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Appendix 1–4

Appendix 1. Patterns in development time, body mass, and mass loss during emergence

Statistical analyses

We analyzed effects of latitude (France/Sweden), feeding treatment (fed/starved), sex (female/male), and their interactions on age and body mass with separate linear mixed-effects models (LMMs). Age and mass were measured on the same individuals at four different time points, hence these repeated measurements are not independent of each other. Therefore, we included the term period, consisting of four levels (start of starvation/end of starvation/end of post-starvation/emergence) as an additional fixed effect, and the identity of the individual as a random effect. This design accounts for the repeated nature of the development time and body mass data. To test the effects of latitude, feeding treatment, sex, and their interactions on proportionate mass loss during the emergence period, we ran a LMM with mass at the start of the emergence period and the duration of the emergence period as covariates.

In all analyses, the family identity (nested in population) and population (nested in latitude) were included as random effects (age and mass models included individual identity as an additional random effect, as explained above). Models fitted with these parameters account for variation due to potential differences among families and populations of a given latitude. To meet model assumptions, age was log-transformed. To prevent overfitting of models, we applied a backwards model selection procedure to each model, starting with a full model including all main fixed effects and their interactions (Zuur et al. 2009). We then assessed the significance of removal of model terms, starting with the highest order interaction, and compared the resulting simpler models with the preceding models based on Akaike's information criterion (AIC). All analyses were conducted in R ver. 3.5.1. (<www.r-project.org>), using the packages '*lme4*' for running mixed-effect models (Bates et al. 2015) and ‘*lmerTest*’ for ANOVA tables with F-tests and p-values using Satterthwaite’s method for denominator degrees of freedom (Kuznetsova et al. 2017).

Results

Development time

French larvae went through all periods faster than Swedish larvae, and this was especially pronounced for the post-starvation period (latitude × period in Table A1, Fig. A1 A). The feeding treatment differentially affected development times in French and Swedish larvae: while previously-starved French larvae extended the post-starvation period compared to non-starved larvae this was not the case in Swedish larvae (latitude × feeding treatment × period in Table A1, Fig. A1 A). As a result, age at emergence was ca 161 days in both starved and non-starved Swedish animals and ca 112 days and 102 days in starved and non-starved French animals, respectively. Males went through all periods faster than females, especially in Swedish larvae. This latitude-dependent sex difference became somewhat smaller when approaching emergence (sex × latitude × period in Table A1). Eventually, males emerged about 16 days (Swedish larvae) and 4 days (French larvae) earlier than females..

Body mass

Swedish animals were consistently heavier than French animals (latitude in Table A1), except at the end of the starvation period where non-starved larvae of both latitudes had the same mass (latitude × feeding treatment × period in Table A1, Fig. A1 B). On average, Swedish adults emerged 5.5 mg heavier than French adults. At the end of the starvation period, mass was ca 6.5 mg lower in starved than in non-starved larvae. This negative effect of starvation on mass became much smaller during the post-starvation period; eventually previously-starved animals were ca 1 mg lighter at emergence (feeding treatment × period in Table A1, Fig. A1 B). Females were consistently heavier than males, and this sex difference changed slightly throughout the periods (sex × period in Table A1, Fig. A1 B). Females emerged ca 3 mg heavier than males.

Mass loss during emergence

During the emergence period, Swedish animals (latitude: $F_{1,3.5}$ = 63.07, p = 0.0058) and previously-starved animals (feeding treatment: $F_{1,642.4}$ = 14.33, p = 0.0002) had a larger proportionate mass loss compared to French animals and non-starved animals, respectively (Fig. A2). Males had a higher proportionate mass loss than females (sex: $F_{1,644.6}$ = 12.02, p = 0.0006).

Discussion

The higher development rates, hence shorter development times, and a lower mass at emergence compared to the Swedish populations are in line with the compound interest hypothesis stating that in populations with relatively short generation times as compared to season length, fitness will be maximized by increasing the number of generations, even at the expense of being small (Fischer and Fiedler 2002). Thermal conditions in Sweden do not allow an extra generation, and therefore selection for large size at maturity to maximize adult fitness should be particularly strong (Roff 1980; Fischer and Fiedler 2002).

Only French larvae extended the post-starvation period when previously starved. The resulting total length of this period was, however, still very short in French animals (ca 7.5 days), especially compared to Swedish animals (40 days). That only the more time-constrained French larvae, that also showed the highest compensatory growth, delayed emergence illustrates the limits of a compensatory growth response and strongly suggests a mass threshold needed to be reached before emergence. Mass at emergence is indeed very important for adult fitness components like survival in damselflies (reviewed by Stoks and Cordoba-Aguilar 2012).

Despite the strong compensatory life history responses, the mass compensation was not complete and previously-starved animals emerged at a slightly lower mass. Mass compensation is often not complete and might be prevented by physiological constraints and will depend on the relative costs and benefits of compensatory growth (Metcalfe and Monaghan 2001). Noteworthy, this net benefit was at higher levels of accelerated growth for French than for Swedish larvae. Our study also confirmed a previous study that animals showing compensatory growth had a higher mass loss during emergence (Stoks et al. 2006). One likely reason for this is that they have a faster metabolism and therefore higher maintenance costs and a faster dissipation of energy reserves (damselflies: Stoks et al. 2006; zebra finches: Criscuolo et al. 2008).

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Table A1. Summary of linear mixed-effect models testing for the effects of latitude, feeding treatment, sex and period on larval development time and adult body mass.

	Development time			Body mass		
	df	F	p	df	F	p
Latitude (Lat)	1,1.9	129.40	0.0079	1,1.9	45.85	0.0219
Feeding treatment (Food)	1,616.3	20.22	< 0.0001	1,623.6	81.17	< 0.0001
Sex	1,613.2	58.07	< 0.0001	1,618.8	208.99	< 0.0001
Lat × Food	1,623.5	12.41	0.0004	1,631.9	2.87	0.0908
Lat × Sex	1,614.7	21.79	< 0.0001			
Period	3,1958.1	10561.56	< 0.0001	3,1942.7	15208.49	< 0.0001
Lat × Period	3,1958.1	1397.08	< 0.0001	3,1942.7	205.96	< 0.0001
Food × Period	3,1958.1	58.04	< 0.0001	3,1942.7	886.98	< 0.0001
Sex × Period	3,1958.1	24.27	< 0.0001	3,1942.9	37.98	< 0.0001
Lat × Food × Period	3,1958.1	49.56	< 0.0001	3,1942.6	71.27	< 0.0001
Lat × Sex × Period	3,1958.1	30.62	< 0.0001			

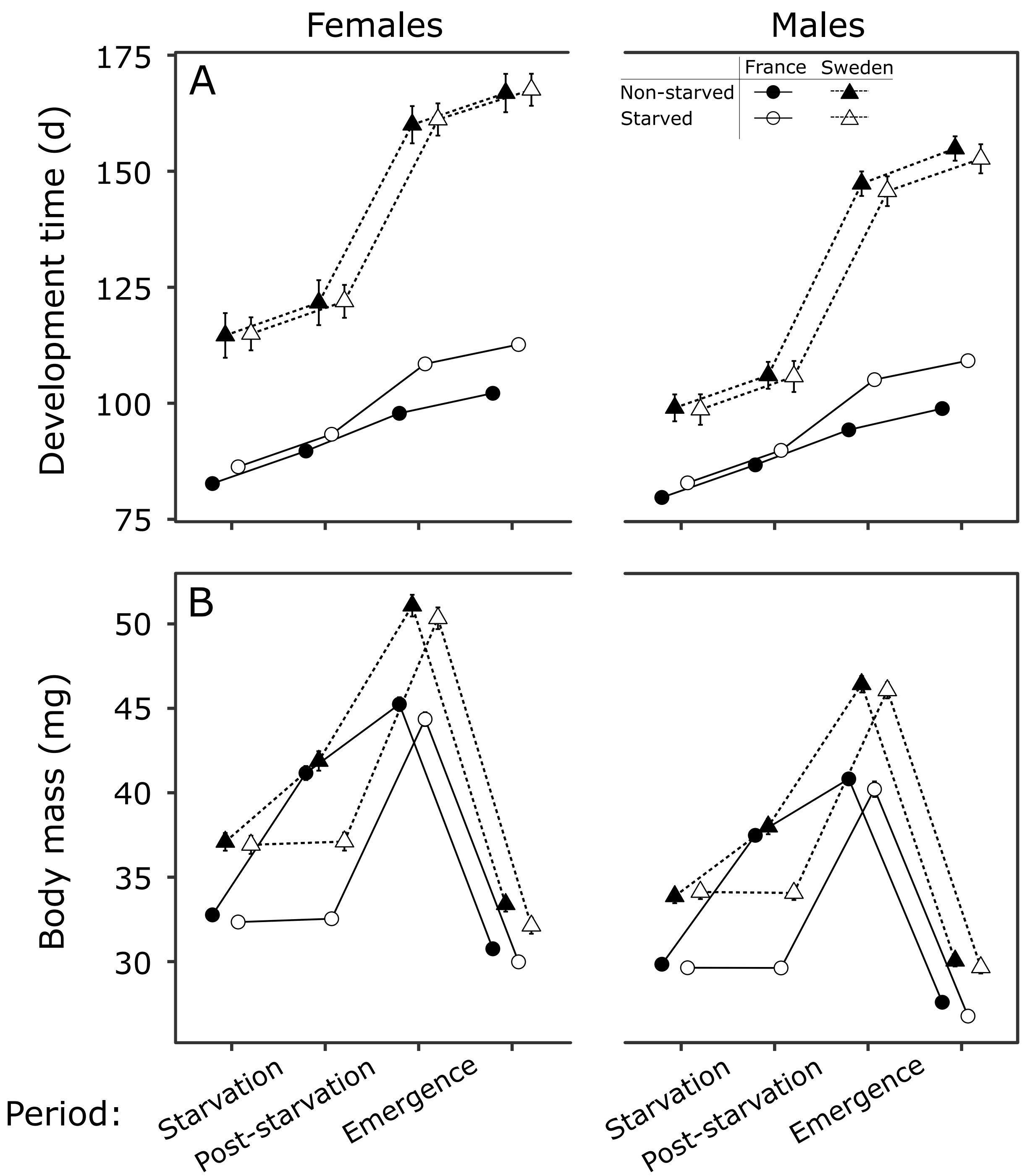

Figure A1. The effects of latitude, feeding treatment and sex on (A) development time and (B) body mass across time periods. Plots on the left represent females, plots on the right represent males. Means are given with 1 SE. Note that error bars are often too small to be visible. Non-starved and starved refer to the feeding status during the starvation period only. Different symbols represent French (circles, connected with solid lines) and Swedish (triangles, connected with dashed lines) populations. Time is expressed as days since hatching. The horizontal axis represent time points where body mass was measured. See Table 1 in the main text for sample sizes.

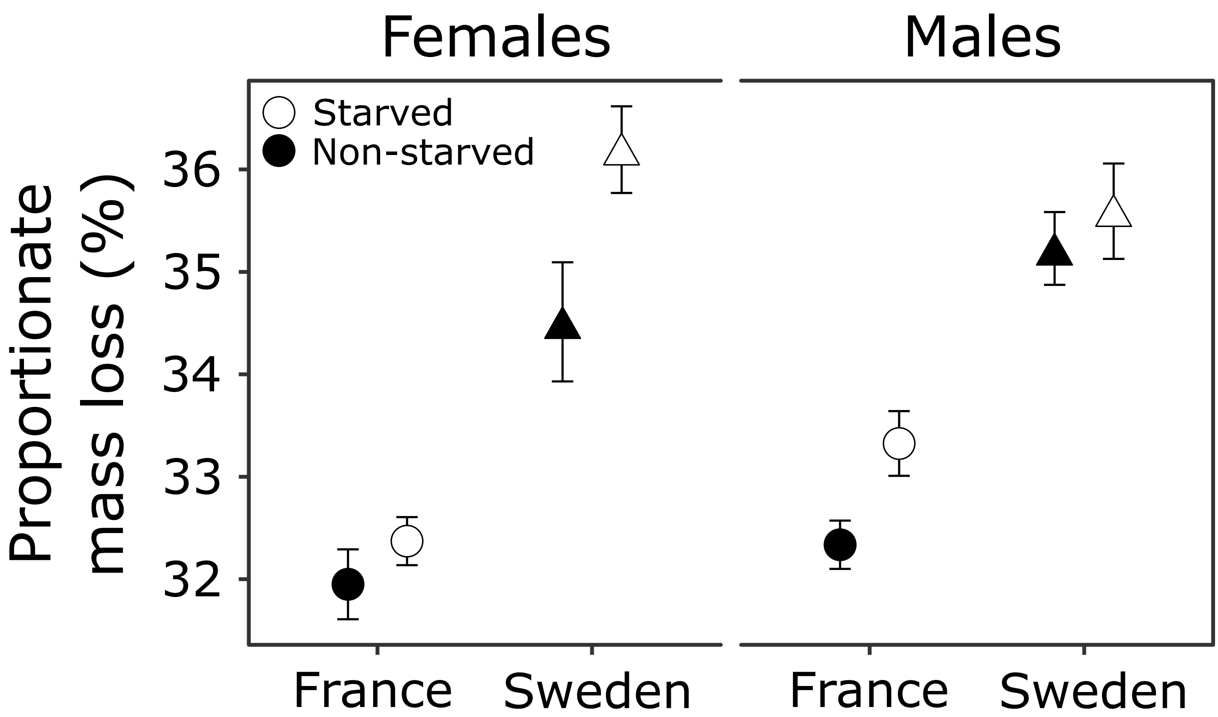

Figure A2. The effects of latitude, feeding treatment and sex on the proportionate mass loss during the emergence period. Means are given with 1 SE. Non-starved and starved refer to the feeding status during the starvation period only. Different symbols represent French (circles) and Swedish (triangles) populations. Plots on the left represent females, plots on the right represent males.

Appendix 2. Assays of the oxidative stress variables

Adults were stored at -80°C until the biochemical assays. Just before running the assays, each adult was homogenized in PBS-buffer (pH 7.4; 1/23 w/v) in a microcentrifuge tube while on ice. Samples were centrifuged for 10 min at 13 200 rpm in a cooled centrifuge at 4 °C and the obtained supernatants were used in the assays. All biochemical response variables were measured spectrophotometrically using a TECAN Infinite M200 spectrophotometer. Each endpoint (except SOD) was assayed in duplicate and the mean of both readings was taken as the value for that individual. We did not run duplicates for SOD as the used kit is expensive and we previously showed high repeatabilities for this assay (De Block and Stoks 2008). Repeatabilities were high for both CAT ($r = 0.72$) and TBARS ($r = 0.97$).

Activity of both antioxidant enzymes was measured following the protocols described in De Block and Stoks (2008). SOD activity was quantified using the SOD Assay Kit-WST (Fluka, Buchs, Austria), which measures the formation of a formazan dye upon reduction of the tetrazolium salt WST-1 with superoxide anions. We added 200 µl WST working solution and 20 µl enzyme working solution to 20 µl supernatant. This mixture was incubated for 20 min at 37°C followed by reading the absorbance at 450 nm. One SOD unit was defined as the amount of sample causing 50% inhibition of the colorimetric reaction per mg protein. CAT activity was quantified based on the protocol of Aebi (1984). 10 µl supernatant was further diluted 16 times with PBS (pH 7.4). To 20 µl of the diluted supernatant, we added 80 µl PBS (pH 7.4) and 100 µl of 20 mM H_2O_2. We measured the removal of H_2O_2 as the reduction in absorbance at 240 nm within 2 min in UV-96 well plates at 30°C. One unit of CAT was defined as the activity of the enzyme that catalyzed the reduction of 1 µmol H_2O_2 per min per mg protein. Note that by expressing SOD and CAT in these units, we corrected for protein content.

Oxidative damage in lipids was quantified based on the formation of malondialdehyde (MDA), the most important substance produced when lipid peroxides decompose (Leibovitz and Siegel 1980). MDA levels were determined using the assay where these react to produce TBARS, which can be measured spectrophotometrically (Ohkawa et al. 1979) (see below for a motivation of this approach). Lipids were first extracted by adding 150 µl of the supernatant in the pellet tube and homogenizing this in 650 µl ice-cold chloroform/methanol (2:1 v/v) with 0.01 (w/v) butylated hydroxytoluene (BHT). Samples were centrifuged 10 min at 13 200 rpm in a cooled centrifuge at 4°C. TBARS were quantified following the protocol described in Barata et al. (2005). To 150 µl of the chloroform fraction we added 32 µl of 8.1% sodium dodecyl sulfate (SDS), 280 µl of 20% acetic acid (pH 3.5) and 280 µl of thiobarbituric acid (TBA). This was mixed and tubes were heated for 60 min at 100°C. After cooling, 200 µl of a mixture of butanol and pyridine (15:1 v/v) was added, mixed vigorously, and centrifuged for 5 min at 13 200 rpm. The supernatant (organic layer) was taken and fluorescence was measured at an excitation/emission wavelength of 530/550 nm. TBARS were expressed in nmol MDA equivalents, derived from an external standard curve of 1,1,3,3-tetramethoxypropane (Oakes and Van Der Kraak 2003).

Total fat was quantified following Bligh and Dyer (1959). To 150 µl of the chloroform fraction we added 500 µl of 50% sulfuric acid (H_2SO_4). Glass tubes were heated for 15 min at 200°C. After cooling, 700 µl of Milli-Q water was added and samples were centrifuged for 5 min at 8000 rpm. The absorbance was measured at 340 nm. Fat concentrations were calculated from standard curves of tripalmitin. Protein content was quantified using the Bradford (1976) method. 1 µl of the original homogenate was diluted in 160 µl Milli-Q water and 40 µl of Bio-Rad Protein Dye reagent concentrate was added and mixed vigorously. Absorbance was read at 595 nm after 5 minutes incubation at 37°C.

Motivation for the TBARS assay

Three potential pitfalls have been identified when using TBARS as an indicator of MDA, hence lipid peroxidation (Hõrak and Cohen 2010):

(1) MDA may be present in ingested food and can be absorbed through the gastrointestinal tract, thus altering background MDA levels in vivo. However, damselfly larvae stop eating a couple of days before emergence to prepare for emergence thus the potential impact of MDA from ingested food will be small in freshly-emerged adults. More importantly, MDA from ingested food cannot explain the pattern in TBARS in our study as, if anything, we would then expect TBARS to be higher in groups where food intake and growth rate during the post-starvation period were higher (French larvae and previously-starved larvae), which was not the case (see results in main text).

(2) A proportion of the MDA determined in the assay may be formed by decomposition of lipid peroxides during the acid heating stage of the assay, which generates further oxidation of the sample matrix with obvious overestimation of the results. Yet, this potential caveat cannot explain any treatment-related patterns in TBARS in our study, as formation of MDA would be random and not be linked to the experimental treatments.

(3) TBARS may be formed from TBA by reacting with other compounds apart from MDA. The assay would thereby overestimate the actual levels of MDA. However, it is considered effective for comparative studies since several other TBA-reactive aldehydes are also products of lipid peroxidation (Hermes-Lima and Storey 1995; Hermes-Lima 2004; Orr et al. 2009). In insects, this assay has indeed been shown to reliably reflect oxidative damage, with TBARS patterns being highly similar to patterns observed for other estimates of oxidative damage in lipids (i.e. MDA) and in proteins (Magwere et al. 2006; Krishnan et al. 2007). Moreover, in a positive control where we exposed animals of a related damselfly to dibenzoylperoxide, a radical initiator, we found, in line with the expected increase in

oxidative damage, an upregulation of the TBARS levels compared to control animals (Lizanne Janssens and Robby Stoks unpubl.). Finally, in another pilot study (associated with Janssens and Stoks 2018) we found the patterns in TBARS levels as here measured using spectrophotometry, and another assay using HPLC (that is more specific for MDA, Monaghan et al. 2009) to be very similar.

Despite these three potential pitfalls with the TBARS assay, we therefore are confident their levels reflect oxidative damage in lipids in our damselfly study system.

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Appendix 3. Additional tables with coefficient estimates

Table A1. Coefficient estimates (and standard errors, SE) from the growth rate model. Reference levels for the calculation of coefficient estimates: Latitude = France; Feeding treatment = non-starved; Sex = female, Growth period = pre-starvation.

	estimate	SE
intercept	0.0379	0.0011
Latitude (Sweden)	-0.0094	0.0008
Feeding treatment (starved)	-0.0018	0.0005
Sex (male)	0.0010	0.0005
Growth period (starvation)	-0.0104	0.0007
Growth period (post-starvation)	-0.0327	0.0007
Latitude (Sweden) : Feeding (starved)	0.0014	0.0008
Latitude (Sweden) : Sex (male)	0.0041	0.0008
Latitude (Sweden) : Period (starvation)	-0.0063	0.0010
Latitude (Sweden) : Period (post-starvation)	0.0027	0.0010
Feeding (starved) : Period (starvation)	-0.0294	0.0007
Feeding (starved) : Period (post-starvation)	0.0108	0.0007
Sex (male) : Period (starvation)	-0.0013	0.0007
Sex (male) : Period (post-starvation)	-0.0002	0.0007
Latitude (Sweden) : Feeding (starved) : Period (starvation)	0.0138	0.0011
Latitude (Sweden) : Feeding (starved) : Period (post-starvation)	-0.0075	0.0011
Latitude (Sweden) : Sex (male) : Period (starvation)	-0.0042	0.0011
Latitude (Sweden) : Sex (male) : Period (post-starvation)	-0.0047	0.0011
Body mass	0.0001	0.0000

Table A2. Coefficient estimates (and standard errors, SE) from the models for oxidative stress variables (SOD, CAT, TBARS) and adult lifespan. Reference levels for the calculation of coefficient estimates: Latitude = France; Feeding treatment = non-starved; Sex = female.

	SOD		CAT		TBARS		Lifespan	
	estimate	SE	estimate	SE	estimate	SE	estimate	SE
intercept	3.8404	0.0312	6.4257	0.1356	-1.9934	1.1817	1.1754	0.3237
Latitude (Sweden)	-0.2021	0.0525	-0.0768	0.0656	0.5739	0.2599	-0.3073	0.0734
Feeding treatment (starved)			-0.0688	0.0315				
Sex (male)			-0.0013	0.0368			-0.3664	0.0644
Latitude (Sweden) : Sex (male)			-0.2033	0.0804				
Mass at emergence			-0.0359	0.0041	0.4544	0.0251	0.0438	0.0105
Fat content					-1.0752	0.2411		

Appendix 4. Additional analysis on the post-starvation growth rate

The higher mortality during post-starvation period in the previously starved larvae (see Results in main text) may have eliminated the weakest, slowest growing animals. Such a 'selective disappearance' (van de Pol and Verhulst 2006) has the potential to inflate the post-starvation growth rate in the previously-starved group, which can be erroneously identified as compensatory growth. To test for this possibility, we simulated an extreme scenario of selective disappearance during the post-starvation treatment, in which the same level of selective disappearance per starvation treatment of the slowest growing control larvae is mimicked. Note that because the starvation treatment did not cause a higher mortality during the starvation treatment (see Results in main text), a scenario of selective disappearing during starvation period could not have been mimicked. Specifically, we removed the slowest growing 12 French larvae (4%) and 23 Swedish larvae (16%) of the control group; mimicking the actual reported mortality during the post-starvation period (as reported in the Results in main text). Using this reduced dataset, we ran a linear mixed-effects model to test effects of latitude (France/Sweden), feeding treatment (fed/starved), sex, and their interactions on post-starvation growth rate. The model included the family identity (nested in population) and population (nested in latitude) as random effects. We used R ver. 3.5.1. (<www.r-project.org>) with the package '*lme4*' for running the model (Bates et al. 2015).

French larvae had faster growth rates than Swedish larvae during the post-starvation period ($F_{1,2.2}$ = 727.04, p < 0.001). Previously starved larvae had higher growths rate compared to the control larvae ($F_{1,644.7}$ = 86.57, p < 0.001), and this compensatory growth was stronger in French than Swedish larvae (Latitude × Feeding treatment, $F_{1,644.7}$ = 86.57, p < 0.001).

Results indicate that even in case of an extreme selective disappearance in the control group, post-starvation growth is still drastically higher in the previously starved larvae. Given that these results fully overlap with the results based on the full dataset (as reported in the main text), we conclude that the here reported compensatory growth of the previously-starved larvae is not driven by selective disappearance.

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