

Fig. S1. Diagram illustrating 20E biosynthesis in insects. In this scheme, cholesterol, obtained from the diet, is converted to cholesterol, 7-dehydrocholesterol, 4 –Diketol, 5 - Diketol, 5b-Ketotriol (2,22dE), 2-Deoxyecdysone (2dE), Ecdysone and finally to 20E. Several enzymes are involved in these conversions, including neverland, CYP307, nm-g/sro, CYP306A1, CYP302A1, CYP315A1, and CYP314, respectively. Black box indicates a pathway that is unknown and possibly involves more than one enzyme.

GGGCGTGAAGATCTGTCTCCTCCAAACTCGCTGAATGGTTATTCAGTGGATAGCTGTGATGGTTCTAAAA
 AGAAAAAGGGACAAGTGCTAGACAACAGGAAGAATTGTGCCTTGTCTGCGGTGATAGGGCGTCTGGTTA
 CCATTACAATGCGTTGACGTGTGAAGGATGCAAAGGCTTCTTCAGGCGGAGCATCACAAAAATGCAGTT
 TACCAATGCAAGTATGGAAATAATTGTGAAATAGATATCTATATGAGAAGGAAATGCCAAGAATGTCGTT
 TAAAAAATGTTTTAAATGTTGGCATGAGG**CCGGAATGCGTAGTACCAGA**ATATCAATGTGCGGTCAAAAG
AAAGGAGAAAAAGCTACAAAAAGACAAAAGATAAAACCAGTATCCACGACAAATGGTTCACCAGAAGCAATA
AAATCAGAATCTGAACCTATAAGGGTCAGTTTTTTCATCCAGTCTGATATCATTGTTAAAAGAGTCACAAA
CCTACCACCGGAGCCAATTCCTTCTGCTCTCTCAAGCTTCACGCTGCCACAACGGAGATGAGGAAGGTGA
GATGGCCAGTAAAGTACCAGTGAACGGTGTAAAACCAGTCAGTCCTGAACAAGAAGAACTTATACATAGA
 CTTGTATATTTCCAAAATGAATATGAACATCCTTCTGAAGAGGAAGTTCGCAAAAT**TAACGCTGGTAACG**
ACGATGAAGAGCAAAGTGATCTCAGGTTTAGGCATATTACCGAGATAACCATATTAACCTGTTCAATTAAT
CGTTGAATTCGCTAAACGTTTACCTGGTTTTGACAAACTACTTAGAGAAGATCAAATTCACACTATTGAAG
GCTTGTTCAAGTGAAGTAATGATGTTGCGAATGGCTAGGAGGTACGATGCCAATCTGATTCAATATTAT
TTGCAAACAATCAACCATATAACCAGAGACTCGTACAGTATGGCCGGTATGGGTGATGTGGTTGAAGATTT
GCTCAGGTTTTGCAGGCAGATGTTCAACATGAAAGTGACAAATGCTGAATACGCCTTGCTTACTGCAATT
 GTTATCTTTTCTGAACGTCCTTCATTAATAGAAGGCTGGAAAGTGGAATAAATTCAGAAATCTATTTAG
 AAGCACTAAAATCCTATGTAGACAACCGGCCGAAACCAAGGCCTCCTACGATTTTCGCCAACTTCTGTC
 AGTTTTAACTGAATTACGTACACTTGAAATCAGAATTCAGAAATGTGTATATCATTAAATTACAAAAT
 AAGAAATTACCACCGTTCCTAGCTGAAATTTGGGACGTAAATGCCTGA

Fig. S2A. The *Rhopr-EcR* gene sequence. The *Rhopr-EcR* (RPRC014174) transcript and the non-overlapping dsRNA fragments are highlighted in yellow. The primers used are bold and underlined.

ATTAATGGAAC TGTTCAC TTACAGGTCTGAGTTTGGTGGAGAACAACCTGAGTCTAGTGGGCCCCGAGT
CGCCCATCGAC **ATGAAGCCGGACACGGCCACACTGCACTGCAGCAGCTTCAGTCCTACACCCACATCCGG**
ACCCACCAGTCCACAAGGGTTCAACATCGTCCCCGAGCAGTGTGCTT **GGCAATGGCAACAAAAGTTCCGGC**
AATGTATATCCACCAAACCATCCGCTCAGTGGCAGTAAACATCTGTGCAGTATCTGTGGAGATCGAGCTT
CCGGCAAACATTATGGAGTGTACAGTTGCGAAGGTTGCAAAGGCTTTTTCAAAGAACCCTCCGCAAAGA
TCTGTCTTATGCTTGTGCGAGAGGACAAAACAGTGTTTAGTGGACAAAAGACAAAGAAACAGATGCCAGTAT
TGTAGATATCAGAAATGTTTATCCATGGGGATGAAGAGGGAAGCTGTACAGGAAGAACGGCAGCGAACGA
AAGAACGAGACCAGAACGAAGTGGAGAGTACCAGTAGTTCCACACGGACATGCCCGTCGAGAGGATACT
CGAAGCAGAACAGAGGGTGGACTGCAAGTTGGAAATGAAAGAGGAGTTCAACTTGGGCCCAATGAGCGAC
ATCATCT **GTCAAGCGACGTACAACCAA**CTTTGGCAGCTGATAGATTGGGCTAAACATATACCGCACTTCA
CATCGCTACCTATCGAAGATCAAGTAACCTTGCTCAGTGCAGGTTGGAATGAGCTGCTTATTGCTGGATT
TTCGCATCGATCGATACTGGCTAAAGAAGGACTGGTGTTAGGTCCCGGAGTGATTGTCAACAGGAACAAC
GCTCATCAGATTGGAGTCGGTCCAATATACGATAGAGTGCTAACAGAATTAGTCTC**CAAGATGAGAGAAA**
TGAAGATGGACAAGACAGAACTTGGATGTCTCAGAACGATCATTCTTTTTAATCCAGAGGTACGTCTCT
CAAGTCTGTCCAAGAAGTGGAACTGCTAAGAGAAAAAGTTTACGCTTCTCTAGAAGAATATACACGCATC
AGCCATCCGAACGAGCCAGGAAGGTTTGCAAACACTCTTGCTAAGGCTGCCATCCTTAAGGTCTATAGGAC
TTAAATGTTTAGAACACCTGTTCTTCTGTAGAGTGGTGGGAGAGGCGCCTGTAGATACATTTCTGGCTCA
GCTCTTAGAATCACCAGAAGTATCTAATCGAATCTAA

Fig. S2B. The *Rhopr-USP* gene sequence. The *Rhopr-USP* (RPRC013330) transcript and the non-overlapping dsRNA fragments are highlighted in yellow. The primers used are bold and underlined

GCAGGGAACCGCCTCCATTGCAAGTCGGTGCGGGACGTGCCAGGACCTCTTACTTTGCCAATCATCGGCA
CCAGGTGGATCTATTATTTTTCAGATACAAG**TGAGCAAAATACACGAAGCTTAT**GCAGATCTGTTTGTGCG
GTACGGCAATGTGGTGGAGAGGAGTCCGTCTGGAACGTGCCCGTGGTGAGCGTGCGGTCCAGGACGGAC
ATCGAGAAGGTGCTGCGCCAGGGCGGCAAGTATCCGATGCGGCCGCCACGGAGGTCATCGCCTACTACC
GCGCCACTCGCCCCGATCGCTACACCAACCTCGGCATCATCAACGAGCAAGGAGAGACCTGGCATCGCCT
AAGAAGTCTCCTGACTCCAGAACTGA**CCTCTTCTAGGACCATGCACA**AGTTCCTGCCGGAAGTACCGTA
GTAGCGGACGACTTCAGTCGATTGGTGGATGCATTCCAGAGAAGGGCCAAACGGATTAGTGGAGGTTTCG
AAGAGCTAGCTTGTGCTATCGGCTTGGAGAGCACTTGTACCTTAATTCTTGGCAAAAGATTGGGATTTCT
AGAGAAAGAAGTTAGCCCTCTTGGCGCAAGAC**TAGCCGAAGCTGTCAAGGAT**CTGTTCTGTGCTTCTAG
GATACATTCTTTGGGCTCCCATTTCTGGAAGTTACTTCCCACGCCAGCGTACAGGAAATTCATGTCTAGTG
AGAACACTATTTACGATGTTATCTCTGAACTAGTGGATTTCAGCTCAGAAACAAGAAGAAGACACTTGGCA
GCTGGACGATGCAGTGCAAAGTGTGTTTCATGGCCATTCTTAGAGCTCCAGGTTTAGACTTAAGAGACAAA
AAAGCTGGAATAATCGATTTTAT**AGCAGCAGGCATCAAGACTT**TGGGCAACACACTTGTATTTCTCTTGT
ATTTGATGGCCAAAATAAACAGTGCCAGGAGAAGCTGTACGAAGAGATCTGGAGATTGGCGCCGAATGG
AGCCTCTCTAGATAGCGCAACTCTGCGTAATGCTCACTACCTCAGAGCCTGCATCATGGAAGCTTTTGA
GTTTTACCCACTGCACCTTGTGTAGCTCGAATTTTGGAAATCAGATGTGCAGCTTGGCGGTTATCACCTTA
AATCTGGATCCGTAGTCCTGTGCCACACTTGGCTCGCATGTATGGACGAAGCAAACCTTTGAGGACGCTAA
AGTATTCTCGCCAGAGAGGTGGCTAGGAAGTGTGAAACTGTTCAACAGGTAATTTCGCATCCCTTTTTG
GTGGTTCCGTTTGGAGTGGGCCGTGCAATGTGTCCTGGCAAGAGATTTCGTGGAAGTGGAGCTTCAAGTAG
TTCTTGGCTTGTATGGTCAGGCAGTTTGAATAGACTTTGAAGGTCATCTGGAAGTGGAAATTTGAATTTCT
GCTCGCACCCAAATCACCTGCCAATTTTTTTGTTAAGGAAAGGTCTAT

Fig. S2C. The *Rhopr-shade* gene sequence. The *Rhopr-shade* (RPRC006945) transcript and the non-overlapping dsRNA fragments are highlighted in yellow. The primers used are bold and underlined

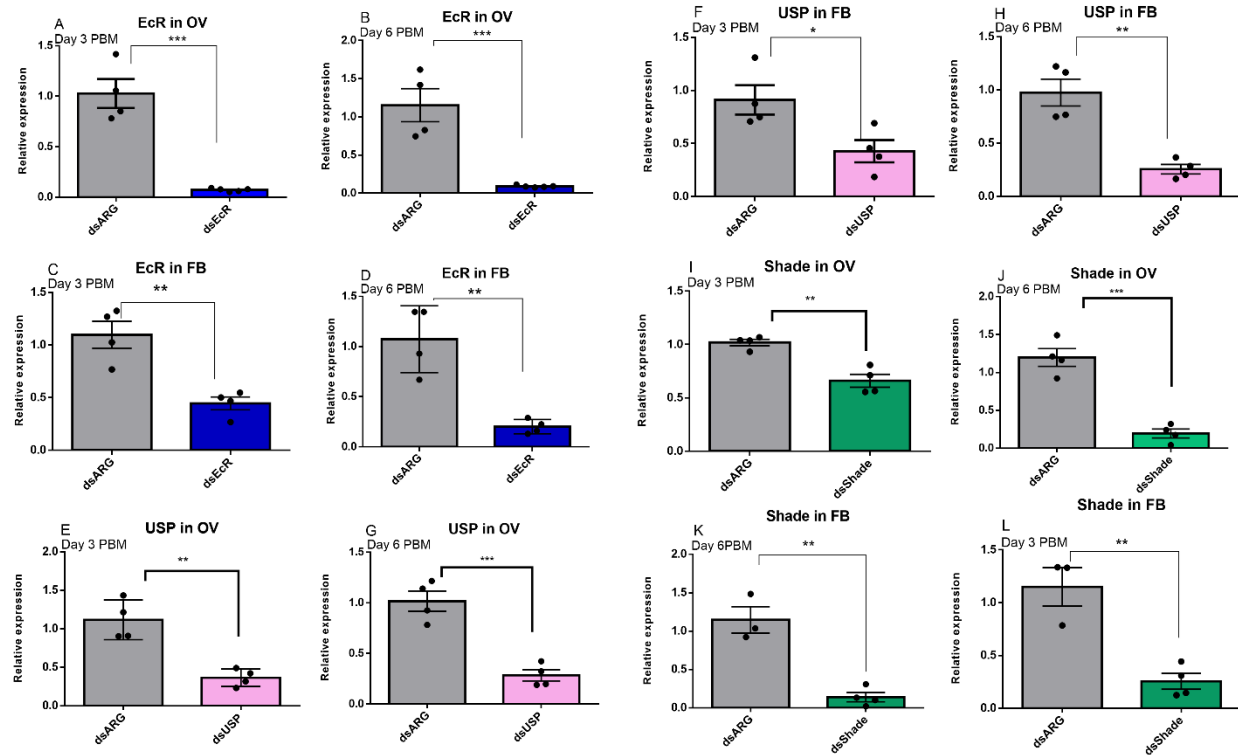


Fig. S3. Efficiency of knockdown of *EcR*, *USP* and *shade* transcripts in the ovary and fat body at 3 and 6 days PBM of adult females *R. prolixus*. Females were injected as described in Materials and Methods. Relative levels of the transcripts were measured in the fat body and ovaries using RT-qPCR. Data indicate means \pm SEM (n=4). *p < 0.05; **p < 0.01; ***p < 0.001. Statistics were performed using Student's t-test.

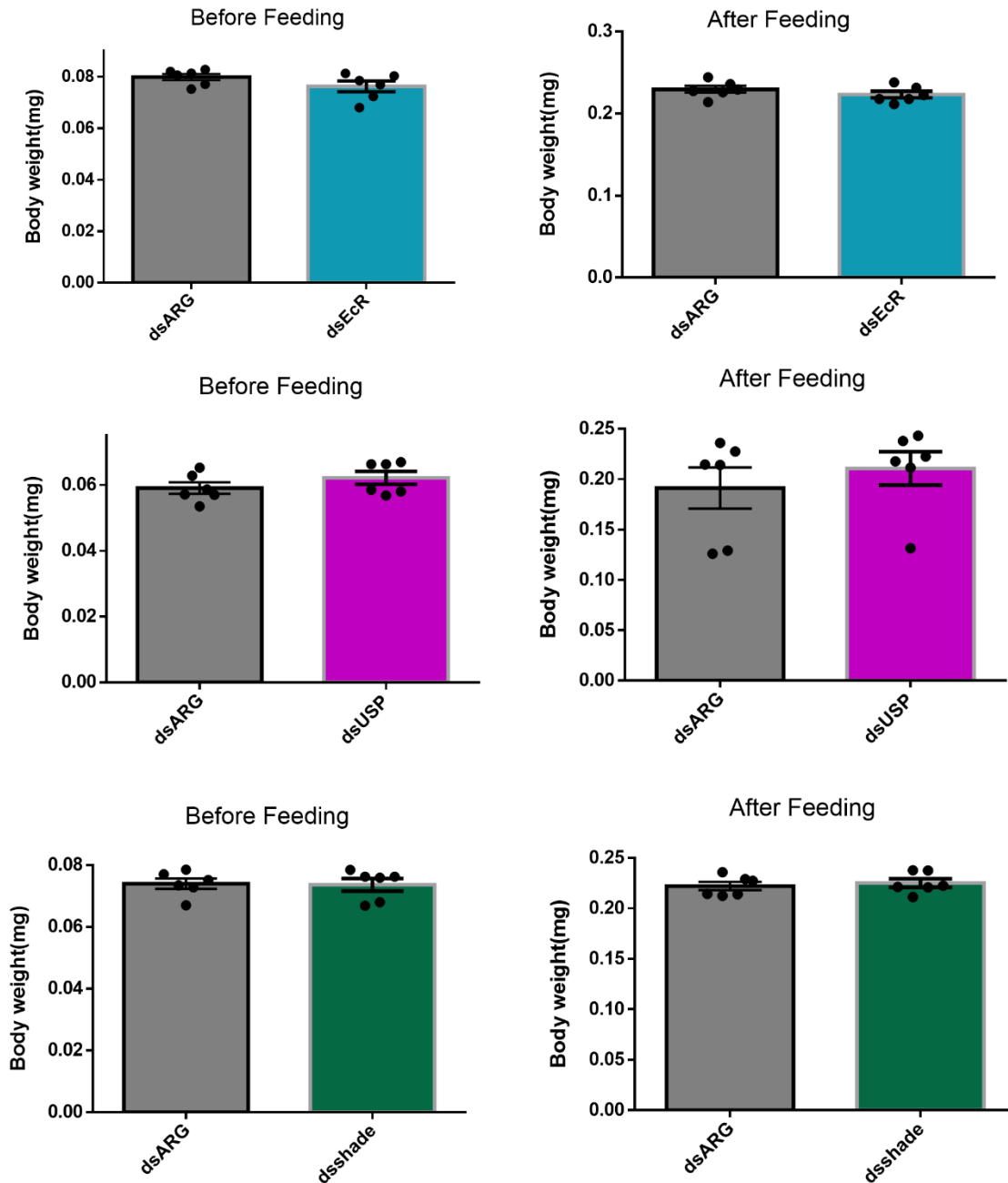


Fig. S4. Knockdown of *EcR*, *USP*, and *shade* transcripts have no effect on the weight of blood meal consumed. The weight of the blood meal did not differ significantly between treatment and control groups in female adults. Data indicate means \pm SEM ($n = 6$). All pairs of data not significant. Statistical analysis was performed using Student's t-test.

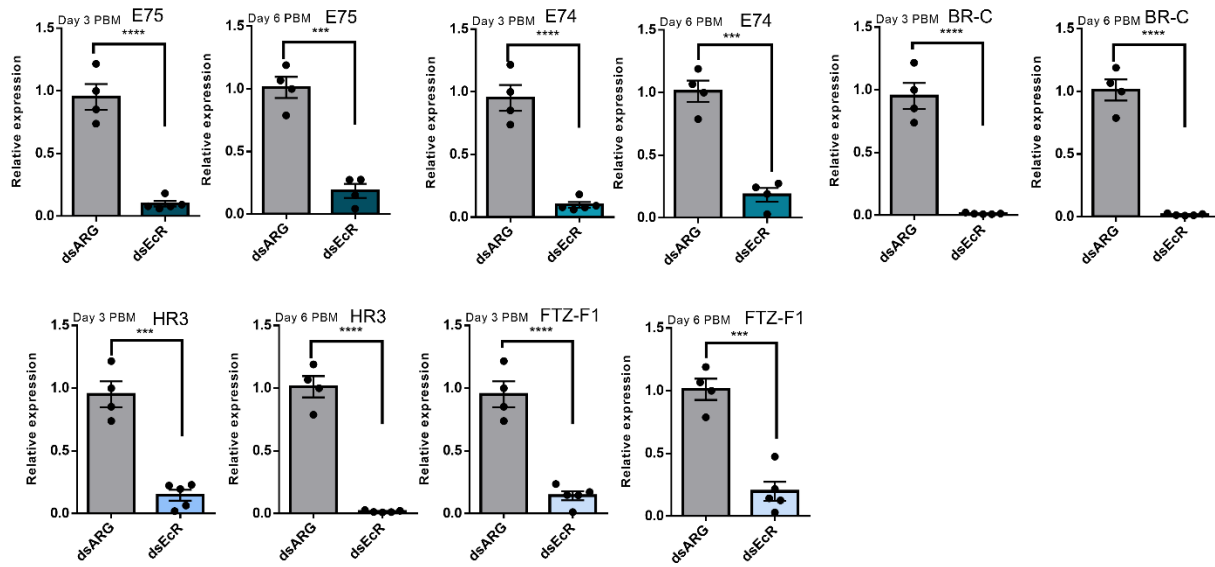


Fig. S5. Knockdown of *EcR* downregulates the transcript levels of *E75*, *E74*, *Br-C*, *FTZ-F1* and *HR3* in the ovary at 3 and 6 days PBM of adult female *R. prolixus*.

Females were injected as described in Materials and Methods. Relative transcript levels were measured using RT-qPCR. Data indicate means \pm SEM ($n = 4$). *** $p < 0.001$; **** $p < 0.0001$. Statistical analysis was performed by Student's t-test.