Evidence for discrete solar and lunar orientation mechanisms in the beach amphipod, Talitrus saltator Montagu (Crustacea, Amphipoda)

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Supplementary Methods

Primer and Taqman MGB probe sequences used in generation of qPCR standards and qPCR assays of Talitrus saltator brain and antennae samples.

T7 phage promoter sequence 5′-TAATACGACTCACTATAGGGAGA-3′

Method	Oligonucleotide name	Fluor/Sequence (5′-3′)
qPCR standards synthesis	TalAK STD F′	TATTTGGGGGCACAATTTGA
	TalAK STD R′	T7-CCAAGGCCAGTTTTCTTGTC
	Talclk STD F′	TTCCTGTTCCTAGACCAGAGG
	Talclk STD R′	T7-ATTCTGGCTTGCTGTTCCAC
	Talcry2 STD F′	TGTTTGGAGGATGTGGACAA
	Talcry2 STD R′	T7-ATGACTTCGATGCCCATCTC
	Talper STD F′	CTCGAGATTCGGTGCATTTT
	Talper STD R′	T7-GCCATCACCTCTTCTTCCAA
	Taltim STD F′	TGCACTTGGCCGGCATA
	Taltim STD R′	T7-ACGGTTCTTCGGGATCTTTT
Taqman™ assay primers and probes	TalAKTaq F′	TCGAGGAACGGCTGTCCTT
	TalAKTaq R′	AGCAGCGTCAACCATTTTGTT
	TalAKTaq Probe	NED-CCTGAGCCAAGACAG
	TalclkTaq F′	TCGGTTACCTGCCATTCGA
	TalclkTaq R′	CAGGTCGTCCACATGGTAGTAATC
	TalclkTaq Probe	FAM-TGCTAGGTACCTCAGGTTA
	Talcry2Taq F′	GCGCTCCCGCAGCTT
	Talcry2Taq R′	GGTCTTCCTCGAAACTCAGAACTG
	Talcry2Taq Probe	FAM-TCAAGGAGTGGAATACG
	TalperTaq F′	GCCTCATGCCTCCATTCTACTG
	TalperTaq R′	CGCAGAGGCTTGGTAGCATT
	TalperTaq Probe	VIC-CGAGTTCGCGAGAAC
	TaltimTaq F′	TGCACTTGGCCGGCATA
	TaltimTaq R′	CTCGTCCGTCGACGTAGACA
	TaltimTaq Probe	VIC-CGGACCTGCTCTTG

Quantitative PCR assays

Quantitative PCR Standards curves
RNA standard curves were generated by *in vitro* transcription using T7phage promoter flanked PCR products (see Table above for primer sequences) as templates. PCRs were done using Bioline MyTaq Red mastermix in 3 x 50µl volumes with the following thermocycling profiles: 95°C for 5 mins activation, followed by 40 cycles of 95°C 15s, 55°C 45s and 72°C 1 min., followed by a 72°C 7 min. final extension. PCR products were pooled, purified using PCR clean-up kits (Bioline, UK) and eluted in 20µl DEPC-treated water. Products were then used for *in vitro* transcription using a T7 MegaShortScript kit (Ambion, Thermo Life Sciences, UK) according to the manufacturer's instructions. Transcription products were purified on 10% 6M urea polyacrylamide gels, stained with ethidium bromide and products of expected size excised and eluted overnight in Probe Elution Buffer (Ambion, Thermo Life Sciences, UK) at RT. RNA was subsequently precipitated in three volumes of ethanol and resuspended in 30µl of water. RNA was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Life Sciences, UK) and copies of RNA calculated using Avogadro's constant/moles. Standard curves in the range 10^{10} to 10^{3} copies were reverse transcribed using Tetro cDNA synthesis reagents (Bioline, UK) and random hexamer primers according to the manufacturer's instructions.

Sample RNA extraction
Sample RNA from *T. saltator* heads (10 per time-point replicate) and antennae was extracted using Qiagen Universal RNA extraction columns following homogenization in Qiazol reagent. On-column DNAse digestion was performed to remove contaminating gDNA according to manufacturer's instructions. 500ng total RNA was reverse transcribed as for standards (above) in 20ul reactions and then diluted 5-fold in DEPC-treated water.

Quantitative PCR
All qPCR reactions were done on an Applied Biosystems QuantStudio 12-Flex 384 well platform using Bioline Sensifast Probe II PCR mix and Taqman MGB probes (see Table above). Quantitative PCR reactions were done in 10µl reactions containing 2µl cDNA and with each target gene assayed in the same reaction as the reference gene *TalAK*. Each primer and probe was used at 0.25µl (2.5µM) per reaction. Cycling conditions were: 95°C 2min (activation) followed by 40 cycles of 95°C 10s, 60°C 15s. Data are expressed as copy number of target gene per copy of the reference gene.