

Critical weight mediates sex-specific body size plasticity and sexual dimorphism in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae)

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Ultimate factors driving insect body size are rather well understood, while—apart from a few model species—the underlying physiological and developmental mechanisms received less attention. We investigate the physiological basis of adaptive size variation in the yellow dung fly *Scathophaga stercoraria*, which shows pronounced male-biased sexual size dimorphism and strong body size plasticity. We estimate variation of a major physiological threshold, the critical weight, which is the mass at which a larva initiates pupariation. Critical weight was associated with sexual size dimorphism and sex-specific plasticity, and is thus a likely target of selection on adult size. Detailed larval growth trajectories derived from individuals raised at two food and temperature treatments further reveal that sex-specific size plasticity is mediated by faster initial growth of males that later becomes reduced by higher male weight loss during the wandering stage. We further demonstrate that integral growth rates, which are typically calculated as simple ratios of egg-to-adult development time and adult weight, do not necessarily well reflect variation in instantaneous growth rates. We illustrate the importance of detailed assessments of ontogenetic growth trajectories for the understanding of adaptive size variation and discuss the mechanistic basis of size determination in shaping sex-specific phenotypic plasticity.

1 | INTRODUCTION

Body size varies dramatically between and within species and contributes greatly to the phenotypic diversity observed across the tree of life. Evolutionary biologists have heavily scrutinized the ultimate drivers of body size variation, revealing an often tight relation of body size to fitness (reviewed in Andersson, 1994; Blanckenhorn, 2000; Chown & Gaston, 2010). Large individuals often leave more offspring, are more successful in acquiring mates, and survive better (Blanckenhorn, 2000; Clutton-Brock, 1988; Honěk, 1993; Shine, 1989). While the ultimate causes of body size variation thus are rather well understood, the proximate mechanisms determining body size remain poorly

investigated apart from a few model species, despite their importance for understanding the evolutionary process (Badyaev, 2002; Chown & Gaston, 2010; Stillwell, Blanckenhorn, Teder, Davidowitz, & Fox, 2010).

In insects, intraspecific body size variation is striking and plastic responses to the environment and sexual dimorphism (SSD) can be extreme, but the underlying physiological and developmental causes have received surprisingly little attention (Badyaev, 2002; Blanckenhorn, 2000; Stillwell et al., 2010). As insect growth is determinate, all structural growth is restricted to the immature stages. It follows that adult size is a function of propagule (i.e., initial) size, growth rate, and the time over which juveniles grow. Nevertheless, body size may only indirectly depend on the rate or duration of growth. At

least as important are the mechanisms that terminate growth at a particular size or point in time (Nijhout, 2003; Nijhout & Davidowitz, 2009; Davidowitz, 2016; see below). Thus, when studying body size variation, be it caused by environmental or genetic processes, the consideration of the complexity of larval growth, as well as mechanisms of size determination, are crucial for the understanding of body size evolution.

Larval growth and the determination of adult size are best understood in *Manduca sexta* and *Drosophila melanogaster* (Mirth & Riddiford, 2007; Nijhout, 2003; Nijhout et al., 2014). In *Drosophila*, after hatching from the egg, larvae start to feed and grow nearly exponentially, leading the larva to moult and expand its cuticle. In their third instar, larvae reach a threshold size called the critical weight (or critical size in the *Drosophila* literature). The attainment of this threshold size is mediated by insulin/insulin-like growth factor signaling acting on the prothoracic gland, which in turn is thought to incite well-described endocrine signaling cascades that lead to the cessation of growth and the onset of pupariation to commence metamorphosis (reviewed by Mirth & Riddiford, 2007; Mirth et al., 2014; Nijhout et al., 2014; Shingleton, 2011). Once the larva has stopped feeding, it enters the wandering stage, during which it empties its gut and prepares for metamorphosis while actively looking for a suitable location for pupariation. It follows that variation in critical weight, marking the start of hormonal interactions leading to metamorphosis, will strongly influence the size of the adult insect. However, since larvae continue to grow until ecdysteroids are secreted, environmental plasticity or sex-specific variation in the terminal growth period (TGP) and the rate of growth during TGP can influence size as well. Variation in sex-specific size plasticity or adult sexual size dimorphism can thus arise from variation in critical weights, differences in the amount of growth during the TGP (which is affected by growth rate and duration), as well as disproportioned weight loss during the subsequent development. Critical weight variation has been shown to affect sexual size dimorphism (Testa, Ghosh, & Shingleton, 2013) and underlies the temperature-size-rule in *D. melanogaster* (Ghosh, Testa, & Shingleton, 2013). In *M. sexta*, in contrast, critical weight is unaffected by temperature (Davidowitz, D'Amico, & Nijhout, 2003; Stillwell & Davidowitz 2010) but nevertheless influences SSD (e.g., Stillwell, Daws, & Davidowitz, 2014). The extent to which these mechanisms apply to other insects is not yet fully clear (Parker & Johnston, 2006). It seems likely, however, that critical weight (or a similar mechanism) is a key player in body size regulation of all holometabolous insects (Callier & Nijhout, 2011; Stieper, Kupershtok, Driscoll, & Shingleton, 2008), although growth variation after the attainment of critical weight could account for adult size variation (e.g., SSD) as well.

The complexity of the larval growth just described is often neglected in organismic biology. This is not a matter of ignorance but rather a necessity given the efforts required in

estimating detailed growth trajectories, especially in comparative and field studies, as well as a consequence of the necessary reductionism when studying life-history evolution (Davidowitz, 2016). Larval growth rate is then often approximated as the ratio of adult size and egg-to-adult development time, thus assuming linear growth. While these estimates are convenient and often the only available data, insect larvae neither grow continuously nor in a linear fashion. Linear growth rates and overall egg-to-adult development times are therefore compound traits that integrate the nonlinear, interactive nature of larval growth, and often the biological meaning ascribed to these measures remains obscure (Tammaru, Esperk, Ivanov, & Teder, 2010). To interpret such growth rate estimates (in the following referred to as “integral growth rates”; Figure 1) in a biologically meaningful way, their comparison to detailed larval growth trajectories is helpful, if not essential.

Here, we study larval growth and physiology in the yellow dung fly *Scathophaga stercoraria* (Linnaeus, 1758) (Diptera: Scathophagidae). This large coprophilous fly (7–13 mm in length; Blanckenhorn, Pemberton, Bussière, Roembke, & Floate, 2010) shows strong body size variation with male-biased sexual size dimorphism (Simmons & Ward, 1991), the adaptive nature of which has been well scrutinized. Body size varies genetically across latitude (Blanckenhorn & Demont, 2004) and (somewhat) altitude (Blanckenhorn, 1997). Plastic responses to habitat depletion (by a “bail-out” response *sensu* Tobler & Nijhout 2010), which is common because dung is an ephemeral habitat, as well as to seasonality and temperature are strong (Blanckenhorn, 1998, 1999, 2009). However, the underlying physiological and developmental mechanisms, the potential targets of selection, have received much less scrutiny (but see Blanckenhorn & Henseler 2005; Blanckenhorn & Llaurens, 2005).

By estimating detailed, individual growth trajectories under different environmental conditions we here aim to reveal the proximate causes underlying sex-specific body size plasticity in the yellow dung fly. We estimate sex-specific critical weight, as opposed to the commonly investigated minimum viable weight, and expect this major size determinant to cause adult body size variation. Additionally, we compare simple integral growth rates to the actual linearized weight increment with age during the initial, quasi-exponential growth phase (=quasi-instantaneous growth rates) to investigate whether the former estimates introduce systematic biases. We conclude by discussing the implications of growth and size determination for the evolution of body size plasticity and sexual size dimorphism.

2 | MATERIALS AND METHODS

Adult *S. stercoraria* were captured on a cattle pasture in Zurich, Switzerland and used to establish an outbred

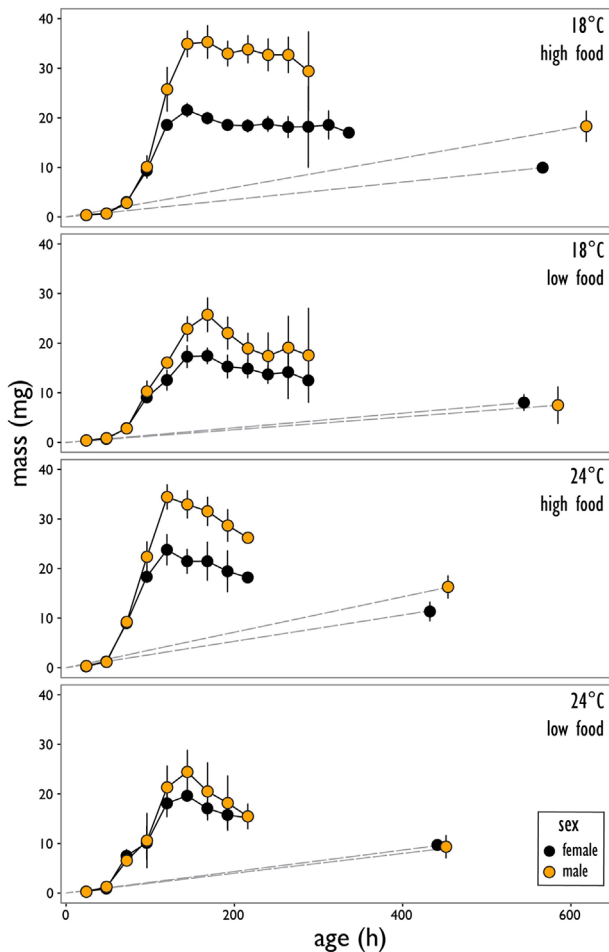


FIGURE 1 Sex-specific larval growth trajectories in different environments characterized by variation in dung quantity and temperature. Points ($\pm 95\%$ CI) represent mean mass of individually-weighted larvae at a given age, starting with newly hatched first instar larvae. Sex-specific mean adult mass is indicated at the mean age at eclosion (isolated points to the right). During early larval development (first ~ 4 days), larval mass increases nearly exponentially. Eventually, larval growth ceases and individuals enter the wandering stage during which weight is lost. Males (indicated in orange) grow faster but reach their peak weight at the same time as females (black dots). Sexual size dimorphism is always present at the larval peak weight but adults are monomorphic under food limitation due to a higher weight loss of males. Broken gray lines indicate integral growth rates derived from adult mass and egg-to-adult development times, illustrating the conceptual difference between instantaneous and integral estimates of growth rate estimates

laboratory culture under standard maintenance procedures (Blanckenhorn et al., 2010).

2.1 | Individual larval growth trajectories

To estimate detailed larval growth trajectories, we followed individual flies throughout their immature stages. Eggs were collected from the laboratory culture and singly placed onto

the surface of a small rectangular dish ($22 \times 22 \text{ mm}^2$) filled with standardized, previously frozen cow dung. To prevent desiccation, the bottom of each dish was filled with a shallow layer of agar (3%). Larvae hatched within 24 hr and were recovered from the opaque substrate with a spatula, rinsed with tap water and dried on filter paper. Clean and dried larvae were then weighed twice to the nearest 0.01 mg and placed back into the same dish. The amount of dung supplied at the start of the experiment therefore, represented the total amount available to a larva to complete its development. The weighing procedure was repeated every 24 hr until larvae underwent pupariation. (Pre)pupae were weighed and checked for adult eclosion every other day. Upon eclosion, adults were killed by freezing, sexed, and weighed. The sex of individuals that died prior to adult eclosion or during the early pupal stages could not be assessed, so these individuals were removed from the data set. To assess plastic responses to environmental variation we used a two-factor design, crossing high and low temperatures (24 vs. 18°C) with unlimited and limited amounts of food (2 vs. 0.7 g of dung per individual). Dung amounts were chosen based on previous findings and personal experience (pers. obs. WUB). Under food limited conditions, young larvae are still able to dwell in and feed on dung in a regular manner. At some point however, nutrients deplete and larvae rummage through an empty cellulose matrix, vainly searching for food.

To assess sex-specific larval growth we measured several parameters. To quantify the speed of growth during the quasi-exponential period, we regressed cube-root transformed weight against age, and calculated the increase in linearized weight with time (slope of the regression in $[\text{mg}^{1/3}/\text{h}]$) for each individual separately. Only data gathered during the time in which larvae grow nearly exponentially was included. Because the relationship between cube-root transformed mass and growing time was approximately linear, the slope of this relationship could be used as an estimate of instantaneous growth rate. To test for differences in instantaneous growth rates between groups, we used a mixed linear model of $\text{mass}^{1/3}$ as a function of age, sex, food quantity, and temperature as fixed effects, their interactions, and the identity of each individual larva as random effect (cf., Blanckenhorn, 1999; Teuschl, Reim, & Blanckenhorn, 2007). In this model, the coefficient of the age term was used to quantify growth rate. Non-significant interaction terms were removed. To ensure that growth was indeed exponential, we here also only analyzed data for the first 96 hr (which represents approximately 2/3rds of the growth period) during which growth followed an exponential trend, irrespective of treatment or sex. To compare instantaneous growth rates to estimates of integral growth (= individual adult mass^{1/3} divided by egg-to-adult development time), we used both estimates simultaneously as dependent variables in a bivariate analysis with the type of growth rate (instantaneous or

integral), sex, food quantity and temperature as explanatory factors and individual identity as random effect.

We further analyzed larval peak mass (maximum weight of the larva), the subsequent weight loss during the wandering phase (difference between larval peak mass and pupal mass), the mass at pupariation, as well as, adult mass using type III ANOVAs with sex, food quantity, temperature, and their interactions as explanatory variables. Measurements of mass were always cube root transformed, and non-significant interactions were removed. We also analyzed the duration of the exponential growth period (estimated graphically from the individual growth trajectory), the age at which growth stopped (age at which a larva reaches its peak mass), the duration of the wandering stage (difference between the age at larval peak mass and age at pupariation), and the ages at pupariation and adult eclosion using analogous type III ANOVAs.

2.2 | Critical weight

In *M. sexta* the critical weight is defined by the minimum mass of a larva at which starvation does not further prolong pupation and is usually estimated by comparing the time to metamorphosis of starved and fed larvae of different weight classes (Nijhout & Williams, 1974). The effect of starvation on the time to pupariation (TTP) is however not universal. Especially in insects inhabiting ephemeral habitats, metamorphosis is induced prematurely under starvation (Blanckenhorn, 1998, 1999; Shafiei, Moczek, & Nijhout, 2001; Stieper et al., 2008; Teder, Vellau, & Tamaru, 2014). These alternative life history strategies therefore, require alternative procedures to identify the weight at which a larva initiates pupariation. In *D. melanogaster*, an alternative “break-point” approach is commonly applied: following the prediction that critical weight should alter the relationship between mass at starvation and the time to pupariation (TTP), plotting the latter two against each other should result in a segmented relationship with a pronounced break-point at the critical weight (due to a change in reaction). Larvae that have not yet reached their critical weight are expected to continue their larval development, while larvae that passed their critical weight should be unaffected or show a so-called “bail-out” response (Tobler & Nijhout, 2010). Crucially, critical weight is different from the minimum viable weight (Davidowitz et al., 2003; Stieper et al., 2008), which is more often reported and sometimes used as a proxy for the critical weight; however, the minimum viable weight refers to the minimum amount of resources necessary to reach the next life stage (Mirth & Riddiford, 2007) and is not directly related to the induction of metamorphosis per se.

Since *S. stercoraria* shortens its development time when food is limited (Blanckenhorn, 1998, 1999), we here apply the break-point method. Analogous to the aforementioned

rearing protocol, individual larvae were placed into small dishes with either limited or unlimited cow dung in a 18°C climate chamber. Over the course of several days, haphazardly chosen 3rd instar larvae (according to the cephaloskeleton morphology: Ferrar, 1987) were removed from their dish, weighed and placed individually into an empty dish equipped only with a thin layer of agar to prevent desiccation. Larvae that underwent such a starvation treatment were monitored every 12 hr to record the timing of either death or pupariation. If larvae successfully pupariated, their time to pupariation (TTP) was calculated as the time between the start of starvation and pupariation. Of the 1,150 larvae starved, only 700 made it to the adult or late pupal stage and could be sexed; data on all other individuals were discarded.

We plotted the time to pupariation (TTP) of each individual larva against its mass at starvation (Figure 2). If larvae have not yet passed their critical weight, pupariation has not yet been induced and larvae may try to forage more in order to reach their critical weight. This prolongs their TTP. Only after a certain time lag will these larvae eventually pupariate (possibly adjusting their target size to the new environment (i.e., modifying their critical weight)). In contrast, the TTP of larvae that have passed their critical weight and already induced metamorphosis, should not be affected by starvation, as JH is already being depleted and ecdysone secretion will inevitably kick in. The reaction of a larva in terms of TTP should thus vary according to its weight at starvation. Prior to reaching their critical weight, larvae are expected to prolong their TTP, which should not happen thereafter. Plotting TTP against larval mass at starvation resulted in an angular relationship with a break point, suggesting the existence of a *Drosophila*-like critical weight. We then fitted a segmented regression to this relationship (using the R-package *segmented*: Muggeo (2008) which also supplies confidence intervals), the break point of which indicates the critical weight (the weight at which the reaction to starvation changes). This was done separately for males and females in both limited and unlimited food conditions (Figure 2).

3 | RESULTS

3.1 | Plastic responses to temperature and food manipulation

Larvae raised at 24°C eclosed earlier than those raised at 18°C ($F_{1,61} = 141.38$, $p < 0.001$) but did not significantly differ in their adult mass ($F_{1,63} = 2.35$, $p = 0.130$). In the high temperature treatment, larvae had higher instantaneous growth rates (GLM with larval mass as dependent variable: age*temperature: $F_{1,196} = 64.78$, $p < 0.001$), but the duration of the exponential growth phase was shorter (main effect:

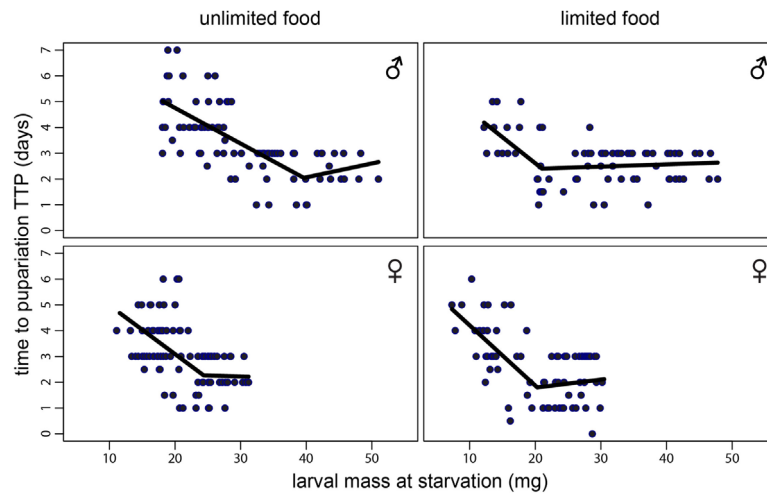


FIGURE 2 Plotting the time to pupariation (TTP) upon starvation against the mass at which larvae were starved results in an angular relationship. This suggests that larvae change their reaction to starvation depending on their size. The peak-points of the segmented regressions thus indicate the critical weights. These vary between sexes and food quantity treatments, suggesting a role of critical weights in shaping sex-specific plasticity (see also Figure 3)

$F_{1,60} = 106.38$, $p < 0.001$). Consequently, 24°C larvae reached their peak weight sooner (age at peak mass, main effect: $F_{1,62} = 13.53$, $p < 0.001$, Figure 1) and showed a shorter wandering stage (main effect: $F_{1,60} = 42.32$, $p < 0.001$), thus pupariating earlier (main effect: $F_{1,60} = 105.00$, $p < 0.001$, Figure 1), and the duration of their pupal stage was shorter than that of larvae raised at 18°C (main effect: $F_{1,61} = 241.45$, $p < 0.001$, Figure 1).

Food quantity had somewhat similar effects: instantaneous growth rates were higher under food limitation (GLM with larval mass as dependent variable: age*food limitation: $F_{1,196} = 13.59$, $p < 0.001$) and larvae terminated their exponential growth phase earlier ($F_{1,60} = 5.21$, $p = 0.026$). Food limitation had no effect on the timing of growth cessation (age at larval peak mass), but it did affect larval peak mass ($F_{1,61} = 27.90$, $p < 0.001$). The wandering stage of food limited larvae was shorter ($F_{1,60} = 5.67$, $p = 0.020$; corrected

for body size) and, as a result, they pupariated earlier. Weight loss during the wandering stage was independent of food quantity, but the lower peak weight of food-limited larvae resulted in lighter pupae ($F_{1,61} = 7.55$, $p = 0.008$). Finally, the duration of the pupal stage did not depend on food limitation but larger individuals took longer to eclose (duration of pupal stage: $F_{1,61} = 28.74$, $p < 0.001$). All AN(C)OVA tables as well as the results of the GLMs are presented in full in the supplementary Tables S1 and S2.

3.2 | Ontogeny of sexual size dimorphism

Overall, the larger males had a higher instantaneous growth rate than females (GLM with larval mass as dependent variable; age*sex interaction: $F_{1,64} = 17.05$, $p < 0.001$), and they also grew for longer in an exponential fashion (sex effect: $F_{1,60} = 20.71$, $p < 0.001$; sex*temperature interaction:

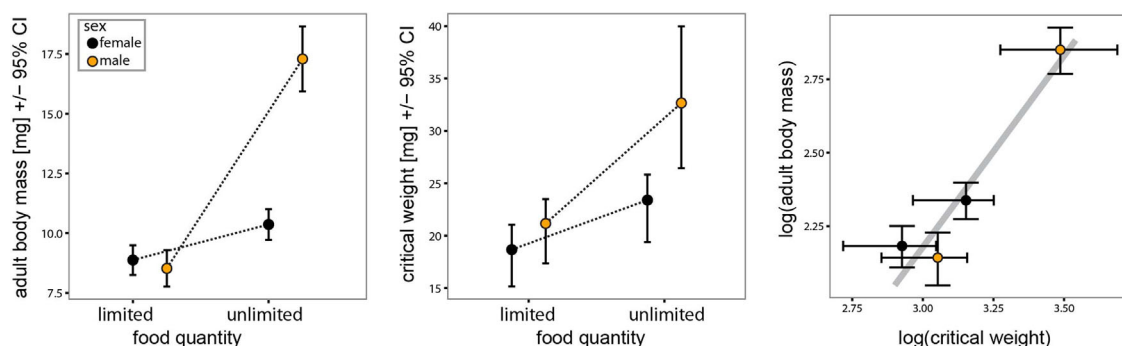


FIGURE 3 Adult body mass (left panel) and critical weight estimates (middle panel) show sex-specific plasticity in response to food availability. Critical weights and body size do not differ at limited food, but males increase their body size and critical weight more strongly than females with increasing food supply. Adult body mass and critical weights correlate strongly ($R^2 > 0.95$, $n = 4$), suggesting that critical weight is a major driver of intraspecific body size variation (log–log plot; right panel)

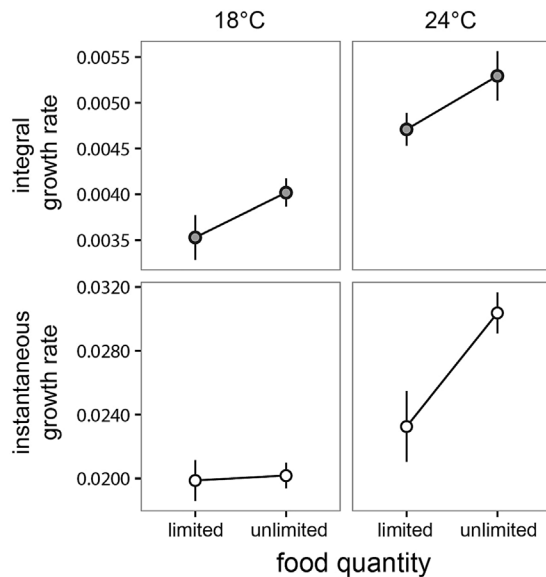


FIGURE 4 Integral growth rates are affected by temperature and food treatments, but there is no interactive effect. Similarly, instantaneous growth rates increase with temperature and food quantity, but in contrast to integral growth rates, instantaneous growth rates react more strongly to food limitation at high (24°C) than at low (18°C) temperature (also see Table S3). These contrasting patterns demonstrate that the two measures can differ substantially across environments such that caution is advised in choosing the appropriate measure when investigating insect growth

$F_{1,60} = 24.72, p < 0.001$). This resulted in a much greater peak mass of males than females once larvae stopped feeding (larval peak mass; $F_{1,61} = 11.09, p = 0.001$, Figure 1). SSD at the larval peak mass was more pronounced at unlimited food (larval peak mass; sex*food interaction: $F_{1,60} = 4.39, p = 0.040$, Figure 1). Surprisingly, independently of body mass, temperature, and food (marginally non-significant), males lost a disproportionate amount of their mass during the wandering stage (weight loss; sex effect: $F_{1,61} = 12.5, p = 0.001$), resulting in a less pronounced SSD in the pupal (pupal mass; sex effect: $F_{1,61} = 32.90, p < 0.001$, Figure 1) and adult stages (adult mass; sex effect: $F_{1,60} = 7.98, p = 0.010$, Figure 1). Even though at peak mass SSD was pronounced in all treatment combinations, adult SSD was only significant at unlimited food conditions (adult mass; sex*food limitation interaction: $F_{1,60} = 9.80, p < 0.001$, Figure 1). Males took overall longer to reach the adult stage (age at eclosion; sex effect: $F_{1,61} = 19.53, p < 0.001$) due to longer pupal development. Males were also more strongly affected by temperature than females (age at eclosion; sex*temperature interaction: $F_{1,61} = 4.01, p = 0.050$), because they spent more time in the larval stage (age at pupariation; sex effect: $F_{1,60} = 5.64, p = 0.021$).

Expectedly, instantaneous growth rates were always higher than calculated integral growth rates. Both estimates

of biological growth correlate strongly ($r = 0.74, p < 0.001$), however. When analyzing differences between the two estimates of growth rates, we found that integral growth rates were generally more strongly affected by food quantity (type*food quantity: $F_{1,63} = 17.10, p < 0.001$). Differences between sexes and temperature treatments were more pronounced when using instantaneous growth rates (sex*type interaction: $F_{1,63} = 32.61, p < 0.001$; temperature*type interaction: $F_{1,63} = 74.34, p < 0.001$). Food quantity only had an effect on instantaneous growth at the high (24°C) but not at low (18°C) temperatures, whereas integral growth rates were always affected by food quantity, irrespective of temperature (type*food quantity*temperature interaction: $F_{1,63} = 28.03, p < 0.001$, Figure 4 and Table S3).

3.3 | Critical weight

While the sexes did not significantly differ in their critical weight at limited food (females: 18.66 mg [15.15, 21.05], males: 21.17 mg [17.35, 23.50]; [2.5th and 97.5th] percentiles are given; Figure 2), males had a higher critical weight at unlimited food (males: 32.67 mg [26.43, 39.98], females: 23.40 mg [19.39, 25.82]). That confidence limits overlap at limited but not at unlimited food in Figure 3 (center) suggests an interaction between sex and environmental conditions.

Apart from critical weight, we could not address the role of the ensuing terminal growth phase due to our measurement interval and sample size limitation. In fact, some estimates of critical weight exceeded the maximal larval peak weight of the averaged trajectory, likely an experimental artifact since critical weight and the growth trajectories were estimated in blocks given the amount work. Also, individual larvae might have been physically stressed by the daily measurements, potentially diminishing size increments. We are however confident in our estimates of growth trajectories, as they are very similar to those of earlier studies not tracking individuals (Blanckenhorn, 1999; Teuschl et al., 2007).

4 | DISCUSSION

S. stercoraria larvae respond to starvation depending on their larval weight, suggesting a *Drosophila*-like critical weight mechanism (cf., Stieper et al., 2008). Critical weight, representing the mass at which pupariation is initiated, was further associated with sexual size dimorphism and condition dependent body size plasticity in response to food availability (Figures 1–3). Therefore, (sex-specific) plasticity in critical weight likely plays a major role in generating body size variation of adult yellow dung flies. The detailed larval growth trajectories documented here reveal that even when adults do not differ in their body size, male and female larvae have dissimilar larval growth schedules. Thus, sexual size

dimorphism varies greatly in its extent throughout juvenile development, and the sexes are likely exposed to different selective pressures during the larval stages. In the following, we discuss the implications of size determination mechanisms and detailed larval growth assessments for the understanding of intraspecific body size variation and sexual size dimorphism.

The universal mechanism initiating metamorphosis, and thus terminating growth in all insects, is an increase in ecdysone titer during the final instar (Nijhout et al., 2014). In *Manduca*, *Bombyx*, and *Drosophila*, intraspecific adult size variation has been attributed in large parts to variation in critical weight, which is the weight (or an unknown correlate thereof; see below) at which a larva initiates ecdysone release (Nijhout et al., 2014). The commonness of the critical weight mechanism in insects is generally assumed, although empirical data are scarce. Our findings in *Scathophaga* advocate in favor of its prevalence. The mere phenotypic presence of a physiological switch-point does however not necessarily imply identical size-sensing mechanisms or homologous endocrine signals. Cuticle tension has been identified as a size-sensing mechanism in two hemipterans (Nijhout, 1984; Wigglesworth, 1940), but holometabolous insects appear to use different triggers. Studies on *D. melanogaster* found that the insulin-dependent growth of the prothoracic gland is associated with critical size sensing (Mirth, Truman, & Riddiford, 2005), while in *M. sexta* oxygen limitation due to the pre-assigned size of the tracheal system has been identified as a major driver in determining critical weight (Callier & Nijhout, 2011; but see Helm & Davidowitz, 2013). In contrast to the actual size-sensing triggers, the consequent endocrine cascades are much better resolved, although the effects of hormones can also vary between species (e.g., the role of JH and ecdysone in *Manduca* vs. *Drosophila* (Mirth et al., 2014)).

The timing of metamorphosis induction in *S. stercoraria* is environmentally plastic and its degree varies between the sexes, which produces severe fitness consequences in the adult stage. In *S. stercoraria* males are the larger sex, which is in stark contrast to *Manduca* and *Drosophila* which show female-biased SSD. The male-biased size dimorphism in the yellow dung fly is ultimately driven by stronger selection on male size relative to female size (Blanckenhorn, 2007, 2009). At the proximate level, we here demonstrated that males induce pupariation at a larger size than females (i.e., at a larger critical weight), in correspondence with the adult SSD. *Scathophaga* further shows an adaptive bail-out response to food limitation (Blanckenhorn, 1999), meaning that larval growth is accelerated yet shortened in order to reach the pupal stage prior to complete habitat depletion. Our data suggest that this response to food depletion is caused by a combination of heightened instantaneous growth rates and a lowered critical weight, which—all else being equal—reduce the larval period as well

as peak weight. In addition, we found sex-specific plasticity in critical weights: males initiate pupariation at a larger size than females at unlimited food only, while the sexes did not differ in their critical weights under food limitation. This again corresponds well to the sex-specific plasticity in adult body size with males being the more plastic sex (Teuschl et al., 2007; Figures 1 and 3). Females thus seem to have a lower target size in good environments, in accordance with the existing SSD and the putative selection pressures leading to it (Blanckenhorn, 2007, 2009), implying that their size is more canalized relative to males. Critical weight and its plasticity thus account to a significant extent for the adaptive body size variation displayed by *S. stercoraria* (Blanckenhorn, 1998; Figure 3), and is therefore a likely target of sexual selection on male size and fecundity selection on female size. Recent findings in *D. melanogaster* suggest that variation in critical weight between sexes is linked to the insulin/insulin-like growth factor signaling pathway (Rideout, Narsaiya, & Grewal, 2015; Testa et al., 2013). Given that insulin is influenced by nutrition (Emlen, Szafran, Corley, & Dworkin, 2006; Shingleton, Das, Vinicius, & Stern, 2005), mediates growth and size, and also interacts with JH and ecdysone (Edgar, 2006), the insulin signaling pathway is a good candidate linking (sex-specific) plasticity to condition in *S. stercoraria*. Nevertheless, its impact on larval growth trajectories requires explicit investigation, particularly in light that *Drosophila* and *Scathophaga* diverged about 50 mya (Wiegmann et al., 2011).

In insects, the evolution of sexual size dimorphism and body size plasticity are primarily studied in adults even though structural body size variation must be induced before the adult cuticle is fully sclerotized. Potential costs of adult size variation thus already arise during the larval or nymphal stages, which is why the mechanisms are crucial to understand the evolution of sexual size dimorphisms and body size in general (Stillwell et al., 2014; Tammaru & Esperk, 2007; Testa et al., 2013; Vendl, Kratochvíl, & Šípek, 2016). SSD has been attributed to variation in the number of instars between the sexes (Esperk, Tammaru, Nylin, & Teder, 2007), sex-specific growth rates (Blanckenhorn et al., 2007; Rohner, Blanckenhorn, & Puniamorthy, 2016; Vendl et al., 2016), variation in development time (Teder, 2014; Rohner et al., 2016), or unequal (post-eclosion) weight loss (Molleman et al., 2011; Testa et al., 2013; see below). We found that in *S. stercoraria* sexual dimorphism in larval peak mass arises due to unequal rates and durations of the initial instantaneous growth, with males growing faster and for a prolonged period. Males were always larger than females prior to the wandering stage. Interestingly however, males also lost significantly more weight after growth ceased (on average: 24.6% in males vs. 21.6% in females; Figure 1). This loss was independent of their body size and especially striking at limited food conditions, with larvae showing strongly male-biased SSD whereas the emerged adults were monomorphic in size,

reaffirming that larval growth trajectories differ between the sexes. Since adult size is under strong selection in males, this weight loss is doubtlessly costly and begs for scrutiny. Most of this weight loss is certainly due to the purging of gut content, but further metabolic costs and the continued development during the wandering stage certainly add to it (Reim, Kaufmann, & Blanckenhorn, 2009). Testa et al. (2013) found that female *Drosophila* larvae, which are larger than males, also lost more weight during the wandering stage. The authors hypothesized that the instantaneous growth rate differences of male and female larvae might be linked to the growth of the imaginal discs, which continue to develop during the wandering stage. If this holds true, imaginal discs of the faster growing sex would generally deplete a larger amount of stored resources. Indeed, on average *S. stercoraria* males grow faster than females. However, weight loss neither correlated with the instantaneous growth rate nor with peak mass in our study, suggesting a different mechanism. Alternatively, sexually dimorphic timing of growth and development of imaginal discs could be responsible for the unequal weight loss of the sexes documented here. Although in *Drosophila* male gonads grow slower than their female counterparts during the late larval stage (Kerkis, 1931), it remains possible that male *S. stercoraria* invest more in growth and development of imaginal tissue during the late larval stages, which could explain why males spend less energy during the pupal stage (Reim et al., 2009).

As demonstrated here and elsewhere (Blanckenhorn, 1999; Tammaru & Esperk, 2007; Tammaru et al., 2010; Teuschl et al., 2007), detailed individual growth trajectories harbor great potential in untangling variation in larval growth. Such assays demand tremendous efforts and may not be applicable in some taxa or certain environments. As a result, integral growth rates estimated from final body sizes and the corresponding egg-to-adult development times are widely in use (Figure 1). Instantaneous growth rates were on average 6.7 ± 0.2 SE times higher than integral rates in females and 7.9 ± 0.3 SE times higher in males. We further found that the two different estimates of growth rate differ in their response to food limitation. Instantaneous growth rates reacted much more strongly to food shortage at high than at low temperatures while the effect of food quantity on integral growth rates was independent of temperature (three-way interaction; Figure 4). This is not an issue of statistical power; rather, the two estimates greatly differ in their conceptual and biological meaning. While instantaneous growth rates estimate the speed of growth during the (more or less) continuous quasi-exponential initial growth phase, integral growth rates integrate not only the total amount of gained weight but also the amount of mass lost throughout juvenile development, including the process of inter-instar molting. Nevertheless, the two measures correlate quite strongly ($r = 0.7$), which implies that the initial rate of growth scales

roughly with the subsequently realized growth. However, as demonstrated here, the shape of growth trajectories can differ consistently between sexes and environments, consequently introducing potentially systematic biases when analyzing integral estimates. Obviously this can, but must not necessarily be problematic. Special caution is surely appropriate if integral growth rates are compared among species, especially if taxa differ in their ecology. Our main point here is that integral growth rates clearly depict a different biological property than instantaneous growth rates, and this inequality may not always be appreciated sufficiently (Esperk et al., 2013; Tammaru & Esperk, 2007).

In summary, we here shed light on the physiological and developmental underpinnings of condition dependent sex-specific body size plasticity and showed that critical weight, a major size determinant, is likely a major driver responsible for sex-specific plasticity and ultimately sexual dimorphism in the yellow dung fly and, probably, insects in general. Our study further suggests that critical weight(-like) mechanisms are indeed common in insects. We also demonstrated the usefulness of detailed larval growth trajectories for the understanding of the ontogeny of sex-specific plasticity in growth, as opposed to simpler estimates of integral growth. Future research should aim at uncovering the proximate causes and the evolvability of sex-specific plasticity in other non-model species in order to obtain a more general understanding of how adult body size variation arises.

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