

Supplementary Information for:

Multiple stressors across ecosystem boundaries: do light pollution and invasive species change the quality of aquatic subsidies to terrestrial predators?

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Table S1. Nitrate (NO_3^-), Ammonium (NH_4^+), Ortho-phosphate (o-PO_4^{3-}), and suspended matter (SM) concentrations* in the water inlet of the RSM during the experiment.

Sampling Date	Experiment Week	$\beta(\text{NO}_3^-)$ [mg L ⁻¹]	$\beta(\text{NH}_4^+)$ [mg L ⁻¹]	$\beta(\text{PO}_4^{3-})$ [mg L ⁻¹]	SM [mg mL ⁻¹]
25/5/2022	1	4.47	0.13	0.11	0.01
1/6/2022	2	3.43	<0.01	0.09	<0.01
8/6/2022	3	3.43	<0.01	0.11	0.01
15/6/2022	4	7.59	<0.01	0.12	<0.01
22/6/2022	5	6.51	<0.01	0.12	0.01
29/6/2022	6	4.63	<0.01	0.12	0.01
6/7/2022	7	4.06	0.13	0.12	0.02

*Measured with a continuous flow analyzer (AutoAnalyzer 3, SEAL Analytical; Bran+Luebbe, Norderstedt, Germany) in accordance with ISO 13395/DIN 38 405, ISO 14256-2, and ISO 14255 standards. Water samples (100ml) were filtered with glass fibre filters and kept at -20°C before measurements. The filters were dried (60°C for 96 h) and weighed, both before and after filtration; the weight difference was used as a measure of Suspended Matter.

Table S2. Number of individuals (n) and mean biomass $\pm$ standard deviation (g, wet weight) of signal crayfish deployed in each flume. The treatments were “Crayfish”: presence of signal crayfish; and “ALAN+Crayfish”: presence of artificial light at night (ALAN) combined with presence of signal crayfish.

Treatment	Flume	Females (n)	Biomass (g)	Males (n)	Biomass (g)
Crayfish	2	12	24 $\pm$ 9	15	21 $\pm$ 5
	5	12	26 $\pm$ 9	15	23 $\pm$ 8
	12	12	29 $\pm$ 14	15	23 $\pm$ 8
	15	12	22 $\pm$ 7	15	28 $\pm$ 13
ALAN+Crayfish	1	12	25 $\pm$ 12	15	23 $\pm$ 9
	8	12	24 $\pm$ 12	15	22 $\pm$ 9
	11	12	24 $\pm$ 9	15	25 $\pm$ 11
	14	12	23 $\pm$ 9	15	25 $\pm$ 13

Table S3. Number of individuals (n) and mean biomass $\pm$ standard deviation (g, wet weight) of signal crayfish retrieved from each flume at the end of the experiment. The treatments were “Crayfish”: presence of signal crayfish; and “ALAN+Crayfish”: presence of artificial light at night (ALAN) combined with presence of signal crayfish.

Treatment	Flume	Females (n)	Biomass (g)	Males (n)	Biomass (g)
Crayfish	2	4	28 $\pm$ 12	3	30 $\pm$ 13
	5	5	22 $\pm$ 10	4	27 $\pm$ 2
	12	4	23 $\pm$ 3	6	35 $\pm$ 12
	15	5	24 $\pm$ 7	6	28 $\pm$ 9
ALAN+Crayfish	1	6	23 $\pm$ 13	9	30 $\pm$ 11
	8	7	21 $\pm$ 8	3	29 $\pm$ 5
	11	4	22 $\pm$ 5	3	37 $\pm$ 9
	14	5	22 $\pm$ 5	6	25 $\pm$ 5

Table S4. Mean biomass (mg) of the identified emergent aquatic insects from flumes without stressors (Control), exposed to the presence of artificial light at night (ALAN), to the presence of signal crayfish (Crayfish), and their combination (ALAN+Crayfish), at one (Week 1), four (Week 4) and six (Week 6) weeks after the initiation of stressors. (Complete $\pm$ SD). Biomass was averaged among flumes, traps and samplings.

	Chironomidae	Tanypodinae	Dixidae	Simuliidae	Psychodidae	Tipulidae	Other Diptera	Ephemeroptera
Week 1								
Control	85 $\pm$ 42	0.4 $\pm$ 0.9	0.2 $\pm$ 0.4	0.6 $\pm$ 0.7	0	0.7 $\pm$ 2	0.6 $\pm$ 0.8	0.1 $\pm$ 0.5
ALAN	76 $\pm$ 38	0.4 $\pm$ 0.7	0.2 $\pm$ 0.3	1 $\pm$ 1.5	0.01 $\pm$ 0.02	2 $\pm$ 5	0.5 $\pm$ 0.6	0.6 $\pm$ 1.3
Crayfish	62 $\pm$ 42	0.2 $\pm$ 0.4	0.2 $\pm$ 0.3	0.8 $\pm$ 1.4	0.01 $\pm$ 0.03	0	0.8 $\pm$ 1.6	0.3 $\pm$ 0.6
ALAN+Crayfish	56 $\pm$ 40	0.2 $\pm$ 0.5	0.2 $\pm$ 0.3	0.5 $\pm$ 1	0.01 $\pm$ 0.04	2 $\pm$ 6	0.6 $\pm$ 0.9	0.1 $\pm$ 0.3
Week 4								
Control	38 $\pm$ 48	4 $\pm$ 6	0.2 $\pm$ 0.3	0.1 $\pm$ 0.2	0	0.2 $\pm$ 1	0.7 $\pm$ 0.7	0
ALAN	36 $\pm$ 53	3 $\pm$ 3	0.3 $\pm$ 0.5	0.1 $\pm$ 0.2	0.03 $\pm$ 0.08	1 $\pm$ 2	1 $\pm$ 0.9	0.04 $\pm$ 0.2
Crayfish	16 $\pm$ 23	1 $\pm$ 1	0.1 $\pm$ 0.3	0.2 $\pm$ 0.3	0.01 $\pm$ 0.03	0	0.6 $\pm$ 1.5	0.04 $\pm$ 0.2
ALAN+Crayfish	23 $\pm$ 29	2 $\pm$ 3	0.2 $\pm$ 0.3	0.1 $\pm$ 0.3	0.03 $\pm$ 0.09	1 $\pm$ 2	0.4 $\pm$ 0.5	0
Week 6								
Control	43 $\pm$ 39	2 $\pm$ 3	0.1 $\pm$ 0.2	0	0.02 $\pm$ 0.1	1 $\pm$ 3	0.4 $\pm$ 0.9	0.05 $\pm$ 0.2
ALAN	21 $\pm$ 27	1 $\pm$ 2	0.1 $\pm$ 0.3	0	0	0.2 $\pm$ 1	0.4 $\pm$ 0.6	0.08 $\pm$ 0.4
Crayfish	32 $\pm$ 32	1 $\pm$ 2	0.1 $\pm$ 0.2	0.2 $\pm$	0.01 $\pm$ 0.02	0	0.7 $\pm$ 1.7	0
ALAN+Crayfish	31 $\pm$ 29	1 $\pm$ 2	0.1 $\pm$ 0.2	0.2 $\pm$	0.10 $\pm$ 0.3	0	0.3 $\pm$ 0.7	0.09 $\pm$ 0.4

Table S5. Analysis of variance (ANOVA) of the linear mixed models for total fatty acid (FA), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, arachidonic acid (ARA; 20:4 ω -6) and eicosapentaenoic acid (EPA; 20:5 ω -3) fluxes ($\mu\text{g m}^{-2} \text{ d}^{-1}$) (log x+1 transformed) from flumes subjected to Artificial light at night (ALAN), and to the presence of signal crayfish (Crayfish) over time (week). Chisq: Chi-square value; Df.: degrees of freedom; Pr(>Chisq): Chi-square test statistic, in bold indicates significant differences ($p < 0.05$).

Response	Predictors	Chisq	Df.	Pr(>Chisq)
Total FA flux	ALAN	0.3982	1	0.528
	Crayfish	0.6082	1	0.435
	Week	113.068	2	<0.001
	ALAN:Crayfish	0.3239	1	0.569
	ALAN:Week	1.2634	2	0.532
	Crayfish:Week	8.2863	2	0.0159
	ALAN:Crayfish:Week	1.7017	2	0.427
SFA flux	ALAN	0.7156	1	0.398
	Crayfish	0.9386	1	0.333
	Week	95.0853	2	< 0.001
	ALAN:Crayfish	0.3781	1	0.539
	ALAN:Week	0.5709	2	0.752
	Crayfish:Week	9.9346	2	0.007
	ALAN:Crayfish:Week	1.5024	2	0.472
MUFA flux	ALAN	1.0459	1	0.306
	Crayfish	0.8452	1	0.358
	Week	97.0605	2	< 0.001
	ALAN:Crayfish	0.5053	1	0.477
	ALAN:Week	0.7533	2	0.686
	Crayfish:Week	7.8349	2	0.020
	ALAN:Crayfish:Week	1.3520	2	0.509

Response	Predictors	Chisq	Df.	Pr(>Chisq)
PUFA flux	ALAN	0.0535	1	0.817
	Crayfish	0.3183	1	0.573
	Week	129.4449	2	< 0.001
	ALAN:Crayfish	0.1227	1	0.726
	ALAN:Week	1.4743	2	0.478
	Crayfish:Week	6.6538	2	0.036
	ALAN:Crayfish:Week	1.5353	2	0.464
ARA flux	ALAN	0.1763	1	0.674
	Crayfish	1.5302	1	0.216
	Week	68.0465	2	< 0.001
	ALAN:Crayfish	0.4379	1	0.508
	ALAN:Week	0.4925	2	0.782
	Crayfish:Week	3.0349	2	0.219
	ALAN:Crayfish:Week	2.4502	2	0.294
EPA flux	ALAN	0.0654	1	0.798
	Crayfish	0.1469	1	0.701
	Week	213.5055	2	< 0.001
	ALAN:Crayfish	0.3637	1	0.546
	ALAN:Week	0.6482	2	0.723
	Crayfish:Week	6.0969	2	0.047
	ALAN:Crayfish:Week	3.5046	2	0.173

Table S6. Mean concentrations $\pm$ standard deviation ($\mu\text{g mg}^{-1}$) of main fatty acids (FA) groups saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) and determined in Chironomidae from flumes without stressors (Control), exposed to the presence of artificial light at night (ALAN), to the presence of signal crayfish (Crayfish), and the combination of both stressors (ALAN+Crayfish), at one (Week 1), four (Week 4) and six (Week 6) weeks after the initiation of stressors.

		Control	ALAN	Crayfish	ALAN+Crayfish
Total FA	Week 1	58 $\pm$ 28	59 $\pm$ 3	55 $\pm$ 12	48 $\pm$ 7
	Week 4	22 $\pm$ 4	24 $\pm$ 6	24 $\pm$ 12	24 $\pm$ 7
	Week 6	15 $\pm$ 6	12 $\pm$ 4	18 $\pm$ 3	21 $\pm$ 6
SFA	Week 1	22 $\pm$ 16	18 $\pm$ 3	18 $\pm$ 5	14 $\pm$ 1
	Week 4	8 $\pm$ 2	7 $\pm$ 3	6 $\pm$ 2	6 $\pm$ 4
	Week 6	6 $\pm$ 1	5 $\pm$ 2	7 $\pm$ 1	8 $\pm$ 3
MUFA	Week 1	12 $\pm$ 6	10 $\pm$ 2	10 $\pm$ 2	9 $\pm$ 1
	Week 4	4 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 1	3 $\pm$ 2
	Week 6	4 $\pm$ 2	3 $\pm$ 1	4 $\pm$ 1	5 $\pm$ 2
PUFA	Week 1	24 $\pm$ 7	31 $\pm$ 3	26 $\pm$ 6	25 $\pm$ 6
	Week 4	10 $\pm$ 1	13 $\pm$ 3	14 $\pm$ 2	14 $\pm$ 1
	Week 6	5 $\pm$ 3	4 $\pm$ 1	6 $\pm$ 1	7 $\pm$ 3
ARA	Week 1	0.8 $\pm$ 0.1	1 $\pm$ 0.3	0.7 $\pm$ 0.2	0.9 $\pm$ 0.2
	Week 4	0.4 $\pm$ 0.1	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1
	Week 6	0.5 $\pm$ 0.3	0.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.8 $\pm$ 0.4
EPA	Week 1	15 $\pm$ 4	23 $\pm$ 2	18 $\pm$ 5	18 $\pm$ 5
	Week 4	3 $\pm$ 0.5	4 $\pm$ 1	3.7 $\pm$ 0.2	4.6 $\pm$ 0.7
	Week 6	2 $\pm$ 2	1 $\pm$ 0.2	1.5 $\pm$ 1	3 $\pm$ 1

Table S7. Analysis of variance (ANOVA) (type III) of the generalised linear model (binomial distribution) of signal crayfish activity (“Active” or “Hidden”) recorded in flumes exposed and non-exposed to artificial night at night (Treat: NoALAN/ALAN) over six weeks (Week). “Active” indicates signal crayfish individuals recorded walking or visible outside hidden places, while “Hidden” refers to individuals inside hidden places or in burrows in the sediment. LR Chisq: Likelihood Ratio Chi-square; Df.: degrees of freedom; Pr(>Chisq): Chi-square test statistic, in bold indicates significant differences (p<0.05).

Predictors	LR Chisq	Df.	Pr(>Chisq)
Treat	0.533	1	0.035
Week	2.328	1	0.173

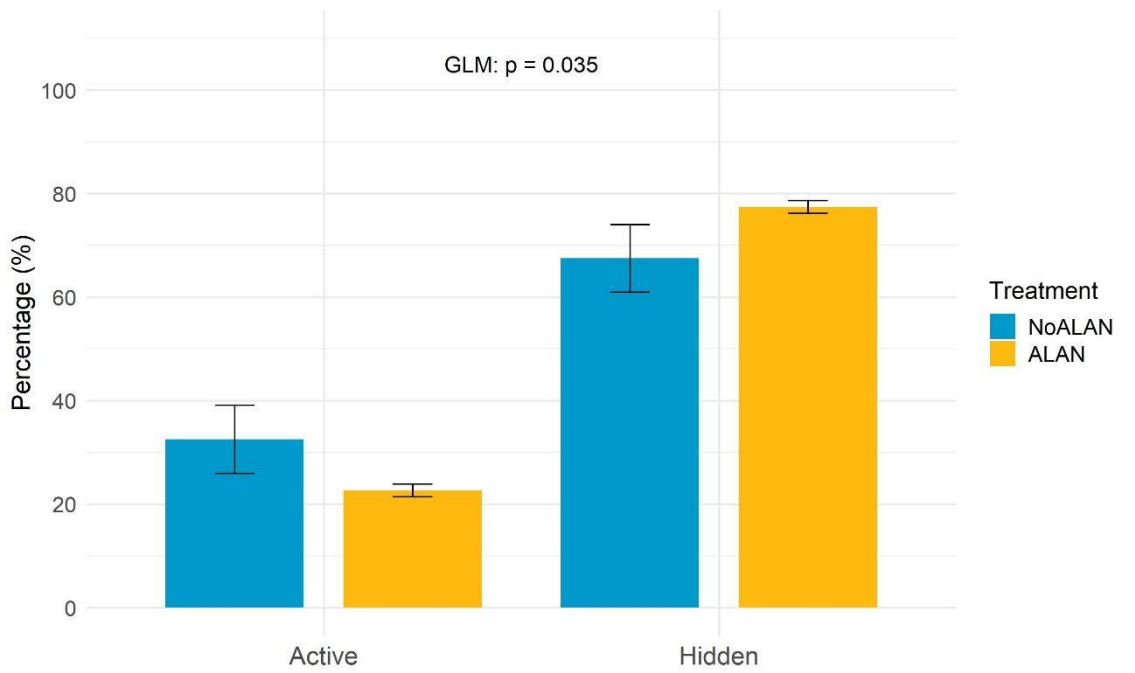


Figure S1. Signal Crayfish activity in flumes exposed (“ALAN”) and non-exposed (“NoALAN”) to artificial night at night. “Active” indicates signal crayfish individuals recorded outside hidden places, while “Hidden” refers to animals inside hidden places or in burrows in the sediment. Crayfish activity was monitored weekly at night. Bars show mean percentage over weeks; error bars indicate standard deviation.

Table S8. Analysis of variance (ANOVA) of the linear models for total fatty acid (Total FA), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, arachidonic acid (ARA; 20:4 ω -6) and eicosapentaenoic acid (EPA; 20:5 ω -3) content (μ g mg⁻¹ dw) in spiders from flumes subjected to Artificial light at night (ALAN), and to the presence of signal crayfish (Crayfish). Df.: degrees of freedom; Sum. Sq: Sum Squares; Mean Sq.: mean squares. F: Fisher statistic.

Response	Predictors	D.f.	Sum. Sq	F	p-value
Total FA content	ALAN	1	879.86	3.53	0.090
	Crayfish	1	96.69	0.39	0.547
	ALAN:Crayfish		48.95	0.20	0.667
	residuals	10	2491.15		
SFA content	ALAN	1	158.35	2.76	0.128
	Crayfish	1	18.43	0.32	0.583
	ALAN:Crayfish	1	28.36	0.494	0.498
	residuals	10	574.08		
MUFA content	ALAN	1	40.86	3.768	0.081
	Crayfish	1	8.910	0.822	0.386
	ALAN:Crayfish	1	0.747	0.069	0.798
	residuals	10	108.43		
PUFA content	ALAN	1	114.21	3.85	0.078
	Crayfish	1	6.53	0.22	0.649
	ALAN:Crayfish	1	0.651	0.022	0.885
	residuals	10	297.06		
ARA content (Square-root transformed)	ALAN	1	0.8477	4.59	0.058
	Crayfish	1	0.4158	2.251	0.164
	ALAN:Crayfish	1	0.0289	0.157	0.701
	residuals	10	1.8471		
EPA content	ALAN	1	8.572	3.4314	0.094

Crayfish	1	0.0002	0.0001	0.993
ALAN:Crayfish	1	0.4535	0.1816	0.679
residuals	10	24.980		

Table S9. Mean concentrations $\pm$ standard deviation ($\mu\text{g mg}^{-1}$) of saturated (SFA) and monounsaturated (MUFA) fatty acids determined in spiders from flumes without stressors (Control), exposed to the presence of artificial light at night (ALAN), to the presence of signal crayfish (Crayfish), and the combination of both stressors (ALAN+Crayfish).

	Control	ALAN	Crayfish	ALAN+Crayfish
SFA	11.2 $\pm$ 8.8	15.2 $\pm$ 8.5	6 $\pm$ 2.3	15.7 $\pm$ 9.7
MUFA	5.8 $\pm$ 3.8	8.8 $\pm$ 4.3	3.7 $\pm$ 0.8	7.6 $\pm$ 3.3
