

**Electronic Supplementary Material for:**  
**Untangling the roles of microclimate, behavior and physiological polymorphism**  
**in governing vulnerability of intertidal snails to heat stress**

Short title: Drivers of vulnerability to heat stress

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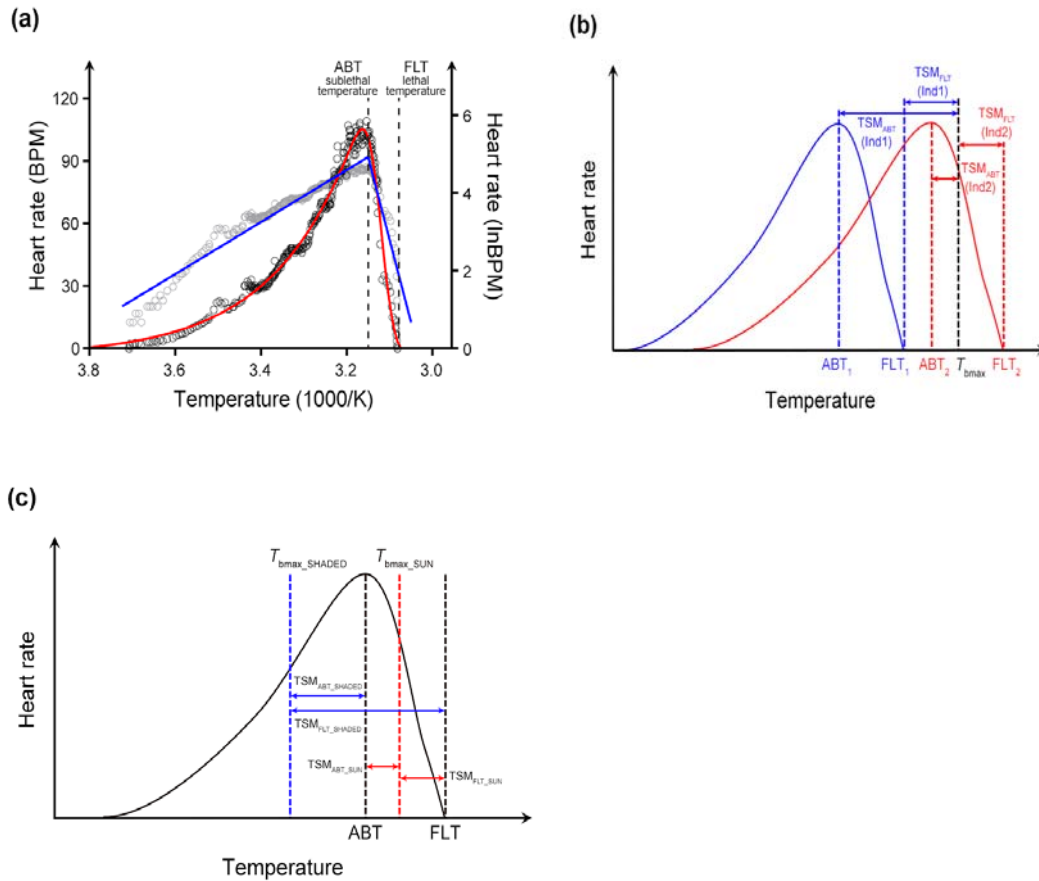
Supplementary information about Heat budget model in

## **2. Materials and methods**

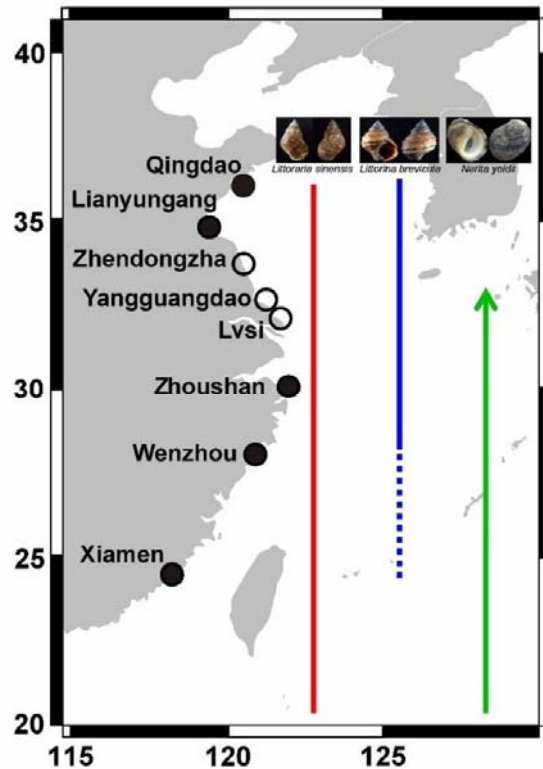
### **(b) Operative temperature estimations**

The heat budget model is based on a steady state equation of heat transfer presented first by Helmuth 1998 and modified by Marshall et al. (2015) for intertidal snails. The model uses hourly measurements of short-wave solar radiation, wind speed and air temperature to estimate heating from solar radiation, and heat loss via convection and infrared radiation. Environmental data were obtained from the National Centers for Environmental Prediction Forecast System Reanalysis (CFSR) at a resolution of  $0.5^\circ \times 0.5^\circ$  for the years 1983-2013. Wind speed reported by CFSR was converted to near-substratum conditions using boundary layer theory (see Helmuth 1999). Calculations of projected and total surface area of the three snail species was estimated by treating animals as spheres. The body lengths used for each of the species are 10mm for *Littoraria sinensis*, 12mm for *Nerita yoldii* and 18mm for *Littorina brevicula*. As a simplifying assumption, rock temperature was assumed to be similar to air temperature and thus serves as a heat sink under extreme conditions when body temperature exceeds air temperature. Estimates of snail temperature in the sun are thus likely underestimates, as rock surface temperature frequently is similar to animal temperature for animals such as snails with a high proportion of surface area in contact with the rock

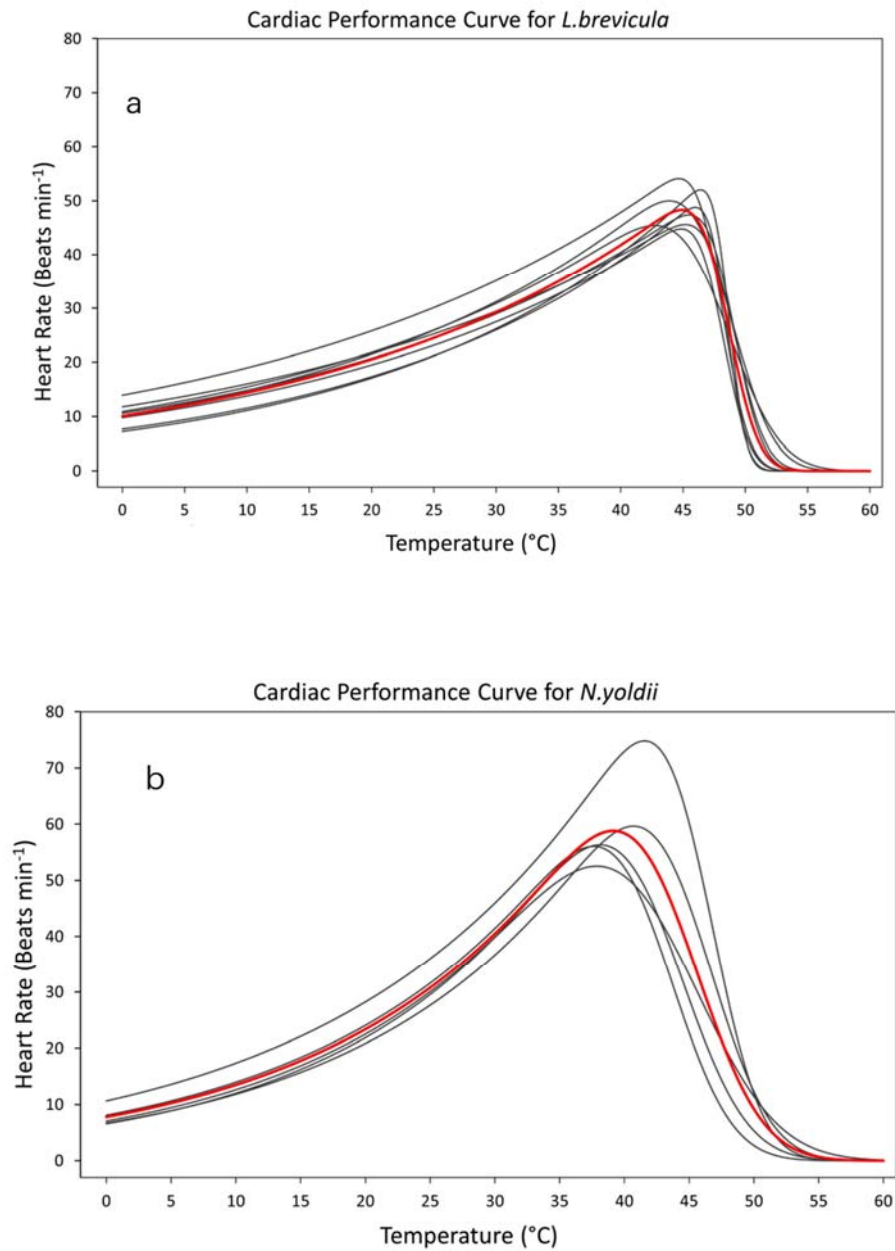
## Supporting Information



**Figure S1 Relationship between cardiac performance (ABT, FLT) and TSM.** (a) Heart rate increases with temperature until the Arrhenius Breakpoint Temperature (ABT) is reached and then decreases rapidly to zero (black circles and the red curve following left y-axis). ABT was calculated by linear regression (blue lines) using data on Arrhenius plots (gray circles following the right y-axis) that lay either above or below the temperature at which the highest heart rate occurred. The temperature at which these two lines intersected was taken as the ABT. FLT is the temperature where heart beat ceases. TSM is defined as the difference between an organism's critical thermal maximum (ABT (nonlethal) or FLT (lethal)) and the highest body temperature that an organism is likely to experience in nature ( $TSM_{ABT} = ABT - T_{bmax}$ ,  $TSM_{FLT} = FLT - T_{bmax}$ ). Due to the physiological polymorphism and microhabitat heterogeneity, TSM varies among (b) individuals and (c) microhabitats.



**Figure S2 Geographic distributions of the three gastropod species, *Littoraria sinensis*, *Littorina brevicula* and *Nerita yoldii* along the Chinese coastline.** Circles indicate sampling sites. Open circles indicate artificial rocky shores and solid circles indicate natural rocky shores. *Littoraria sinensis* (red line) is widely distributed along the Chinese coastline, *L. brevicula* (blue line) is mainly distributed to the north of Wenzhou, although it appears seasonally in the winter and spring further south (dashed blue line) and *Nerita yoldii* (green line) was formerly recorded south of Zhoushan but the species' range is now expanding northward. *L. sinensis* and *L. brevicula* were collected from eight sites, Qingdao (36.05°N, 120.35° E, QD), Lianyungang (34.76° N, 119.37° E, LYG), Zhendongzha (34.20° N, 120.30° E, ZDZ), Yangguangdao (32.52° N, 121.42° E, YGD), Lvsi (32.11° N, 121.59° E, LS), Zhoushan (29.92° N, 122.41° E, ZS), Wenzhou (27.86° N, 121.18° E, WZ), Xiamen (24.43° N, 118.15° E, XM), whereas *N. yoldii* were only present at five sites, namely YGD, LS, ZS, WZ and XM. Animals were transported back to the State Key Laboratory of Marine Environmental Science, Xiamen University, within 24 h. Individuals were randomly allocated into plastic containers (L × W × H: 30 cm × 17 cm × 25 cm) with a density of ~1.0 individual / cm<sup>2</sup> and acclimated at 16°C in common garden conditions for 60 to 282 days. Animals were daily sprayed for 1 h with fresh seawater (=low tide) but otherwise remained emersed (high tide). Snails were fed on natural biofilm (bacteria, diatoms, cyanobacteria and other algae), cultured on plastic plates (20 cm × 20 cm) for two weeks before being presented to the snails.



**Figure S3 The highly variable cardiac performance curves for different populations of (a) *Littorina brevicula* and (b) *Nerita yoldii*.** Heart rate curves were generated using exponentially modified Gaussian model (EMG) when individuals were exposed to increasing temperature at a rate of 6°C h<sup>-1</sup> from 16°C to the temperature at which the heartbeat ceased (FLT). Each black line depicts heart rate curves generated for all individuals in each population and the red lines depict the heart rate curves generated for all the individuals of a species.

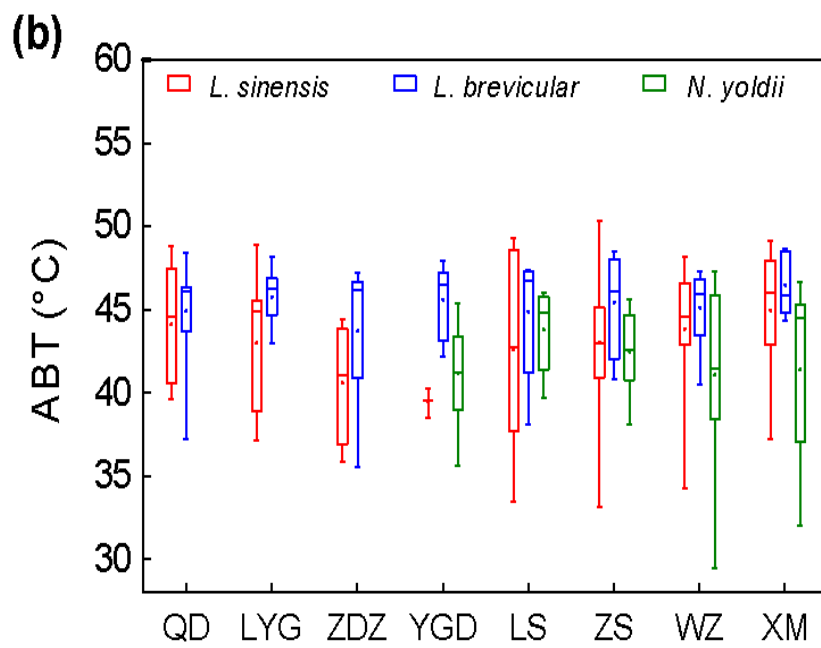
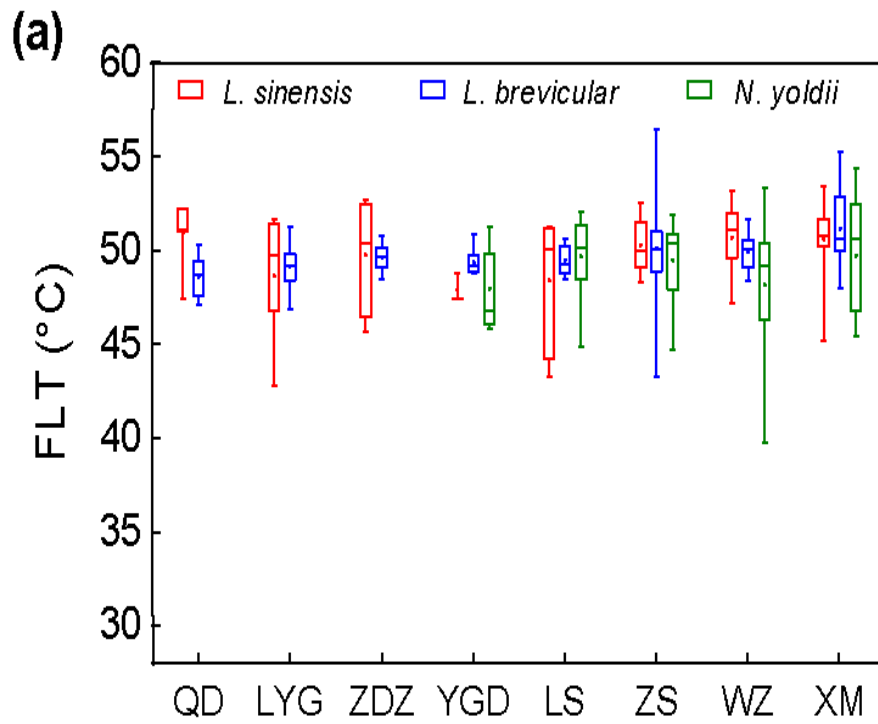
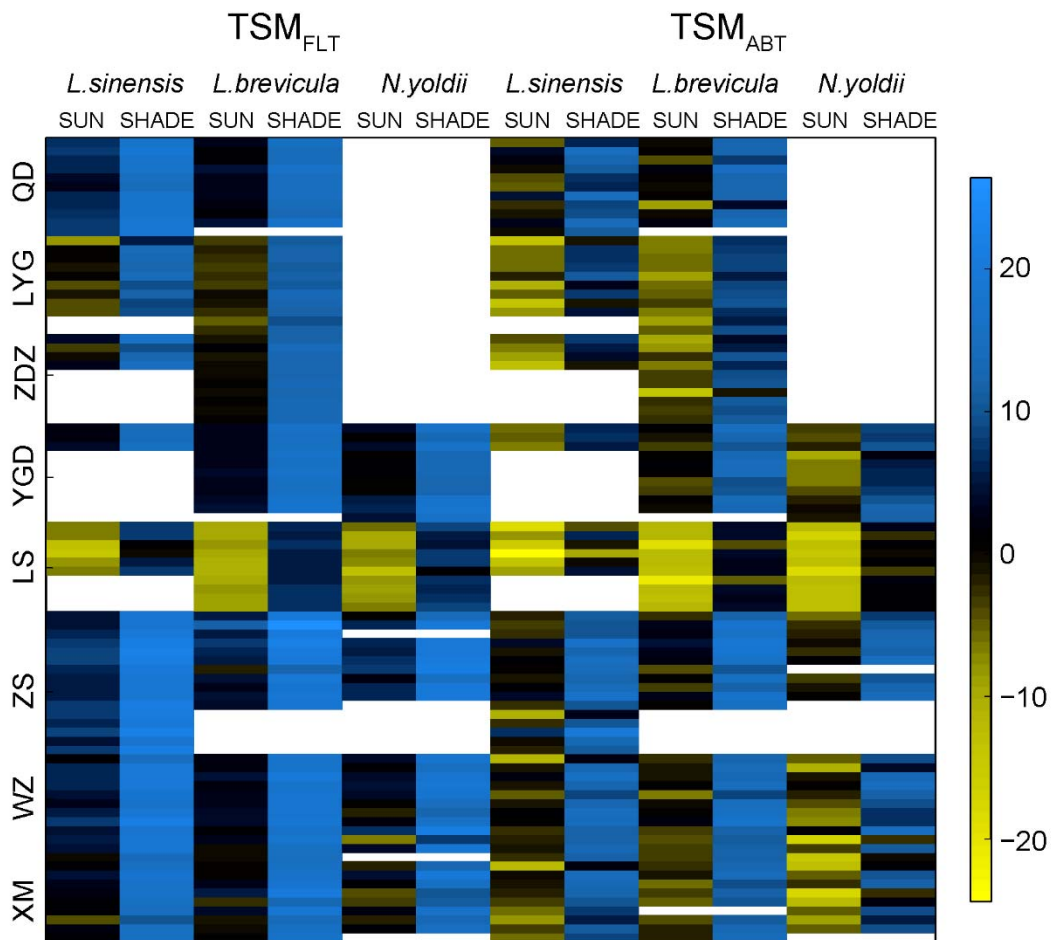


Figure S4 (a) Flat Line temperature (FLT) and (b) Arrhenius breakpoint temperature (ABT) of different populations of *Littoraria sinensis*, *Littorina brevicular*, *Nerita yoldii*.



**Figure S5.** Heat map of thermal safety margin (TSM) of *Littoraria sinensis*, *Littorina brevicula*, *Nerita yoldii*. The color scale bar indicates TSM levels, defined as the difference between an organism’s critical thermal maximum (FLT and ABT) and the highest temperature that an organism is likely to experience in nature. With TSM values  $>0$  indicating organism’s critical thermal maximum exceed the highest operative temperature, 0 indicating no TSM, and values  $<0$  indicating the highest operative temperatures exceed the critical thermal maximum.

**Table S1. Deviation of temperature functions of heart rate,  $f(T)$ , of populations from single individuals.** F-test of generalized additive models (GAM) of heart rate to temperature between individuals and populations were generated to describe the individual differences.

	<i>L. sinensis</i>			<i>L. brevicula</i>			<i>N. yoldii</i>		
	d.f.	<i>F</i> -value	<i>P</i> -value	d.f.	<i>F</i> -value	<i>P</i> -value	d.f.	<i>F</i> -value	<i>P</i> -value
$f(T)$ for all individuals in QD	16.62	97.63	< 2E-16	12.26	87.03	< 2E-16			
Deviation from $f(T)$ for single individual in QD	9.0927	4.2046	1.89E-05	18.762	20.921	< 2.20E-16			
	11.452	17.504	< 2.20E-16	9.7395	4.5401	2.71E-06			
	8.6788	71.257	< 2.20E-16	8.5522	9.6305	5.19E-14			
	4.7941	36.474	< 2.20E-16	10.457	18.908	< 2.20E-16			
	3.5893	30.309	< 2.20E-16	18.982	4.6941	7.29E-11			
	6.4792	11.258	3.07E-13	13.064	8.7085	< 2.20E-16			
	7.1404	5.6362	1.47E-06	11.727	74.26	< 2.20E-16			
	3.6637	19.337	1.41E-14	18.581	8.8663	< 2.20E-16			
	7.5153	14.267	< 2.20E-16	11.252	45.568	< 2.20E-16			
	14.172	98.541	< 2.20E-16	19.653	77.206	< 2.20E-16			
12.913	48.424	< 2.20E-16							
$f(T)$ for all individuals in LYG	15.39	248.8	< 2E-16	17.22	63.24	< 2E-16			
Deviation from $f(T)$ for single individuals in LYG	11.007	18.693	< 2.20E-16	11.607	16.959	< 2.20E-16			
	20.232	27.923	< 2.20E-16	9.0809	27.684	< 2.20E-16			
	15.911	39.544	< 2.20E-16	15.266	10.099	< 2.20E-16			
	19.311	28.712	< 2.20E-16	1.826	75.808	< 2.20E-16			
	13.398	12.143	< 2.20E-16	6.9355	17.104	< 2.20E-16			
	20.956	12.058	< 2.20E-16	15.728	32.065	< 2.20E-16			
	15.608	17.479	< 2.20E-16	9.1755	31.097	< 2.20E-16			
	5.7321	12.181	4.88E-13	7.8549	32.38	< 2.20E-16			
	9.3015	30.122	< 2.20E-16	15.279	6.5194	3.61E-14			



				11.872	128.95	< 2.20E-16			
				18.419	21.328	< 2.20E-16			
<b>f(T) for all individuals in ZDZ</b>	17.22	99.13	< 2E-16	15.95	67.22	< 2E-16			
	22.24	61.212	< 2.20E-16	8.6395	29.708	< 2.20E-16			
	12.458	17.867	< 2.20E-16	6.2855	32.463	< 2.20E-16			
	20.246	32.279	< 2.20E-16	6.3088	10.54	4.82E-12			
	18.974	4.5305	6.42E-10	8.2368	10.407	9.04E-15			
Deviation from f(T) for single individuals in ZDZ				11.205	29.741	< 2.20E-16			
				7.7516	9.6956	5.69E-13			
				6.3822	37.481	< 2.20E-16			
				6.4261	54.497	< 2.20E-16			
				11.693	136.2	< 2.20E-16			
				6.9189	4.728	3.03E-05			
<b>f(T) for all individuals in YGD</b>	12.31	235.5	< 2E-16	18.87	262.4	< 2E-16	15.55	109.9	< 2E-16
	25.202	13.778	< 2.20E-16	11.323	14.438	< 2.20E-16	13.253	14.058	< 2.20E-16
	22.921	116.62	< 2.20E-16	12.991	45.477	< 2.20E-16	8.6646	30.364	< 2.20E-16
	14.374	32.213	< 2.20E-16	12.755	70.076	< 2.20E-16	10.982	18.127	< 2.20E-16
				9.3981	27.547	< 2.20E-16	11.837	55.712	< 2.20E-16
				17.066	2.6991	0.00019	9.1243	29.713	< 2.20E-16
Deviation from f(T) for single individuals in YGD				19.322	3.2311	2.32E-06	6.6367	57.954	< 2.20E-16
				19.879	11.643	< 2.20E-16	12.069	10.977	< 2.20E-16
				7.2351	136.09	< 2.20E-16	6.4506	19.641	< 2.20E-16
				5.194	59.953	< 2.20E-16	6.2078	26.995	< 2.20E-16
				8.5749	11.886	< 2.20E-16	10.622	20.397	< 2.20E-16
							8.7588	7.5935	8.37E-11

f(T) for all individuals in LS	18.96	47.92	< 2E-16	16.29	181.5	< 2E-16	18.49	269.6	< 2E-16
	6.042	22.966	< 2.20E-16	16.586	52.386	< 2.20E-16	2.1028	14.727	2.42E-07
	14.303	15.354	< 2.20E-16	10.177	37.709	< 2.20E-16	12.345	52.015	< 2.20E-16
	9.197	109.3	< 2.20E-16	12.194	11.646	< 2.20E-16	19.449	57.499	< 2.20E-16
	8.2521	20.271	< 2.20E-16	10.934	34.857	< 2.20E-16	16.217	28.767	< 2.20E-16
Deviation from f(T) for single individuals in LS	16.67	52.629	< 2.20E-16	14.892	8.7139	< 2.20E-16	8.8244	23.954	< 2.20E-16
	9.8021	17.433	< 2.20E-16	17.836	62.655	< 2.20E-16	10.672	28.744	< 2.20E-16
				8.5646	28.763	< 2.20E-16	14.79	37.937	< 2.20E-16
				5.1799	17.605	< 2.20E-16	4.3263	7.3092	3.61E-06
				4.9745	11.08	1.51E-10	9.4244	14.802	< 2.20E-16
				11.841	10.779	< 2.20E-16	15.614	13.395	< 2.20E-16
f(T) for all individuals in ZS	18.86	228	< 2E-16	16.11	110.4	< 2E-16	18.25	213.8	< 2E-16
	7.1615	16.441	< 2.20E-16	9.018	54.038	< 2.20E-16	6.8492	83.082	< 2.20E-16
	6.5764	19.289	< 2.20E-16	10.61	19.968	< 2.20E-16	9.2764	22.54	< 2.20E-16
	11.422	5.9093	6.86E-10	16.568	55.656	< 2.20E-16	3.725	58.361	< 2.20E-16
	6.845	2.0329	0.04901	15.002	16.308	< 2.20E-16	6.0315	72.138	< 2.20E-16
	12.058	14.835	< 2.20E-16	3.3613	34.982	< 2.20E-16	12.469	109.6	< 2.20E-16
	5.4275	6.999	6.65E-07	14.116	13.622	< 2.20E-16	12.342	9.3564	< 2.20E-16
Deviation from f(T) for single individuals in ZS	9.4538	134.74	< 2.20E-16	12.219	6.954	9.15E-13	8.5493	8.5823	2.88E-12
	10.227	144.1	< 2.20E-16	10.327	22.45	< 2.20E-16	10.791	38.409	< 2.20E-16
	2.9131	64.539	< 2.20E-16	13.185	42.059	< 2.20E-16	14.685	7.0296	4.55E-15
	8.2369	47.739	< 2.20E-16	10.879	62.779	< 2.20E-16	5.0041	41.624	< 2.20E-16
	7.6646	26.267	< 2.20E-16						
	3.5598	55.956	< 2.20E-16						
	11.199	12.147	< 2.20E-16						



**Table S2. Deviation of temperature functions of heart rate,  $f(T)$ , of species from geographical populations.** F-test of generalized additive models (GAM) of heart rate to temperature between species and populations were generated to describe the variance of populations.

	<i>Littoraria sinensis</i>			<i>Littorina brevicula</i>			<i>Nerita yoldii</i>		
	d.f.	F-value	P-value	d.f.	F-value	P-value	d.f.	F-value	P-value
$f(T)$ for each species	15.37	948.1	< 2E-16	18.96	620.2	< 2E-16	16.8	520.3	< 2E-16
Deviation from $f(T)$ QD	12.925	19.29	< 2.20E-16	19.858	7.9654	< 2.20E-16			
Deviation from $f(T)$ LYG	10.707	10.639	< 2.20E-16	8.9512	32.121	< 2.20E-16			
Deviation from $f(T)$ ZDZ	13.804	9.1993	< 2.20E-16	13.15	56.344	< 2.20E-16			
Deviation from $f(T)$ YGD	8.4078	20.141	< 2.20E-16	11.439	30.852	< 2.20E-16	12.801	54.085	< 2.20E-16
Deviation from $f(T)$ LS	20.364	38.261	< 2.20E-16	11.394	17.257	< 2.20E-16	19.905	69.953	< 2.20E-16
Deviation from $f(T)$ ZS	20.672	58.333	< 2.20E-16	10.975	23.35	< 2.20E-16	19.905	69.953	< 2.20E-16
Deviation from $f(T)$ WZ	17.152	24.399	< 2.20E-16	14.619	7.4385	< 2.20E-16	15.51	17.772	< 2.20E-16
Deviation from $f(T)$ XM	23.106	6.3642	< 2.20E-16	7.9794	22.413	< 2.20E-16	12.466	13.947	< 2.20E-16

**Table S3 Summary of modelled 30-year body temperatures ( $T_b$ ) of the three species (*Littoraria sinensis*, *Littorina brevicula* and *Nerita yoldii*) along the Chinese coastline.**

Site	Return Time to ABT (> 45 °C) (days)	# of days over ABT (>45 °C)	Max $T_b$ in sun	Max $T_b$ in shade	95% tile in sun	95% tile in shade
<i>Littoraria sinensis</i>						
Qingdao	N/A	1	44.58	33.68	32.15	26.26
Lianyungang	6.13	36	50.81	37.87	32.58	26.95
Zhendongzha	9.98	109	48.67	36.63	32.56	27.18
Yangguangdao	6.77	173	44.94	33.17	32.48	27.66
Lvsi	4.07	757	57.45	43.41	36.94	29.88
Zhoushan	N/A	2	43.67	30.52	32	27.72
Wenzhou	8	2	45.72	32.18	34.46	28.3
Xiamen	11.45	28	49.01	34.93	37.32	29.18
<i>Littorina brevicula</i>						
Qingdao	N/A	1	45.97	33.68	32.53	26.26
Lianyungang	8.45	14	51.8	37.87	32.97	26.95
Zhendongzha	10.1	16	49.63	36.63	32.9	27.18
Yangguangdao	N/A	2	46.16	33.17	32.78	27.66
Lvsi	7.01	398	58.83	43.41	37.37	29.88
Zhoushan	N/A	0	45.12	30.52	32.23	27.72
Wenzhou	16	7	47.04	32.18	34.81	28.3
Xiamen	21.78	14	50.22	34.93	37.82	29.18
<i>Nerita yoldii</i>						
Qingdao	N/A	N/A	45.07	33.68	32.29	26.26
Lianyungang	N/A	N/A	51.17	37.87	32.72	26.95
Zhendongzha	N/A	N/A	49.01	36.63	32.68	27.18
Yangguangdao	15	71	45.33	33.17	32.58	27.66
Lvsi	5	526	57.94	43.41	37.08	29.88
Zhoushan	3	10	44.19	30.52	32.09	27.72
Wenzhou	7.94	72	46.19	32.18	34.59	28.3
Xiamen	3.57	907	49.44	34.93	37.5	29.18

**Table S4. Percentages of individuals whose thermal limits are lower than its predicted maximum operative temperature and are thus expected to experience mortality ( $\geq 1$  times). These predictions are to show percentages of individuals for which physiological impairment is predicted ( $T_b > ABT$ ) and percentages of individuals threatened with lethal temperatures ( $T_b > FLT$ )**

Species/ Site	TSM <sub>ABT_SUN</sub>	TSM <sub>ABT_SHADED</sub>	TSM <sub>FLT_SU</sub> N	TSM <sub>FLT_SHADE</sub> D
<i>Littoraria sinensis</i>	77.14	8.57	21.43	1.43
Qingdao (QD)	54.55	0.00	0.00	0.00
Lianyungang (LYG)	100.00	22.22	66.67	0.00
Zhendongzha (ZDZ)	100.00	25.00	25.00	0.00
Yangguangdao (YGD)	100.00	0.00	0.00	0.00
Lvsi (Lvsi)	100.00	50.00	100.00	16.67
Zhoushan (ZS)	56.25	0.00	0.00	0.00
Wenzhou (WZ)	70.00	0.00	0.00	0.00
Qingdao (QD)	90.91	0.00	18.18	0.00
<i>Littorina brevicula</i>	70.73	3.66	36.59	0.00
Qingdao (QD)	30.00	0.00	0.00	0.00
Lianyungang (LYG)	100.00	0.00	100.00	0.00
Zhendongzha (ZDZ)	100.00	10.00	50.00	0.00
Yangguangdao (YGD)	40.00	0.00	0.00	0.00
Lvsi (Lvsi)	100.00	20.00	100.00	0.00
Zhoushan (ZS)	27.27	0.00	9.09	0.00
Wenzhou (WZ)	70.00	0.00	0.00	0.00
Qingdao (QD)	100.00	0.00	30.00	0.00
<i>Nerita yoldii</i>	90.00	10.00	32.65	0.00
Yangguangdao (YGD)	90.91	0.00	0.00	0.00
Lvsi (Lvsi)	100.00	30.00	100.00	0.00
Zhoushan (ZS)	77.78	0.00	0.00	0.00
Wenzhou (WZ)	80.00	10.00	20.00	0.00
Qingdao (QD)	100.00	10.00	44.44	0.00