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Experimental study on the impact of continuous and dynamic artificial light at night on moths originating from different skyglow conditions

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Biodiversity is negatively affected by light pollution, caused by artificial light at night (ALAN). Light-emitting diodes facilitate new lighting technologies to mediate the negative effects of ALAN, such as dynamic ALAN where light intensity can be adjusted to traffic density. Organisms living in highly light-polluted areas may show adaptations to mitigate the negative effects of ALAN. In a split-brood rearing experiment, larvae of two moth species (*Ochropleura plecta* and *Agrotis exclamationis*) originating from low-medium and high-medium skyglow populations were grown under either continuous ALAN, dynamic ALAN or control-dark conditions. We tested for ALAN effects on larval mortality, feeding behaviour, development and body mass, and whether effects depended on skyglow levels in the population of origin. Contrary to previous studies, we found either no or positive effects of ALAN on larval development, with similar or stronger effects of dynamic ALAN compared to continuous ALAN. For *A. exclamationis*, we showed evidence for faster development, increased growth rate and higher body mass under ALAN. This could reduce larval exposure to parasites and increase fecundity. We found no evidence for evolutionary responses in low-medium or high-medium skyglow larvae. Our results show that ALAN does not affect larval development the same way in all species.

Keywords Caterpillar, Development, Dynamic lighting, Lepidoptera, Light pollution, Phenotypic plasticity

Mankind is increasingly illuminating the nocturnal landscape by introducing artificial lighting at night (ALAN) in the environment, mainly in the form of public lighting[1]. The light pollution this causes is not limited to the direct vicinity of the light, but is also reflected by the atmosphere and clouds, creating skyglow[2]. Skyglow is much weaker in intensity than direct light pollution, but causes ALAN to spread up to several kilometres from its source in a cumulative way based on local clusters of lights.

The mounting evidence on negative effects of ALAN on biodiversity[3,4] combined with high costs of public lighting has prompted the question whether and how the use of ALAN can be better regulated[5,6]. Limitations in the legal framework make it difficult to obtain a common policy on ALAN regulation. For example, the European policy framework requires that there is evidence of negative ALAN effects on populations of protected species (i.e., species on the Annexes of the Habitat and Birds Directive) or that the party responsible for the ALAN emission causes intentional harm to organisms, which is often hard to prove[7]. Some countries have developed binding laws, while others stick to non-binding guidelines for reducing light pollution[8]. From a biodiversity perspective, policy strategies for darkening areas surrounding nature reserves[9,10] and creating dark corridors[11,12] are increasingly proposed and defended. However, such ALAN mitigation measures are typically not isolated from politically difficult trade-offs with social and economic factors or arguments, of which safety (or at least safety perception) is usually considered the most important[6]. The transition from classic light sources (e.g., sodium) to light emitting diodes (LED) led to an increase of short-wavelength light, potentially increasing damage to biodiversity[13]. However, it has also stimulated higher flexibility of ALAN regulation[5]. In places where the traffic volume is limited but lighting remains necessary, two main types of mitigation measures have been adopted. First, the light spectrum can be changed towards longer wavelengths like amber or red light (e.g.[14]). This so-called ‘bat-friendly’ light has the purpose to reduce attractive or repulsive effects of streetlights to flying insects and thus bats[15]. Secondly, there are dynamic streetlights whose intensity can be adjusted according to the operational need for illumination depending on the amount or presence of traffic or passers-by. So-called ‘smart streetlights’ are an example. These streetlights have a motion detection system that only switches on when

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a person or vehicle passes and switches off again after a fixed, typically short, period of illumination. This should significantly reduce exposure time to ALAN. However, a recent study showed that even a short exposure to ALAN can cause behavioural changes in adult moths[16]. To what extent the ecological effects of dynamic ALAN differ from continuous ALAN is still poorly understood.

Here we experimentally tested the impact of both types of ALAN on the growth and larval development of moths. Moths are an emblematic species group when it comes to light pollution, since most species are nocturnal and show a typical flight-to-light response[17,18]. ALAN does not only cause direct behavioural effects in adult moths, but may also affect larval development. Previous studies have mainly shown negative effects of ALAN on larval development, including increased larval mortality[19], diapause inhibition[19–22], faster development[19,20,22] and reduced growth rate[23]. Eventually, ontogenetic exposure to ALAN may lead to a reduction in adult body size[19,20,23], which in turn may have negative effects on fecundity, particularly in females[24,25], and on mobility (e.g.[26]).

When exposure to ALAN is accompanied by fitness costs in inherited traits for a significant part of a population, it has the potential to drive evolutionary responses[27]. Altermatt & Ebert[28] showed that ermine moths originating from urban, light-polluted areas had a weaker flight-to-light response compared to conspecifics of rural, dark areas, suggesting an adaptive response against the negative effects of flight-to-light. It was recently shown that these differences related to changes in wing morphology[29]. For intraspecific variation in adult feeding and mate calling behaviour no such adaptive responses have been found, even though both traits were negatively affected by ALAN[30]. Similar studies on adaptative potential relative to larval development are currently lacking. These previous studies collected moths close to streetlights[28,30]. For species where the majority of adults are assumed to stay close to the host plant on which they grew up as larvae, like in the case of ermine moths[31], this can indeed be useful. However, for species that show higher mobility, it is likely that the general density of public lighting at the landscape scale, which is reflected in the skyglow level, is a more important driver of evolutionary processes than individual light sources. Merckx et al.[22] showed ALAN at skyglow intensity is sufficient to constrain larval growth and inhibit diapause induction in *Chiasmia clathrata*. Therefore, chronic exposure to high skyglow levels has the potential to drive adaptive responses that could enhance larval development under ALAN.

In this study, we tested for differences in the impact of ALAN on larval development of moths whose mother originated from either high-medium or low-medium skyglow areas, allowing to examine both local adaptation and plastic constraints. In a split-brood breeding experiment, larvae originating from populations under high-medium and low-medium skyglow levels were reared under either continuous ALAN, dynamic ALAN or dark night conditions. We tested the treatments in two generalist moth species, *Ochropleura plecta* and *Agrotis exclamationis* (Noctuidae), which are both still common so they are an interesting complement to previous studies that used more specialised or less common study species[19,20,22,23]. Moreover, this choice allowed us to sample females in reasonable numbers and well distributed over our study regions. Noctuidae are particularly attracted to short-wavelength ALAN, which is relevant given the current shift towards LED streetlights that usually emit more short-wavelength light. Previous studies of ALAN on larval survival found either no effect[22,23] or negative effects[19]. ALAN exposure usually accelerates larval development, either through diapause inhibition[19], but also independently of such a diapause inhibition[20,22]. When the impact is not compensated by a higher growth rate, it leads to reduced final body mass, as shown in previous studies[19,20,23]. Dynamic ALAN significantly reduces the exposure time compared to continuous ALAN and can therefore be expected to have a weaker effect on larval development. If an adaptive response is present in high-medium skyglow larvae, we predict higher larval survival, growth rate and final body mass under ALAN conditions compared to low-medium skyglow larvae.

Materials and methods

Study species

For our experimental study, we selected two noctuid species: the heart and dart *Agrotis exclamationis* L. and the flame shoulder *Ochropleura plecta* L. (Lepidoptera: Noctuidae). Adults of both species drink nectar[32] and can be considered as, at least to a significant extent, income breeders[33]. *Agrotis exclamationis* is a common and widespread species in the temperate and Mediterranean areas of the Palearctic region (GBIF.org. https://www.gbif.org/). It is present in several kinds of open habitats, including agricultural areas, and flies in two generations a year (from May to October in Belgium[32]). The larvae are nocturnal and, during the day, they hide in litter or soil. They are able to feed on a wide range of herbaceous plants and grasses, including crops. In autumn, full-grown larvae make a cocoon in the soil to hibernate.

Ochropleura plecta is a Holarctic moth species that is common and widespread in the temperate and subpolar zones, but rarer in the Mediterranean zone (GBIF.org. https://www.gbif.org/). It is characterised as a habitat generalist, being present in both open habitats and woodlands. It has two to three generations a year (from April to October in Belgium[32]). Larvae feed on a large variety of herbaceous plants during the night and hide on the food plant during the day. They hibernate as pupae in the soil.

Female sampling and pre-experimental breeding conditions

Females were sampled in two regions in Belgium that differ significantly in skyglow (Supplementary Fig. S1); Central Belgium with relatively high-medium skyglow levels (SQM: 19.50–20.43 mag./arc sec^2, sky brightness: 727–1711 µcd/m^2) and Southern Belgium with relatively low-medium skyglow levels (SQM 20.82–21.30 mag./arc sec^2, sky brightness: 326–507 µcd/m^2) (data from http://www.lightpollutionmap.info[2]). They were captured with a Skinner light trap with a mercury vapour lamp and LedTraps with a 5050 UV LED strip[34] from the end of May to the beginning of July 2023. Females were transferred to the lab and kept in plastic boxes (115 × 115 × 60 mm) with 1-mm holes for aeration, a 1.5-mL tube with 10% honey-water solution and paper towel for oviposition. For *A. exclamationis*, eggs from 23 females were obtained (11 families from the high-medium skyglow region, 12 from the low-medium skyglow region). For *O. plecta*, eggs from 12 females were obtained (6 families from each region). We did not test for genetic relatedness between the females originating from the same sampling site, but both species are common, widespread species of reasonable mobility; hence, the probability of having caught related females is assumed to be low. Eggs and resulting larvae were reared in groups of *c.* 10 larvae/box in a climate chamber under a day-night cycle of LD 16:8 and at a day and night temperature of 23 °C and 18 °C, respectively. Larvae were fed a mixture of *Plantago lanceolata* and *Taraxacum officinale* leaves until the start of the experiment.

Split-brood breeding experiment

For each family of *A. exclamationis* and *O. plecta*, 6 to 12 and 6 to 24 larvae, respectively, were transferred to individual boxes with aeration holes and paper towel in a climate room under a day-night cycle of LD 16:8 and at a day and night temperature of 23 °C and 18 °C with measured mean temperatures in the rearing boxes reaching 24.3 °C and 19.9 °C. The larvae were equally divided over two climate rooms with each three light treatments; (i) continuous ALAN from a single LED light (Philips 1.4 W, 2700 K; 9.10–46.7 lx) during the whole night; (ii) dynamic ALAN from a single LED light with one-minute pulses of light and variable time of darkness mimicking dynamic streetlights with movement detection (on: 10.3–44.2 lx, off: 0.16–0.23 lx); the time scheme was programmed on Raspberry Pi Zero W (Raspberry Pi Foundation) and is shown in Supplementary Table S1; (iii) control treatment with dark nights (0.03–0.16 lx). Every day, the larvae were checked for moulting and were fed with fresh pieces of wild-picked *P. lanceolata* leaves. The size of a single piece of food plant was fixed and the number of pieces was adjusted to the amount eaten during the previous day. A plant mister was used to increase relative humidity (above 75%). Paper towel was replaced and faeces removed regularly. Larvae were weighed with a microbalance (Mettler MT5 microbalance; precision ± 10^{-5} g) at the beginning of the experiment and then every 5 or 7 days for *A. exclamationis* and *O. plecta*, respectively. Full-grown larvae moulted to a pre-pupal stage in which they usually construct a cocoon underneath the paper towel where they pupate. Pupae were weighed four days after pupation and adults were weighed on the day of eclosion. Mass loss upon eclosion was determined as (1 – adult mass/pupal mass) · 100[35]. Growth rate was only calculated for *A. exclamationis*, since *O. plecta* had a very short development time. Overall exponential growth rate for *A. exclamationis* was calculated as (maximal larval mass/larval mass at start experiment) ^ (1/day of maximal larval mass) – 1. Initial growth rate was calculated in the same way for the first 10 days of the experiment and final growth rate for the last 10 days before maximal larval mass was reached. Adults were provided with a tube of honey-water solution for five days, after which they were stored in the freezer (–21 °C). After daily handling, the position of the rearing boxes was randomized.

Statistical analysis

First, we tested for differences in survival, larval development, feeding behaviour and body mass between *O. plecta* and *A. exclamationis*, after which we tested for the effects of ALAN treatment, skyglow origin and sex, as well as their two-way interactions, on these factors for both species separately. Non-significant two-way interactions were removed from the models. Differences in mortality were tested using generalized linear mixed models (GLMM) with a binomial error distribution. GLMMs with a Poisson error distribution were used to test for differences in duration of the larval and pupal period. Larval body mass at the start of the experiment was included as a covariate for duration of the larval period. Feeding frequency was tested using a GLMM with a binomial error distribution and the amount of eaten food was tested using a GLMM with a gamma error distribution. Differences in body mass and mass loss upon eclosion were tested using linear mixed models (LMM). Duration of the larval period and the interaction effects with ALAN treatment, skyglow origin and sex were also included to test for differences in body mass and feeding behaviour. LMMs were used to test for differences in exponential growth rate in *A. exclamationis*. In all LMM and GLMM models, climate room was included as a covariate and family nested within sampling site was included as a random factor. In case there was a significant treatment effect, a post-hoc test with Benjamini-Hochberg correction was performed to compare the different treatments. Sample sizes are provided in Supplementary Tables S2 and S3. All analyses were performed in R 4.4.2 (R Development Core Team, R Foundation for Statistical Computing, Austria. http://www.R-project.org/). We reported our results in line with the good practice of the language of evidence[36].

Results

Except for larval survival, we found moderate to very strong evidence for differences in all developmental variables and body mass between *O. plecta* and *A. exclamationis* (Supplementary Table S4). Therefore, further analyses were performed for both species separately as to avoid collinearity effects between species identity and developmental or body mass traits.

Ochropleura plecta

Development and feeding behaviour

The ALAN treatments had no effect on larval survival of *O. plecta* and also the skyglow level at the population of origin had no significant effect on larval survival (Table 1). The duration of the larval period was not affected by ALAN treatment, skyglow level at the population of origin or sex, but larvae of lower mass at the start of the experiment showed a longer development time (very strong evidence; Table 1). We found moderate evidence for higher feeding frequency in males than in females. The difference between the sexes was only significant under dynamic ALAN ($t = 2.94$, $P = 0.004$; Fig. 1a; Table 1). Female larvae ate more than male larvae (weak evidence; Fig. 1b) and the amount of food eaten was positively related to the duration of the larval period (very strong evidence; Table 1).

	O. plecta	**χ^2**	***P***	***A. exclamationis***	**χ^2**	***P***
Larval survival	Treatment	1.03	0.598	Treatment	0.84	0.656
	Skyglow	0.24	0.624	Skyglow	1.75	0.186
	Room	0.83	0.361	Room	4.24	**0.039**
Duration of larval period	Treatment	0.31	0.856	Treatment	26.49	<**0.001**
	Skyglow	0.24	0.627	Skyglow	1.39	0.239
	Sex	0.99	0.320	Sex	6.46	**0.011**
	Larval mass at start	29.44	<**0.001**	Larval mass at start	27.93	<**0.001**
	Room	0.00	0.968	Room	17.32	<**0.001**
				Skyglow x Sex	13.39	<**0.001**
Feeding frequency	Treatment	4.45	0.108	Treatment	4.05	0.132
	Skyglow	0.26	0.609	Skyglow	0.00	0.960
	Sex	5.79	**0.016**	Sex	0.52	0.473
	Duration of larval period	0.87	0.350	Duration of larval period	3.94	**0.047**
	Room	3.22	0.073°	Room	2.67	0.102
				Treatment x duration of larval period	5.54	0.063°
Amount eaten	Treatment	1.28	0.528	Treatment	3.07	0.216
	Skyglow	0.64	0.424	Skyglow	2.64	0.104
	Sex	2.73	0.098°	Sex	0.65	0.420
	Duration of larval period	84.52	<**0.001**	Duration of larval period	200.74	<**0.001**
	Room	2.56	0.110	Room	0.19	0.667
Maximal larval mass	Treatment	4.52	0.104	Treatment	7.12	**0.029**
	Skyglow	0.32	0.571	Skyglow	0.194	0.659
	Sex	0.80	0.370	Sex	0.00	0.981
	Duration of larval period	1.37	0.241	Duration of larval period	29.68	<**0.001**
	Room	1.32	0.250	Room	3.05	0.081°
Pupal mass	Treatment	6.75	**0.034**	Treatment	16.58	<**0.001**
	Skyglow	1.12	0.290	Skyglow	0.22	0.638
	Sex	0.03	0.872	Sex	3.21	0.073°
	Duration of larval period	7.88	**0.005**	Duration of larval period	57.78	<**0.001**
	Room	0.39	0.534	Room	2.09	0.148
				Treatment x Sex	8.71	**0.013**
Adult mass	Treatment	3.77	0.152	Treatment	12.42	**0.002**
	Skyglow	0.96	0.327	Skyglow	0.33	0.565
	Sex	23.72	<**0.001**	Sex	23.21	<**0.001**
	Duration of larval period	3.19	0.074°	Duration of larval period	47.14	<**0.001**
	Room	0.81	0.368	Room	5.60	**0.018**
Mass loss at eclosion	Treatment	2.60	0.272	Treatment	5.34	0.069°
	Skyglow	0.44	0.508	Skyglow	0.40	0.527
	Sex	39.30	<**0.001**	Sex	72.80	<**0.001**
	Room	0.10	0.747	Room	0.31	0.578

Table 1. Statistical output for differences in larval survival and development for *Ochropleura plecta* and *Agrotis exclamationis*. All tests are based on D.f. = 1, except for ALAN-treatment effects where D.f. = 2. Significant *p*-values (< 0.05) are given in bold, whereas statistical trend values reflecting weaker evidence (< 0.1) are indicated with a ° symbol.

Body mass

Maximal larval mass was not affected by ALAN treatment, skyglow level at the population of origin, sex or duration of the larval period (Table 1). Pupal mass tended to be higher under continuous ($t = 2.43$, $P = 0.050$) and dynamic ALAN ($t = 1.94$, $P = 0.081$) than under the control treatment (Fig. 1c). We also found strong evidence for a positive relationship between pupal mass and duration of the larval period (Table 1). Mass loss at eclosion was higher in males than in females (very strong evidence; Fig. 1d) leading to a higher adult body mass in females than in males (very strong evidence; Fig. 1e), which tended to increase with duration of the larval period (weak evidence; Table 1).

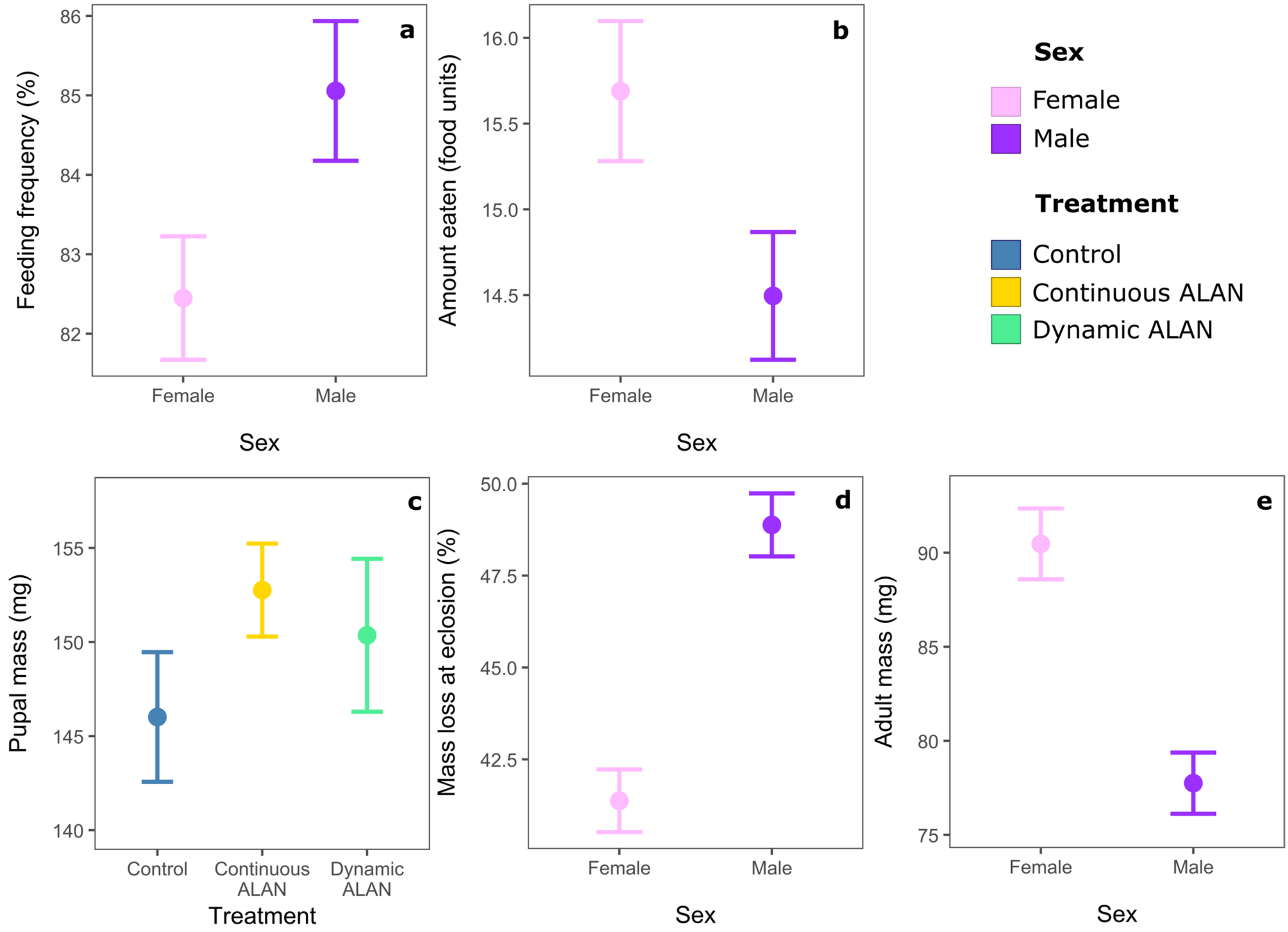

Fig. 1. **(a,b,d,e)** Raw mean values ± SE of feeding frequency, amount eaten, mass loss at eclosion and adult mass in female and male *Ochropleura plecta*. **(c)** Raw mean values ± SE of pupal mass under the ALAN treatments.

Agrotis exclamationis

Development and feeding behaviour

Larval survival was not affected by ALAN treatment or skyglow at the population of origin (Table 1). Larvae under dynamic ALAN developed faster than larvae in the control treatment ($t = 5.04$, $P < 0.001$) and continuous ALAN ($t = 3.33$, $P = 0.003$; Fig. 2a). Larvae originating from high-medium skyglow populations developed faster than larvae from low-medium skyglow populations and this difference was stronger in males than in females (strong evidence; Fig. 2b; Table 1). Larvae of lower mass at the start of the experiment showed a longer larval development time (Table 1). Feeding frequency decreased with duration of the larval period and this effect was stronger under continuous ALAN (moderate evidence; Fig. 2c). The amount of food eaten was positively related to duration of the larval period (very strong evidence; Table 1).

Body mass

Maximal larval mass was higher under dynamic ALAN than under the control treatment ($t = 2.56$, $P = 0.031$; Fig. 2d) and increased with duration of the larval period (very strong evidence; Table 1). While pupal mass tended to be higher for males than females under the control treatment ($t = 1.78$, $P = 0.077$) and continuous ALAN ($t = 1.94$, $P = 0.055$), the opposite was found under dynamic ALAN ($t = -1.72$, $P = 0.087$; Fig. 2e). Pupal mass also increased with duration of the larval period (very strong evidence; Table 1). Mass loss at eclosion tended to be lower under continuous ALAN than under the control treatment ($t = 2.26$, $P = 0.065$) and was higher in males than in females (very strong evidence; Fig. 2f). Adult body mass was higher under continuous ($t = 3.32$, $P = 0.003$) and dynamic ALAN ($t = 2.60$, $P = 0.016$) than under the control treatment, higher in females than in males (very strong evidence; Fig. 2g) and increased with the duration of the larval period (very strong evidence; Table 1).

Larval growth rate in A. exclamationis

We found moderate evidence for higher overall growth rate under dynamic ALAN than under the control treatment ($t = 2.48$, $P = 0.043$; Fig. 3a). This tended to be the case for initial growth rate ($t = 2.29$, $P = 0.061$; Fig. 3c)

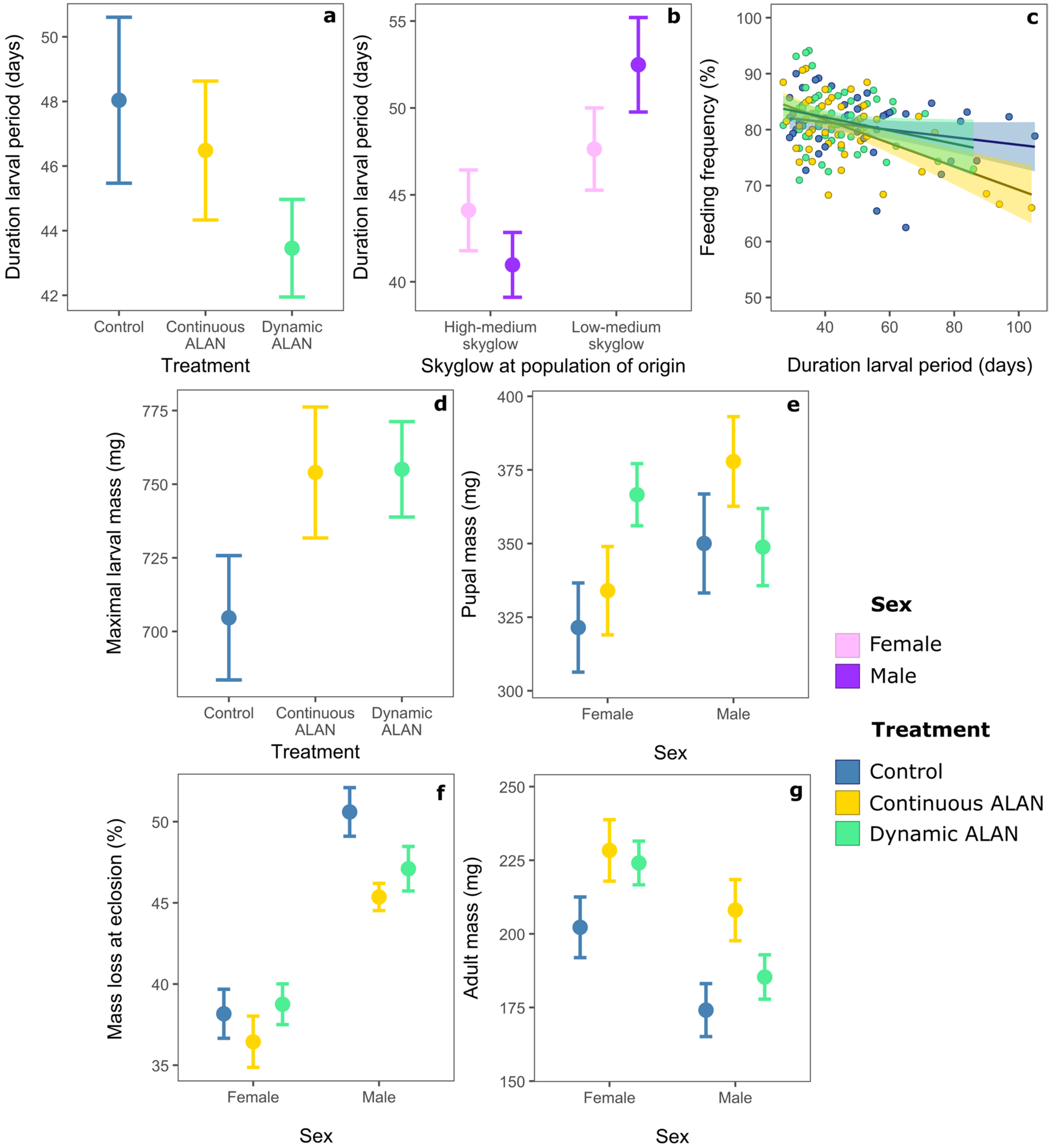

Fig. 2. **(a,d)** Raw mean values ± SE of duration of the larval period and maximal larval mass in *Agrotis exclamationis* under the ALAN treatments. **(b)** Raw mean values ± SE of duration of the larval period in females and males originating from high-medium and low-medium skyglow populations. **(c)** Raw values and linear regression lines (and 95% confidence intervals: shaded area) of feeding frequency in function of duration of the larval period under the ALAN treatments. **(e-g)** Raw mean values ± SE of pupal mass, mass loss at eclosion and adult mass in females and males under the ALAN treatments.

rather than growth rate at the end of the larval period ($t = 0.87$, $P = 0.663$; Table 2). We found strong evidence for higher initial growth rate in males than in females (Fig. 3d; Table 2). For overall growth rate, no sex difference was found for larvae originating from high-medium skyglow populations, but males had a lower growth rate than females when originating form low-medium skyglow populations (moderate evidence; Fig. 3b; Table 2). Growth rate at the end of the larval period was not affected by ALAN treatment, skyglow level at the population of origin or sex (Table 2).

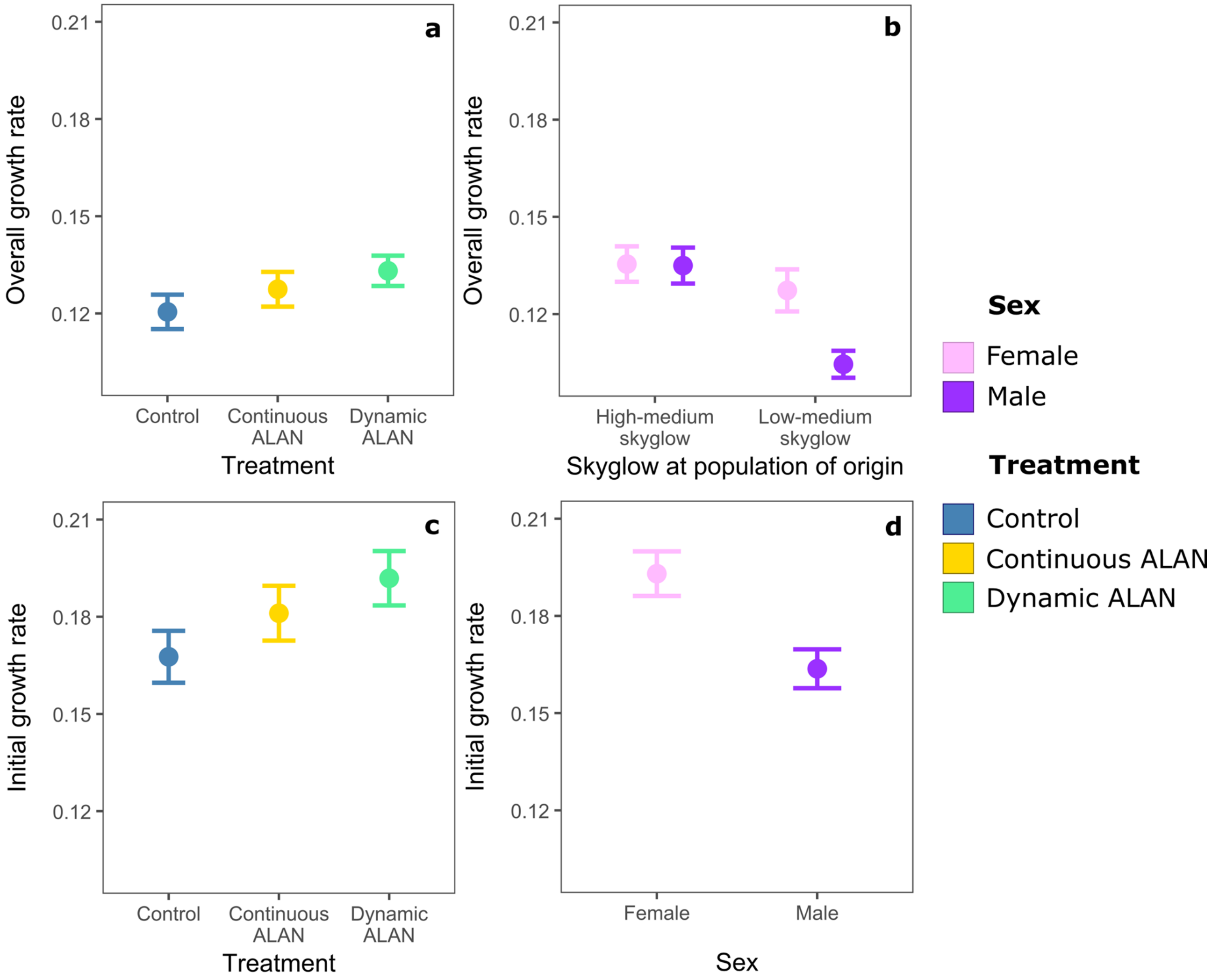

Fig. 3. **(a**, **c)** Raw mean values ± SE of overall and initial exponential growth rate in *Agrotis exclamationis* under the ALAN treatments. **(b)** Raw mean values ± SE of overall growth rate in females and males originating from high-medium and low-medium skyglow populations. **(d)** Raw mean values ± SE of initial growth rate in females and males.

Discussion

In this study, we investigated whether larval growth and development in two noctuid moths were affected by continuous and dynamic ALAN and whether ALAN effects varied between populations with different skyglow levels. For *A. exclamationis*, our results show evidence for faster larval development and higher growth rate under dynamic ALAN and increased body mass under both types of ALAN. For *O. plecta*, only pupal mass tended to increase under ALAN. The observed differences were mainly sex-related, while the skyglow level of the population of origin had hardly any effect on larval development. We discuss the implications of our results for the studied species and how they relate to previous studies, how the effects of dynamic ALAN compare to continuous ALAN, the lack of effects of skyglow levels at the population of origin, and we consider the extent to which our results can be extrapolated to other moth species.

For larvae, it is often advantageous to have a shorter development time, since this reduces the risk of parasitism[37,38] and provides competitive advantages when food sources are limited[39,40]. When this is coupled with increased body mass, as we found for *A. exclamationis*, it can also have benefits for fecundity[24,25] and mobility[41]. In contrast to previous studies in moths, where fast larval development was often coupled with lower body mass[19,20,22], we found no to even positive effects of ALAN on larval development. Recent studies on moth communities indeed show that most species are negatively affected by light pollution, however, there are also species that show positive correlations with ALAN[42,43]. This suggests that not all species respond in the same way to light pollution. For example, studies in other insect groups have shown no to positive effects of ALAN on juvenile development[44–46], although negative effects may still be present at the physiological level and in other life stages[45–47].

Growth rate is one of the important factors determining larval development time and body mass[48]. We show evidence for increased growth rate under dynamic ALAN. It seems that this difference was mainly observable

	A. exclamationis	χ^2	***P***
Exponential growth rate (overall)	Treatment	6.23	**0.044**
	Skyglow	0.90	0.342
	Sex	0.01	0.909
	Room	7.86	**0.005**
	Skyglow x Sex	4.42	**0.035**
Exponential growth rate (initial)	Treatment	5.31	0.070°
	Skyglow	1.80	0.179
	Sex	8.81	**0.003**
	Room	1.35	0.246
Exponential growth rate (final)	Treatment	1.12	0.572
	Skyglow	0.57	0.450
	Sex	0.34	0.562
	Room	0.61	0.433

Table 2. Statistical output for differences in exponential growth rate for *Agrotis exclamationis*. All tests are based on D.f. = 1, except for treatment effects where D.f. = 2. Significant *p*-values (< 0.05) are given in bold, whereas statistical trend values reflecting weaker evidence (< 0.1) are indicated with a ° symbol.

at the beginning of the experimental treatment, although the evidence for this is rather weak. Previous studies have shown that ALAN effects on growth rate can already occur soon after the start of exposure[23,49]. Later in development, differences in growth rate were no longer significant, but these were possibly masked by interindividual variation in development time.

Our dynamic ALAN treatment reduced the exposure time by two-thirds compared to the continuous ALAN treatment and was therefore expected to have a weaker effect on larval development (as previously demonstrated for flight-to-light[50]). Yet dynamic ALAN affected the duration of the larval period, growth rate and maximal larval mass more strongly than continuous ALAN. Moreover, both ALAN conditions showed similar effects on adult body mass. These results indicate that dynamic ALAN can at least have as strong effects as continuous ALAN, which is in line with a previous study that showed stronger negative effects of part-night exposure to ALAN on aphid colonies compared to full-night ALAN[51]. If this would also be the case in species negatively affected by ALAN, it would mean that dynamic streetlights are not necessarily better for moths[52], at least when it comes to larval development, but this remains to be tested.

An evolutionary response in larval development under ALAN would require negative or positive fitness consequences[27]. For *O. plecta*, we found no or only slightly positive ALAN effects and, consequently, there was a lack of adaptive response in this species. On the other hand, our results suggest positive effects of ALAN in *A. exclamationis*. However, also for this species, we did not show evidence for an adaptive response in moths originating from high-medium skyglow populations. We found barely any evidence for effects of skyglow levels at the population of origin per se. Possibly, populations from high-medium and low-medium skyglow areas may be genetically more strongly connected to each other than we assumed. In fact, our low-medium skyglow populations were only 15–35 km away from areas with high-medium skyglow levels. For good flying species like *A. exclamationis*, and to a somewhat lesser extent *O. plecta*[53], these distances are certainly passable. It is therefore not inconceivable that gene flow between these populations occurs to a greater or lesser extent. It would therefore be interesting to compare the responses of different populations in combination with population genetic work (e.g.[54], but local adaptation can also be maintained despite gene flow[55]. Moreover, our study area (i.e., Belgium) did not allow to select sampling sites with actual zero or low skyglow levels[2]. Finally, it should be noted that, although we selected sites without significant local differences in urbanisation levels, we could not fully control for other potentially confounding factors that differ between our sampled regions (cf.[42,56]) like altitude, temperature and vegetation. Future studies should therefore aim to disentangle the impact of these environmental variables as much as possible by including several regional replicates, which would require a much larger research effort[57].

The more hidden lifestyle of *A. exclamationis* caterpillars may limit both potential harmful and positive effects of ALAN, while *O. plecta* larvae are likely to experience higher exposure[32,58]. Yet, while we showed evidence for multiple ALAN effects on larval development in *A. exclamationis*, effects in *O. plecta* were mostly absent. However, body mass response to ALAN went in the same direction for both species, although non-significant for *O. plecta*. It should therefore be noted that for practical reasons, *O. plecta* larvae were exposed to the experimental treatments at a higher starting mass compared to *A. exclamationis*. As a result, the experimental period was *c.* two to three times shorter for *O. plecta* than for *A. exclamationis* which may have led to the lack of significant differences in the former species.

One of the ways ALAN potentially acts on moth ontogeny is by changing food consumption[59]. For larvae that show a nocturnal activity peak, ALAN can be expected to disturb feeding, while diurnal larvae could in turn see their activity period extended. Our study species have larvae that are mainly nocturnal, but despite faster development under ALAN, they also had a higher body mass, suggesting that feeding behaviour also changes under ALAN. Larvae that developed faster had a higher feeding frequency, although feeding frequency did not differ between ALAN treatments. However, we showed that faster-developing larvae in total consumed a lower quantity of food, yet it is possible that they consumed larger amounts per day or exhibited higher levels of energy efficiency, but this remains to be tested.

Depending on the ability to still take up nutrients during the adult stage, the body mass at the end of ontogeny determines to a greater or lesser extent the reproductive and dispersal capacity of the adult[33]. Adults are often very motile making them sometimes difficult to weigh, so it is often opted to measure maximal larval mass or pupal mass instead of adult mass[20,22,23]. Maximal larval mass is often a less precise measurement, because larvae are typically weighed only once or twice a week, which means the actual day of maximum mass can be missed. In turn, pupal mass is usually measured at a fixed number of days after pupation, making it a more precise and therefore better proxy for adult mass. However, during the pupal period and adult eclosion, some body mass is also lost and this is usually more in males than in females[35]. In our study, we see for maximal larval mass in *A. exclamationis* that only dynamic ALAN leads to higher mass compared to the control treatment; for pupal mass, continuous ALAN also tends to lead to higher mass than the control treatment and male mass tends to be higher than in females. But under continuous ALAN, less mass was lost during eclosion which ultimately caused adult mass under continuous ALAN to be slightly higher than under dynamic ALAN. Moreover, males lost *c.* one-fourth more mass than females, resulting in higher adult mass in females compared to males. This indicates that conclusions should always be treated with some caution when using proxies for final body mass.

Our results suggest that exposure to ALAN during development has a rather positive effect on our study species, especially *A. exclamationis*. However, in contrast to previous studies that mainly showed negative ALAN effects on larval development[19,20,22,23], this study involves two highly generalist species that are still abundant in residential areas[32]. These species seem well adapted or adaptable to changes associated with urbanisation, but this is usually only true for a limited amount of insect species[60,61]. Moreover, we used two species of the same family (Noctuidae), and negative effects on larval development were found in two other species of this family[20,23]. This means that our results cannot simply be extrapolated to other moth species, but at the same time they also show that not all species are necessarily negatively affected by ALAN or at least not in the same way.

While the amount of evidence for negative effects of ALAN on larval development in moths is increasing, our study shows that this cannot be generalized across all species. We show evidence for faster larval development and increased body mass under continuous and dynamic ALAN conditions in moths originating from low-medium and high-medium skyglow regions. Further studies are now warranted to explore the mechanisms behind the differences in response between species.

Data availability

All data and R code used for the analyses are available from Dryad Digital Repository: https://doi.org/10.5061/dryad.r4xgxd2qm.

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Author contributions

E.V.d.S., M.L., L.L. and H.V.D. contributed to the conceptualisation of this study and the experimental design. E.V.d.S., M.L. and L.L. performed the experimental work and collected the data. E.V.d.S. analysed the data, visualised the results and wrote the original draft. E.V.d.S., R.A.W. and H.V.D. substantially revised the manuscript and all authors approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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