Social context rapidly reshapes chemical signalling and endocrine profiles in two mouse subspecies

Supplementary Information

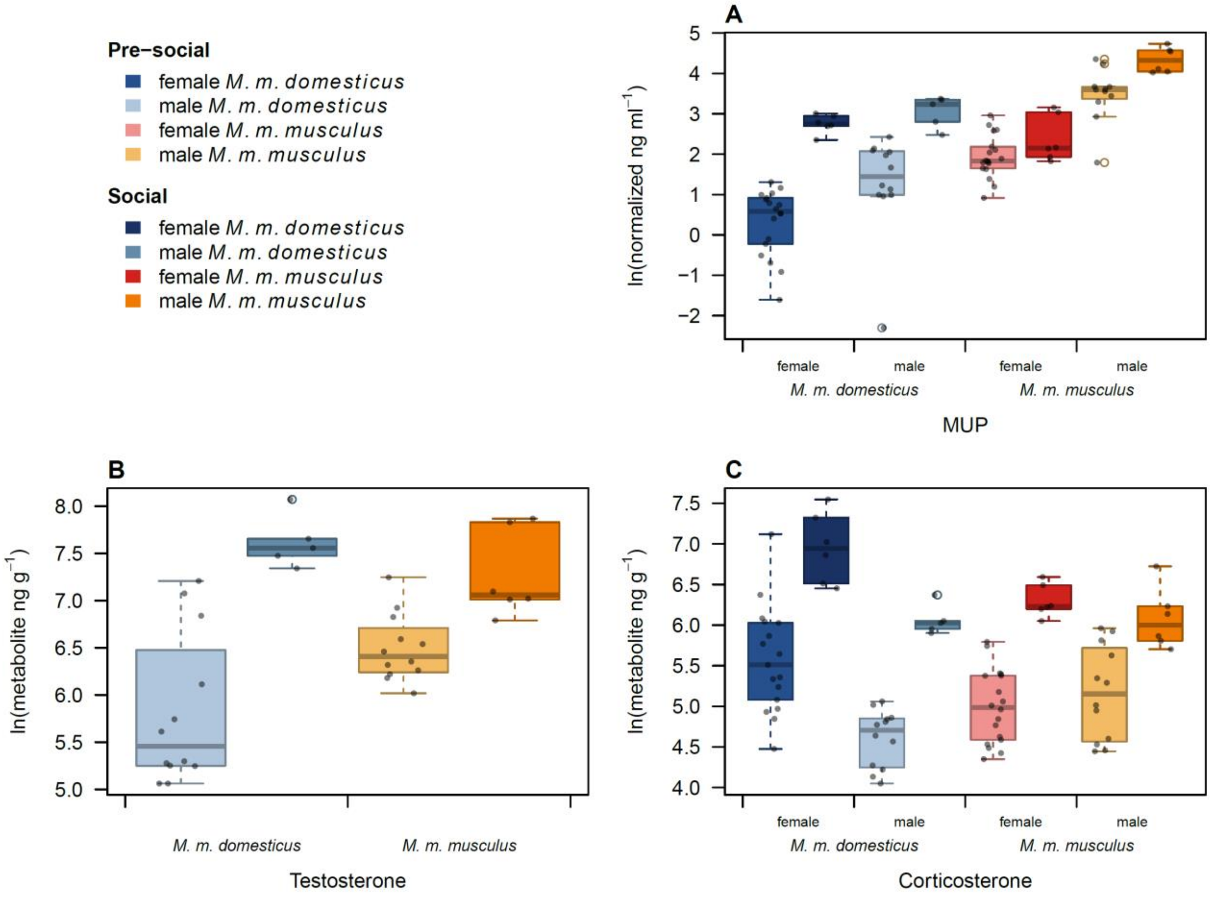

Fig. S1. Measured concentrations of MUPs (A), testosterone (B), and corticosterone (C) in the Initial phase. Desaturated colours represent singly housed mice before enclosure entrance (pre-social context), whereas saturated colours show mice socialised for 8 days in the enclosure (social context). Points are individual measurements, boxplots show interquartile range (Q1– Q3), with the bold horizontal line indicating the median. Whiskers extend to the most extreme data points within 1.5 × interquartile range (IQR), values outside this range are shown as empty circles.

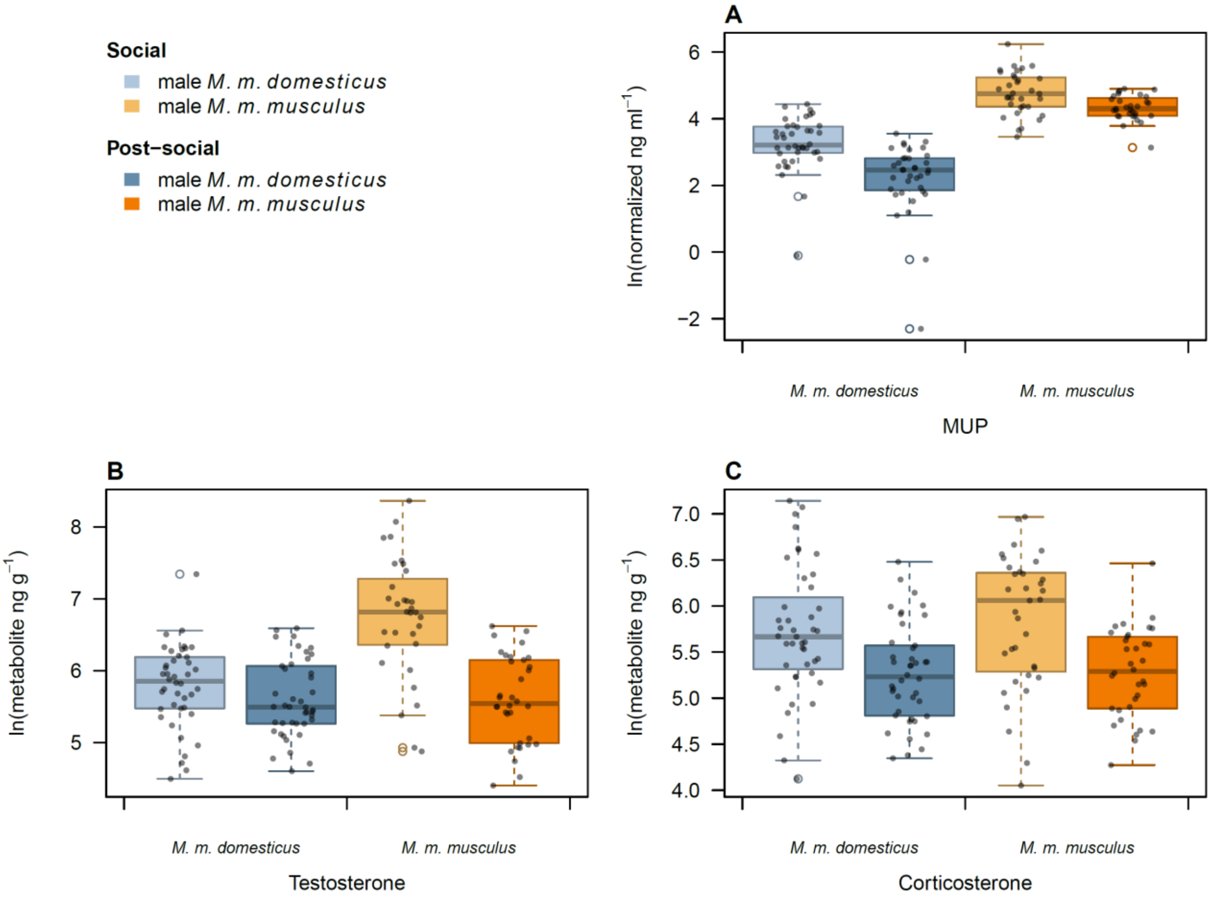

Fig. S2. Measured concentrations of MUPs (A), testosterone (B), and corticosterone (C) exiting the Social phase and entering the Isolation phase. Desaturated colours represent mice on the final day in the enclosure (social context), whereas saturated colours depict individuals 24 h after transfer to solitary housing (post-social context). Points are individual measurements, boxplots show the median (horizontal bar) and IQR. Whiskers extend to the most extreme data points within 1.5 × IQR, values outside this range are shown as empty circles.

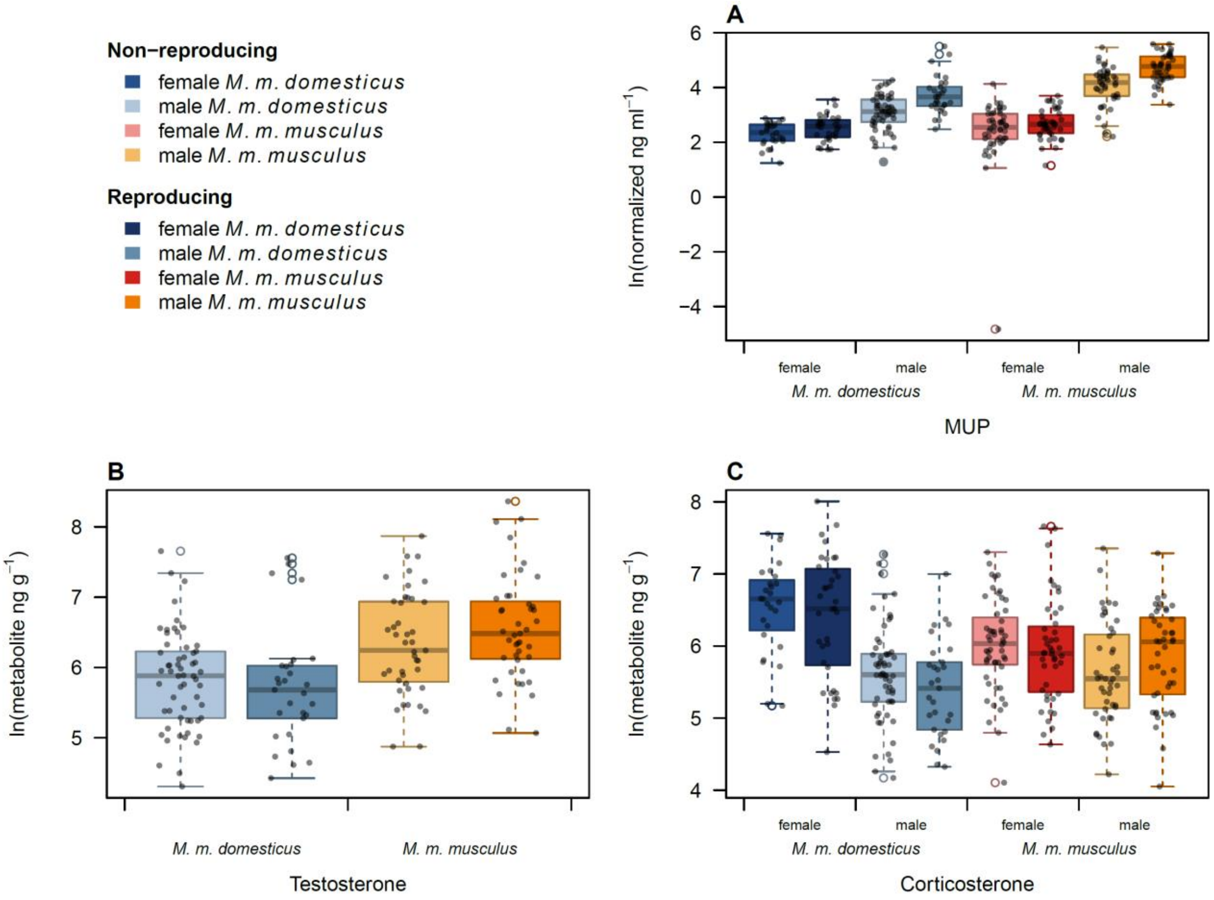

Fig. S3. Concentrations of MUPs (A), testosterone (B), and corticosterone (C) during the Social phase. Desaturated colours represent non-reproducing mice (i.e., those to which no offspring could be assigned within the 44-day window surrounding sampling events). Saturated colours display reproducing mice (≥ 1 assigned offspring). Points are individual measurements, whereas boxplots depict the median (horizontal bars) and IQR. Whiskers extend to the most extreme data points within 1.5 × IQR, values outside this range are shown as empty circles.

Table S2. Predictor estimates from GLS models evaluating changes in MUP, testosterone, and corticosterone (Δ) when animals were transferred from the social conditions to isolation. Each model includes subspecies and current reproductive output, treated either as a binary variable (currently reproducing vs. not reproducing; upper part of the table) or as a continuous variable (current number of assigned offspring, CRO; lower part of the table). Residual heteroscedasticity was modelled using variance functions (*varExp* for continuous predictors and *varIdent* for group-specific variance) within the *nlme* framework. Columns report the estimate, standard error (SE), degrees of freedom (df), *t*-value, and *p*-value for each predictor. Note that Δ values were not log-transformed in these models.

Agent	**Effect**	**Estimate**	**SE**	**df**	***t***	***P***
ΔMUP	Subspecies (*musculus*)	-36.93	16.42	63	-2.249	*0.0280*
	Reproduction (yes)	-29.89	17.04	63	-1.754	0.0843
ΔTestosterone	Subspecies (*musculus*)	-563.34	125.25	72	-4.498	*<0.001*
	Reproduction (yes)	-251.27	229.31	72	-1.096	0.2768
ΔCorticosterone	Subspecies (*musculus*)	-8.22	68.06	72	-0.121	0.9042
	Reproduction (yes)	-46.60	72.13	72	-0.646	0.5203
ΔMUP	Subspecies (*musculus*)	-39.08	16.66	63	-2.346	*0.0221*
	Reproduction (CRO)	-1.81	2.26	63	-0.799	0.4273
ΔTestosterone	Subspecies (*musculus*)	-706.33	157.89	72	-4.474	*<0.001*
	Reproduction (CRO)	-2.07	28.840	72	-0.072	0.9430
ΔCorticosterone	Subspecies (*musculus*)	-35.953	67.544	72	-0.532	0.5962
	Reproduction (CRO)	5.791	8.068	72	0.718	0.4752

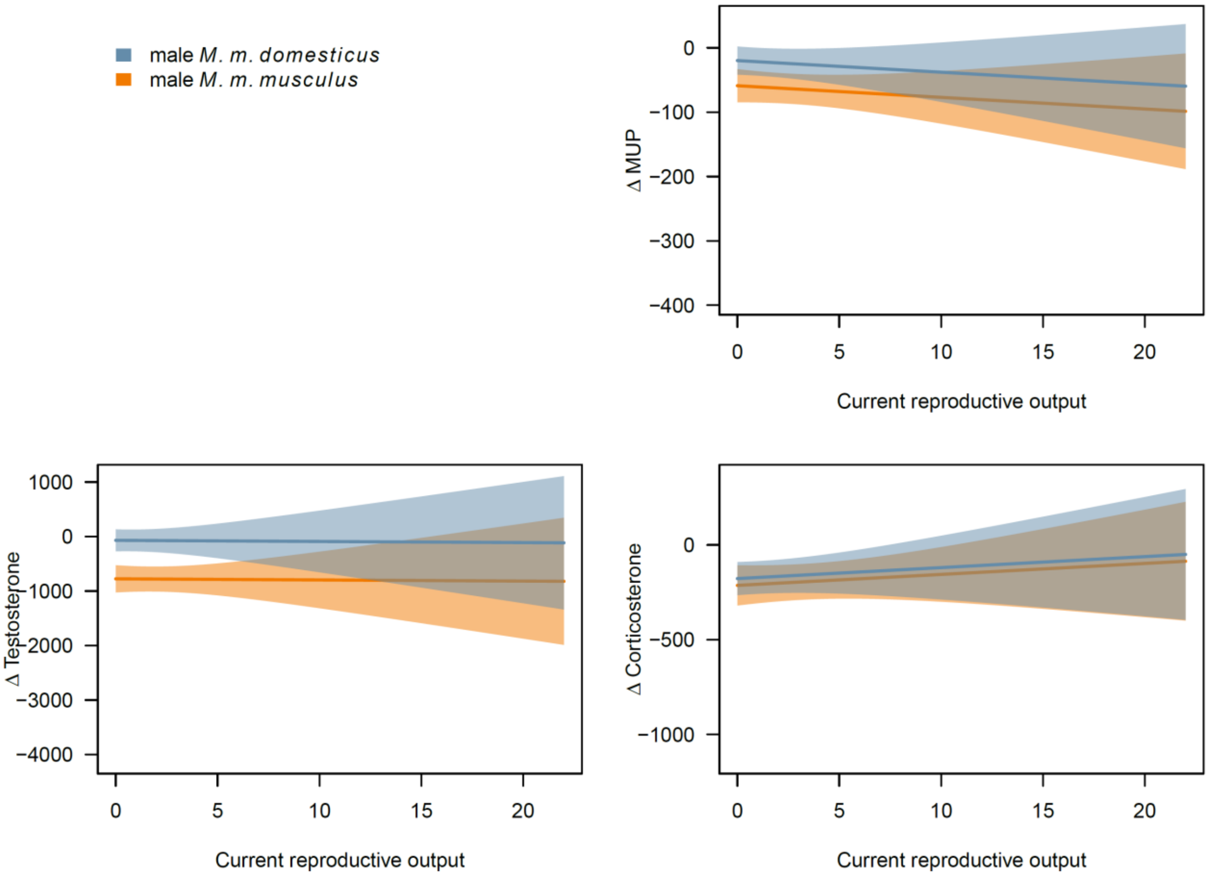

Fig. S4. Changes (Δ) in MUPs, corticosterone, and testosterone concentrations following the transition from the social experiment to isolation, plotted against current reproductive output (number of assigned offspring within the assessment window). Lines represent model predictions from GLS models including subspecies and current reproductive output as fixed effects, and shaded areas indicate 95% confidence intervals (± 1.96 SE). Colours distinguish subspecies (*M. m. domesticus* and *M. m. musculus*); Δ values are shown on the original scale.

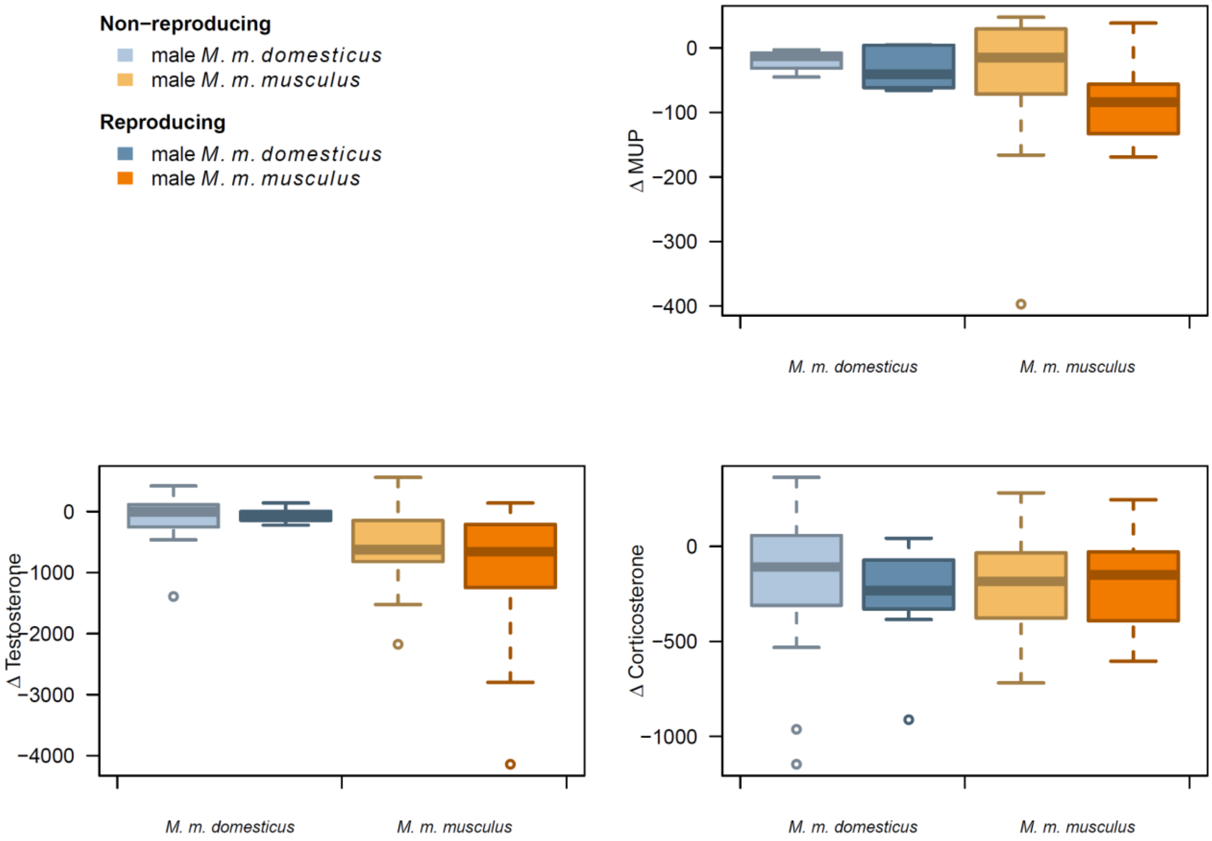

Fig. S5. Graphical representation of measured ΔMUPs, ΔCorticosterone, and ΔTestosterone changes following the transition from the social experiment to isolation, with animals grouped by subspecies and reproductive status (currently reproducing vs. currently not reproducing). Desaturated colours indicate non-reproducing individuals, whereas more saturated colours represent reproducing individuals. Boxes display the median and the first and third quartiles (Q1, Q3), with the bold horizontal line marking the median. Tukey whiskers extend to the most extreme data points within 1.5 × IQR, and more distant values are plotted as empty circles.