The following supplement accompanies the article

Interactive effects of ocean warming and acidification on sperm motility and fertilization in the mussel *Mytilus galloprovincialis*

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Table S1. Water parameters of experimental treatments.

Parameter	Mean	SE/ Range
Temperature °C: control	18.1	± 0.06
Temperature °C: elevated	24.1	± 0.09
pH: ambient	8.0	± 0.02
pH: - 0.02	7.8	± 0.004
pH: - 0.04	7.6	± 0.004
pCO_2* (µatm): ambient pH	456	(450 – 460)
pCO_2* (µatm): - 0.02 pH	777	(767 - 784)
pCO_2* (µatm): - 0.04 pH	1292	(1275 - 1304)
Dissolved oxygen (%)	90	± 2.2
Salinity (ppt)	34.8	± 0.01
A_T (μmol kg^{-1})	2316	(2286 - 2336)

As we did not measure total alkalinity [A_T] during our experiment, we calculated an estimated range of values based on data sourced from IMOS Ocean Portal (https://imos.aodn.org.au/imos123/), Takahashi et al. (2014), and Foster et al. (2014); each directly comparable to the region and time our seawater was collected. Corresponding A_T ranges from across these sources were used to calculate possible ranges for the reported levels of pCO_2 (* partial pressure of CO_2) using CO2SYS software (Pierrot et al. 2006) with the constants of Mehrbach et al (1973) as refitted by Dickson and Millero (1987). We accept that there will be some level of error in these estimations, however, the differences in pCO_2 between each treatment are much greater than the calculated ranges.

Table S2. Eigenvalues and percentage of variance explained by two principal component analyses (PCAs) on sperm motility parameters at Time 1 and Time 2.

	Time 1			Time 2		
Principal component	**Eigenvalue**	**% Variance**	**Cumulative % Variance**	**Eigenvalue**	**% Variance**	**Cumulative % Variance**
1	3.95	56.4	56.4	3.85	55.0	55.0
2	2.18	31.1	87.5	2.15	30.7	85.7
3	0.60	8.6	96.0	0.84	12.0	97.6
4	0.24	3.4	99.4	0.11	1.6	99.2
5	0.03	0.4	99.8	0.04	0.6	99.9
6	0.01	0.1	99.9	0.01	0.1	100.0
7	<0.01	0.1	100.0	<0.01	<0.1	100.0

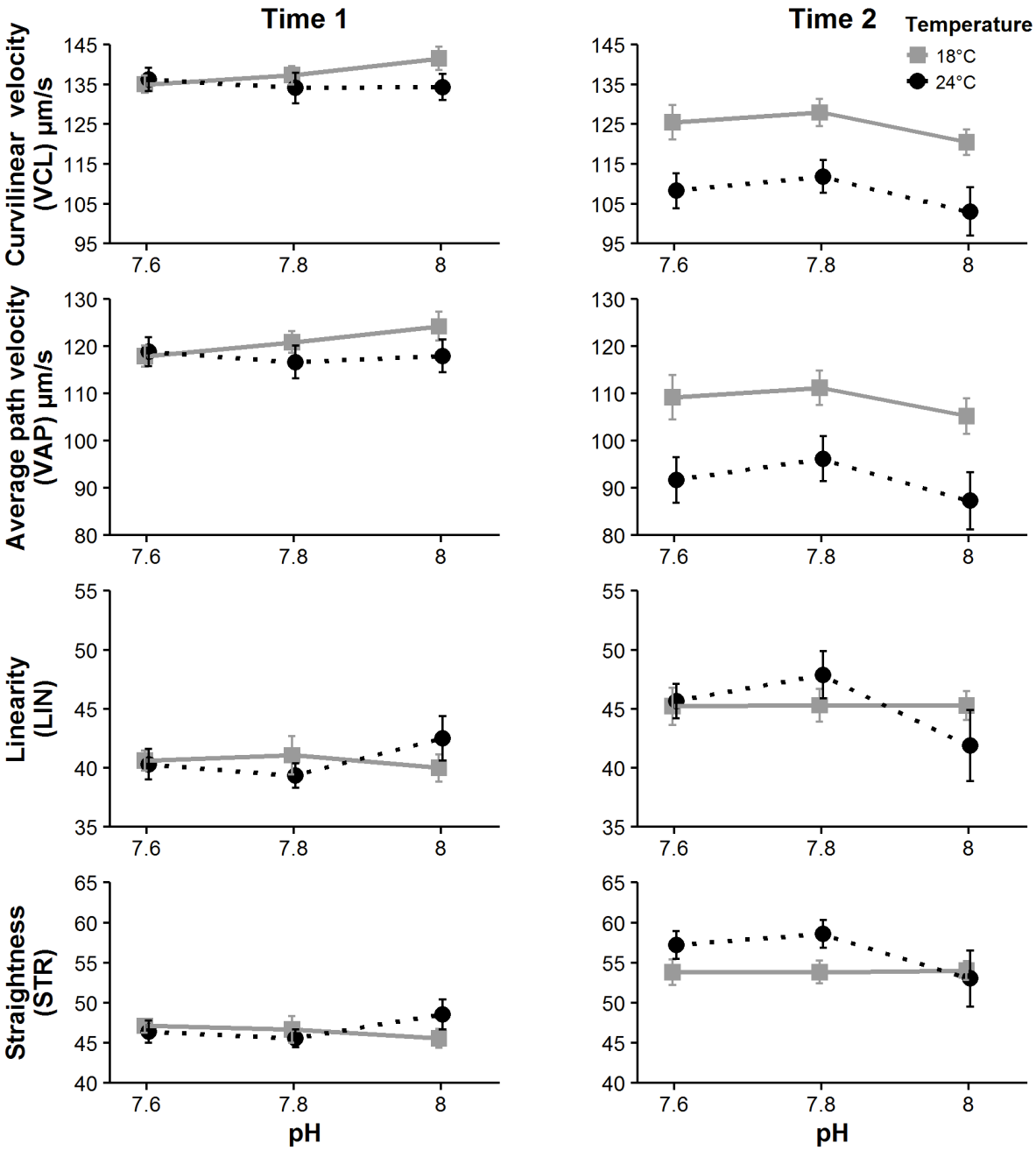

Fig S1. Effects of temperature and pH on the raw parameters of sperm motility across two time points. VCL = the curvilinear velocity along the sperm's path; VAP = smoothed average sperm path velocity; LIN = sperm linearity - the ratio of VSL/VCL; STR = sperm straightness - the ratio of VSL/VAP (VSL = the average velocity on a straight line from start to endpoint of the sperm's path). Time 1 is 20 min in treatment; Time 2 is 2 h in treatment. Light grey lines (■) indicate 18 °C, while black dotted lines ( ) indicate 24 °C (n = 14; ± SE).

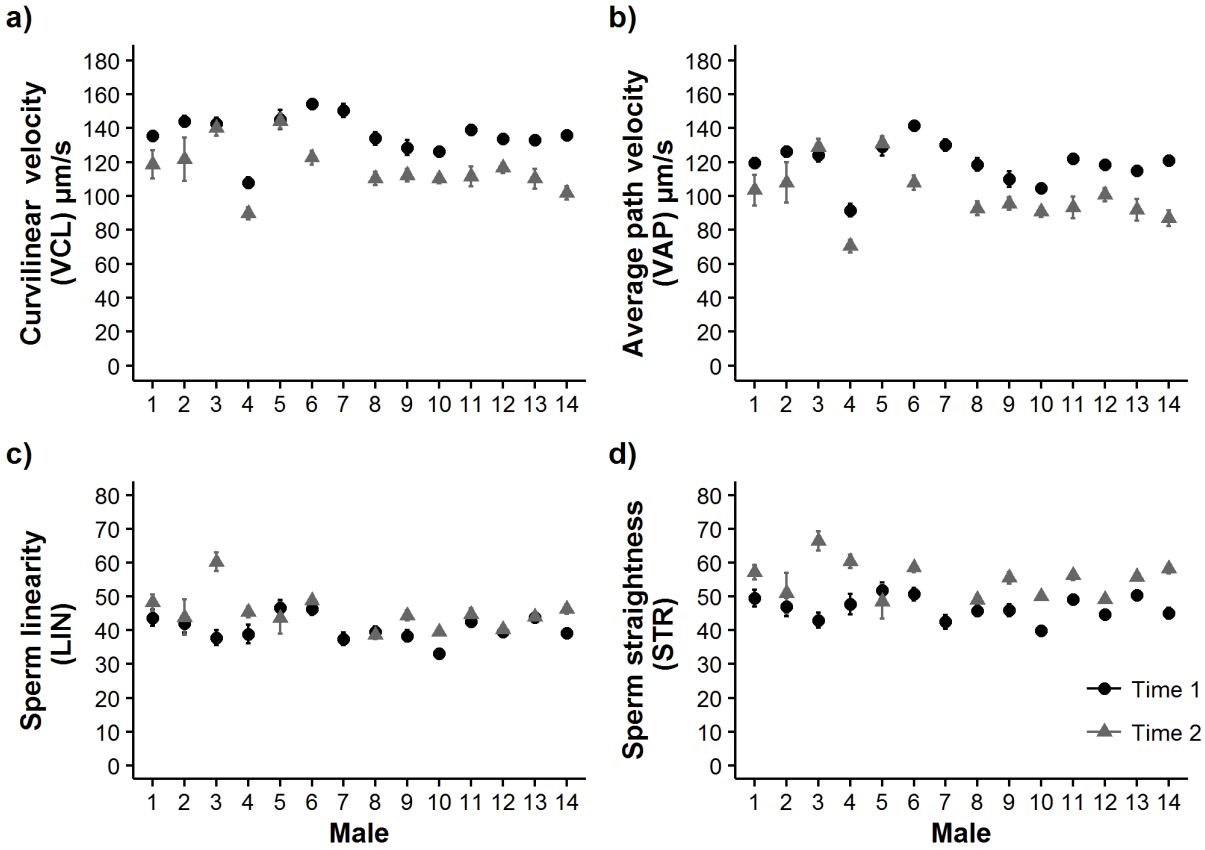

Fig S2. Sperm motility parameters among males across two time points, averaged across all treatments. VCL = the curvilinear velocity along the sperm's path; VAP = smoothed average sperm path velocity; LIN = sperm linearity - the ratio of VSL/VCL; STR = sperm straightness - the ratio of VSL/VAP (VSL = the average velocity on a straight line from start to endpoint of the sperm's path). Black points ( ) indicate Time 1 (20 min in treatment); grey points ( ) indicate Time 2 (2 h in treatment; n = 14; ± SE).

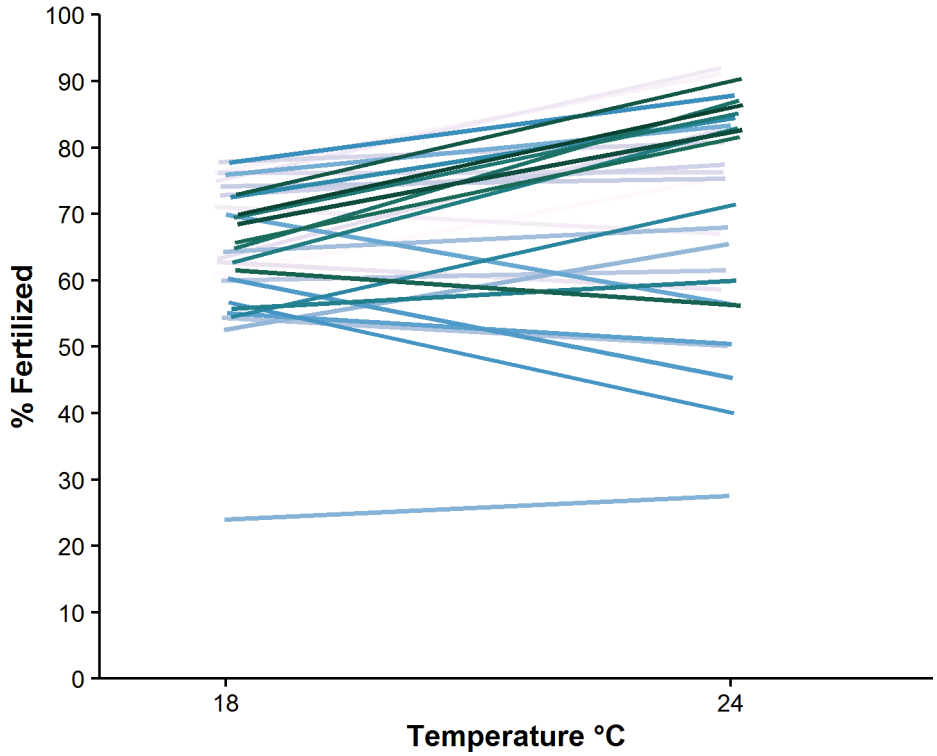

Fig S3. Male-by-temperature interaction plots on fertilization rates across each temperature. Thus, each line is a different sire family (n = 32), represented by different shades for clarity.

References

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