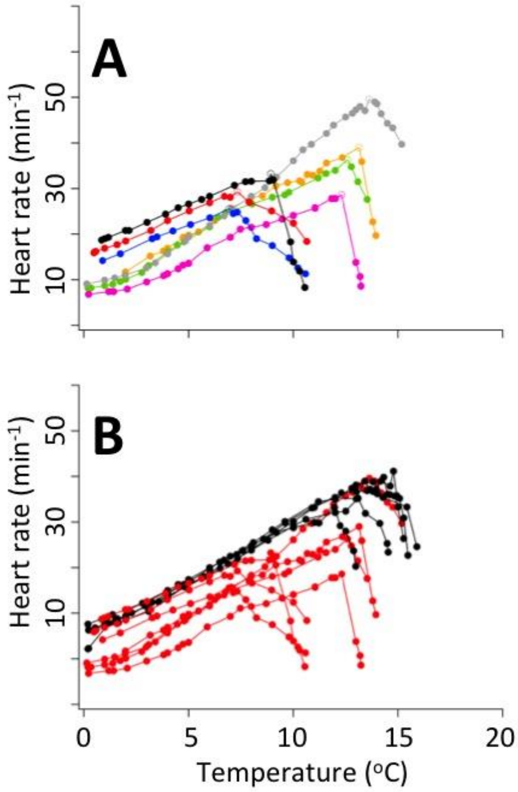

Figure S1. Quality control of individual *C. aceratus* suitability for analysis of f_H and HRV, using objective assessment of thermal sensitivity with minimally invasive measures. Assuming that f_H is the most labile variable, based on our existing data for these species and prior knowledge of stress in fishes, the consequences of incorporating all gathered data can be profound. A) All individuals recovered well from surgery and displayed none of the external signs of stress over the next 48-72 h, e.g. banded pigmentation, and hence were considered suitable for use in subsequent investigations. Note that those individuals with intact sinus rhythm but which started the thermal ramp with higher resting f_H demonstrated the lowest BPT, and CT_{MAX}, hence inclusion would underestimate thermal tolerance in this species. B) Heart rates of intact icefish (red circles) show an overlap of the relatively tachycardic individuals with atropinised icefish (black circles) during a thermal ramp, suggesting one contributor to greater thermal sensitivity is an unusually low vagal tone.

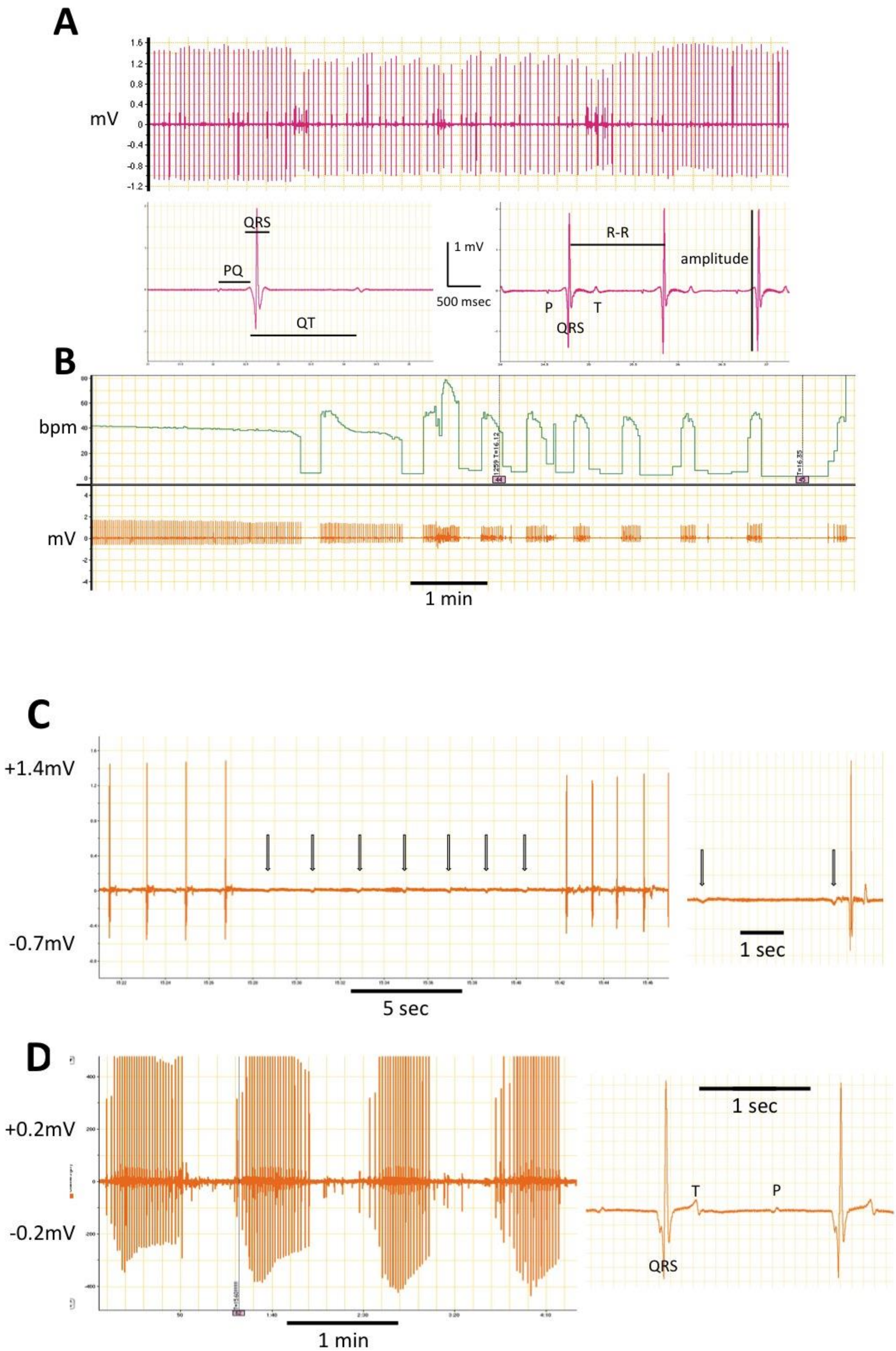
A
mV
1.6
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
QRS
PQ
QT
1 mV
500 msec
R-R
amplitude
P
T
QRS
B
bpm
mV
1 min
C
+1.4mV
-0.7mV
5 sec
1 sec
D
+0.2mV
-0.2mV
1 min
1 sec
T
P
QRS

Figure S2. Screenshots of live recordings showing variability in cardiac electrical activity during a thermal ramp. A) *N. coriiceps* showing variable f_H at 7.6°C, transiently increasing HRV as a result of prolonged R-R interval before normal sinus rhythm was restored – insert, individual waveforms at -1.01°C and +14.6°C, respectively, indicating the distinct cycle durations evident; B) *N. coriiceps* showing atropine periodicity prior to death at 16.1°C, with progressively longer periods of asystole; C) ECG trace from *N. coriiceps* showing conduction failure at 16°C, 7 P-waves are indicated – insert, expanded view showing 2 P-waves without intervening QRS; D) *C. aceratus* with ventricular tachyarrythmias of 42 bpm at 15.8°C – insert, expanded view showing normal ECG waveform during elevated f_H.

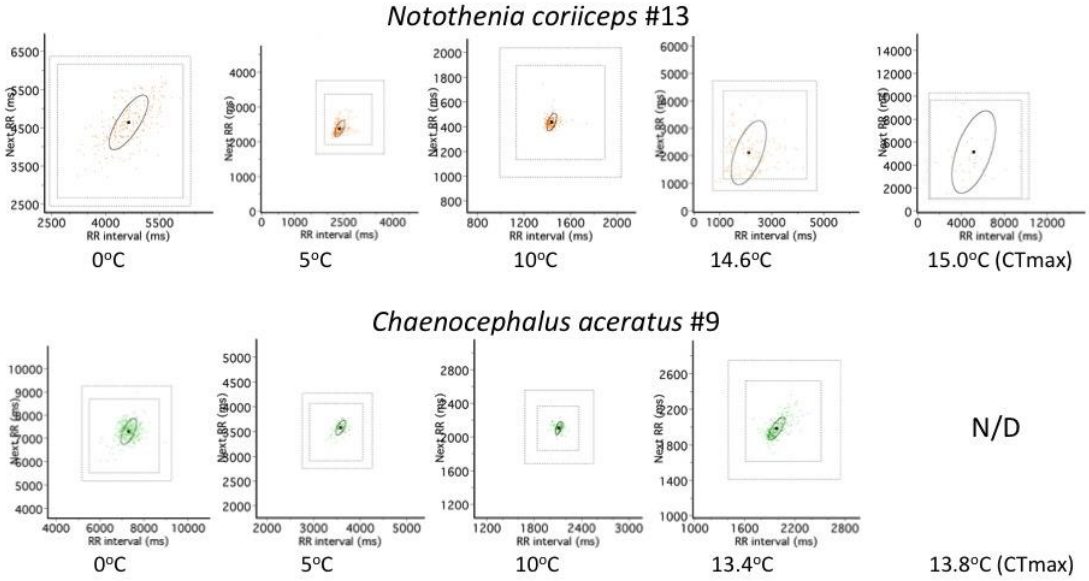

Figure S3. Representative Poincare plots - a plot of R-R(n+1) against R-R(n) -of individual *N. coriiceps* (top) and *C. aceratus* (bottom). Note the progressive narrowing of R-R interval spread with elevated temperature, followed by widening as CT_{MAX} is approached. The collapse of *C. aceratus* sinus rhythm rapidly after peak f_H (BPT) makes analysis difficult around CT_{MAX}.

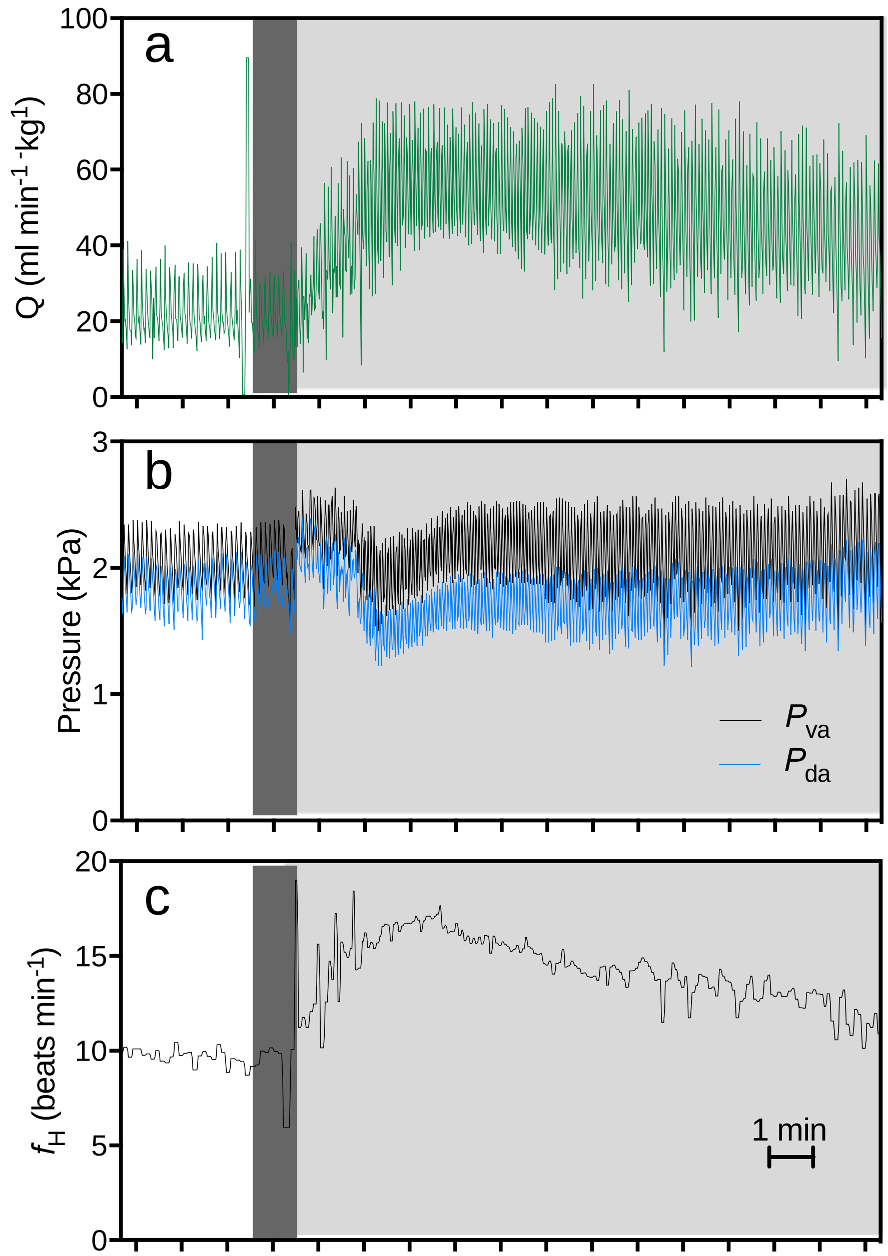

Figure S4. An original and representative trace of cardiovascular parameters before and during activity at 0.8°C. The dark grey zone represents the period during which the animal was agitated, the light grey zone represents the ensuing period of activity. (a) $\dot{Q}$, cardiac output; (b) P_{da}, dorsal aortic pressure; P_{va}, ventral aortic pressure; (c) f_H, heart rate.

Table S1. Heart rate variability (analysis of changes in beat-to-beat intervals with time) indices during recovery and thermal ramp test.

Click here to Download Table S1

Mean±SD(n); **P*<0.05, ***P*<0.001 *vs. C. aceratus*. NB: few icefish reached 15°C, CT_{MAX} was consistently lower than this.

Abbreviations: NN, interbeat intervals obtained from the R-R series (ms); SDNN, standard deviation of NN intervals (ms); Ratio, (standard deviation of all NN intervals / standard deviation of differences between adjacent NN intervals); RMSSD, square root of the mean of the sum of squared differences between adjacent NN intervals; CVNN, coefficient of variation (ratio of SDNN to mean NN) of NN (%); NN_{150}, proportion of adjacent NN intervals that differ by more than 150ms (%); Total power, power in the analysis region (ms^2); LF:HF, ratio between LF power and HF power.

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