

Effect of Acute Seawater Temperature Increase on the Survival of a Fish Ectoparasite

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Supplementary Material

Tests of Cox model assumption of proportionality

We tested the Cox model assumption of proportionality using the Global test statistic in the function “coxph” and “cox.zph” in the package “coxme” and graphically using a smoothed spline plot of the Schoenfeld residuals relative to time. For the Great Barrier Reef data, while the Global test statistic was significant for the unfed gnathiids ($P < 0.0001$, Table S3), only one covariate showed some evidence of proportionality ($P = 0.0154$) and furthermore its Pearson's product-moment correlation coefficient (ρ) was relatively low (Table S3); more importantly, the slope of the scaled Schoenfeld residuals versus time plot for unfed gnathiids was nearly zero and straight indicating the assumption of proportional hazards was indeed met (Figure S4) (Therneau and Grambsch 2000). Furthermore, as “significant” proportionality tends to make no difference to the interpretation of the data for large samples sizes (Therneau and Grambsch 2000), and since the sample size of unfed gnathiids was relatively large ($n = 1113$), this suggests that our interpretation of these data is robust. The Global test statistic was not significant for the fed gnathiid model ($P = 0.979$, Table S4), and this was also supported by the Schoenfeld residuals plot (Figure S5).

Tables

Table S1. Philippines; Average water temperature of aquaria for five treatments over three aquarium replicates for (a) Trial 1 and (b) Trial 2.

(a)	Treatment	Mean $\pm$ SE	(b)	Treatment	Mean $\pm$ SE
	28°C	28.1°C $\pm$ 0.09		30°C	29.9°C $\pm$ 0.04
	30°C	30.1°C $\pm$ 0.03		32°C	31.8°C $\pm$ 0.11
	32°C	31.7°C $\pm$ 0.12		33°C	32.8°C $\pm$ 0.04
	34°C	33.7°C $\pm$ 0.09		34°C	33.9°C $\pm$ 0.08
	36°C	35.9°C $\pm$ 0.13		35°C	34.9°C $\pm$ 0.07

Table S2. Philippines; Sample size of larval gnathiid isopods < 2 mm and >2 mm in length in five different temperature treatments over three aquarium replicates for (a) Trial 1 and (b) Trial 2.

(a)	Treatment		Gnathiids <2mm n per treatment		Gnathiids >2mm n per treatment		All gnathiids n per treatment	
	28°C		36		40		76	
	30°C		39		40		79	
	32°C		33		40		73	
	34°C		30		40		70	
	36°C		30		40		70	
	Total		168		201		369	

(b)

Treatment	Gnathiids <2mm n per treatment	Gnathiids >2mm n per treatment	All gnathiids n per treatment
30°C	31	33	64
32°C	31	32	63
33°C	27	32	59
34°C	32	36	68
35°C	32	31	63
Total	153	165	318

Table S3. Great Barrier Reef; Tests of proportionality, using function “cox.zph” in library “coxme” in R 3.2.5, for full model for unfed gnathiid survival among temperature treatments and juvenile stages. Bolded values are ones mentioned in main text.

	rho	chisq	p
Temperature 31C	-0.01616	0.294	5.88E-01
Temperature 32C	0.000973	0.0011	9.74E-01
Headwidth	-0.00992	0.1187	7.30E-01
Stage 2	-0.01199	0.1483	7.00E-01
Stage 3	-0.03169	1.2621	2.61E-01
Temperature 31C x Headwidth	0.011911	0.1595	6.90E-01
Temperature 32C x Headwidth	-0.00408	0.0193	8.90E-01
Temperature 31C x Stage 2	0.065325	5.8432	1.56E-02
Temperature 32C x Stage 2	0.03805	1.8805	1.70E-01
Temperature 31C x Stage 3	0.018777	0.4265	5.14E-01
Temperature 32C x Stage 3	-0.00878	0.0898	7.64E-01
Headwidth x Stage 2	0.012901	0.1737	6.77E-01
Headwidth x Stage 3	0.029838	1.1113	2.92E-01
Temperature 31C x Headwidth x Stage 2	-0.06579	5.87	1.54E-02
Temperature 32C x Headwidth x Stage 2	-0.03813	1.8722	1.71E-01
Temperature 31C x Headwidth x Stage 3	-0.01961	0.4583	4.98E-01
Temperature 32C x Headwidth x Stage 3	0.003162	0.0116	9.14E-01
GLOBAL	NA	73.8811	< 0.0001

Table S4. Great Barrier Reef; Tests of proportionality, using function “cox.zph” in library “coxme”, for full model of fed gnathiid survival among temperature treatments and juvenile stages. Bolded values are ones mentioned in main text.

	rho	chisq	p
Temperature 31C	0.027477	6.60E-02	0.797
Temperature 32C	0.037964	7.90E-02	0.779
Headwidth	0.0568	1.54E-01	0.694
Stage 2	0.000286	5.09E-06	0.998
Stage 3	0.02118	3.81E-02	0.845
Temperature 31C x Headwidth	-0.02383	4.90E-02	0.825
Temperature 32C x Headwidth	-0.03168	5.24E-02	0.819
Temperature 31C x Stage 2	0.056878	2.45E-01	0.62
Temperature 33C x Stage 2	0.085775	9.70E-01	0.325
Temperature 31C x Stage 3	-0.06044	3.03E-01	0.582
Temperature 32C x Stage 3	-0.01034	8.76E-03	0.925
Headwidth x Stage 2	0.000233	3.27E-06	0.999
Headwidth x Stage 3	-0.02234	4.02E-02	0.841
Temperature 31C x Headwidth x Stage 2	-0.05241	2.11E-01	0.646
Temperature 32C x Headwidth x Stage 2	-0.08808	9.66E-01	0.326
Temperature 31C x Headwidth x Stage 3	0.054199	2.45E-01	0.62
Temperature 32C x Headwidth x Stage 3	0.010703	8.85E-03	0.925
GLOBAL	NA	7.33E+00	0.979

Table S5. Philippines Tests of proportionality, using function “cox.zph” in library “coxme” in R 3.2.5, for full model for Trial 1 gnathiid survival among temperature treatments. Bolded values are ones mentioned in main text.

	rho	chisq	p
Temperature 30C	-0.0136	0.0630	8.02E-01
Temperature 32C	0.000973	0.0011	7.56E-02
Temperature 34C	-0.00992	0.1187	2.14E-01
Temperature 36C	-0.01199	0.1483	7.86E-01
GLOBAL	NA	9.3134	5.37E-02

Table S6. Philippines; Tests of proportionality, using function “cox.zph” in library “coxme” in R 3.2.5, for full model for Trial 2 gnathiid survival among temperature treatments. Bolded values are ones mentioned in main text.

	rho	chisq	p
Temperature 32C	0.0786	1.7556	0.185
Temperature 33C	0.0422	0.5065	0.477
Temperature 34C	-0.0183	0.0945	0.759
Temperature 35C	0.0258	0.1902	0.663
GLOBAL	NA	3.4151	0.491

Table S7. Great Barrier Reef; Summary output for full model for unfed gnathiid survival among temperature treatments and juvenile stages for Cox model. Bolded values are ones mentioned in main text.

	coef	exp(coef)	se(coef)	z	p
Temperature 31C	1.781933	5.94E+00	1.230345	1.45	0.15
Temperature 32C	2.944345	1.90E+01	1.220448	2.41	0.016
Headwidth	-4.62464	9.81E-03	5.382691	-0.86	0.39
Stage 2	-2.32931	9.74E-02	2.567027	-0.91	0.36
Stage 3	-2.11335	1.21E-01	2.359538	-0.9	0.37
Temperature 31C x Headwidth	-7.58628	5.07E-04	7.1727	-1.06	0.29
Temperature 32C x Headwidth	-11.9827	6.25E-06	7.050043	-1.7	0.089
Temperature 31C x Stage 2	-2.91421	5.42E-02	4.328255	-0.67	0.5
Temperature 32C x Stage 2	-6.77908	1.14E-03	4.038398	-1.68	0.093
Temperature 31C x Stage 3	-2.97919	5.08E-02	3.000398	-0.99	0.32
Temperature 32C x Stage 3	-5.37649	4.62E-03	3.021687	-1.78	0.075
Headwidth x Stage 2	7.283235	1.46E+03	12.12401	0.6	0.55
Headwidth x Stage 3	6.320849	5.56E+02	9.547878	0.66	0.51
Temperature 31C x Headwidth x Stage 2	16.38334	1.30E+07	20.15396	0.81	0.42
Temperature 32C x Headwidth x Stage 2	34.94723	1.50E+15	18.71507	1.87	0.062
Temperature 31C x Headwidth x Stage 3	14.09394	1.32E+06	12.25296	1.15	0.25
Temperature 32C x Headwidth x Stage 3	25.74954	1.52E+11	12.29105	2.09	0.036

Table S8. Great Barrier Reef; Summary output for full model for fed gnathiid survival among temperature treatments and juvenile stages for Cox model.

	coef	exp(coef)	se(coef)	z	p
Temperature 31C	17.93922	6.18E+07	8.165565	2.2	0.028
Temperature 32C	19.75536	3.80E+08	6.986636	2.83	0.0047
Headwidth	27.04453	5.56E+11	30.25091	0.89	0.37
Stage 2	-1.34936	2.59E-01	8.579537	-0.16	0.88
Stage 3	-12.3012	4.55E-06	14.78514	-0.83	0.41
Temperature 31C x Headwidth	-89.2034	1.82E-39	44.54095	-2	0.045
Temperature 32C x Headwidth	-95.1268	4.86E-42	37.8657	-2.51	0.012
Temperature 31C x Stage 2	-15.8059	1.37E-07	12.28354	-1.29	0.2
Temperature 32C x Stage 2	-17.6102	2.25E-08	13.18383	-1.34	0.18
Temperature 31C x Stage 3	-2.82112	5.95E-02	17.55895	-0.16	0.87
Temperature 32C x Stage 3	7.876065	2.63E+03	16.61263	0.47	0.64
Headwidth x Stage 2	-10.692	2.27E-05	42.65298	-0.25	0.8
Headwidth x Stage 3	22.35396	5.11E+09	58.57617	0.38	0.7
Temperature 31C x Headwidth x Stage 2	90.52706	2.07E+39	61.27706	1.48	0.14
Temperature 32C x Headwidth x Stage 2	95.24606	2.32E+41	64.17521	1.48	0.14
Temperature 31C x Headwidth x Stage 3	41.22042	7.98E+17	72.55342	0.57	0.57
Temperature 32C x Headwidth x Stage 3	9.002369	8.12E+03	66.85154	0.13	0.89

Table S9. Great Barrier Reef; Analysis of (i) deviance tables (Type II tests) and (ii) summary outputs for unfed gnathiid survival for separate Cox models for each juvenile stage. These were used to interpret a significant interaction between headwidth and juvenile stage in the full model. Tests of proportionality were non-significant for stage one ($P = 0.0566$) and significant for stage two ($P = 0.0141$) and three ($P = 0.0020$). However, slopes of the scaled Schoenfeld residuals versus time plots were nearly zero and straight (plots not provided) indicating the assumption of proportional hazards was indeed met (Therneau and Grambsch 2000). Bolded values are ones mentioned in main text. * $P < 0.05$, *** $P < 0.001$.

(a) Stage 1

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	87.75	2.2e-16	***
	Headwidth	1	22.33	2.294e-06	***
	Temperature x Headwidth	2	4.49	0.1058	

(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	1.933090	6.91e+00	1.229055	1.57	0.1200
	Temperature 32C	3.575502	3.57e+01	1.222040	2.93	0.0034
	Headwidth	3.575502	7.75e-03	5.413998	-0.90	0.3700
	Temperature 31C x Headwidth	8.196986	2.75e-04	7.165240	-1.14	0.2500
	Temperature 32C x Headwidth	-14.930648	3.28e-07	7.050043	-2.12	0.0340

(b) Stage 2

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	37.1323	8.646e-09	***
	Headwidth	1	1.2710	0.2596	
	Temperature x Headwidth	2	0.9916	0.6091	

(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	-0.3781194	6.85e-01	4.146366	-0.09	0.93
	Temperature 32C	-2.5148027	8.09e-02	3.884596	-0.65	0.52
	Headwidth	1.8988111	6.68e+00	10.948025	0.17	0.86
	Temperature 31C x Headwidth	5.6178545	2.75e+02	18.807654	0.30	0.77
	Temperature 32C x Headwidth	17.3571173	3.45e+07	17.471996	0.99	0.32

(c) Stage 3

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	51.0717	8.127e-12	***
	Headwidth	1	4.0200	0.04496	*

Temperature x Headwidth	2	0.8324	0.65954
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(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	-0.7595495	4.69e-01	2.779882	-0.27	0.78
	Temperature 32C	-1.3731740	2.53e-01	2.868779	-0.48	0.63
	Headwidth	2.5812093	1.32e+01	8.060813	0.32	0.75
	Temperature 31C x Headwidth	4.9717164	1.44e+02	10.085983	0.49	0.62
	Temperature 32C x Headwidth	9.4902545	1.32e+04	10.446274	0.91	0.36

Table S10. Great Barrier Reef; Analysis of (i) deviance tables (Type II tests) and (ii) summary outputs for fed gnathiid survival for separate Cox models for each juvenile stage. These were used to interpret the significant interaction between temperature and juvenile stage in the full model. Tests of proportionality were all non-significant (all $P \geq 0.28$). Bolded values are ones mentioned in main text. * $P < 0.05$, *** $P < 0.001$.

(a) Stage 1

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	3.8300	0.1473	
	Headwidth	1	3.9779	0.0461	*
	Temperature x Headwidth	2	3.8382	0.1467	

(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	14.34852	1.70e+06	8.479400	1.69	0.091
	Temperature 32C	15.73310	6.80e+06	7.450357	2.11	0.035
	Headwidth	21.10865	1.47e+09	33.535586	0.63	0.530
	Temperature 31C x Headwidth	-72.42310	3.52e-32	45.936027	-1.58	0.110
	Temperature 32C x Headwidth	-77.35271	2.55e-34	40.079802	-1.93	0.054

(b) Stage 2

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	24.7229	< 0.0001	***
	Headwidth	1	0.1653	0.6843	
	Temperature x Headwidth	2	0.1746	0.9164	

(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	-1.3151315	2.68e-01	9.112425	-0.14	0.89
	Temperature 32C	1.5224684	1.52	11.670059	0.13	0.90
	Headwidth	0.6278073	1.87e+00	29.431537	0.02	0.98
	Temperature 31C x Headwidth	16.6429892	1.69e+07	41.847233	0.40	0.69
	Temperature 32C x Headwidth	2.0085683	7.45e+00	53.933735	0.04	0.97

(c) Stage 3

(i)		Df	Chisq	Pr(>Chisq)	
	Temperature	2	14.0035	0.0009103	***
	Headwidth	1	0.5840	0.4447629	
	Temperature x Headwidth	2	4.0336	0.1330806	

(ii)		coef	exp(coef)	se(coef)	z	p
	Temperature 31C	23.88180	2.35e+10	19.73292	1.21	0.230
	Temperature 32C	40.23727	2.98e+17	20.06580	2.01	0.045
	Headwidth	80.75554	1.18e+35	66.58529	1.21	0.230
	Temperature 31C x Headwidth	-77.72307	1.76e-34	71.80455	-1.08	0.280
	Temperature 32C x Headwidth	-130.10014	3.15e-57	72.18782	-1.80	0.072

Table S11. Great Barrier Reef; Analysis of (i) deviance tables (Type II tests) and (ii) summary outputs for fed gnathiid survival for separate Cox models for each temperature treatment. These were used to interpret the significant interaction between temperature and headwidth in the full model. Tests of proportionality were all non-significant (all $P \geq 0.55$). Bolded values are ones mentioned in main text. *** $P < 0.001$.

(a) 29°C temperature

(i)		Df	Chisq	Pr(>Chisq)	
	Stage	2	15.4751	0.0004361	***
	Headwidth	1	3.1519	0.0758383	
	Stage x Headwidth	2	0.9823	0.6119294	

(ii)		coef	exp(coef)	se(coef)	z	p
	Stage 2	3.857200	4.73e+01	9.52347	0.41	0.690
	Stage 3	-7.345043	6.46e-04	15.18176	-0.48	0.630
	Headwidth	63.697990	4.61e+27	38.40159	1.66	0.097
	Stage2 x Headwidth	-45.726213	1.38e-20	49.29586	-0.93	0.350
	Stage3 x Headwidth	-12.255316	4.76e-06	62.90875	-1.19	0.850

(b) 31°C temperature

(i)		Df	Chisq	Pr(>Chisq)	
	Stage	2	3.9569	0.1383	
	Headwidth	1	0.4232	0.5154	
	Stage x Headwidth	2	3.4687	0.1765	

(ii)		coef	exp(coef)	se(coef)	z	p
	Stage 2	-19.92332	2.23e-09	9.866755	-2.02	0.043
	Stage 3	-13.97628	8.51e-07	11.312943	-1.24	0.220
	Headwidth	-70.39055	2.69e-31	38.688269	-1.82	0.069

Stage2 x Headwidth	92.09379	9.90e+39	49.499703	1.86	0.063
Stage3 x Headwidth	61.85845	7.32e+26	50.581288	1.22	0.220

(c) 32°C temperature

(i)		Df	Chisq	Pr(>Chisq)	
	Stage	2	10.8339	0.004441	***
	Headwidth	1	3.2423	0.071760	
	Stage x Headwidth	2	4.5000	0.105399	

(ii)		coef	exp(coef)	se(coef)	z	p
	Stage 2	-26.56103	2.92e-12	12.09114	-2.20	0.028
	Stage 3	-6.15366	2.137e-03	10.85020	-0.57	0.570
	Headwidth	-85.55179	7.00e-38	43.89213	-1.95	0.051
	Stage2 x Headwidth	119.06989	5.15e+51	58.77401	2.03	0.043
	Stage3 x Headwidth	40.00691	2.37e+17	50.18167	0.80	0.430

Table S12. Philippines; Summary output for full model for Trial 1 gnathiid survival among temperature treatments for Cox model. Bolded values are ones mentioned in main text.

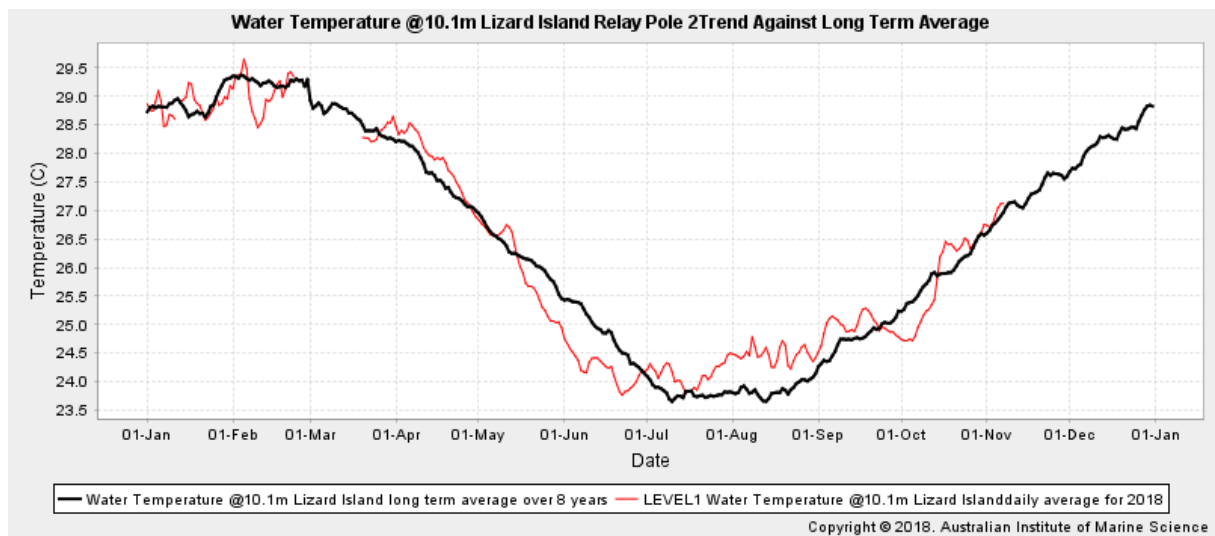
	coef	exp(coef)	se(coef)	z	p
Temperature 30C	1.235503	1.131507	0.1743966	0.71	0.48
Temperature 32C	2.944345	1.508107	0.1818816	2.26	0.024
Temperature 34C	-4.62464	1.395315	0.1810765	1.84	0.066
Temperature 36C	-2.32931	2.463726	0.1978712	4.56	0.000052

Table S13. Philippines; Summary output for full model for Trial 2 gnathiid survival among temperature treatments for Cox model. Bolded values are ones mentioned in main text.

	coef	exp(coef)	se(coef)	z	p
Temperature 32C	0.3633410	1.438126	0.1989315	1.83	0.0680
Temperature 33C	0.2831514	1.327306	0.1973084	1.44	0.1500
Temperature 34C	0.4242918	1.528508	0.1922528	2.21	0.0270
Temperature 35C	0.5511882	1.735314	0.1993638	2.76	0.0057

Supplementary figures

(a)



(b)

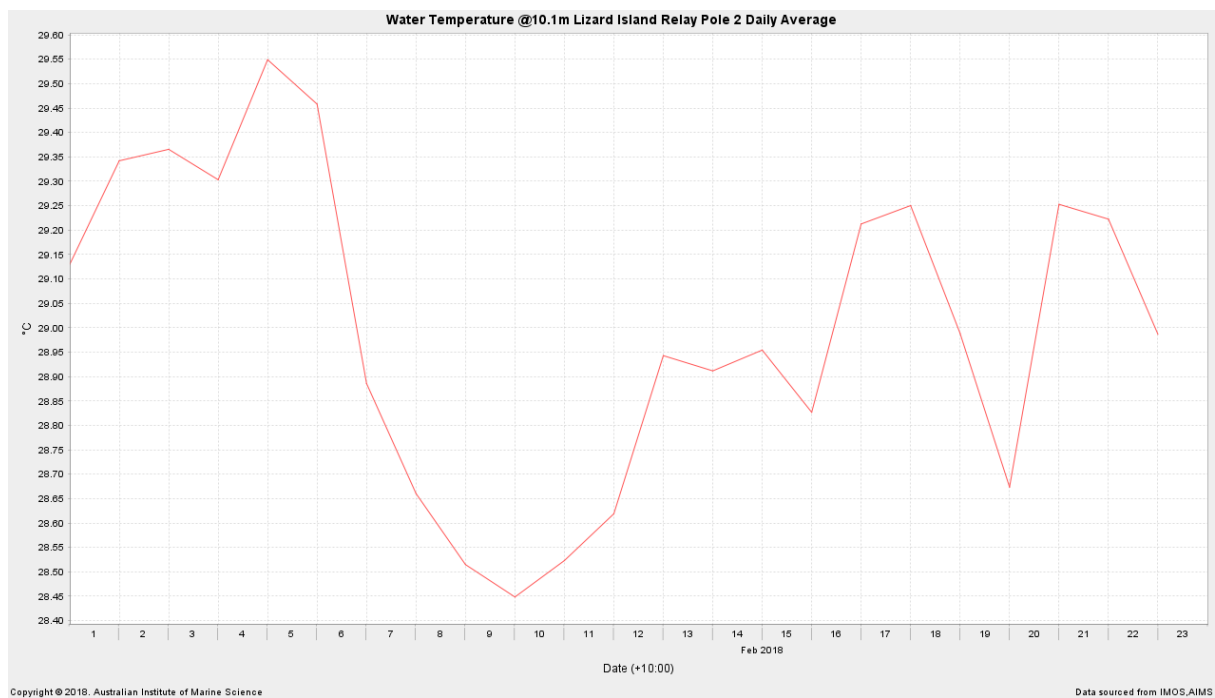


Figure S1. Seawater temperature for Great Barrier Reef data. a) Average temperatures in 2018 against long term average water temperatures (8 years). b) Actual daily temperatures for February 2018 (only February 1 to 23 was available). Source: Australian Institute of Marine Science. Accessed 13.11.2018 <http://data.aims.gov.au/aimsrtids/yearlytrends.xhtml>.

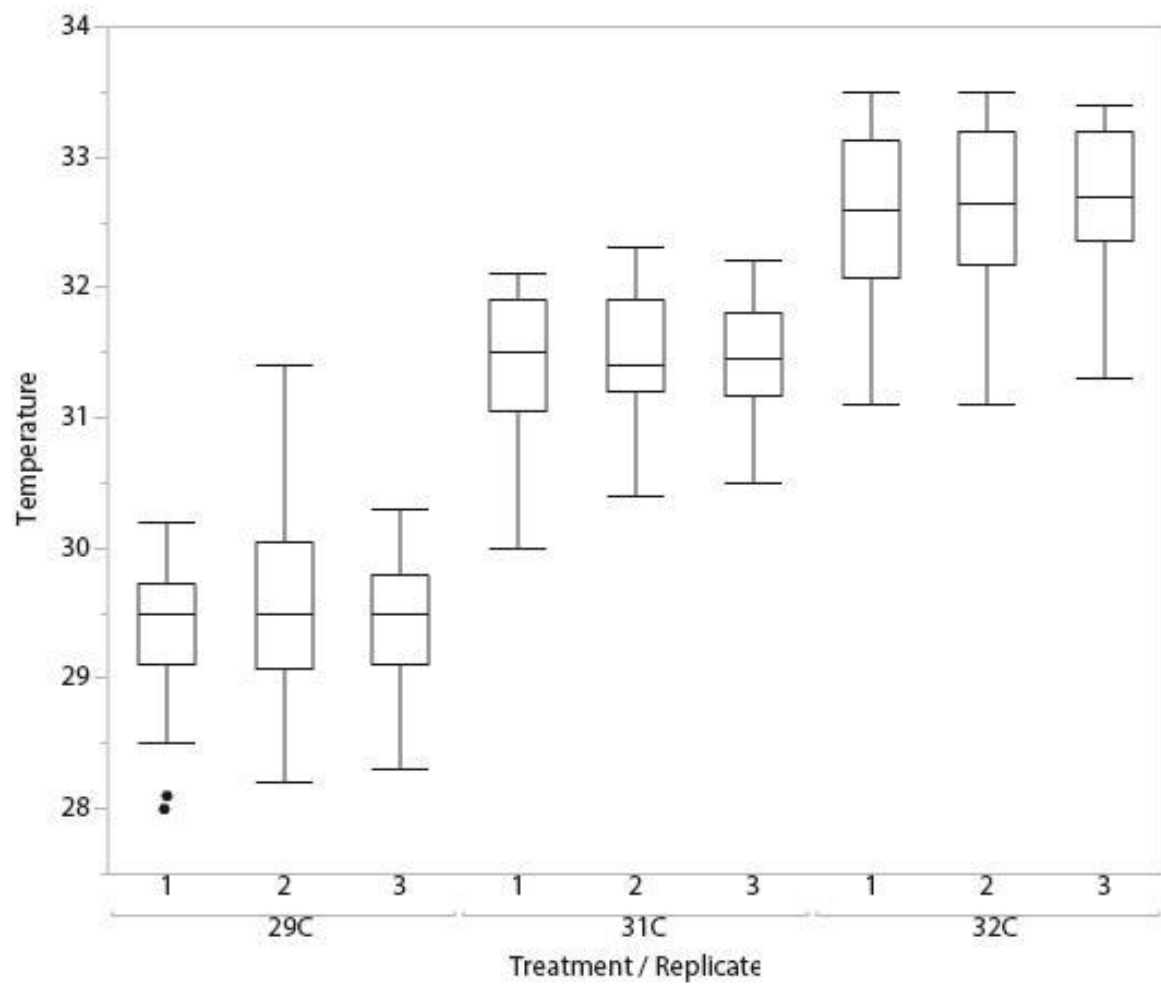
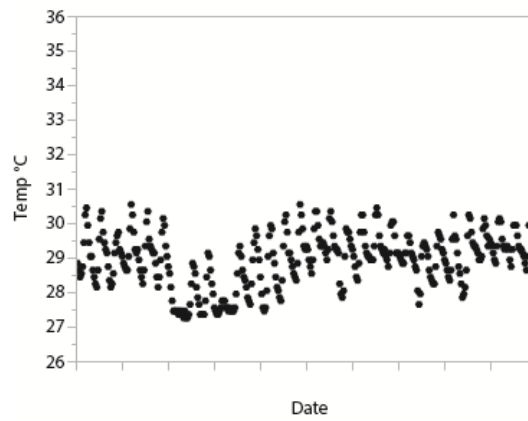
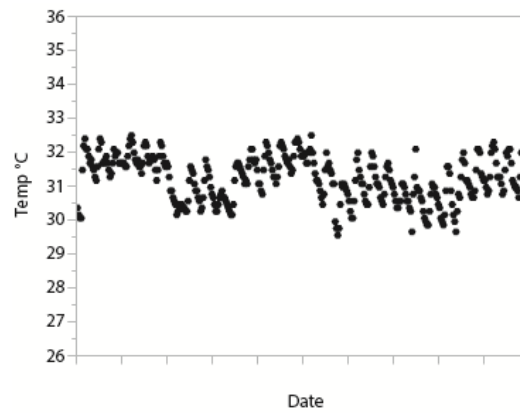


Figure S2. Great Barrier Reef; Temperatures, measured using a handheld device at 12:00 h, for three replicate aquaria per temperature treatment.

(a) 29°C



(b) 31°C



(c) 32°C

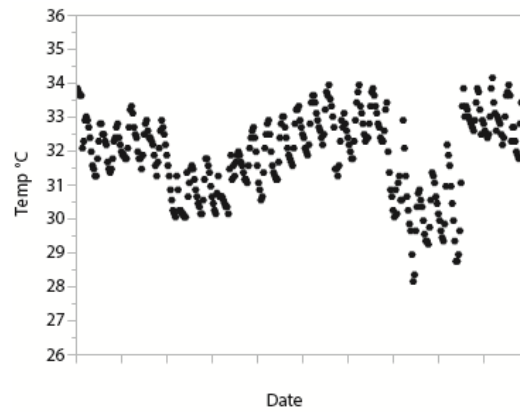


Figure S3. Great Barrier Reef; Water temperatures in an aquarium over duration of study for each of the temperature treatments between February 1 and March 2 2018. Temperatures were measured using HOBO Pendant temperature/light loggers, UA-002-08. Loggers were calibrated against the temperature probe which was calibrated to 26°C, 28°C and 30°C and NATA (National Association of Testing Authorities, Australia) certified.

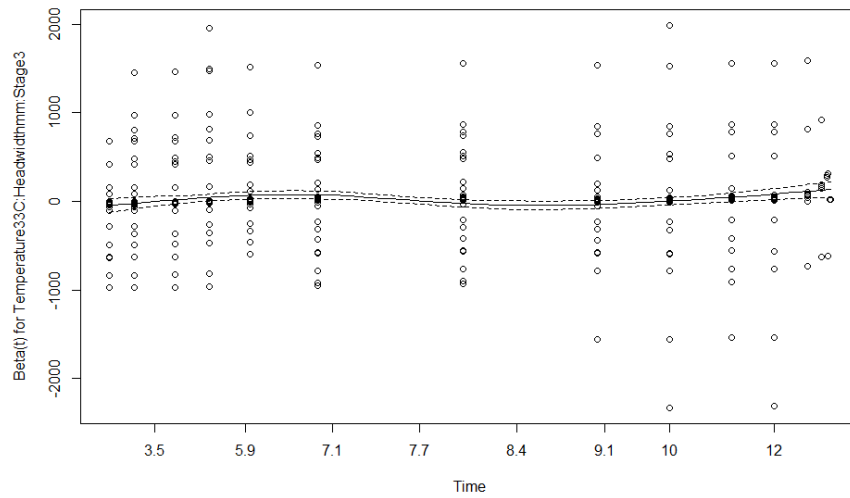


Figure S4. Great Barrier Reef; Scaled Schoenfeld residual plot for full model testing unfed gnathiid survival relative to time (days), obtained using the function “cox.zph”, transform = “km”. Smoothed spline with 90% confidence intervals.

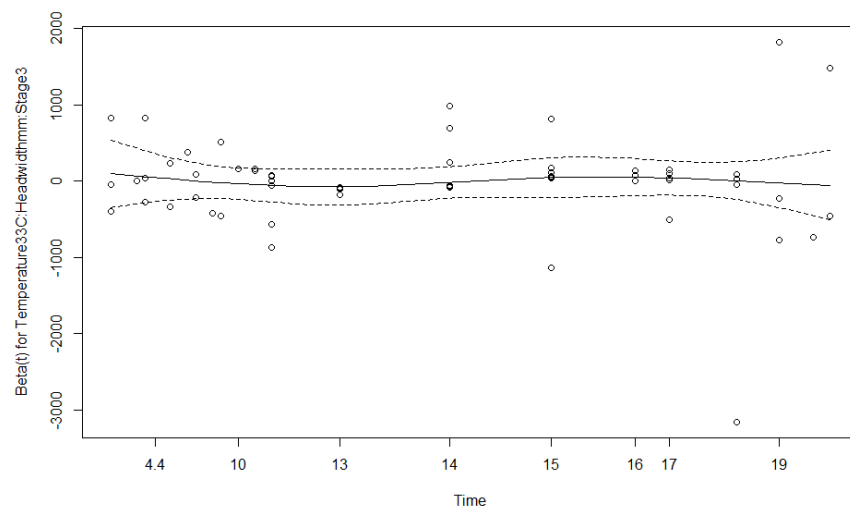


Figure S5. Great Barrier Reef; Scaled Schoenfeld residual plot for full model testing fed gnathiid survival relative to time (days), obtained using the function “cox.zph”, transform = “km”. Smoothed spline with 90% confidence intervals.

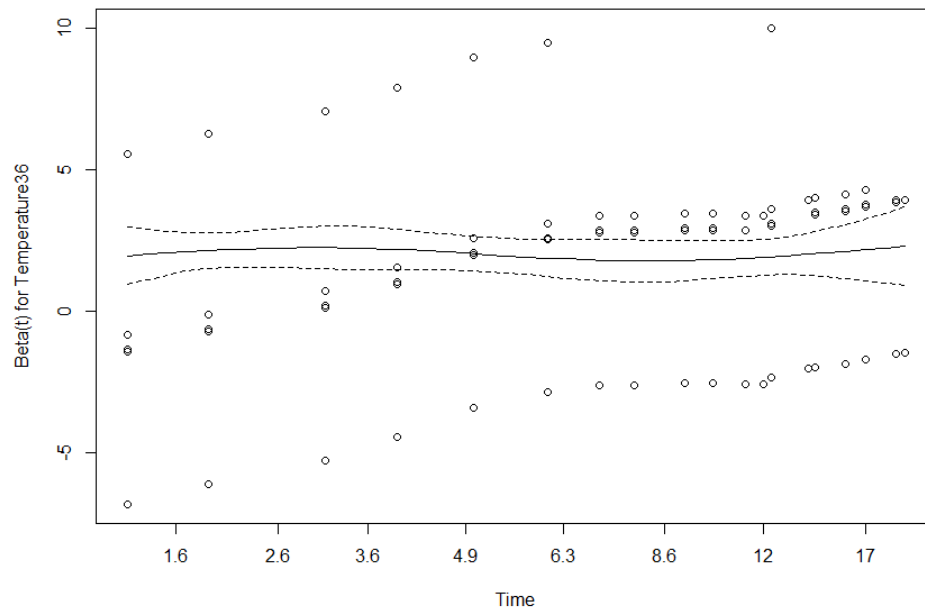


Figure S6. Philippines; Scaled Schoenfeld residual plot for full model testing gnathiid survival relative to time (days), obtained using the function “cox.zph”, transform = “km”. Smoothed spline with 90% confidence intervals for Trial 1

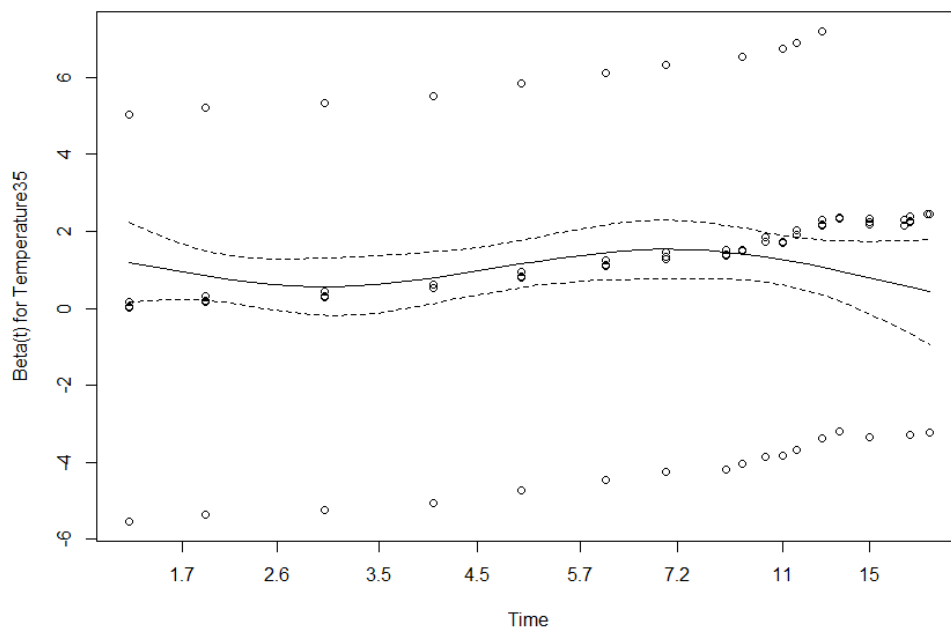


Figure S7. Philippines; Scaled Schoenfeld residual plot for full model testing gnathiid survival relative to time (days), obtained using the function “cox.zph”, transform = “km”. Smoothed spline with 90% confidence intervals for Trial 2.