Effects of larval diet quality on the growth and development of immature stages of *Telostylinus angusticollis* (Diptera : Neriidae)

Amy K. Hooper[A] *and Russell Bonduriansky* (iD)[A,B]

[A]School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney,
NSW 2052, Australia.
[B]Corresponding author. Email: r.bonduriansky@unsw.edu.au

Abstract. Nutrient abundance during development has profound effects on adult morphology, life history and behaviour in many insects, but effects of nutrition on juvenile development are less well known. We investigated how larval diet quality affects patterns of growth, development and survival of larvae and pupae in the neriid fly *Telostylinus angusticollis* (Enderlein). We reared flies on two larval diets varying in nutrient concentration ('rich' versus 'poor') that have been shown previously to affect a wide range of adult traits in this species. We found that nutrient concentration affected larval growth trajectories, with individuals reared on the rich diet exhibiting greatly accelerated growth and reaching a larger body size. By contrast, we found no evidence that diet affected timing of development at the pupal stage, suggesting that developmental constraints may prevent variation in pupal development rate. Although overall mortality during the immature stages was not affected by larval diet, we found some evidence that individuals reared on a poor diet might experience higher larval mortality, whereas individuals reared on a rich diet might experience higher mortality during emergence from the puparium. Our results enhance understanding of the effects of nutrition on growth, development, and life history.

Introduction

Environmental quality during development has important implications for phenotypic variation across many adult traits. This has been shown in studies that examine environmental (e.g. diet) effects on the development of various adult traits, including morphology (Boggs and Niitepõld 2016), behaviour (Raguso *et al*. 2007) and survival (Boggs and Freeman 2005). These effects can also be transgenerational (Bonduriansky and Head 2007; Vijendravarma *et al*. 2009; Valtonen *et al*. 2012), and could therefore have important fitness consequences. This is especially true for holometabolous insects, in which resources are accumulated during the larval stage and then allocated to adult traits during the pupal stage to produce an adult morphology that is fixed after pupal eclosion.

How diet affects growth and development has important consequences for understanding how adult traits evolve. Larval diet is an important determinant of adult body size, with increased larval diet quality being associated with increased body size in many insect species (Chapman 1998). Decreased dietary quality during the larval stage has also been shown to delay development in insects (Tikkanen *et al*. 2000; Kaspi *et al*. 2002; Couret *et al*. 2014). This interplay between diet, total development time, and the evolution of body size has been investigated extensively (Davidowitz and Nijhout 2004; Kingsolver and Huey 2008; Nijhout *et al*. 2010).

While several studies have investigated effects of nutrition on larval development, effects on pupal development (i.e. metamorphosis) have received little attention. Some evidence suggests that larval and pupal stages respond differently to nutrient abundance. For example, in *Drosophila melanogaster* (James and Partridge 1995) and the green lace wing *Mallada basalis* (Ye *et al*. 2015), most variation in development time arises during the larval stage, while pupal development occurs at a relatively fixed rate. However, because adult morphology develops during the pupal stage, species that exhibit strongly plastic, nutrient-dependent development of adult morphologies (such as secondary sexual traits) might also exhibit nutrient-dependent variation in the rate and patterns of pupal development. For example, in horned beetles (*Onthophagus taurus*), males reared on a rich diet produce elaborate horns as weapons, and males but not females take longer to develop when reared on a higher-quality diet (Hunt and Simmons 1997). Because diet does not affect time to pupation in this species (Schwab *et al*. 2017), the development of elaborate male secondary sexual traits appears to involve a prolongation of pupal development.

The pupal stage is a closed system where no further resource intake is possible. Developmental processes during the pupal stage therefore mediate resource allocation trade-offs that shape adult morphology (Klingenberg and Nijhout 1998; Nijhout and Emlen 1998). Detailed knowledge of the effects of nutrient abundance on the progression of pupal development could thus provide insights into such trade-offs (Emlen 2000; Tomkins *et al*. 2005; Fry 2006).

The Australian neriid fly *Telostylinus angusticollis* (Enderlein), a native of coastal New South Wales and southern Queensland, exhibits strong developmental plasticity to variation in larval diet. When larvae are provided with a nutrient-rich diet, individuals emerge as larger adults, and males also develop larger secondary sexual traits (Bonduriansky 2007; Sentinella *et al*. 2013; Cassidy *et al*. 2014). Increased nutritional content in larval diet results in higher reproductive output in females (Adler *et al*. 2013), and enhanced combat performance in males (Hooper *et al*. 2017). Adult males reared on a rich larval diet also exhibit more rapid teneral development and accelerated ageing (Hooper *et al*. 2017). Yet, despite the importance of larval nutrition for adult phenotype and performance in *T. angusticollis*, the progression and plasticity of juvenile development have never been described in detail in this species.

The aims of this study are two-fold. First, we describe the immature stages of *T. angusticollis* from egg to teneral adult. Previous studies have shown that increased resources during the larval stage result in accelerated juvenile development in this species (Bonduriansky 2007; Bonduriansky and Head 2007; Hooper *et al*. 2017). However, it is unclear whether this effect results from changes in development rate at the larval or pupal stages, or involves changes in the relative timing of particular developmental events. Hence, the second aim of this study was to determine how larval diet affects rates and patterns of growth and development.

Materials and methods

The laboratory stock used in this study was descended from ~30 female and ~30 male individuals collected from a naturally occurring population at Parsley Bay Reserve in Sydney, Australia (33°51′09″S, 151°16′43″E), and maintained for several generations in the laboratory as a large outbred population. Stock adults were provided with Petri dishes of brown sugar and live yeast, and a substrate of moist cocopeat providing elevated humidity and *ad libitum* access to water. Larvae developed in the 'rich' larval diet medium (see below). In stock cages, females were able to oviposit on the same batch of larval medium over several days, and larvae therefore experienced a range of nutritional conditions. To determine effects of larval diet on development, we established two diet treatments ('rich' and 'poor') that have previously been shown to result in a significant difference in adult body size (Sentinella *et al*. 2013). The rich larval diet consisted of 32.8 g of protein (Nature's Way soy protein isolate) and 89 g of carbohydrate (brown sugar, Coles brand) per 500 mL of reverse-osmosis water and 170 g of coco peat. The poor diet consisted of 5.5 g protein and 14.8 g carbohydrate for the same amount of water and coco peat. Larval medium was thoroughly homogenised using a hand-held electric beater (Homemaker brand, model HM409, Kmart Australia), then frozen at −18°C until day of use (ProfiLine freezer, Liebherr Australia, Adelaide, Australia). During development, all animals were kept at 25°C on a 12-h light–dark cycle.

Larval development

Stock females were allowed to lay eggs on standard oviposition medium. To track larval development, eggs were transferred individually to 10 g of rich (*n* = 38) or poor (*n* = 35) larval

medium. The containers were checked every 24 h and each larva was photographed using a Leica DFC digital camera mounted on a Leica MS5 stereomicroscope. Photographs were later analysed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The linear measurement function of ImageJ was used to measure total body length, and the length of the sclerotised section of mouthparts (mouth hooks). All images were taken at a consistent magnification for each trait type (40× for body size measurements in first-instar larvae, 20× for body size measurements in second-instar larvae, and 10× for body size measurements in third-instar larvae and for determination of pupal development stage), allowing us to use linear measurements to quantify and analyse trait sizes across all focal individuals. Measurements were converted from pixels to millimetres using calibrations based on a stage micrometer. As a proxy for larval body length, the length of the trachea between the anterior and posterior spicules was measured, as this tended to coil with larval body movement, rather than stretch, and hence was less variable than absolute body length. This measure of body length was used to calculate growth rates (in mm day^{-1}) within each instar. Mouth hooks were measured from the lateral view, where the curve is visible (see Results). Any deaths during development of the larval stage were also recorded. Only individuals that successfully moulted to the next instar were included in analysis of growth rate (see below).

Pupal development

To rear individuals for pupal development assays, 20 eggs were transferred to each of nine containers of 100 g rich larval medium and nine containers of 100 g poor medium. These containers were checked daily for new pupae, and the newly pupated individuals were set aside so that the age of each pupa could be known at the time of dissection. To track pupal development, individuals had to be dissected, which required destructive sampling and precluded obtaining longitudinal data on individuals. At each day during pupal development, a subsample of pupae was dissected, and their pupal stage of development classified (*n* = 8–12 for each day and diet combination). We developed a classification key for the different stages of pupal development (see Results).

Statistical analyses

Body length was analysed using a linear mixed-effects Gaussian model with diet, day of measurement, and their interaction entered as fixed effects, with individual larval ID entered as a random effect to account for repeated measures. To analyse development of mouth hook size, we first calculated the average mouth hook length for each individual during each instar. Neriid mouth hooks exhibit a characteristic size and morphology within each larval instar (Vinasco Mondragon and Carrejo 2016), so variation within an instar for a given individual represents measurement error. Average mouth hook length per instar was then analysed using a mixed-effects linear Gaussian model with instar, diet, and their interaction as fixed effects, and individual larval ID as a random effect. Growth rate per instar (calculated as mm day^{-1}) and days of development per instar were analysed using models structured similarly to the model used for mean mouth hook length. Sex was not included in models of larval growth because we were unable to determine sex at the larval

stage. Mixed models were fitted using the 'lmer' function in the R package *lme4* (Bates *et al*. 2015), and *P*-values were calculated using the package *LmerTest* (Kuznetsova *et al*. 2017) in R 3.3.2 (R Development Core Team 2008).

Survival during development from egg to adult emergence was analysed using a Cox proportional-hazards model to test treatment effects on mean mortality rate (hazard), using the *survival* package (Therneau 2015) in R. Diet was entered as a predictor variable. Individuals that emerged successfully as adults were considered to have survived the juvenile development phase, while individuals that died before adult emergence were treated as censored data. We also used a Fisher's exact test within each stage of development to determine whether the proportion of individuals that died during each stage was affected by diet. Means ± standard error are reported where appropriate.

Results

Eggs

The eggs of *T. angusticollis* are similar to those of other neriid species previously described (Berg 1947; Olsen and Ryckman 1963). Eggs are elongate, fusiform, and white in colour, and ~0.85 mm in length (Fig. 1). Fine longitudinal grooves are present on the surface of the chorion. Each egg has an elongate anterior filament, which protrudes from the substrate after the egg is laid, and probably functions in respiration (Wigglesworth 1972). Hatching occurs 47 ± 1.44 h after oviposition. All viable eggs hatched by four days after oviposition. Larvae emerge head first near the anterior filament, where the chorion is split along two seams approximately one-quarter of the way down the egg. The proportion of eggs hatched was 83.5% (*n* = 164).
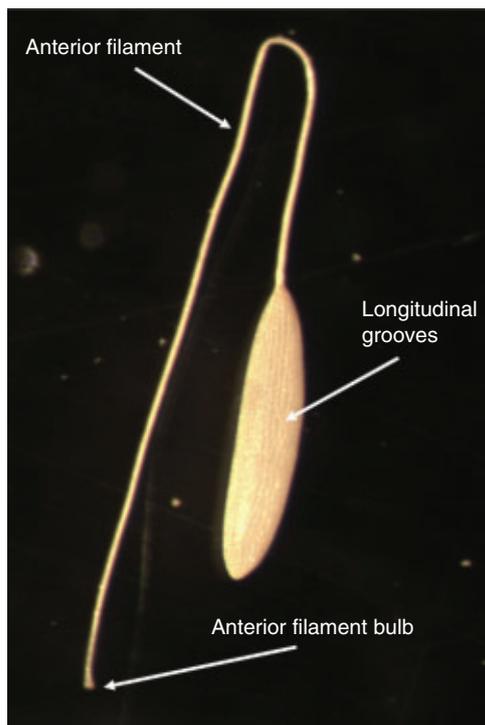
Larvae

In the wild, larvae are often observed beneath the bark of *Acacia longifolia* and *Erythrina × sykesii* trees. They have a particularly strong association with areas that are damaged by beetles boring into the bark, as females lay eggs into the beetle holes (authors' pers. obs.). Larvae probably feed on the rotting wood tissue or the bacteria or fungus growing on it, as has been previously described in the closely related tropical neriid species *Telostylinus lineolatus* (Berg 1947).

Like most other Cyclorrhapha, larvae of *T. angusticollis* lack thoracic legs (apodus), and have a greatly reduced head capsule (acephalous), with a cephalopharangeal skeleton withdrawn into the body and mouth parts that are reduced to mouth hooks (Heming 2003). Larvae are cylindrical, elongate, and muscidiform (i.e. cylindrical, with tapered anterior and blunt posterior: Fig. 2), and similar to other described species of Neriidae in shape (Fig. 2) (Berg 1947; Olsen and Ryckman 1963). Each larval stage of *T. angusticollis* has 12 segments (Fig. 2*e*): 1 small anterior pseudothoracic segment (which contains the mouthparts), 3 thoracic segments, and 8 abdominal segments. Thoracic segments are characterised by a lack of a spinulose area (Fig. 2*e*). The abdominal segments are the largest body segments, and make up most of the larval body. These segments are characterised by a spinulose area present on the anteroventral surface. The prothoracic (anterior) spicules are present at the posterior end of the first thoracic body segment. Posterior spicules are located on the final (eighth) abdominal segment.

Mouth parts of each larval stage consist of paired mouth hooks, which are sclerotised and darkly pigmented (Fig. 2*b, d, f*). The internal cephaloskeleton is not visible from the outside, and hence was not measured. Changes in mandible size and morphology (Fig. 2) indicate that *T. angusticollis* goes through three instars before pupation (mean instar durations: first: 3.5 days; second: 3.7 days; third: 10.0 days). Diet did not affect the number of instars during development (i.e. inspection of morphological features in larvae developing in rich and poor larval diets showed that larvae on both diets passed through three instars), nor length of mouth hooks for each instar (Fig. 3*a*; Table 1).

Larvae reared on the rich diet experienced higher growth rates during each larval stage (Fig. 3*b*; Table 1). For body length, we detected a significant interaction between day of development and larval diet (Table 2). There was also a significant interaction between instar and diet on the number of days of development, indicating that larvae reared on a poor diet spent longer developing in the later instars (especially in the third instar) compared with larvae reared on a rich diet (Fig. 3*c*; Table 1). For individuals that successfully emerged as adults, the average number of days from hatching to pupation was 15.2 (±1.01) days for the poor diet and 12.75 (±0.54) days for the rich diet. After approximately Day 5 of larval development, larvae reared on a rich diet attained a greater body length than larvae reared on a poor diet, and this difference persisted until pupation (Fig. 3*d*).

Fig. 1. Egg of *Telostylinus angusticollis.* Eggs are ~0.9 mm long, and the anterior filament is ~3 mm long. The image was taken at 40× magnification.

Fig. 2. Anatomy of *Telostylinus angusticollis* larvae. First instar larvae (*a*), with mouthparts (indicated by arrow, *b*); second instar larvae (*c*), with mouthparts (*d*); third instar larvae with anatomy labelled (*e*) and mouthparts (*f*).

Prepupae

When the larvae stop feeding, ~12 h before pupation, they enter the prepupal phase of development and often disperse from the larval substrate, similar to the prepupal behaviour of several other families of Cyclorrhaphous Diptera (Robertson 1936; Fraenkel and Bhaskaran 1973; Denlinger and Zdarek 1994). During this stage, larvae also exhibit jumping behaviour, similar to behaviour previously described in piophilid larvae

(Bonduriansky 2002). This behaviour begins with the larva clamping the posterior end of its body with its mouthparts. The body then becomes turgid, and when the mouthparts release, the body is flung up to 20 cm. This behaviour can be induced by gentle pinching, simulating a predator attack, which suggests that this behaviour might function to avoid predation.

Pupal development is generally similar to that previously described in other Cyclorrhaphous Diptera (Fraenkel and Bhaskaran 1973; Denlinger and Zdarek 1994; Barros-Cordeiro
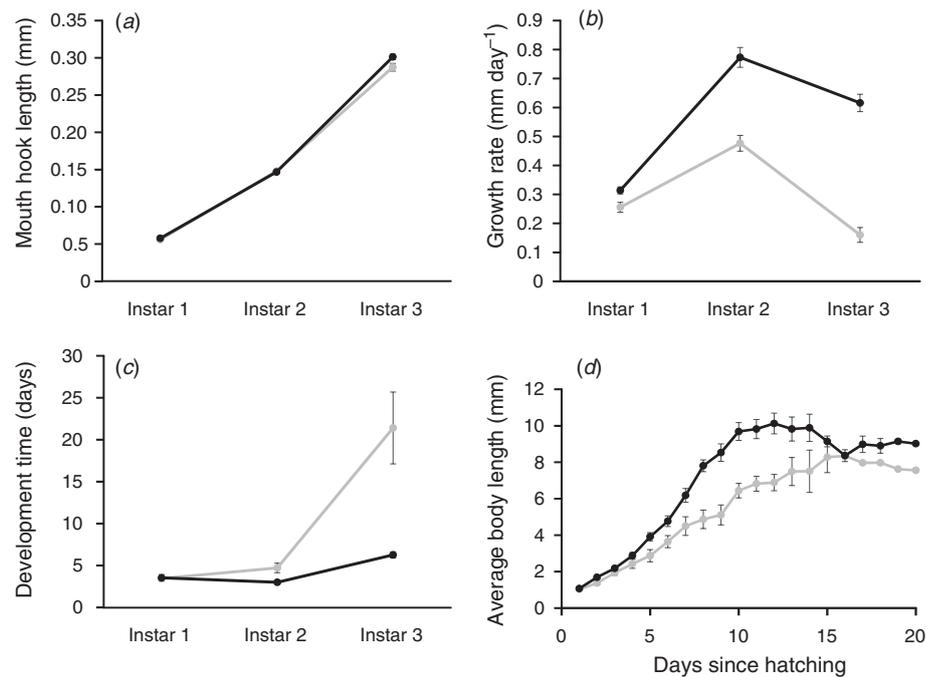
Fig. 3. Growth parameters (means ± standard error) of *Telostylinus angusticollis* larvae reared on either a rich (black line) or poor (grey line) diet: mouth hook length per instar (*a*), growth rate per day per instar (*b*), development time per instar (*c*), and average body length per day since hatching (*d*).

Table 1.   Effects of diet and larval instar on larval growth parameters
Bold denotes significant effects (*P* < 0.05)

	Mouth hook length			Days of development per instar			Growth rate (mm/day)		
	Estimate	s.e.	*P*	Estimate	s.e.	*P*	Estimate	SE	*P*
Intercept	**−0.065**	**0.006**	**<0.001**	**−4.993**	**1.730**	**0.005**	0.249	0.138	0.078
Diet	−0.010	0.009	0.275	**6.650**	**2.182**	**0.003**	**0.294**	**0.097**	**0.004**
Instar	**0.115**	**0.003**	**<0.001**	**7.050**	**0.937**	**<0.001**	0.060	0.076	0.435
Diet × instar	0.008	0.004	0.067	**−5.683**	**1.119**	**<0.001**	0.075	0.087	0.395
Random effects									
Individual ID	1.010×10^{-20}			0			0		
Residual	0.00036			11.21			0.04293		

et al. 2016). Immediately before formation of the pupal case, the anterior segments of the body retract, reducing the body length by 30.1% ± 1.24 for individuals reared on the poor diet, and 21.5% ± 1.19 on the rich diet. Hence, pupae from individuals reared on the rich diet are larger (males: 10.41 ± 0.04 mm; females: 8.13 ± 0.05) than those reared on the poor diet (males: 5.81 ± 0.04 mm; females: 5.76 ± 0.04 mm). The anterior spicules are now located at the very end of the body (Denlinger and Zdarek 1994). The cuticle hardens, and continues to darken to a deep brown colour for ~4 days. The pupae are elongate and dorso-ventrally curved (Fig. 4*a*), but the extent of the curvature varies between individuals. External features of the larvae, including body segments, spinulose areas, and anal plates, are also visible on the exterior of the puparium.

The prepupal stage proceeds for ~24 h following formation of the pupal case. During this time, many larval features are present, such as the trachea, and the sclerotised larval mouth hooks

Table 2.   Effects of diet and day of development on larval body length
Bold denotes significant effects (*P* < 0.05)

	Larval body length		
	Estimate	s.e.	*P*
Intercept	**1.347**	**0.258**	**<0.001**
Diet	**−0.837**	**0.365**	**0.0236**
Day	**0.367**	**0.017**	**<0.001**
Diet × day	**0.438**	**0.025**	**<0.001**
Random effects			
Individual ID		1.716	
Residual		0.807	

(Fig. 4*b*; stage I). A large gas bubble also develops in the centre of the pupal case during the prepupal phase, as it does in *Drosophila* (Robertson 1936).

Fig. 4.   Stages of pupal development of *Telostylinus angusticollis*: (*a*) outside view of pupal case; (*b*) prepupa, stage I; (*c*) phanerocephalic stage, stage II; (*d*) orange eyes, approximately Day 6, stage III; (*e*) brown eyes, approximately Day 8, stage IV; increasing pigmentation of appendages (*f*: stage V; *g*: stage VI), approximately 10–12 days, stages. Pupae at each stage are shown in dorsal, side, and/or ventral view. Pupae are ~6–8 mm in total length.

Pupae

Approximately two days after the pupal case forms, the invaginations of the head and thoracic segments become visible, indicating the phanerocephalic stage of development (Fraenkel and Bhaskaran 1973) and the start of metamorphosis (Fig. 4*c*). Approximately one day later, opaque eyes are visible in the pupa, indicating the transition to pharate adulthood.

At approximately Day 5 after formation of the pupal case, the adult thoracic segments, body appendages and external genitalia (on the males) become well defined, though still soft and not sclerotised or pigmented. Legs and wings are folded along the ventral side of the pupal body. Antennae are folded underneath the ventral side of the head. Genitalia are contained within a capsule at the posterior end of the body (Fig. 4*c–g*). After this point, the body appendages, including legs, antennae and wings, become more sclerotised and distinct (Fig. 4*d–e*). At approximately Day 6 the eyes begin to darken to a light orange colour (Fig. 4*d*, stage III), and at Day 8 markings become visible on the dorsal side of the thorax and the eyes become dark brown (Fig. 4*e*, stage IV). At this point, legs and wings can be separated

from the body, indicating increased sclerotisation. At Days 9–10 after the pupal case forms, ~3 days before emergence, pigmentation in the external features begins (Fig. 4*f*; stage V). Pigmentation starts on the thorax, and proceeds on the antennae, legs and wings. The last features to be pigmented are the head and the posterior end of the abdomen (Fig. 4*g*; stage VI).

Diet had no apparent effects on the rate or pattern of pupal development (Fig. 5). There was no difference in pupation duration between diets ($t_{4.2} = 0.240$, $P = 0.822$). At ~12.7 days after the onset of pupation, adults emerge from the puparium at the anterior end. The puparium is ruptured at the first four body segments on the ventral side, and this section of the puparium is completely removed, similar to other described neriid species (Berg 1947).

The approximate mean durations of development for each immature stage are shown in Table 3.

Survival during development

There was no effect of larval diet on mean overall (egg-to-adult) juvenile mortality (i.e. hazard) rate (hazard ratio = 0.68; 95%
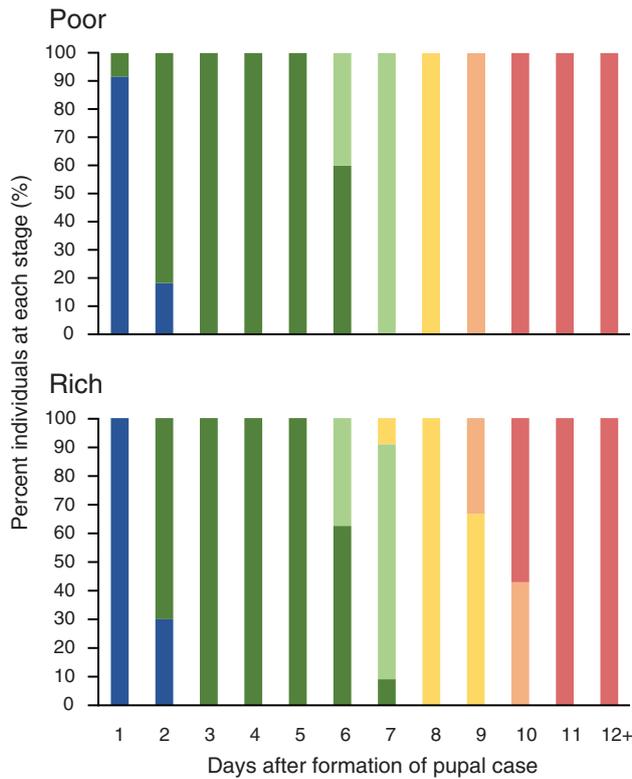
Poor

Rich

Fig. 5. Proportion of *Telostylinus angusticollis* pupae at each stage of development for each day after pupation, demonstrating the similar progression of pupal development for poor (top panel) and rich (bottom panel) larval diets. Dark blue, stage I; dark green, stage II; light green, stage III; yellow, stage IV; orange, stage V; red, stage VI.

confidence intervals: lower, 0.316, upper, 1.467; *P* = 0.326) (Fig. 6). In both diet treatments, >40% of larvae died during the first five days of larval development, after which there was a reduction in larval mortality rate. Patterns of mortality suggest that a higher proportion of poor-diet individuals died during the third instar, and that more rich-diet individuals died during the pupal phase (Fig. 7). Additionally, all imagos that died during emergence were reared on the rich diet. However, these patterns were not statistically significant, potentially due to low power (Fig. 7).

Discussion

Overall, development of *Telostylinus angusticollis* is similar to that of other neriid species (Berg 1947; Olsen and Ryckman 1963; Vinasco Mondragon and Carrejo 2016) and, more broadly, other Cyclorrhaphous Diptera (Fraenkel and Bhaskaran 1973; Denlinger and Zdarek 1994). However, while we found that larval diet has a large effect on growth and development rate during the larval phase, our results suggest little effect of larval diet on the rate or progression of pupal development. These results are surprising, considering that these diets produce significant differences in body shape and expression of secondary sexual traits in males (Bonduriansky 2007), and that these traits are produced during the pupal phase. In the beetle

Table 3. Approximate mean durations (in hours) of each developmental stage, and total development, for individuals reared on nutrient-poor and nutrient-rich larval diets

Stage	Larval diet	
	Poor	Rich
Egg	47	47
First-instar larva	72	72
Second-instar larva	96	60
Third-instar larva	504	144
Prepupa	24	24
Pupa	305	305
Total	1048	652
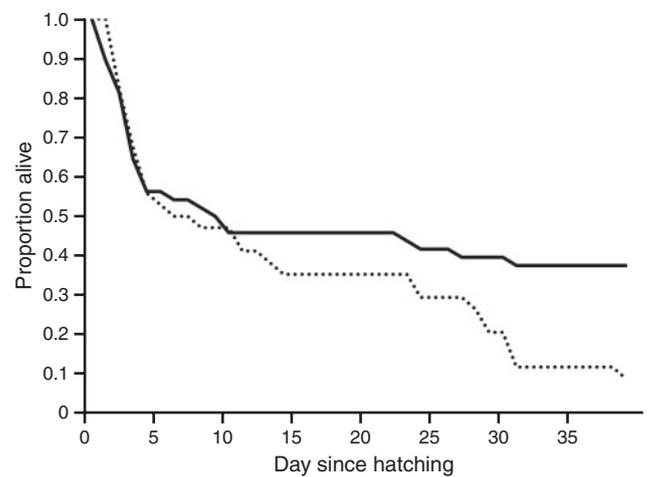
Fig. 6. Survival of larvae reared on poor (dotted line, *n* = 34) and rich (solid line, *n* = 48) larval diets.
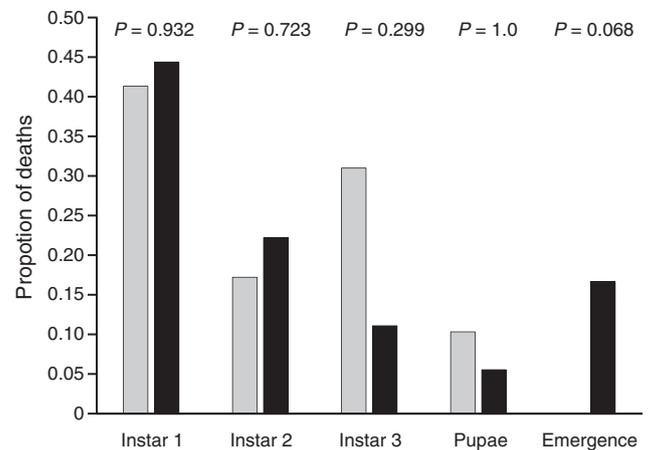
Fig. 7. Proportion of preadult deaths that occurred at each developmental stage for larvae reared on either a rich (black) or poor (grey) diet. *P*-values above each pair of bars represent results of Fisher's exact test to compare the proportion of individuals that died at each stage.

Onthophagus taurus, another holometabolous species where males reared on a rich larval diet produce exaggerated secondary sexual traits, pupal development is extended in males (Hunt and

Simmons 1997; Schwab *et al*. 2017). Our results contrast with these findings, but are consistent with patterns previously shown in *Drosophila melanogaster*, where variation in development time arises largely due to variation in the duration of the larval stage rather than the duration of the pupal stage (James and Partridge 1995). This suggests that there may be developmental constraints in Diptera that prevent variation in the development of the pupal stage.

In contrast to the diets of some other holometabolous species (Wigglesworth 1972; Ali *et al*. 1990; Bentancourt *et al*. 2003), larval diet did not change the number of instars or mouth hook size during development in *T. angusticollis*. However, diet had a large effect on larval development and growth rates, with rich-diet larvae exhibiting greatly accelerated growth and development and reaching a larger body size relative to larvae reared on a poor diet. The lack of an effect of larval diet on mouth hook size suggests that dietary nutrients have differential effects on growth of different body parts, with larvae reared on a nutrient-rich larval diet exhibiting increased body length and width but not increased head size relative to larvae reared on a nutrient-poor larval diet. It is not known whether larval diet affects juvenile hormone profiles in this species, such that pupation is initiated at different critical body sizes or masses in larvae reared on different nutrient concentrations. These results for overall larval growth rate are consistent with previous evidence of large effects of larval diet on egg-to-adult development rate in this species (Bonduriansky 2007; Bonduriansky and Head 2007). Our results are also consistent with patterns from several other species in which increased resources are associated with accelerated larval development rate and increased adult body size (see Awmack and Leather 2002 for a review). These include holometabolous taxa, such as the Mediterranean fruit fly *Ceratitis capitata* (Kaspi *et al*. 2002), the mosquito *Aedes aegypti* (Couret *et al*. 2014), the moth *Operophtera brumata* (Tikkanen *et al*. 2000), as well as hemimetabolous taxa, such as the cricket *Teleogryllus commodus* (Hunt *et al*. 2004). In *T. angusticollis*, the growth rate of larvae reared on a rich diet is particularly accelerated relative to that of larvae reared on a poor diet during the second and third instars. High growth rates during the later stages of larval development suggest that this may be an important stage for investment in adult morphology via resource allocation to imaginal discs, as occurs in other species (Moczek and Nijhout 2004). Because *T. angusticollis* adults are sexually dimorphic in body size (with males larger, on average, than females) when reared on nutrient-rich larval diets (Bonduriansky 2007), the growth rate of male larvae may be more sensitive to nutrient abundance than the growth rate of female larvae, and the diet effect on mean growth rate may thus be driven by male responses. However, because we were not able to determine the sex of larvae, we were not able to test for an interaction of larval diet and sex on development rate.

It is generally assumed that a faster growth rate is advantageous because it increases probability of reaching adulthood, and this is likely true in *T. angusticollis*, where larvae are vulnerable to ant predation (authors' pers. obs.). Hence, accelerated growth rate may be beneficial for *T. angusticollis* in the wild. However, rapid growth is likely to have important implications for adult physiology and performance. Accelerated somatic growth has been associated with increased oxidative damage (Alonso-Alvarez *et al*. 2007) and elevated metabolic rate (Criscuolo *et al*. 2008), which may impair adult performance (Metcalfe and Monaghan 2003). Faster growth rates may also be subject to physiological constraints that predispose quickly built, larger bodies to increased developmental errors (Blanckenhorn 2000; Metcalfe and Monaghan 2003). In fact, larger *T. angusticollis* males have been shown to be more susceptible to somatic damage, suggesting that accelerated growth is associated with a more fragile body (Adler *et al*. 2016). Higher susceptibility to somatic damage may also contribute to accelerated actuarial and reproductive ageing in males reared on a rich larval diet (Hooper *et al*. 2017). (These effects have not yet been investigated in *T. angusticollis* females.) Hence, despite potential net advantages of accelerated development, this strategy may also impose some costs on individuals that develop on a rich diet. We do not yet know how the nutrient composition of our experimental larval diets compares with nutrient composition encountered by larvae in natural populations of this species. It is possible that our nutrient-rich larval diet has very high protein content by comparison with natural larval food, and that this excess dietary protein imposes some costs that are rarely experienced by wild flies.

In poor-diet individuals, we found much greater variation in development time during the third instar. This also coincided with higher mortality rates during the third instar for poor-diet larvae, which may indicate that individuals on a poor diet may struggle to reach a critical weight required to pupate successfully, as has been demonstrated in the tobacco hornworm *Manduca sexta* and in *Drosophila melanogaster* (Robertson 1963; Nijhout and Williams 1974; Nijhout 1975). However, contrary to studies of several other species (e.g. see Ali *et al*. 1990; Shanower *et al*. 1993; Boggs and Freeman 2005), decreased diet quality did not increase overall larval mortality during development. Moreover, we found that rich-diet individuals had a near-significantly higher probability of death during pupal eclosion, which may indicate a potential mortality cost to individuals of a larger body size. It has been previously shown that the development of secondary sexual traits can interfere with moulting in a waterstrider (Arnqvist 1994), and such traits might also interfere with emergence in holometabolous species (although our sample size may have been insufficient to detect this effect in this study). Our results suggest that larval nutrition might have complex effects on patterns of larval and pupal mortality, necessitating further investigation.

Conclusions

We have described the immature stages and development of *Telostylinus angusticollis* for the first time, and quantified the effects of diet on growth and development. We found that diet had large effects on larval growth, which may expose larger individuals to costs in adulthood. By contrast, we found no evidence that diet affected timing of pupal development, which may indicate developmental constraints on the pace and pattern of metamorphosis. Although overall egg-to-adult survival rates were similar for both larval diet treatments, our results suggest that diet-dependent mortality could occur during certain phases of larval and pupal development.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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