**The combined effects of reactant kinetics and enzyme stability explain the temperature dependence of metabolic rates**

J.P. DeLong, J.P. Gibert, T.M. Luhring, G. Bachman, B. Reed, A. Neyer, K.L. Montooth

Figure S1. **A**. Plot of the Arrhenius factor () – the proportion of the potential reaction rate *A*0 that can occur given the kinetic state of the reactants – over a wide temperature range, showing the rapid rise at lower temperatures and a tapering off at very high temperatures. **B**. The Arrhenius factor over a narrower range, showing that the factor is extremely small over the biologically relevant temperature range. At 37°C (i.e., normal human body temperature) the Arrhenius equation predicts that only about 10-9% of the potential reaction rate given by *A*0 can occur. This suggests that metabolic rates should be vanishingly low in the biologically relevant temperature range, and only with a massive supply of substrates can the reaction proceed.

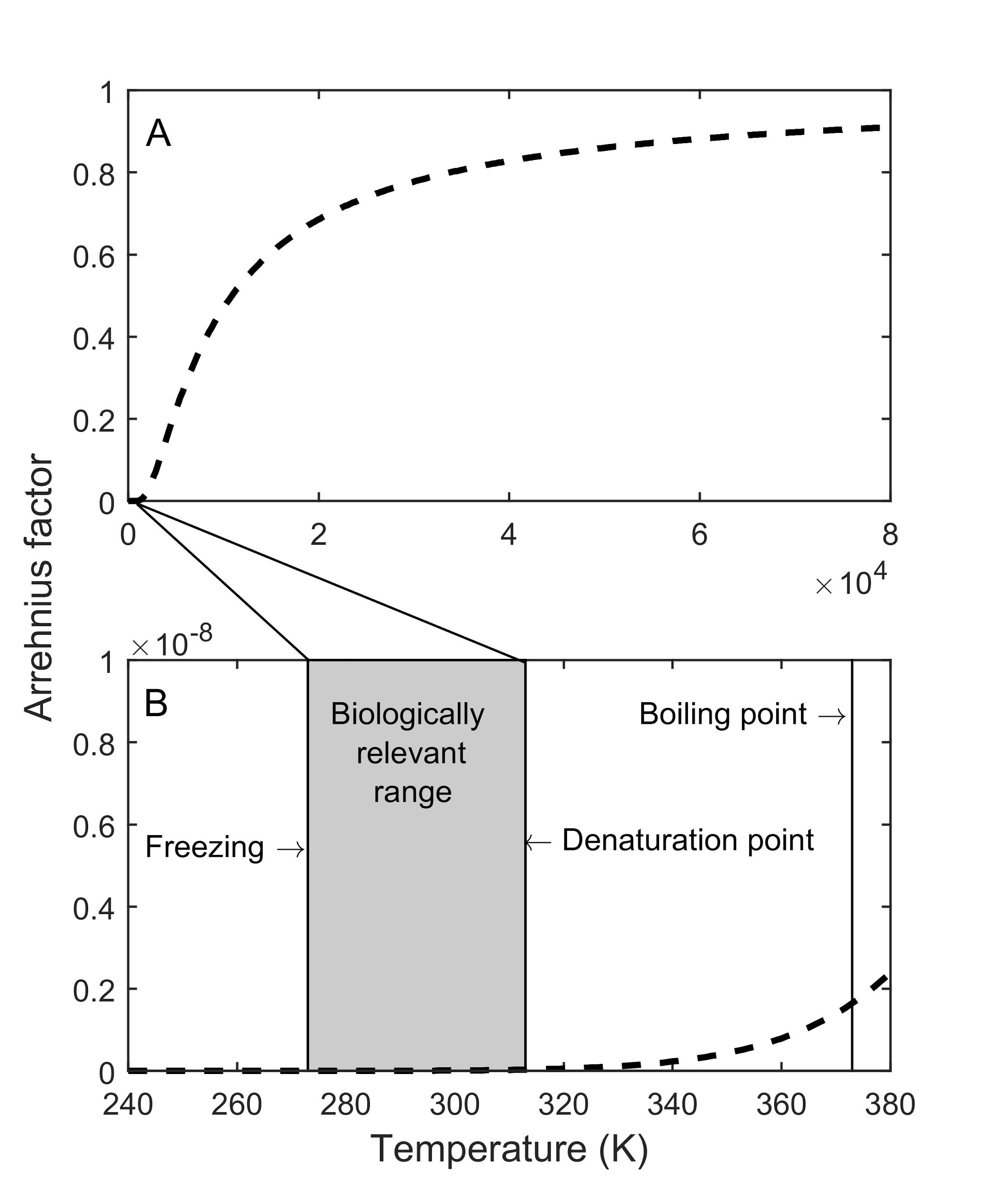


Table S1. Parameters and CIs for fits to data sets.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Taxon** | **Scientific name** | **Median *R*2** | **ln*A*** | **95% down** | **95% up** | ***E*b** | **95% down** | **95% up** | ***E*Δ*H*** | **95% down** | **95% up** |
| Issartel, et al, 2005 | Amphipod | *Nephargus verei* | 0.85 | 20.85 | 0.004 | 157.3 | 0.80 | 0.20 | 4.54 | 2.59 | 1.53 | 7.07 |
| Issartel, et al, 2005 | Amphipod | *Gammarus fossarum* | 0.66 | 0.26 | 0.019 | 72.7 | 0.17 | 0.16 | 2.08 | 1.88 | 1.44 | 3.85 |
| Issartel, et al, 2005 | Amphipod | *Nephargus rhenorhodanensis* | 0.89 | 10.10 | 0.002 | 152.4 | 0.43 | 0.16 | 4.24 | 1.60 | 1.06 | 5.42 |
| Heiman and Knight 1975 | Stonefly | *Acroneuria californica* | 0.82 | 0.17 | 0.015 | 10.2 | 0.12 | 0.11 | 0.38 | 2.26 | 1.90 | 2.69 |
| Alexander Jr. and McMahon, 2004 | Zebra mussel | *Dreissena polymorpha*, 5°C | 0.54 | 18.79 | 0.008 | 150.3 | 0.73 | 0.21 | 4.29 | 2.17 | 1.42 | 5.81 |
| Alexander Jr. and McMahon, 2021 | Zebra mussel | *Dreissena polymorpha*, 25°C | 0.39 | 20.62 | 0.002 | 150.8 | 0.81 | 0.24 | 4.40 | 2.33 | 1.60 | 5.99 |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Scientific name** | ***E*Δ*C*p** | **95% down** | **95% up** | ***T*m** | **95% down** | **95% up** | ***T*opt** | **95% down** | **95% up** |
| Issartel, et al, 2005 | *Nephargus verei* | 0.09 | 0.08 | 0.12 | 311.5 | 307.8 | 328.3 | 293.0 | 292.4 | 293.8 |
| Issartel, et al, 2005 | *Gammarus fossarum* | 0.11 | 0.09 | 0.15 | 304.7 | 303.7 | 306.6 | 291.0 | 290.3 | 291.7 |
| Issartel, et al, 2005 | *Nephargus rhenorhodanensis* | 0.07 | 0.06 | 0.08 | 309.8 | 307.7 | 315.1 | 294.6 | 293.8 | 295.7 |
| Heiman and Knight 1975 | *Acroneuria californica* | 0.25 | 0.21 | 0.30 | 304.0 | 303.7 | 304.5 | 295.8 | 295.4 | 296.2 |
| Alexander Jr. and McMahon, 2004 | *Dreissena polymorpha*, 5°C | 0.08 | 0.07 | 0.11 | 314.0 | 313.5 | 314.7 | 296.9 | 295.6 | 298.1 |
| Alexander Jr. and McMahon, 2021 | *Dreissena polymorpha*, 25°C | 0.08 | 0.06 | 0.09 | 320.3 | 319.3 | 321.5 | 299.8 | 298.7 | 301.0 |

Matlab code for data simulation and analysis:

clear; clc; clf;

[num text] = xlsread('TPC\_data\_set\_theory\_paper','Metabolic data');

% read in columns

temp\_all = num(:,8)+273.15;

MR\_all = num(:,17);

MR\_SE\_all = num(:,18);

for z = 1:6 % run for 6 data sets

if z == 1 % Niphargus rhenorhodanensis

species = '\itNiphargus rhenorhodanensis';

temp = temp\_all(1:9);

mr = MR\_all(1:9);

mr\_SE = MR\_SE\_all(1:9);

sample\_size = 10;

elseif z == 2 % Niphargus virei

species = '\itNiphargus virei';

temp = temp\_all(10:17);

mr = MR\_all(10:17);

mr\_SE = MR\_SE\_all(10:17);

sample\_size = 10;

elseif z == 3 % Gammarus fossarum

species = '\itGammarus fossarum';

temp = temp\_all(18:26);

mr = MR\_all(18:26);

mr\_SE = MR\_SE\_all(18:26);

sample\_size = 10;

elseif z == 4 % Acroneuria californica

species = '\itAcroneuria californica';

temp = temp\_all(44:51);

mr = MR\_all(44:51);

mr\_SE = MR\_SE\_all(44:51);

sample\_size = 3;

elseif z == 5 % 5C acclimated Dreissena polymorpha

species = '5C acclimated \itDreissena polymorpha';

temp = temp\_all(27:34);

mr = MR\_all(27:34);

mr\_SE = MR\_SE\_all(27:34);

sample\_size = 12;

elseif z == 6 % 25C acclimated Dreissena polymorpha

species = '25C acclimated \itDreissena polymorpha';

temp = temp\_all(28:43);

mr = MR\_all(28:43);

mr\_SE = MR\_SE\_all(28:43);

sample\_size = 12;

end

% data transformations

lnmr = log(mr);

mr\_SD = mr\_SE.\*sqrt(sample\_size); % calculate SE from SD

for i = 1:length(mr) % create modeled data temp vector

temp\_matrix(1:sample\_size,i) = temp(i);

end

temp\_mod = reshape(temp\_matrix,[sample\_size\*length(temp),1]); % turn matrix into column vector

num\_bootstraps = 1000; % how many bootstrapped data sets to use

rsquares = NaN(num\_bootstraps,1); % open an empty vector to put in rsquareds

parameters = NaN(num\_bootstraps,4); % open an empty vector to put in parameters

topt = NaN(num\_bootstraps,1); % open an empty vector for topt

tref = NaN(num\_bootstraps,1); % open an empty vector for tref

num\_fits = 0; % start fitting index at 0

for j = 1:1000 % start bootstrap

if num\_fits < num\_bootstraps+1 % keep fitting if you haven't done them all

% create modeled data set

for i = 1:length(mr)

mr\_matrix(:,i) = mr(i) + mr\_SD(i).\*randn(sample\_size,1); % generate data for each temperature

end

mr\_mod = reshape(mr\_matrix,[sample\_size\*length(temp),1]); % reshape data set for fitting

lnmr\_mod = log(mr\_mod); % lake log

% find tref

index = (find(mr == max(mr))-1):length(mr); % find right side of TPC

temp\_tref = temp\_matrix(:,index); % temp for right side based on above index

temp\_tref\_X = reshape(temp\_tref,[sample\_size\*length(index),1]);

mr\_tref = mr\_matrix(:,index); % mr for right side based on above index

mr\_tref\_X = reshape(mr\_tref,[sample\_size\*length(index),1]);

ft\_ref = fittype( 'poly2' ); % set up fit as second order polynomial

opts = fitoptions( 'Method', 'LinearLeastSquares' ); % choose method

[fitresult1, gof1] = fit( temp\_tref\_X, mr\_tref\_X, ft\_ref, opts ); % Fit model to data

% figure( 'Name', 'untitled fit' ); % plot fit with data (best to leave this commented unless exploring)

% h = plot( fitresult1, temp\_tref, mr\_tref );

% xlabel tempk

% ylabel mr

fit\_roots = roots(coeffvalues(fitresult1)); % find roots of fitted polynomial

tref(j) = max(real(fit\_roots)); % take real parts where necessary

trefs = num2str(tref(j)); % switch to string to include below

% fit real TPC curve

ft = fittype(['lnA - (Ea - deltaH\*(1 - x/',trefs,') - deltaCp\*(x - ',trefs,' - x\*log(x/',trefs,')))/(x\*8.617e-5)'], 'independent', 'x', 'dependent', 'y' );

opts = fitoptions( ft ); % Set up options

opts.Display = 'Off';

opts.Lower = [-Inf 0 -Inf 0];

opts.MaxFunEvals = 6000000;

opts.MaxIter = 4000000;

opts.StartPoint = [0.1 1 0.001 0.01];

opts.Upper = [Inf Inf Inf Inf];

try

[fitresult, gof] = fit( temp\_mod, lnmr\_mod, ft, opts );

catch ME

end

rsquares(j) = gof.rsquare; % log the rsquare value

num\_fits = num\_fits + 1; % index the fitting

coeffs = coeffvalues(fitresult); % pull out the fitted parameters

parameters(j,:) = coeffs; % log them

overall\_curve = feval(fitresult,temp\_mod); % calculate the fitted curve for this bootstrap

temps\_for\_fit = min(temp\_mod):1:max(temp\_mod); % set temperatures

curve2(j,:) = feval(fitresult,temps\_for\_fit)'; % calculate the fitted curve for the set temps

topt(j) = (coeffs(1) - coeffs(3) + coeffs(2)\*tref(j))/coeffs(2); % calculate topt

curve2(curve2(:,1)==0,:) = [];

% figure(2);clf(2);

% box on; hold on;

% plot(temp\_mod,lnmr\_mod,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

% plot(temp\_mod,overall\_curve,'-k','LineWidth',2);

end

end

topt(topt(:,:)==0) = []; % clear out bad fits with zeros

curve\_up = prctile(curve2(:,:),97.5); % pull out curves from bootstrapped fits

curve\_down = prctile(curve2(:,:),2.5);

curve\_med = prctile(curve2(:,:),50);

% compile parameters with confidence intervals and print to excel sheet

lnA = prctile(parameters(:,4),50);

lnA\_97\_5 = prctile(parameters(:,4),97.5);

lnA\_2\_5 = prctile(parameters(:,4),2.5);

Ea = prctile(parameters(:,1),50);

Ea\_97\_5 = prctile(parameters(:,1),97.5);

Ea\_2\_5 = prctile(parameters(:,1),2.5);

deltaH = prctile(parameters(:,3),50);

deltaH\_97\_5 = prctile(parameters(:,3),97.5);

deltaH\_2\_5 = prctile(parameters(:,3),2.5);

deltaCp = prctile(parameters(:,2),50);

deltaCp\_97\_5 = prctile(parameters(:,2),97.5);

deltaCp\_2\_5 = prctile(parameters(:,2),2.5);

tref\_med = prctile(tref,50);

tref\_97\_5 = prctile(tref,97.5);

tref\_2\_5 = prctile(tref,2.5);

topt\_med = prctile(topt,50);

topt\_97\_5 = prctile(topt,97.5);

topt\_2\_5 = prctile(topt,2.5);

% find closest part of median curve to plot ci's on topt

length(temps\_for\_fit)

for q = 1:length(temps\_for\_fit)

val = temps\_for\_fit(q) %value to find

tmp\_low(q) = abs(topt\_2\_5-val)

tmp\_hi(q) = abs(topt\_97\_5-val)

end

[idx idx] = min(tmp\_low); %index of closest value

lnmr\_med\_low = curve\_med(idx); %closest value

[idx idx] = min(tmp\_hi); %index of closest value

lnmr\_med\_hi = curve\_med(idx); %closest value

parameters\_out(z,:) = [median(rsquares) lnA lnA\_2\_5 lnA\_97\_5 Ea Ea\_2\_5 Ea\_97\_5 deltaH deltaH\_2\_5 deltaH\_97\_5 deltaCp deltaCp\_2\_5 deltaCp\_97\_5,...

tref\_med tref\_2\_5 tref\_97\_5 topt\_med topt\_2\_5 topt\_97\_5];

figure(2);

subplot(6,3,z\*3-2);

box on;

jbfill(min(temp\_mod):1:max(temp\_mod),curve\_up,curve\_down,[0.69 0 1],[0.69 0 1],0.5,0.5); hold on;

l1 = plot(temp\_mod,lnmr\_mod,'ok','MarkerFaceColor',[0.7 0.7 0.7]);

l2 = plot(temp,lnmr,'o','MarkerEdgeColor',[0 0.53 1],'MarkerFaceColor',[0 0.53 1]);

l3 = plot(temps\_for\_fit,curve\_med,'-k','LineWidth',1);

l4 = plot([topt\_2\_5 topt\_97\_5],[lnmr\_med\_low lnmr\_med\_hi],'-','Color',[1 0.6 0.2],'LineWidth',3);

xlim([270 330]);

if z == 1

legend([l1 l2 l3 l4],'Sample data set','Mean values','EAAR model','95% CIs on {\itT}\_{opt}',...

'Location','SouthEast');

elseif z == 4

ylabel( 'ln(Metabolic rate (W))','FontSize',12);

end

temps\_for\_effects = 270:330;

deltaH\_effect = deltaH.\*(1 - temps\_for\_effects./tref\_med);

deltaCp\_effect = deltaCp.\*(temps\_for\_effects - tref\_med - temps\_for\_effects.\*log(temps\_for\_effects./tref\_med));

Ec\_of\_T = deltaH\_effect + deltaCp\_effect;

net\_Ea = Ea - (Ec\_of\_T);

eaar\_factor = exp(-(net\_Ea)./(temps\_for\_effects.\*8.617e-5));

arrhenius\_factor = exp(-(Ea)./(temps\_for\_effects.\*8.617e-5));

subplot(6,3,z\*3-1);

box on; hold on;

h1 = plot(temps\_for\_effects,deltaCp\_effect,'-','Color',[0.8 0.2 0],'LineWidth',2);

h2 = plot(temps\_for\_effects,deltaH\_effect,'-','Color',[0.4 0 0.2],'LineWidth',2);

h3 = plot(temps\_for\_effects,Ec\_of\_T,'-','Color',[0.4 0.2 1],'LineWidth',2);

h4 = plot(temps\_for\_effects,net\_Ea,'-','LineWidth',2,'Color',[0.5 0.5 0.5]);

h5 = plot([min(temps\_for\_effects) max(temps\_for\_effects)],[Ea Ea],'--','LineWidth',2,'Color',[0.5 0.5 0.5]);

h6 = plot(tref\_med,0,'ok','MarkerFaceColor','k');

xlim([270 330]);

title(species);

if z == 1

legend([h1 h2 h3 h4 h5 h6],'\DeltaCp effect','\DeltaH effect','{\itE}{\_c}({\itT})','Net {\itE}{\_a}',...

'{\itE}{\_b}','{\itT}\_{m}','Location','SouthOutside');

elseif z == 4

ylabel( 'Energy (ev)','FontSize',12);

elseif z == 7

xlabel( 'Temperature (K)','FontSize',12);

end

subplot(6,3,z\*3);

box on; hold on;

g1 = plot(temps\_for\_effects,eaar\_factor,'-k','LineWidth',2);

g2 = plot(temps\_for\_effects,arrhenius\_factor,'--k','LineWidth',2);

xlim([270 330]);

if z == 2

legend([g1 g2],'EAAR factor','Arrhenius factor','Location','NorthEast');

elseif z == 4

ylabel( 'Proportion of reaction','FontSize',12);

end

% clear these variables as they need to be reused and set at new sizes

% for each data set

clear('temp\_matrix')

clear('mr\_matrix')

clear('temp\_mod')

clear('temps\_for\_fit')

clear('curve2')

clear('tmp\_hi')

clear('tmp\_low')

figure(3); % figure to plot parameter estimates and CIs

plot\_index = -1\*z+7;

subplot(1,6,1);

hold on; box on;

plot([lnA\_2\_5 lnA\_97\_5],[plot\_index plot\_index],'-k');

plot(lnA,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('lnA');

xlim([0 100]);

ylim([0 7]);

set(gca,'YTick',[]);

subplot(1,6,2);

hold on; box on;

plot([Ea\_2\_5 Ea\_97\_5],[plot\_index plot\_index],'-k');

plot(Ea,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('{\itE}{\_b}');

xlim([0 3]);

ylim([0 7]);

set(gca,'Ytick',[]);

subplot(1,6,3);

hold on; box on;

plot([deltaH\_2\_5 deltaH\_97\_5],[plot\_index plot\_index],'-k');

plot(deltaH,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('\DeltaH');

xlim([0 8]);

ylim([0 7]);

set(gca,'Ytick',[]);

subplot(1,6,4);

hold on; box on;

plot([deltaCp\_2\_5 deltaCp\_97\_5],[plot\_index plot\_index],'-k');

plot(deltaCp,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('\DeltaCp');

xlim([0 0.3]);

ylim([0 7]);

set(gca,'Ytick',[]);

subplot(1,6,5);

hold on; box on;

plot([tref\_2\_5 tref\_97\_5],[plot\_index plot\_index],'-k');

plot(tref\_med,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('{\itT}\_m');

xlim([290 330]);

ylim([0 7]);

set(gca,'Ytick',[]);

subplot(1,6,6);

hold on; box on;

plot([topt\_2\_5 topt\_97\_5],[plot\_index plot\_index],'-k');

plot(topt\_med,plot\_index,'ok','MarkerFaceColor',[0.5 0.5 0.5]);

xlabel('{\itT}\_{opt}');

xlim([280 320]);

ylim([0 7]);

set(gca,'Ytick',[]);

end

figure(3);subplot(1,6,2); plot([0.65 0.65],[0 7],'--k');

gtext('\itN. rhenorhodanensis')

gtext('\itN. virei')

gtext('\itG. fossarum')

gtext('\itA. californica')

gtext({['5C acclimated'],['\itD. polymorpha']})

gtext({['25C acclimated'],['\itD. polymorpha']})