

What keeps insects small?—Size dependent predation on two species of butterfly larvae

David Berger · Richard Walters · Karl Gotthard

Received: 18 January 2006 / Accepted: 28 March 2006 /
Published online: 27 September 2006
© Springer Science+Business Media B.V. 2006

Abstract Insect size usually increases greatly in the latter stages of development, while reproductive value increases strongly with adult size. Mechanisms that can balance the benefits associated with increased growth are poorly understood, raising the question: what keeps insects from becoming larger? If predation risk was to increase with juvenile size, it would make an extension of development very risky, favouring smaller final sizes. But field measures of juvenile mortality seldom show any general patterns of size dependence. We here therefore try to estimate a mechanistic relationship between juvenile size and predation risk by exposing the larvae of two closely related butterflies to a generalist invertebrate predator in a laboratory experiment. Predation risk increased with larval size but was not affected by the species-specific growth rate differences. These results indicate that predation risk may increase with the size of the juvenile even when predators are relatively small. By basing a model simulation on our data we also show that size dependent predation of the kind found in this study has potential to stabilise selection on body size in these species. Thus, these findings suggest that more detailed studies of the size dependence of predation risk on juvenile instars will increase the understanding of what it is that keeps insects small.

Keywords Life history · Body size · Growth rate · Juvenile mortality · Age- and size at maturity · Predation risk · Lepidoptera · Model

Introduction

Selection on growth trajectories and optimal body size is a central focus of life history research and is often addressed with the age- and size at maturity trade-off as centre of discussion; large adult size is associated with high reproductive potential, while the attainment of a larger size might increase juvenile mortality risk (Stearns

D. Berger (✉) · R. Walters · K. Gotthard
Department of Zoology, Stockholm University, 10691 Stockholm, Sweden
e-mail: david.berger@zoologi.su.se

1992; Roff 2002). One of the most commonly suggested agents of juvenile mortality is predation. Increased foraging activity is assumed dangerous because it is likely to make prey easier to detect while an extension of the juvenile growth period exposes the individual to predators for a longer time. Strong predation pressures are therefore predicted to select for non-maximised growth and smaller body sizes of prey organisms (Case 1978; Lima and Dill 1990; Kozłowski 1992; Stearns 1992; Roff 2002). Observations of non-maximised somatic growth rates in animal taxa are common (Case 1978; Calow 1982; Arendt 1997; Gotthard 2001) indicating that there are indeed costs associated with growth and foraging activity that influence the adaptive growth patterns of prey. Theoretical models concerned with optimal body size indicate that the adaptive growth responses of juvenile organisms can be highly dependent on the relationship between juvenile size and mortality (see examples Sibly 1985; Grand 1999; Lundvall et al. 1999). For example, higher predation risk to large individuals is predicted to select for a shorter growth period and a smaller body size. These models illustrate that it is essential to estimate the relationship between prey size and predation risk in order to make accurate predictions about optimal growth trajectories and final size.

Insect growth takes place during the larval phase and directly determines the size of the adult. Weight gain of the larvae is usually proportional to the size of the growing individual (Gotthard et al. 1994, 1999, 2000; Nylin et al. 1995, 1996; Gotthard 1998; D'Amico et al. 2001; Margraf et al. 2003; Nijhout 2003; Esperk and Tammaru 2004). So, if feeding activity remains constant, the absolute size of an individual will increase in exponential fashion throughout development. Hence, the size increase will be particularly high in the final stages of growth. Since adult size has been shown to be strongly correlated with both male mating status and female fecundity in many insect orders (for reviews see Honek 1993; Blanckenhorn 2000), even a short extension of the growth period may result in large fitness pay-offs to adults. Surprisingly, there exists very little evidence of costs associated with juvenile growth (Blanckenhorn 2000). The overall question we want to address is therefore; what keeps these organisms from evolving towards larger sizes?

The potential effects of size dependent predation in insects have received relatively little attention and have seldom been the main concern of general models of optimal body size in organisms with deterministic growth (but see Sibly et al. 1985). On the other hand, growth rate (i.e. foraging rate) and the length of the growth period have often been used as single model predictors of predation risk to juveniles (see examples Roff 1981; Ludwig and Rowe 1990; Abrams et al. 1996; Abrams and Rowe 1996). If there was no relationship between prey size and predation risk, the instant risk of being killed by a predator would most likely be constant throughout development. Alternatively, if predation risk was to increase with the size of the prey, the danger of foraging would become greater and greater for each day of growth. The cumulative risk of predation would then increase very fast, especially in the end of the development period, and have the potential to stabilise the benefits of prolonged proportional juvenile growth. Hence, a general positive relationship between juvenile size and predation risk could be a part of the explanation to what is keeping insects small.

Under the assumption that larval growth is close to optimal, the relationship between growth-associated costs and benefits suggested by empirical studies offers an unsatisfying explanation to the observed juvenile growth strategies of insects. We know of very few earlier studies trying to quantify the relationship between larval size

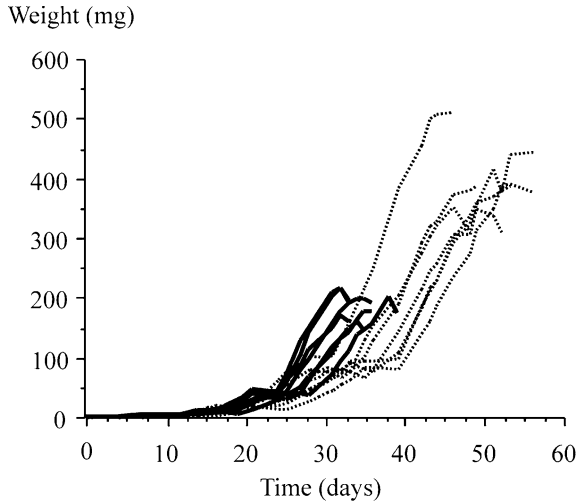
and predation risk. We here try to do just that by exposing the larvae of two satyrid butterflies to the generalist invertebrate predator *Picromerus bidens* (Linnaeus) (Pentatomidae) in a laboratory experiment. We used two populations of the small but fast growing *Pararge aegeria* (Linnaeus), from Sweden and Madeira respectively, and compared them to their larger and slower growing close relative *Pararge xiphia* (Fabricius) endemic to Madeira. This enabled us to study potential size dependent predation in the wide larval size-span present in the *Pararge* genera and at the same time look at possible effects of the growth rate difference between the species. Our results show a strong and relatively constant positive relationship between predation risk and juvenile size, while the difference in growth rate between the species seemed to be of minor importance. In order to evaluate the consequences that size dependent predation of the type found in this study can have for optimal body size, we also perform a simple model simulation based on our data. We conclude that it is possible that predation from invertebrates generally increases with larval size and if so, is likely to be an important factor for the evolution of insect body size.

Methods

Study species

P. xiphia and *P. aegeria* are two closely related species of butterfly belonging to the subfamily Satyrinae (Nymphalidae). Their general ecology is relatively similar and the typical habitat of both species is woodlands. However, *P. xiphia* is endemic on the island of Madeira while *P. aegeria* is widespread throughout Europe including Madeira (Tolman 1997) where it for the first time was spotted in 1976 (Owen et al. 1986). Before pupal moult, both species go through four larval stages during which the solitary living green larvae use different grass species as food plants making them very cryptic against their natural background. In *P. xiphia* there is however a colour polymorphism in the fourth and final instar where some larvae develop into a brown morph at the entry into the final instar. The larvae of *P. aegeria* hatch at a weight of about 0.4 mg and can become up to 250 mg before pupation, while *P. xiphia* larvae hatch at weights of about 0.8 mg and can weigh up to 500 mg before pupation (see Fig. 1). In general, *P. xiphia* grow much slower than *P. aegeria* (personal observations, see Figs. 1 and 2). The pupal mass of *P. xiphia* is nearly the double of *P. aegeria* pupae (unpublished data, compare Fig. 1). Potential female fecundity has been shown to increase strongly with size in *P. aegeria* (Karlsson and Wickman 1990, Gotthard et al. *submitted manuscript*). Even so, studies have shown that growth rates in northern populations of *P. aegeria* are highly flexible in response to the light regime (Nylin et al. 1989, 1993; Gotthard et al. 1994; Nylin et al. 1995) demonstrating that the larvae often adaptively reduce growth rates rather than grow at maximum potential. In this study we used two populations of *P. aegeria* collected in southern Sweden and on Madeira respectively, and one population of *P. xiphia* from Madeira. Using *P. aegeria* from the genetically different populations of Sweden and Madeira (see Gotthard et al. 1994) increases the potential for results of great generality as the regional comparison controls for effect on predation risk caused by other factors than size- and growth rate differences. Both populations of *P. aegeria* were started with eggs from 10 wild caught females from each location, while the population of *P. xiphia* was started from 5 females in 2003 and 5 females in 2004.

Fig. 1 Growth curves of seven larvae of *P. xiphia* (dashed lines) and seven larvae of *P. aegeria* (Mad) (full lines). Weight increase of both species is close to exponential and very high in the latter stages of development. Individuals of *P. aegeria* reach larval weights around 200 mg before pupation, while *P. xiphia* individuals reach weights around 400 mg



The populations were reared in three different cultivations on the grass species *Dactylis glomerata* (Linnaeus) under a light regime set to 14 h light followed by 10 h dark in a constant temperature of 17°C.

Picromerus bidens (Linnaeus) is a heteropteran bug belonging to the Pentatomidae and is abundant in most parts of Europe from late May, as first instar larvae, to October as adults. This bug is a generalist predator on most cryptic as well as aposematic insect larvae but mainly hunts larvae of the Lepidoptera and Chrysomelidae (Coleoptera) (Southwood and Leston 1959). It seems to be most abundant on shrubs, flowering plants and grass in attachment to woodlands (Southwood and Leston 1959). Our observations indicate that a likely way for *P. bidens* to localise prey could be to detect larval bite marks on foliage by use of the antennae. The *P. bidens* used in this experiment were collected from two different geographical populations in the eastern part of Sweden. In 2003, 31 bugs were caught and used in experiments while 27 bugs were used in 2004. In order to control the hunger level of the bugs, no predator was fed the day before a trial, or used in an experiment for two consecutive days.

Growth rate measurements

In order to get a more precise estimate of the differences in body size and growth rates between the two species, seven individuals each of *P. xiphia* and *P. aegeria* (Mad) were reared on *D. glomerata* individually in 0.5 l plastic jars in a climate cabinet. The rearing conditions were set to 17°C under a diurnal cycle of 14 h of light and 10 h of dark. The larvae were weighed in close intervals from hatching to pupation in order to measure and compare the growth rates, development times and weights at metamorphosis for each instar of the two species. Since weight increase during juvenile larval growth has been shown to be next to exponential for these species, measures of the relative growth rates for the instars of each species used in the data analysis were obtained by the formula: $[\text{Ln}(\text{end weight}) - \text{Ln}(\text{start weight})]/\text{growth period}$, (see Gotthard et al. 1994). The end- and start weights were typically chosen within an interval of 3–5 days for each instar in order to escape pre- and post-moulting periods that often slow growth. In this way the values of the

relative growth rates in the instars are more likely to be representative of the growth rates of the individuals used in the predation trials since no moulting larvae were included in these experiments.

Predation trials

To estimate predation risk associated with larval size and growth rate, a laboratory experiment was performed during the period August to October in the consecutive years of 2003 and 2004. Each trial was conducted in an equilateral cage with the side of 50 cm placed in the laboratory. Six to 14 trials, depending on the access to predators, were performed in parallel on each day of experiments. The cages were illuminated with lamps (60 W) placed 30 cm above each cage. The temperature in the cages varied between 22°C and 27°C during the experiments. A single potted plant of *D. glomerata* with a height and diameter of 30 cm was placed in the centre of each cage. At 16.00–18.00 h the day before the trials the larvae were weighed and singly put on one plant. At 10.00 h the following morning the trials were started by placing one *P. bidens* at the bottom of each plant to hunt the larva, so that each cage contained one plant with one larva and one predator. This procedure allowed the larvae at least 16 h to settle at a preferred position on the host plant before the predator was introduced. The trials went on for the six following hours after the introduction of the predator, during which observations of predation events were conducted every 30 min. Since *P. bidens* feed for relatively long time on its prey that hang attached to its proboscis, 30 min was assumed to have been a short enough interval to guarantee the detection of all predation events taking place during the six hours. Therefore, all cases where the larva was not found after the trial were excluded from the data analysis. The disappearances of these larvae might have been due to migration of big larvae about to start metamorphosis or small larvae failing to settle on the plant after handling by the observer the day before. Out of in total, 314 trials, 29 trials were removed from the statistical analyses due to missing larvae and missing or poorly conditioned predators. The larval instars were evenly represented in the analysed data as well as in the removed data. The predators were used repeatedly until no longer in condition to hunt while each larva was only used once. On each day of trials larvae with a weight difference as big as possible were used to control for variation between experimental days. It was not possible to use all three populations simultaneously on each day of experiments, but larvae of *P. aegeria* and *P. xiphia* were used together on 24 out of 39 days.

Model

In order to quantify and compare the effects on optimal size of size dependent predation as opposed to a constant mortality risk throughout development, we construct a simple fitness model based on data for the two species from the experiments. Fitness (w) is calculated as survival to metamorphosis at a certain size, $l(x)$, multiplied by potential fecundity as a function of size, $m(x)$, (Eq. 1). Optimal larval size at the onset of metamorphosis is predicted when assuming both size dependent predation and a constant mode of predation risk at different levels of predator presence.

$$w = l(x) m(x) \quad (1)$$

Larval mass at time t is predicted by fitting an exponential growth function to the available data on the seven individuals of each species observed for the growth and body size calculations, (Eq. 2).

$$\text{larval mass}(t) = \omega \exp^{rt}. \tag{2}$$

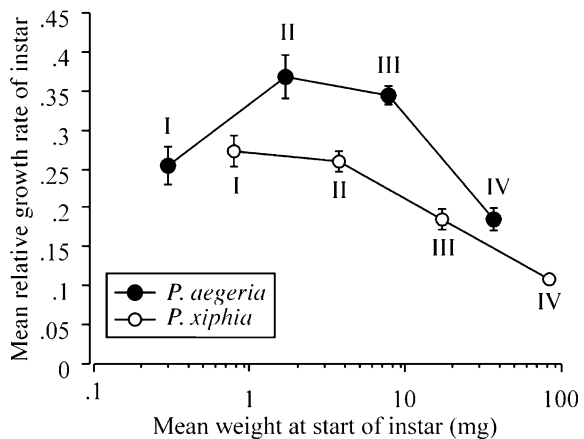
Where r is the species specific growth coefficient, t the development time in days and ω the hatch weight, which was set to the observed averages of 0.8 mg for *P. xiphia* and 0.4 mg of *P. aegeria*. Since the relative growth rate slows down in the fourth instar we modelled growth rates in the first three instars by calculating the growth coefficients r_{1-3} based on the size increase from hatching to the onset of the fourth instar for each species (see the section on relative growth rate measurements above). Notice that the growth rates in the first three instars presented in Fig. 2 does not correspond to this measure because we here do not exclude the period of moult between instars in our calculations. At the average size at which each species enter instar four we changed the coefficient in the model to a value given by calculating the growth coefficient r_4 based on the size increase that occurs in the fourth instar, excluding the moulting period (see fourth instar Fig. 2). Thus, we make the assumption that individuals in the fourth instar can continue their growth at this point at an unchanged rate for an indefinite number of extra developmental days.

Fecundity–larval size relationships were derived for each species from unpublished data on egg weight produced per day during the first week of adult life. The relationships were based on 18 females in *P. xiphia* and 42 females for *P. aegeria*, (data on *P. aegeria* available in Gotthard et al. *submitted*). The linear relationships between size and fecundity are calculated as:

$$m(x) = k \omega \exp^{rx} + c \tag{3}$$

Where k is the slope coefficient describing how fecundity increases with larval size and c is the intercept. The fecundity–size relationships for these two species were estimated as: $0.028 * (\text{larval mass}) - 4.86$, ($R^2 = 0.30$, $n = 18$, $P < 0.05$) in *P. xiphia*, and: $0.046 * (\text{larval mass}) - 5.06$, ($R^2 = 0.30$, $n = 42$, $P < 0.001$) in *P. aegeria*. Given

Fig. 2 Relative growth rate (mean + SE) for each instar of the seven larvae of *P. xiphia* and *P. aegeria* (Mad) plotted against the average weight of the individuals at the onset of each instar. The growth rates are calculated during the feeding periods of each instar and excludes moulting to better represent the growth rates of the larve used in the predation trials



by the equations, the minimum larval size that supported reproduction was 119 mg for *P. aegeria* and 188 mg for *P. xiphia*.

The relationship between larval size and daily predation risk was estimated by fitting a logistic curve to the data from the predation experiment. The size dependent predation risk (u^*) is given by Eq. 4:

$$u^* = e^{a+b * \text{Ln}(\text{larval mass})} / \left[1 + e^{a+b * \text{Ln}(\text{larval mass})} \right] \quad (4)$$

where a and b are constants. The constant daily predation risk (u) was assumed equal to the mean daily mortality rate calculated over all trials of the predation experiment. In the experiment there was always a predator present in the vicinity of the prey, a situation probably not that common in nature where one would expect predation levels to fluctuate greatly in space and time. Therefore the predation risk was modelled for different levels of predator presence (p) by multiplying both the constant and the size dependent predation risk by values of p between 0.1 and 1. This also enabled evaluation of the consequences of the two predation modes for size at metamorphosis under different levels of predation. In earlier field studies on *Pieris brassicae* (Kristensen 1994) and *P. aegeria* larvae (Karlsson, unpublished data), average daily mortality was about 14 and 12% respectively (see also Gotthard 2000), so it seems reasonable to expect low values of predator presence (p) to give closer approximation of natural levels of predator presence. The larval survival, $l(x)$ to time t could then be given by:

$$u^* : \quad l(x) = (1 - U_1^*P) \times (1 - U_2^*P) \times (1 - U_3^*P) \times \dots \times (1 - U_t^*P) \quad (5)$$

$$u : \quad l(x) = [1 - \text{daily average mortality} * p]^t \quad (6)$$

Statistical procedures

Difference in body size between the species was compared with the Student's T -test by using the pupal weights of the seven individuals of each species used in the growth rate measurements. Growth rate differences were analysed with a Repeated measures ANOVA model, with [INSTAR] as the repeated measure, [SPECIES] as a grouping variable and [RELATIVE GROWTH RATE] as the dependent variable. The mortality risk for larvae of different weights belonging to the three populations were analysed with the Cox proportional hazards regression (Cox 1972), which allows handling of censored data (i.e. the larvae that were not found by the predators during the experiment and thereby could not be noted for a time of death). This analysis was carried out by using [LARVAL WEIGHT] as a continuous predictor, [POPULATION] as a grouping variable and [TIME OF DEATH] as the response variable. Both cage- and year effects were controlled for but these variables were later removed from the analysis since they did not have any significant effects on the model. Larvae surviving the whole predation period (6 h) were coded as censored observations. For the model, the relationship between larval size and predation risk was estimated with a logistic regression and the fecundity size relationships were tested with a simple regression analysis. The Cox proportional hazards regression was carried out in the data analysis software Stata 8.2 (Statacorp 2003) while the

t-tests, ANOVA repeated measures, regression analysis' and the logistic regression were computed in Statistica 5.5 (StatSoft Inc. 1999).

Results

Body size and growth rate

The pupae of *P. xiphia* weighed more than the pupae of *P. aegeria* ($t_{12} = -8.54$, $P < 0.001$), (average pupal weight of *P. aegeria* = $148.1 \text{ mg} \pm 6.2$ (SE), *P. xiphia* = $280.5 \text{ mg} \pm 14.2$ (SE)). Results showed that *P. xiphia* grow at a slower rate in comparison to *P. aegeria* ($F_{1:12} = 27.4$, $P < 0.001$) (Fig. 2). There was also a significant effect of instar on growth rate ($F_{3:36} = 39.4$, $P < 0.001$) with a decrease in relative growth rate in the final instar of both species (Fig. 2).

Predation risk

All observed attacks by *P. bidens* were successful. During both observations and rearing, no predator was ever found to disregard a larva that it had detected; hence, it is likely that detection always resulted in death of the larva in the experiment. The effect of larval weight on time of death was highly significant, with larger larvae having a higher risk of being killed (hazard ratio = 1.0038, $z_{285} = 4.92$, $P < 0.001$), while there were no significant differences in mortality between the populations (hazard ratio = 1.1588, $z_{285} = 1.19$, $P = \text{n.s.}$) (Figs. 3, 4). The analysis of mortality differences between the two colour morphs in the fourth instar of *P. xiphia*, showed that individuals of the green morph suffered higher predation in comparison to individuals of the brown morph (hazard ratio = 2.2053, $z_{50} = 2.29$, $P < 0.05$). Because of the difference between the two morphs, the relationship between larval weight and mortality was analyzed again after including only brown or only green fourth instar *P. xiphia* larvae respectively. This however, did not change the results from the first analysis. The relationship between larval weight and mortality was significant in the analysis using the green fourth instar larvae (hazard ratio = 1.0060, $z_{255} = 6.08$, $P < 0.001$) as well as in the analysis using the brown fourth instar larvae (hazard ratio = 1.0035, $z_{265} = 4.05$, $P < 0.001$), while there still were no significant differences between the populations when using either green (hazard ratio = 1.1865, $z_{255} = 1.32$, $P = \text{n.s.}$) or brown (hazard ratio = 1.1075, $z_{265} = 0.79$, $P = \text{n.s.}$) larvae in the analysis.

Model

The growth coefficient, r_{1-3} , was calculated to 0.13 ± 0.004 (SE) for *P. xiphia*, and to 0.19 ± 0.005 (SE) for *P. aegeria*, while r_4 was 0.11 ± 0.005 (SE) for *P. xiphia* and 0.19 ± 0.014 (SE) for *P. aegeria* ($n = 7$ in all cases). The regression coefficients for the relationship between larval size and daily predation risk in the experiment were calculated to $a = 1.267$ and $b = -0.4614$, (Larval weight: $z_{292} = -6.71$, $P < 0.001$). The average daily mortality in the experiment based on all individuals was 52%. Since no population differed significantly from the others in the survival analysis, the relationships were assumed for both species.

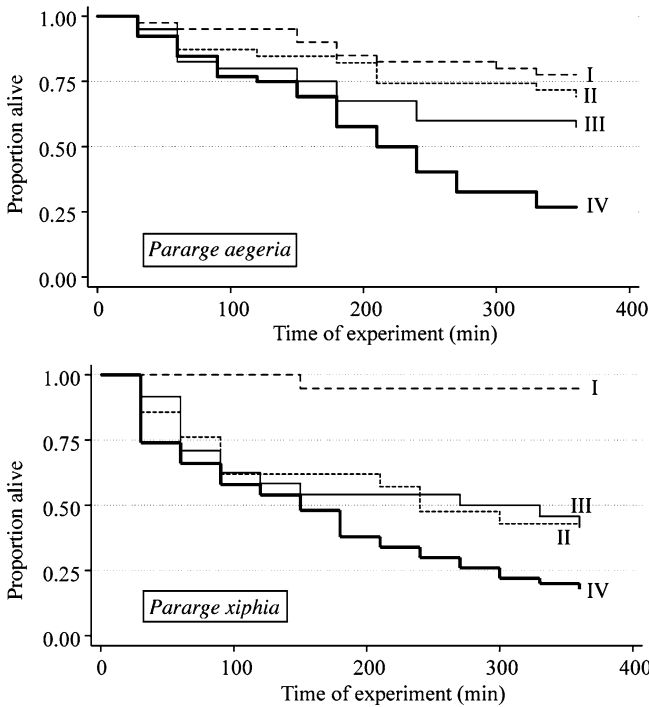


Fig. 3 Survival curves of the four instars of the two *P. aegeria* populations combined (top) and *P. xiphia* (bottom) during the 360 min trial of predation. Instar survival recorded every 30 min. Survival curves for each instar (I–IV) are based on samples of between 19 and 52 individuals

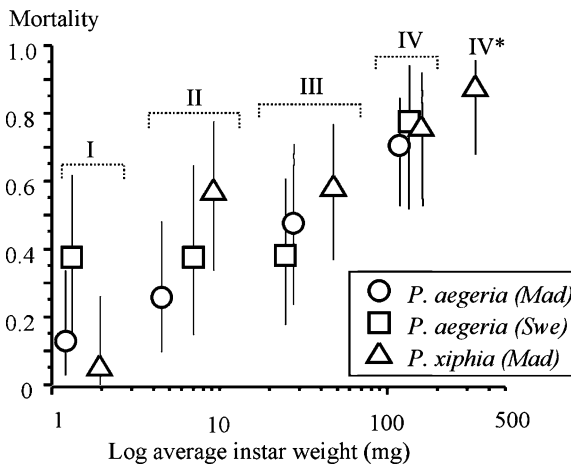


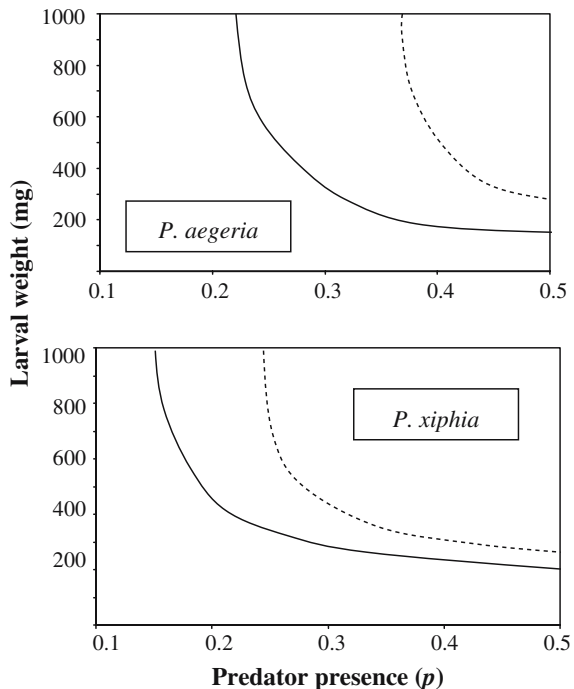
Fig. 4 Summary of the mortality of all instars of *P. xiphia* (triangle), *P. aegeria* (Mad) (circle) and *P. aegeria* (Swe) (square) at the end of the 360 min trial period. Mortality values are given with 95% confidence intervals. Individuals of *P. xiphia* in the fourth instar weighing over 250 mg is represented by the triangle marked IV* while the triangle IV shows the mortality of the fourth instar *P. xiphia* larvae weighing below 250 mg. Sample sizes of *P. aegeria* (Mad) instar I: 17, II: 16, III: 21, IV: 18, *P. aegeria* (Swe) instar I: 23, II: 23, III: 19, IV: 34, and *P. xiphia* instar I: 19, II: 21, III: 24, IV: 21, IV*: 29

For all ranges of predation levels, the size dependent mode of predation predicted smaller body sizes than the constant predation risk mode (Fig. 5). In both species optimal size at metamorphosis differed little between the two predation modes when predator presence was above $p = 0.5$. In these cases optimal size was instead mostly dependent on minimum larval size that could support reproduction because the extremely high mortality rates favoured early metamorphosis. Since *P. aegeria* grows faster than *P. xiphia* and gain more in size each day, a higher predator presence was needed to balance selection on size in *P. aegeria* because there were no extra mortality costs associated with high growth rates incorporated into the model. The strongest difference between the two modes of predation was seen in a predator presence of about 0.15–0.25 for *P. xiphia* and about 0.20–0.40 for *P. aegeria* (Fig. 5).

Discussion

In insects, experimental evidence of costs associated with a large body size is in shortage while there seems to be strong selective forces favouring an increase in adult size (Blanckenhorn 2000). This imbalance of costs and benefits along with observations of proportional insect growth, where a brief extension of larval development time results in a substantial increase in final size, make it hard to understand why insects do not prolong growth. In our study, predation risk increased with larval size. Furthermore, the form of the positive relationship between size and predation risk was very similar between the three studied populations and relatively constant throughout larval development (a weight span of 1–429 mg in this study). If predation risk in general increases with larval size, extended larval growth will be

Fig. 5 Model predictions of optimal larval size at metamorphosis for different levels of predator presence (p) in *P. aegeria* (above) and *P. xiphia* (below). A full line illustrates the relationships under the size dependent predation mode, while dashed lines show the relationships under the constant predation risk mode



associated with a continuously escalating instant mortality risk. Such a relationship between size and predation risk radically alters the cost–benefit relationship of attaining a larger size, since it would make increased growth late in development very dangerous.

To our knowledge, very few studies have attempted to quantify the relationship between juvenile size and predation risk in insects. Some field studies indicate that predation imposed by vertebrates generally increases with the instar of the larva (Atlegrim 1992; Parker 1993; Kristensen 1994; Dial and Roughgarden 1995; Gunnarsson and Hake 1999; Hooks et al. 2003). However, predation risk imposed by invertebrates is likely to be a strong selective force. This is supported by the results of the field study by Kristensen (1994) investigating predation on larvae of the butterfly *Pieris brassicae*. In the experiment, individuals in cages exposed only to invertebrate predators suffered 69% mortality during development from egg to prepupa. However, results from the few studies found that have estimated size dependent predation from invertebrates are mixed, with predation risk both increasing (Fincke et al. 1997; Lang et al. 1999) and decreasing (Kristensen 1994; Palokangas and Neuvonen 1992) with larval instar. It has also been argued that field studies quantifying mortality rates related to juvenile size might not accurately estimate the changes in predation risk (Ludwig and Rowe 1990; Abrams and Rowe 1996). Recorded mortality figures are instead likely to be a combined result of both predation risk and prey behaviours that shift adaptively. For example, prey behaviour might change over development in response to both shifting predation pressures and changed future reproductive value, which is likely to be higher for older and larger juveniles (Werner and Anholt 1993; Grand 1999). Thus, when generalising from field mortality data it is important to consider what the size-specific mortality is relative to the size-specific foraging rate by the prey. Our quantification of growth rate differences between the instars indicates that, if anything, risk taking might be lower in the final instars (relative growth rates decrease in the final instar of both species, see Fig. 2). Although this study cannot get around the problem with shifting prey behaviour, we were however still able to detect strong effects of larval size on survival by keeping predator abundance high and equal for all larvae as well as keeping the larval size classes equally represented.

In our model simulation, the size dependent mode of predation predicted smaller optimal sizes than the constant predation risk mode for both species. Hence, the main important conclusion that can be drawn from the simulation is that size dependent predation has greater potential to balance selection on body size compared to when predation risk is independent of size. The difference in effect between the two predation modes were almost negligible at high predator presence ($p > 0.5$) since high mortality rates in general favour minimisation of the development period. This is in agreement with more general models (e.g. Kozłowski and Wiegert 1987). Since the daily mortality in our experiment was very high (52%), it is questionable whether high values of p generate good representations of average natural predation levels. For example, at a value of p equal to 0.25, (yielding a daily mortality rate of 13%), for a species growing to the size of 200 mg and at the same rate as *P. aegeria*, only two in one hundred individuals survive to metamorphosis. For a species like *P. aegeria*, where females can maximally lay a few hundreds of eggs (Karlsson and Wickman 1990), this average daily mortality rate seems like an upper boundary for what would be reasonable to expect. These assumptions of predation levels are also supported by approximations of daily mortality levels of 14 and 12% from earlier

field studies on butterfly larvae (see Kristensen 1994; Gotthard 2000). We have a more incomplete picture of the life history of *P. xiphia*, but due to the longer development time and larger size of this species, our model predicts that such high levels of predator presence are even more unlikely for this species. The lowest level of predator presence at which the constant mode of predation still balanced selection on increased size was for *P. aegeria* equal to 0.40 and for *P. xiphia* 0.25, while the same values for the size dependent mode were 0.25 and 0.16 respectively. Thus, a constant mode of predation risk does not seem to balance selection for size increase under what can be assumed common levels of predation occurring in nature, whereas the size dependent mode has a much stronger potential (Fig. 5).

It is important to keep in mind that the differences in size–fecundity relationships and growth rates between the two species have only been approximated and that the model cannot take all factors affecting final size into account. Thus, it is unlikely that the model should predict true values of optimal size for the species. For example, a difference between the species is the size–fecundity relationships, these will set the limit for the size at which reproduction can start. Since reproduction in *P. aegeria* is supported by a smaller final size than in *P. xiphia*, minimum size at maturity is smaller in *P. aegeria*. Also, because *P. aegeria* has the fastest growth rate, i.e. a higher increase in body mass per time unit, and no costs of fast growth was incorporated into the model, higher predation levels were required to stabilise selection on body size in this species. In our simulation we estimated the fecundity relationships by fitting linear regressions to our data, which, brings about two important consequences. First of all, fecundity will change at the same *rate* as growth. Thus, modelling growth as exponential will make fecundity increase in an exponential fashion with development time. Second, the relationship is assumed to be constant throughout size ranges beyond those observed in real populations. In nature, fecundity–size relationships are likely to at some point change shape. For example, limiting resources such as food plants, temperatures appropriate for oviposition, and factors as longevity and predation on adults can make the fecundity benefits of size increase follow a pattern of diminishing returns (Leather 1988). Earlier data on female body size and fecundity in *P. aegeria* (Karlsson and Wickman 1990) shows this relationship to be positively allometric, but more recent findings indicate that the strength of this relationship might be condition dependent, (Gotthard et al. *submitted manuscript*). The rate at which size increase translates into fecundity benefits is of outermost importance for selection on final size. The model based on our results does however show that size dependent predation has potential to stabilise selection from fecundity benefits, even when these increase exponentially and indefinitely with development time.

Foraging is often assumed to increase predation risk and this trade-off is held as one of the most important reasons to why an increase in body size at some point no longer will be profitable (Abrahams and Dill 1989; Lima and Dill 1990; Ludwig and Rowe 1990; Abrams 1991; Abrams et al. 1996; Abrams and Rowe 1996; Arendt 1997; Abrams and Schmitz 1999). Feeding activity has been shown potentially dangerous to larvae of the Lepidoptera (see Bernays 1997). Likewise, an earlier experiment by Gotthard (2000) with *P. aegeria* larvae showed that high growth rates increase predation risk from *P. bidens*. However, the experiment by Gotthard (2000) was designed to keep variation in prey size to a minimum. We show in this study that the actual size of the active larva might be of great importance in terms of predation risk. The relatively large effect of larval size was strengthened by the fact that, even

though *P. aegeria* grows faster in general compared to *P. xiphia*, there was no difference in mortality between the populations when controlling for size differences. Movement of the head and mouth parts while feeding, the position on the host plant and the bite marks on the foliage left from foraging, are probably important differences between foraging and resting insect larvae (Bernays 1997) and it seems likely that the effects of activity and prey size on predation risk are inter-related in shaping the optimal growth trajectories of insect larvae. For example, the same increase in activity might not result in the same increase in predation risk for a smaller individual compared to a larger one. It seems probable that a small insect larva sitting exposed while feeding on its host plant might not be in the range of detection by some predators, while the same situation for a large larva might be much more dangerous.

The generality of a relationship between the size of a given prey and predation risk is often questioned since it is likely to be dependent on the characteristics of both the prey and predator. Because we in this experimental study only used one single predator, the results should not be seen as an exact measure of the predation risks inferred on insect larvae throughout their development. However, *P. bidens* is a generalist predator hunting a wide variety of insect larvae (Southwood and Leston 1959) much in resemblance to other ground patrolling invertebrate predators, and Pararge larvae represent a general type of solitary cryptic insect larvae without any special adaptations to avoid certain predators. Thus, the general characteristics of both predator and prey do make the type of relationship found likely to be applicable to many other insect species in nature. Nevertheless, we find it important to point out that our conclusion from the simulation results is not that these perfectly mirror the real situation. Rather, the model shows the important general differences and implications for optimal size when assuming size dependent mortality as opposed to assuming a constant predation risk throughout development.

Conclusions

The decision of when to terminate growth should depend on what relative changes in mortality risk and reproductive value that continued growth at a specific developmental state will bring about (Kozłowski and Wiegert 1986). The question this study is concerned with is: what relative increase in predation risk might face a large larva prolonging growth in contrast to an individual of the same size instead choosing to enter metamorphosis? Thus, the objective has been to investigate how larval size increases the risk of being detected by potential generalist invertebrate predators. By doing this we have shown that continued growth at already large sizes may be very costly in terms of increased predation risk. Our model also shows that this type of size dependence has potential to stabilise selection on body size under naturally occurring predation levels. The results suggest that closer investigations of size–predation risk relationships between insect larvae and their predators in nature are needed as these relationships can have large impact on the evolution of growth trajectories and optimal size in insects.

Acknowledgements We would like to thank Christer Wiklund and Darrell Kemp for their labours in collecting adult butterflies. We are also grateful to S. Nylin, W. Blanckenhorn and other, anonymous reviewers for sharing their useful comments on earlier versions of the manuscript. This research was financed by a grant from the Swedish Research Council to K. G.

References

- Abrahams MV, Dill LM (1989) A determination of the energetic equivalence of the risk of predation. *Ecology* 70:999–1007
- Abrams PA (1991) Life-history and the relationship between food availability and foraging effort. *Ecology* 72:1242–1252
- Abrams PA, Leimar O, Nylin S, Wiklund C (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *Am Nat* 147:381–395
- Abrams PA, Rowe L (1996) The effects of predation on the age and size of maturity of prey. *Evolution* 50:1052–1061
- Abrams PA, Schmitz OJ (1999) The effect of risk of mortality on the foraging behaviour of animals faced with time and digestive capacity constraints. *Evol Ecol Res* 1: 285–301
- Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. *Q Rev Biol* 72:149–177
- Atlegrim O (1992) Mechanisms regulating bird predation on a herbivorous larva guild in boreal coniferous forests. *Ecography* 15:19–24
- Bernays EA (1997) Feeding by Lepidopteran larvae is dangerous. *Ecol Entomol* 22:121–123
- Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small? *Q Rev Biol* 75:385–407
- Calow P (1982) Homeostasis and fitness. *Am Nat* 120:416–419
- Case TJ (1978) On the evolution and adaptive significance of postnatal growth rates in terrestrial vertebrates. *Q Rev Biol* 53:243–282
- Cox DR (1972) Regression models and life tables. *J R stat soc B* 34:187–220
- D'Amico LJ, Davidowitz G, Nijhout HF (2001) The development and physiological basis of body size evolution in an insect. *Proc R Soc Lond B Biol Sci* 268:1589–1593
- Dial R, Roughgarden J (1995) Experimental removal of insectivores from rain-forest canopy – Direct and indirect effects. *Ecology* 76:1821–1834
- Esperk T, Tammaru T (2004) Does the 'investment principle' model explain moulting strategies in lepidopteran larvae? *Physiol Entomol* 29:56–66
- Finck OM, Yanoviak SP, Hanschu RD (1997) Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. *Oecologia* 112:244–253
- Gotthard K (1998) Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: investigating an adaptive reaction norm. *J Evol Biol* 11:21–39
- Gotthard K (2000) Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. *J Anim Ecol* 69:896–902
- Gotthard K (2001) Growth strategies of ectothermic animals in temperate environments. In: Atkinson D, Thorndyke M (eds) *Environment and animal development – genes, life histories and plasticity*. BIOS Scientific Publishers Limited
- Gotthard K, Nylin S, Wiklund C (1994) Adaptive variation in growth-rate – life-history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* 99:281–289
- Gotthard K, Nylin S, Wiklund C (1999) Seasonal plasticity in two satyrine butterflies: state-dependent decision making in relation to daylength. *Oikos* 84:453–462
- Gotthard K, Nylin S, Wiklund C (2000) Individual state controls temperature dependence in a butterfly (*Lasiommata maera*). *Proc R Soc Lond B Biol Sci* 267:589–593
- Grand TC (1999) Risk-taking behaviour and the timing of life history events: consequences of body size and season. *Oikos* 85:467–480
- Gunnarsson B, Hake M (1999) Bird predation affects canopy-living arthropods in city parks. *Can J Zool* 77:1419–1428
- Honek A (1993) Intraspecific variation in body size and fecundity in insects – a general relationship. *Oikos* 66:483–492
- Hooks CRR, Pandey RR, Johnson MW (2003) Impact of avian and arthropod predation on lepidopteran caterpillar densities and plant productivity in an ephemeral agroecosystem. *Ecol Entomol* 28:522–532
- Karlsson B, Wickman PO (1990) Increase in reproductive effort as explained by body size and resource-allocation in the speckled wood butterfly, *Pararge-aegeria* (L). *Func Ecol* 4:609–617
- Kozłowski J (1992) Optimal allocation of resources to growth and reproduction – implications for age and size at maturity. *Trends Ecol Evol* 7:15–19
- Kozłowski J, Wiegert RG (1986) Optimal allocation of energy to growth and reproduction. *Theor Pop Biol* 29:16–37
- Kozłowski J, Wiegert RG (1987) Optimal age and size at maturity in annuals and perennials with determinate growth. *Evol Ecol* 1:231–244

- Kristensen CO (1994) Investigations on the natural mortality of eggs and larvae of the large white *Pieris-Brassicae* (L) (Lep-Pieridae). *J Appl Entomol* 117:92–98
- Lang A, Filser J, Henschel JR (1999) Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. *Agric Ecosyst Environ* 72:189–199
- Leather SR (1988) Size, reproductive potential and fecundity in insects – Things aren't as simple as they seem. *Oikos* 51:386–389
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation – a review and prospectus. *Can J Zool* 68:619–640
- Ludwig D, Rowe L (1990) Life-history strategies for energy gain and predator avoidance under time constraints. *Am Nat* 135:686–707
- Lundvall D, Svanback R, Persson L, Bystrom P (1999) Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. *Can J Fish Aquat Sci* 56:1285–1292
- Margraf N, Gotthard K, Rahier M (2003) The growth strategy of an alpine beetle: maximization or individual growth adjustment in relation to seasonal time horizons? *Func Ecol* 17:605–610
- Nijhout HF (2003) The control of body size in insects. *Dev Biol* 261:1–9
- Nylin S, Gotthard K, Wiklund C (1996) Reaction norms for age and size at maturity in Lasiommata butterflies: Predictions and tests. *Evolution* 50:1351–1358
- Nylin S, Wickman PO, Wiklund C (1989) Seasonal plasticity in growth and development of the speckled wood butterfly, *Pararge aegeria* (Satyridae). *Biol J Linn Soc* 38:155–171
- Nylin S, Wickman PO, Wiklund C (1995) Life-cycle regulation and life-history plasticity in the speckled wood butterfly – Are reaction norms predictable? *Biol J. Linn Soc* 55:143–157
- Nylin S, Wiklund C, Wickman PO, Garciabarras E (1993) Absence of trade-offs between sexual size dimorphism and early male emergence in a butterfly. *Ecology* 74:1414–1427
- Owen DF, Shreeve TG., Smith AG (1986) Colonization of Madeira by the speckled wood butterfly, *Pararge aegeria* (Lepidoptera: Satyridae), and its impact on the endemic *Pararge xiphia*. *Ecol Entomol* 11:349–352
- Palokangas P, Neuvonen S (1992) Differences between species and instars of Phratora leaf beetles (Coleoptera, Chrysomelidae) in the probability of being preyed on. *Ann Zool Fenn* 29:273–278
- Parker MS (1993) Size-selective predation on benthic macroinvertebrates by stream-dwelling salamander larvae. *Arch Hydrobiol* 128:385–400
- Roff DA (1981) On being the right size. *Am Nat* 118:405–422
- Roff DA (2002). *Life history evolution*. Sinauer Associates, Inc., USA
- Sibly RM, Calow P., Nichols N (1985) Are patterns of growth adaptive? *J Theor Biol* 112:553–574
- Southwood TRE, Leston D (1959) *Land and water bugs of the British isles*. Frederick Warne & Company LTD, London
- Statacorp (2003) *Stata Statistical Software: Release 8.2*. College Station, TX: Stata Corporation
- StatSoft, Inc. (1999) *STATISTICA for Windows*. Statsoft East 14th Street, Tulsa
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, Oxford
- Tolman T (1997) *Butterflies of Europe*. Princeton University Press, Princeton and Oxford
- Werner EE, Anholt BR (1993) Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *Am Nat* 142:242–272