment, which account for two strong interactions in our analysis, and suggest an intriguing hypothesis. Late individuals at low food levels actually had the slowest development rate and the largest mass at emergence. These results are exactly the opposite of our expectations. The large mass can be explained by the slow development, but slow development is the opposite of predictions from theory (e.g., Ludwig and Rowe 1990, Rowe and Ludwig 1991, Abrams et al. 1996). Moreover, it is the opposite to results found in all other relevant treatments in these current experiments, and in past experiments with damselflies and a variety of other taxa (e.g., Newman 1988*a, b,* Nylin et al. 1989, Blanckenhorn 1998, Johansson and Rowe 1999).

Our current hypothesis for these unexpected results is that the specific conditions resulted in a delay of emergence to the next season, or ''cohort splitting'' (Norling 1984, Martin et al. 1991). A similar phenomenon, referred to as ''diapause development,'' is common in insects with holometabolic development. Here, individuals forgo emerging the present season, enter a diapause, and overwinter to emerge next season (Nylin et al. 1989, Wiklund et al. 1991, Blanckenhorn 1998). Cohort splitting, though not focused upon explicitly, has also been reported for fish (Treasurer 1981). Current theory, which uniformly predicts accelerated development of time-constrained individuals, assumes a univoltine life history without overlapping generations (e.g., Ludwig and Rowe 1990, Rowe and Ludwig 1991, Abrams et al. 1996). This means that it forces all individuals to emerge (or mature) in the current season. It should be applied with some caution to cases in which cohort splitting is possible. Although *L. sponsa* typically overwinters in the egg stage (Jödicke 1997), there is some evidence that larvae also can overwinter. This evidence is based on the rare presence of very large larvae in samples collected early in autumn (Valtonen 1982) and late in winter (Warren 1988). Of course, overwintering will greatly increase total prereproductive mortality, and all else staying equal, decrease the intrinsic rate of increase (*r*). However, at low enough growth and a short enough growing season, overwintering will be favored. We suggest our low-food, time-constrained treatment favored cohort splitting. The probability of this occurring in the field is probably very low, given the high foraging activity of *L. sponsa* (Jeffries 1990, Pickup and Thompson 1990, Stoks 1998, Johansson 2000). Additionally, the survival probability of such late odonate larvae is low because of cannibalism by earlier hatched, larger larvae (Fischer 1961, Van Buskirk 1992, Anholt 1994). If cohort splitting did occur, why did the larvae emerge after about 150 d, which, in the wild, would be equivalent to emerging in late October early November? We suggest that this resulted from the lack of declining temperatures in our experiment. Induction of winter diapause in developing odonates appears to require a temperature cue as well as a photoperiod cue (Norling 1984). In the absence of a temperature cue, as in our experiment, a winter diapause was prevented and larvae therefore emerged.

Future directions

Our experiments suggest two areas of future research. First, we suggest that future experiments should further explore the direct and behaviorally mediated life history responses to predation risk. Our results, and those of past studies (e.g., Skelly and Werner 1990, Peckarsky et al. 1993, Ball and Baker 1996), suggest that there is no direct response of prey development rate to predation risk, despite the predictions of theory. This cannot be explained by a fixed relationship between growth and development. We have shown here and elsewhere (Johansson and Rowe 1999) that late individuals can accelerate development independent of growth rate. An alternative explanation for the absence of direct effects of predation risk on development is that we did not have enough overlap in growth rates between the predator treatments, to distinguish different development rates for individuals growing at the same rate. Future experiments could resolve this by increasing the range of food levels and thereby the zone of overlapping growth rates among treatments. Second, we have suggested that food-limited, late individuals may opt for overwintering in the larval stage by delaying rather than accelerating development. The potential of cohort splitting requires further experimentation in this and other taxa. A first line of inquiry would be to determine if there is some threshold size for achieving fecundity at emergence, which would promote delaying emergence to the next growing season. We know that fecundity and mating success generally increase with size in odonates (Sokolovska et al. 2000) and other animals (Andersson 1994). Indeed, studies on several odonate species suggest that larvae have a critical size above which rapid growth and emergence follow. If larvae are below this critical size, development is slowed down, and cohort splitting occurs (Norling 1984, Martin et al. 1991, Gurney et al. 1992). A second line of inquiry would be to determine if our result is repeatable, and over what range of resource and time pressure. The prediction is that the fraction of individuals that delay development would be an increasing function of resource and time limitation.

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