Title: A Cold Limit to Adaptation in the Sea

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Abstract

Temperature affects biological functions by altering reaction rates. Physiological rates usually double to treble for every 10°C rise, and x1-x4 encompasses normal biological functions. However, in polar marine species inhabiting temperatures around 0°C many processes are slowed beyond the Arrhenius relationships for warmer water species. Growth, embryonic development, Specific Dynamic Action (SDA) duration, and time to acclimate to altered temperature, are all x5-x12 slower in species living near 0°C than at 10°C. This cold marine physiological transition to slower states is absent, however, in oxygen consumption and SDA factorial scope; processes where capacity is related to aerobic scope. My opinion is that processes involving significant protein modification are impacted, and protein synthesis or folding problems cause the slowing of rates beyond expected temperature effects.

Temperature effects on the rate of biological processes

For over 100 years, temperature has been generally accepted as the strongest driver of the rate of biological functions. Early studies showed that process rates ranging from alcoholic fermentation to frog development followed relationships proposed by Arrhenius (see Box 1) and van't Hoff and increased x2-x3 for every 10°C temperature rise (a Q_{10} of 2-3). This "rule" is still a mainstay of temperature biology, being quoted widely in studies from

molecular biology through biochemistry to physiology and ecology, and in all major texts [1] and reviews [2] that address temperature effects on biological systems.

Investigations of development at low temperature have revealed a markedly larger temperature effect than the normal biological range, but these studies have generally not identified underlying mechanisms. Probably the first such investigations were in the 1960s on echinoderms and copepods, where development rate slowed dramatically around 0°C [3,4]. This result was confirmed by other studies [5-7] and extended to bivalve and gastropod molluscs, where the Q_{10} for brooding species at temperatures below 5°C was reported as 35.1 [8]. Other biological functions have also been demonstrated to proceed slowly in polar species, including growth [9,10], the time required to complete processes associated with feeding [11], the time required to complete gametogenesis [12,13], arm regeneration in ophiuroids [14], and the time required to acclimate to elevated temperature [15]. However, the fact that this slowing is outside the normally accepted Arrhenius relationships has rarely been identified.

In this article I analyse routine performance of biological processes across temperatures from tropical to polar latitudes and show that temperate and tropical species follow Arrhenius relationships and the expected Q_{10} of 2-3, but that polar species do for some processes but not for others. Data in the literature are of sufficient quality to allow good comparisons for embryonic development, growth, routine oxygen consumption and the time required to complete post-prandial metabolic processes, the Specific Dynamic Action of feeding (SDA). More limited data are also available to show the impact of low temperature on arm regeneration in brittle stars and for acclimation (see Glossary) to elevated temperature in marine ectotherms, but neither of these sets of data encompass

47 the whole tropical to polar temperature regime. The processes deviating from Arrhenius
48 relationships at low temperature (embryonic development, growth, SDA duration and
49 regeneration in brittle stars) all require significant protein synthesis, whereas those that
50 follow Arrhenius (oxygen consumption and SDA peak rise) do include the costs of protein
51 synthesis, but also incorporate large elements of other processes, e.g. homeostasis,
52 membrane pumping, muscular activity, that are not limited by protein synthesis. A signal
53 should be present in oxygen consumption measures, because energetic costs of protein
54 synthesis form part of total metabolism, but it will be smaller and harder to detect than in
55 development, growth etc. It is my opinion that there is a cold marine physiological transition
56 to slower rates in marine species living at temperatures near 0°C and problems with protein
57 synthesis and folding at low temperature are the cause of the observed marked slowing of
58 rates.

59 **Embryonic development**

60 Several studies have demonstrated that early development of marine invertebrates slows
61 markedly at extreme low temperatures compared to temperate and tropical species,
62 including time from fertilisation to hatching in echinoids and asteroids [3-5, 16], isopods
63 [17], bivalve molluscs and brooding duration in gastropod molluscs [8]. Most studies have
64 showed a slowing of at least five to ten times compared to temperate species. A wide-scale
65 survey of data for brooding period with temperature in gastropod molluscs demonstrates a
66 marked slowing at temperatures around 0°C (Fig. 1a). Tropical species living at 25°C-30°C
67 require 0.5-4 weeks to develop from fertilisation to hatching, and in temperate species
68 living at 10°C-20°C this is 1-18 weeks. For species living at temperatures near 0°C the
69 brooding period ranges from 26-100 weeks. However, 2 of the 3 shortest polar brooding

times are for unusual species with mixed reproductive strategies where release occurs months before settlement and onset of the juvenile phase, which is normally achieved at brood release [8]. For these species, the full development times to juvenile stage have been used. An Arrhenius plot reveals a coherent relationship for temperate and tropical species, but values for polar species living near 0°C are significantly slower compared to times predicted from the relationship for temperate and tropical species ($t=-6.73$, 8df, $P<0.0001$)(Fig 1b). This not only confirms the conclusions drawn previously [3-5] that development rates in polar species are slow, but shows they are slowed beyond the normally accepted effects of temperature on biological systems.

Growth

Growth rates in polar species living permanently near 0°C have been recognised as slow for over 4 decades, but comprehensive comparisons from the tropics to the poles are rare or absent. Single species studies have generally identified rates x2-x5 slower than temperate counterparts in invertebrates [18-20] and fish [21], and a few studies have reported an order of magnitude slower growth in Antarctic species [22]. There are some reports of relatively rapid growth in cold-water species including some ascidians [23], bryozoans [24] and sponges [25]. These fast rates are still, however, more than x5 slower than fast growing phylogenetically related temperate species. Some studies have also shown a dramatic effect of small temperature changes on growth rates in laboratory experiments. For instance in the scallop *Adamussium colbecki*, a rise from 0°C to 3°C increased growth rates approximately tenfold [26]. A problem when analysing growth across latitudes and temperature ranges is identifying suitable comparisons. Analyses using small numbers or comparing fast rates in one area with slow or average rates elsewhere are clearly flawed. A

larger scale survey of growth rates for a taxon where many studies from tropical to polar latitudes exist, such as echinoid echinoderms reveals an increase of growth rate with temperature (Fig 1c). For each temperature regime in Fig 1c (e.g. polar, cool temperate, temperate) there is a range of growth rates with the slowest species growing x8-x10 slower than the fastest (the temperature or regional specific variation in growth). Why the range from slowest to fastest should be consistent across latitudes remains to be explained. An Arrhenius plot of the echinoid growth rates produces a strong linear relationship for temperate and tropical species (Fig 1d). However, rates for polar species living near 0°C are significantly slower than the extrapolation from the relationship for temperate and tropical species ($t=-2.83$, 4df, $P=0.047$). As for embryonic development, growth at temperatures near 0°C does not fit expected Arrhenius relationships.

Oxygen consumption

Oxygen consumption (MO_2) is a measure of immediate energy use under aerobic conditions. Ectotherm MO_2 increases with temperature across latitudes in fish [27] and invertebrates [20,28]. Nearly 100 years ago August Krogh [29] noted that polar species are active at low temperatures, whereas temperate species cooled to low temperatures are not. From this he postulated that polar species should have raised metabolic rates to compensate for the effect of low temperature. Early Antarctic studies in the 1950's and 1960's produced data to support this contention and the concept of "Metabolic cold Adaptation" (MCA) was proposed [30,31]. Later studies found both support for this hypothesis, especially from within species comparisons of populations living at different temperatures, and evidence against, mainly in multiple species analyses across wide latitudinal and temperature ranges [27,28]. A review of routine oxygen consumption data for marine bivalve molluscs from the

poles to the tropics with habitat temperature reveals an increasing curve, ostensibly similar to that for growth rate (Fig 2a), and metabolic rates double to treble with each 10°C temperature rise [27,28]. When the MO_2 data in Fig. 2a are transformed to an Arrhenius relationship the majority of values for polar species fall below the extended relationship for temperate and tropical species. However, they overlap the line and there is no significant reduction in MO_2 beyond Arrhenius predictions for species living near 0°C (t=-2.11, 13df, P=0.055) (Fig 2b). These data do not support the MCA hypothesis, which would predict polar species metabolic rates to be above the Arrhenius line for temperate and tropical species. Furthermore because data for polar species are not significantly lower than the extended Arrhenius relationship for temperate and tropical species there is no clear reduction in MO_2 at low temperature.

Specific Dynamic Action of feeding (SDA)

SDA is a measure of the metabolic costs of processing food and the associated post-absorptive functions (see Box 2). There have been several investigations of SDA in polar species, and these have generally reported similar peak heights to lower latitude species, but longer SDA durations [11,34,35]. An analysis of marine ectotherm SDA peak height data from tropical to polar latitudes shows there is no relation between peak height and environmental temperature (Fig 3a; ANOVA, $F_{1,110}$=0.85, P=0.358). SDA peak height has been argued to be related to aerobic scope, especially for sessile taxa (Box 2 [11]). The data here thus indicate that in marine invertebrates and fish the **factorial** aerobic capacity associated with feeding varies little with temperature from the tropics to the poles, ranging from values marginally above 1 to between 6 and 7, although values predominantly lie in the range 1.5-4.0. The peak rise is, however, a factorial metric related to pre-feeding MO_2,

and as resting and standard metabolic rates rise with temperature, the energy available for work during the SDA increases with temperature, even when the factorial rise is constant (Box 2 Fig.2, [11]). This explains part of the relationship between SDA duration and temperature (Fig 3b). Here SDA duration increases gradually as temperature declines from 30°C to 5°C. Below 5°C duration increases markedly, similar to the slowing of development and growth. An Arrhenius analysis shows SDA duration in polar species is significantly longer than predictions from extrapolating the relationship for temperate and tropical species ($t=7.13$, 7df, $P<0.0001$) (Fig 3c). Most of the energy used in the SDA is allocated to post-absorptive processes, primarily the manipulation of absorbed materials into storage and/or growth [11,32,34,35]. Much of the SDA duration increase beyond Arrhenius expectations at low temperature is likely due to increased costs in the remodelling of absorbed materials.

Q_{10} values

A clear impression of the scale of the change in rate of the above metrics across temperatures can be obtained using the Q_{10} metric, which calculates the temperature induced alteration in a biological rate scaled to an equivalent 10°C temperature change. When the physiological rates investigated here are averaged for 5°C temperature blocks and each block compared with adjacent temperatures, Q_{10} values are in the expected 1-4 range for temperatures between 5°C and 30°C for development rate, growth, MO_2 and SDA duration (Table1). However, the values for the comparisons of rates for species living below 5°C with those at 5-10°C are much higher for development (12.1), growth (5.1) and SDA duration (6.3). These very high Q_{10} values indicate that a factor other than temperature driven alterations in enzyme mediated systems is occurring. However, for MO_2, although the

highest Q_{10} value is for the lowest temperature comparison, the value obtained (3.2) is well within expectations.

Several other characteristics have been investigated at low temperature and these might allow comparisons with temperate and tropical species, but data are insufficient for Arrhenius and Q_{10} analyses across latitudes similar to those above.

Regeneration, acclimation, egg size, oogenesis and activity

Brittle stars can regenerate arms lost to predators or physical damage. Regeneration can be very common, with over 90% of individuals in some populations exhibiting regeneration [36]. Arrhenius analyses are not possible because of limited data. However, after arm loss there is a lag phase before regeneration begins, which lasts a few days in temperate species, but 5-7 months in the two Antarctic species studied to date [36,37]. Q_{10} values for the slowing of this lag phase range from around 15 to >20 [36,37]. Reported Q_{10} values for regeneration rate between polar and temperate species are also high [37]. Even for such limited data the high Q_{10} values suggest that at polar marine temperatures regeneration is slowed outside the normally expected Arrhenius relationship.

Acclimation is the resetting of steady physiological state following an environmental change. This response takes 2-6 months in Antarctic marine invertebrates compared to a few days or weeks for warmer water species, a slowing by up to x20 [38].

Egg size in polar marine invertebrates is much larger than at lower latitudes, in isopods [39], amphipods [40] and nudibranch molluscs [41]. Egg and embryo mass is usually x3-x6 larger in Antarctic species compared to temperate relatives, and nudibranch embryos were around x35 heavier than temperate relatives [41].

183 Gamete development also takes much longer in polar species than temperate comparators.
184 Generally oogenesis takes 18-24 months in benthic marine invertebrates living permanently
185 around 0°C, compared with 2-6 months for cool temperate species [42]. Taking the means
186 of these values gives a comparison of 4 vs 21 months for a temperature rise of around 10°C
187 (Q_{10}=5.25). Data are insufficient to accurately calculate the slowing between 5-10°C, but the
188 slowing around 0°C is large.

189 Several investigations have studied activity in low temperature species, including burying in
190 clams and anemones, swimming in scallops and fish, drilling in predatory snails and
191 locomotion in limpets [20], and more recently righting in echinoderms and gastropods [43].
192 Only two activities have been demonstrated to be fully compensated for low temperature,
193 being carried out at similar rates to temperate relatives or similar ecotypes, and the reasons
194 for this compensation have been identified. These are sustained swimming in fish, where
195 mitochondrial volume density in the pectoral muscles is increased by x2-x3 compared to
196 temperate fish [44]; and burying in the clam *Laternula elliptica* where the organ responsible
197 for burying is x3-x5 larger [45]. Polar species living near 0°C generally perform activities x2-
198 x10 slower than species at 10-20°C, and Q_{10} values average 2-4 [20], mostly within the
199 normally expected range.

200

201 **What causes the slowing of physiological rate at low temperatures?**

202 **The processes that are slowed and the ones that are not**

203 Evaluating the above observations together, allows us to see that the physiological
204 processes not demonstrating a sharp decline in rate around 0°C, MO_2, SDA peak and

activity, are all associated with aerobic systems, aerobic scope, and immediate ATP requirements, which appear to follow the expected patterns of temperature effects on biological systems throughout the whole temperature range in the sea. The processes that appear heavily impacted: embryonic development, growth, regeneration, SDA duration, acclimation to temperature change, egg size and oogenesis all involve the synthesis or remodelling of significant quantities of protein. These processes are also dependent on ATP supply, and a signal in routine MO_2 should be present, but other processes, e.g. cellular homeostasis also require ATP and these might not be affected in the same way at low temperature, which could mask any small signal from protein synthesis costs. It should be noted here that in the Arrhenius analysis of MO_2 low temperature species were close to significantly lower than expected (P=0.055).

Proteins, protein synthesis and Heat-Shock Proteins

Proteins become more unstable both above and below an optimum point, and at both high and low temperatures the instability is caused by unfolding [46]. Heat denaturation occurs mainly because of increased thermal motion (enthalpy) of polar residues. However, low temperature unfolding is less intuitive, as it is accompanied by a decrease in entropy [47]. Furthermore cold denaturation only occurs in aqueous solutions, and is markedly affected by the density of water [48]. Seawater density changes more at temperatures near and below 0°C than at any other similar temperature range in the Oceans.

Several strands of evidence indicate that ectotherms living at temperatures around 0°C experience difficulty synthesising proteins that fold to the fully functional state. These include the lack of the usual HSR in some species, often high constitutive HSP production (Box 3) and gene duplication events to produce “extra” HSP70 proteins. The evolution of

additional *HSP70* genes and high levels of constitutive production of HSPs would indicate higher proportions of poorly formed proteins that require HSPs to reach the fully functional state and/or the presence of degraded proteins that need to be removed from the cell. Whilst the energetic cost implications of this elevated production (compared with temperate or tropical species) have yet to be quantified in Antarctic species, an energetic burden has been demonstrated in other species [60].

Polar marine ectotherms synthesise less than 1% of their body protein per day compared to 4-6% for temperate species [61,62], which could be due to any one of several factors, including ecological such as seasonality or overall resource availability. However, the proportion of protein retained, as opposed to broken down and recycled is only 15%-20% compared to 25%-95% (mean = 52%) in temperate species, indicating a smaller proportion of functional proteins is made [61]. Evidence of difficulty making proteins at low temperature also comes from analyses of RNA:protein ratios, which are significantly higher in polar than temperate or tropical species [62]. RNA concentration is a measure of the signal needed to produce proteins. The higher the RNA signal compared to the amount of protein produced, the more problems are expected during protein synthesis on the ribosome and folding [62].

Good evidence of high levels of denatured proteins in polar marine species also comes from studies showing high levels of ubiquitination in species living near 0°C. Ubiquitin binds to malformed or denatured proteins, which is the signal within the cell to begin the process of degradation and recycling of the amino acids. Recent studies on Antarctic fish have shown that ubiquitination levels are significantly higher than for fish at lower latitudes [63].

Concluding remarks

There are five strong pieces of evidence that the production of functional proteins is significantly more difficult for polar marine species: Proteins are markedly less stable at low polar temperatures [46-48]; Antarctic species often have additional HSP70 proteins and several species display higher levels of constitutive expression of at least one *HSP70* family compared to warmer water species [52-55]; Proportions of synthesised proteins retained as opposed to recycled are significantly higher in low temperature species [61]; RNA:protein ratios are higher at low temperatures [62]; Protein ubiquitination levels are high in cold polar species [63].

The result of these difficulties is a chronic increase in HSP production at extreme low temperature. When combined with a slowed capacity to produce fully functional proteins, this is the likely explanation for the cold marine physiological transition to markedly slowed rates in a range of processes in polar marine species, which is achieved through diverted resources and limited protein supply. This factor also explains extended gametogenesis times and very large egg size, because of increased energetic losses as more proteins are recycled. Problems producing functional proteins forms a pervasive effect that overrides other factors when temperatures fall to near 0°C in the sea.

It is clear that progress in this field is now limited by lack of understanding of the mechanisms that impair the production of functional proteins in cold polar species and research needs are prescient on, for example, detailed investigations of protein folding at low temperatures and the role of low temperature seawater density and viscosity changes in this process.

The meta-analyses presented here on the effects of temperature on biological functions of marine animals demonstrate that tropical and temperate species follow expectations from the relationships developed by Arrhenius and van't Hoff over 100 years ago. They also show that Antarctic species living near or below 0°C do not, and are slowed well beyond expectations. Functions reflecting aerobic processes, however, are not affected in this way. It is clear that Antarctic marine species have been unable to fully adapt their growth, development and SDA duration to polar conditions, and that there is a low temperature limit to adaptation in the sea. There is strong evidence indicating that this limit is brought about by the effects of low temperature on protein synthesis. What causes this sudden change in ability to make functional proteins at temperatures around zero remains unknown. The implications of the low temperature limit to adaptation for capacities to respond to environmental change are amongst the next major questions that need to be addressed in this field (see outstanding questions box).

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438

Table 1. Q_{10} coefficients for physiological rates comparing adjacent 5°C temperature blocks. Development is the time taken for brooding gastropod molluscs to reach the free living juvenile stage (Fig 1a). Growth is Richards or von Bertalanffy K coefficient for echinoids (Fig 1c). MO_2 is bivalve mollusc oxygen consumption (Fig 2a). SDA duration is the time taken to complete the SDA following feeding for marine ectotherms (Fig 3b).

	Temperature comparison blocks (°C)				
	<5 vs 5-10	5-10 vs 10-15	10-15 vs 15-20	15-20 vs 20-25	20-25 vs 25-30
Development	12.1	3.25	2.10	1.47	2.23
Growth (K)	5.08	1.60	1.16	1.90	3.13
MO_2	3.21	2.69	1.67	1.15	2.62
SDA duration	6.34	1.95	1.29	1.09	0.79

447 Text for box 1

448

449 **Temperature, biological processes and the Arrhenius relationship**

450

451 The rates that chemical and biological processes function at increase with temperature. This
452 is because the molecules involved in the relevant reactions have more energy at higher
453 temperatures. Any reaction involves populations of molecules interacting with each other,
454 and only a proportion of those molecules have sufficient energy to complete the reaction,
455 and this is called the activation energy. At higher temperatures a larger proportion of the
456 molecules present are above the activation energy and the reaction proceeds faster (Fig 1).
457 Over 100 years ago two scientists made dramatic breakthroughs in understanding this effect
458 of temperature on reaction rate. Van't Hoff worked from empirical data and identified that
459 chemical reactions usually double to treble for each 10°C rise in temperature.

460 Arrhenius also worked from an empirical basis and recognised that the rate of reactions
461 depended on the energy state of the molecules involved in the reaction, where only those
462 molecules above a certain energy state could complete the reaction. He called this energy
463 threshold the activation energy (E_a), and realised that at higher temperatures a larger
464 proportion of molecules present are above the activation energy, making reaction rates
465 faster (Fig. 1). Arrhenius then went on to derive an equation relating chemical reaction rate
466 to temperature:

467

468 $$k = PZ \exp\left(\frac{Ea}{RT}\right)$$

Where *k* is the rate constant, P a measure of the reactivity of molecules, Z is the collision factor, E_a the activation energy, R the gas constant and T absolute temperature.

Rearranging the Arrhenius equation above shows a plot of log rate against 1/T should produce a straight line with a slope of $E_a/4.575$, which allows activation energies to be calculated from empirical data.

Both the van't Hoff Q_{10} and Arrhenius relationship are used widely in analyses of the effect of temperature on biological rates. There are problems with this approach, in that both assume that only temperature changes in the system, and they do not allow for interactions of many factors, such as multiple reactions running concurrently in a cell. Because of this it may be viewed as surprising that biological systems predominantly fit Arrhenius relationships when temperature changes, but a very large body of data published over the last 100 years has demonstrated that biological rates predominantly do follow the Arrhenius relationship over the normal biological temperature range.

Box 1 Figure I

Figure legend. A population of molecules at a given temperature has a range of energy states that, at normal biological temperatures approximates a normal distribution, but as temperature decreases on the Kelvin scale the distribution becomes progressively positively or right skewed. At higher temperatures, as shown here, the energy state is higher because of increased kinetic energy in the system, and the energy of a given molecule can be calculated from the Maxwell-Boltzmann distribution. For any reaction a molecule requires

sufficient energy to complete the reatction and this is celled the activation energy (E_a). At any temperature only a portion of the molecules have sufficient energy to complete the reaction, as shown by the shaded area. At higher temperatures a larger proportion of the molecules in the population are above the E_a and this is why chemical and biological reaction rates are faster at higher temperatures.

Text for Box 2:

Temperature effects on the Specific Dynamic Action of feeding (SDA)

Animal metabolic rates rise after feeding for a period before returning to pre-feeding levels (Fig. 1). The metabolic rise is usually measured as oxygen consumed and reflects increased costs associated with the handling and digestion of food and a range of post-absorptive functions, including breakdown and synthesis of proteins, transport of absorbed materials and growth [11,32]. The two main SDA components commonly reported are peak height and the response duration. The peak height is measured as the factorial rise over pre-feeding metabolic rate where e.g. a doubling of metabolism produces a peak height of 2 and a trebling 3 (Box 2, Fig. 1). The duration is the time from the first elevation of metabolism following feeding to the return to pre-feeding levels. The area under the curve is a measure of the total energy used in digestive and post-absorptive processes associated with a meal, and is called the SDA coefficient.

There is evidence that the maximum SDA peak height is set by the capacity of the oxygen delivery system, and is analogous to aerobic scope for sedentary or slow moving animals [11]. This evidence includes that the maximum metabolic rate achieved in the SDA peak was the same as the maximum metabolic rate measured in experiments where specimens were warmed to their upper temperature limits. This was shown for both the limpet *Nacella concinna* and the brachiopod *Liothyrella uva*. The former had an SDA peak of 2.3 at 0°C while the latter had one of 1.6. When both were exposed to elevated temperatures to

522 evaluate upper temperature limits the respective rise to maximum over routine metabolism
523 at 0°C was 2.4 and 1.6 [11,33]. Thus two mechanisms for raising metabolic rate produced
524 the same two maxima, which was interpreted as indicating the SDA peak was a limit set by
525 the aerobic delivery system [11]. This limit is probably reached due to the combination of
526 several processes working in tandem. After the peak metabolic rates remain elevated for
527 some time, and during this period growth continues to the end of the SDA duration [11,34],
528 even though digestive processes have ceased. The growth and protein synthesis elements
529 of the rise in metabolism thus account for only part of the SDA peak, but are a larger part of
530 the overall SDA coefficient.
531
532 Metabolic rates of ectotherms decline with environmental temperature across latitudes
533 [27,28] (this article Fig. 2). Because SDA peak height is related to routine or standard
534 metabolic rate factorially, and this relationship does not change with environmental
535 temperature (this article Fig. 3a) the maximum amount of energy generated at peak is less
536 at lower temperatures (Box 2, Fig. 2). Thus, for example, if routine metabolic rate doubles
537 for a 10°C rise in environmental temperature and the SDA peak rise is 2.2 then the energy
538 produced at peak is 4.4 units at the warmer temperature compared to 2.2 at the lower
539 temperature. This means that SDA processes can be completed more rapidly at the warmer
540 temperature and results in an extended duration as temperature declines.
541
542
543 Box 2 Figure I
544

Figure legend. Schematic SDA. a: metabolic rate (as oxygen consumed) rises after feeding to a peak value and then declines back to pre-feeding levels. The measure of peak height is factorial and in the example shown the peak reached is 2.2 times higher than pre-feeding metabolic rate. The routine measures made of SDA are factorial peak height and duration (time from initial rise above pre-feeding metabolic rate to return to that level). 0 indicates the day of feeding. b: At lower temperatures the factorial peak height remains the same, but the duration increases.

Box 2 Figure II

Figure Legend. Schematic diagram of the effect of temperature on the absolute amount of power available at the SDA peak. The curve shown is for a hypothetical taxon with a routine oxygen consumption rate of 1 µmol O_2 h^{-1} at 0°C. Oxygen consumption rises with temperature across the range to tropical values around 30°C. The increase used is a Q_{10} of 2.27, which is the mean of the values for bivalve mollusc MO_2 across latitudes in main text Table 1. An SDA peak of x2 is used for illustration. This doubles metabolic rates for work, and at 5°C 1.6 µmol O_2 h^{-1} of equivalent power is available, whereas at 20°C this rises to 5.1 µmol O_2 h^{-1}. Although the factorial SDA peak rise is constant more power is available at higher temperatures for work. Fig based on [11].

568

569 **Text for Box 3**

570

571 **The Heat-Shock Response in Antarctic Ectotherms**

572

573 The only claimed universal response to stress in organisms is the heat-shock response (HSR),
574 where heat-shock proteins (HSP) are produced in response to thermal challenges [49] and a
575 range of other stressors, including dehydration in plants and insects [50]. The HSPs are a
576 large family of proteins with many different forms in different species, with a range of
577 functions. In normal cells they help mis-folded proteins to attain or regain their functional
578 states. They also target degraded proteins and regulate their removal from the cell, helping
579 to prevent the formation of cytotoxic aggregates [51]. The best studied of these are the
580 70kDa family, the HSP70 proteins.
581 Investigations of the HSR in Antarctic marine ectotherms began in 2000 when Hoffman *et al.*
582 [52] showed the fish *Trematomus bernacchii* lacked the classic HSR. Further studies
583 demonstrated that most, if not all Antarctic fish cannot up-regulate HSP70 in response to
584 external stresses, probably because a mutation in the promoter region of the *HSP70* gene
585 prevented HSF1 binding and later transcription [46]. However, they do all permanently
586 express the form of HSP70 which is normally produced in response to stress in other species
587 [52-54].
588 The invertebrates are more complex. Two species, the clam *Laternula elliptica* and the
589 limpet *Nacella concinna* both have an HSR, but these proteins are only induced in
590 laboratory experiments at 8°C-10°C and 15°C respectively [55], significantly above any
591 temperatures experienced by these species for millions of years. However, there might be

592 an HSR in the natural environment, as *N. concinna* exhibited induction of different HSP70
593 family members in response to changes associated with the spring sea-ice thaw, exposure
594 during tidal cycles and chronic low level thermal challenges [56,57]. Investigations of two
595 Antarctic krill species (*Euphasia superba* and *E. crystallorophias*) showed gene duplication
596 events resulting in multiple forms of HSP70 in both species, and a relatively weak HSR [58].
597 The Antarctic amphipod *Paraceradocus gibber* and the starfish, *Odontaster validus*, were
598 both reported to have no demonstrable thermal HSR [54,59]. Not all the invertebrates
599 studied above have, however, have shown high constitutive (permanent) expression of
600 *HSP70* genes, but these studies only investigated a limited range of HSP family members.
601 The paradigm that life at permanent low temperature requires elevated constitutive
602 expression of one or more HSP70s remains to be disproved.
603 There are, therefore, three groups of cold polar ectotherm in terms of HSR (Fig 1). These
604 are species that lack an HSR as any increase in HSP production in response to thermal
605 challenges, but that have high constitutive HSP levels (all Notothenioid fish investigated to
606 date and some invertebrates). The second group are invertebrates that lack an HSR and
607 appear not to constitutively produce any HSPs. The third group have an HSR, but their
608 induction is complex and difficult to predict. This latter group, like the fish might
609 constitutively produce one or more HSP70 family members.
610
611 Box 3 Figure I.
612
613 Figure legend. Schematic showing the different types of heat shock response and HSP
614 production in Antarctic marine ectotherms. To the left is the group that have no HSR, but
615 have high constitutive HSP levels. The middle group are species that lack an HSR and have

not been demonstrated to have increased constitutive levels of HSPs. The right hand group have an HSR that is not induced in experiments until temperatures exceed any experienced by these species for millions of years, and they seem to have high constitutive production of some HSPs.

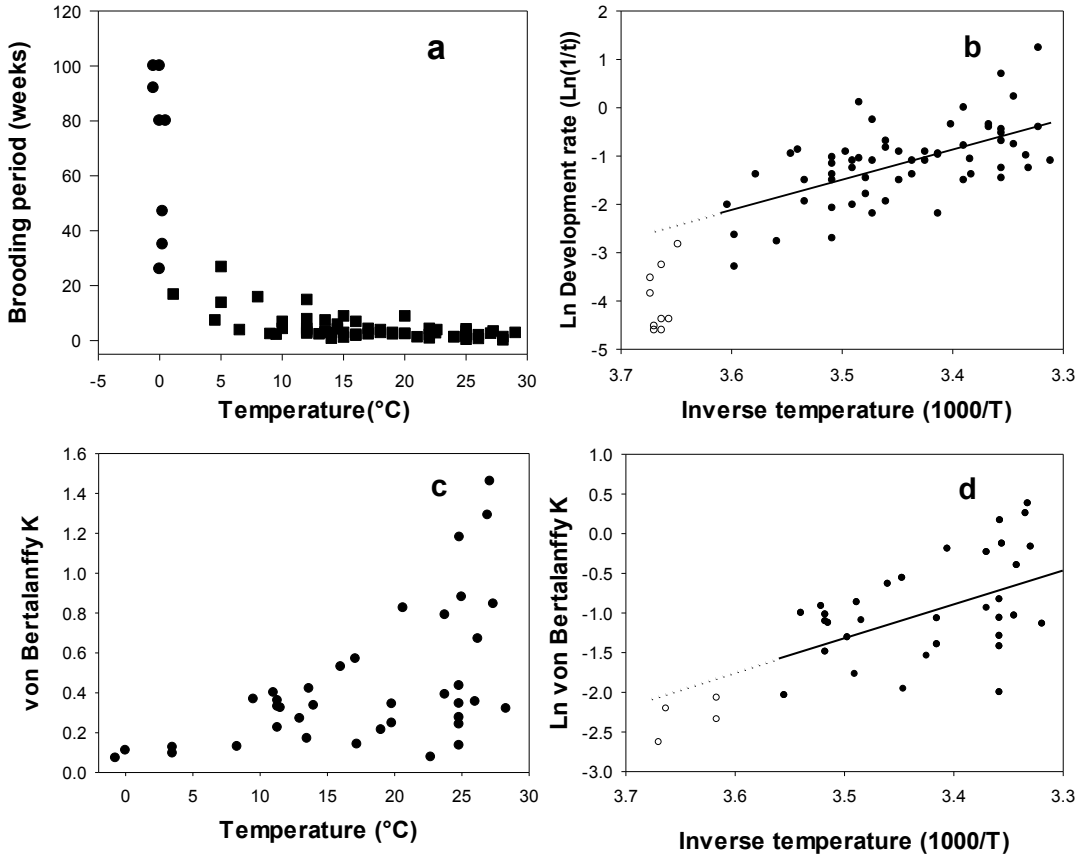
Figure legends:

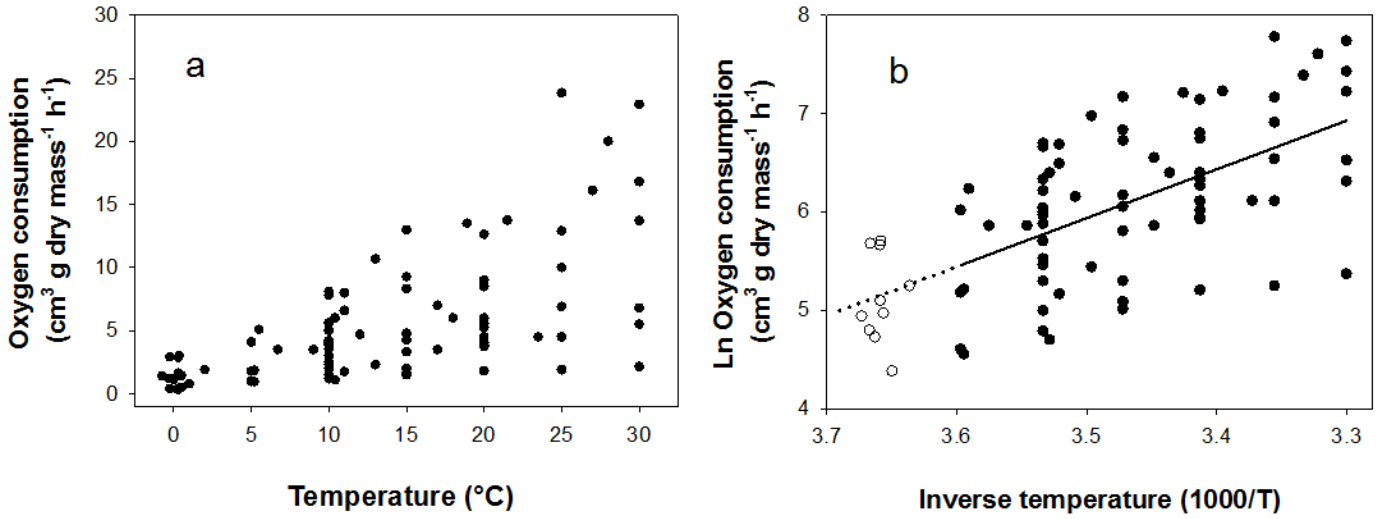
Figure 1. Development, growth, and regeneration rates at ambient temperatures for tropical to polar species. 1a: Time from brood initiation to release ($\frac{1}{\text{development rate}}$) for brooding gastropod molluscs. In most cases release is of crawling juveniles, but for 2 Antarctic species, *Torellia mirabilis* and *Marseniopsis mollis* release is of veliger larvae and development time to juvenile is approximately double that of brooding *per se* [8]. Data shown for these species is the full development period to juvenile. Circles denote Antarctic species, squares denote non-Antarctic gastropods. 1b: Arrhenius plot of Ln developmental rate to juvenile stage for brooding gastropod molluscs. Open circles denote Antarctic, closed circles denote Temperate and tropical gastropods. Fitted line is for temperate and tropical species (brooding rate ($\frac{1}{\text{weeks}}$) = 20.37 - 6.25($\frac{1000}{T}$); $r^2 = 0.36$, F = 32.4, 58 df, P<0.001) where T is absolute temperature; 1c: Richards or von Bertalanffy K coefficients for echinoids from tropical to polar latitudes plotted against habitat temperature. 1d: Arrhenius plot of K for echinoid growth rate. Solid line is the relationship for temperate and tropical species (Ln K = 13.72 - 4.297($\frac{1000}{T}$); $r^2 = 0.27$, F = 25.1, 66 df, P<0.001); dotted line is an extension of this relationship to polar temperatures. For Figs 1a and 1c raw data and sources are given in supplementary tables 1 and 2. Each data point represents a single species, and where there is more than 1 record in the literature the value plotted is the mean of rate and temperature.

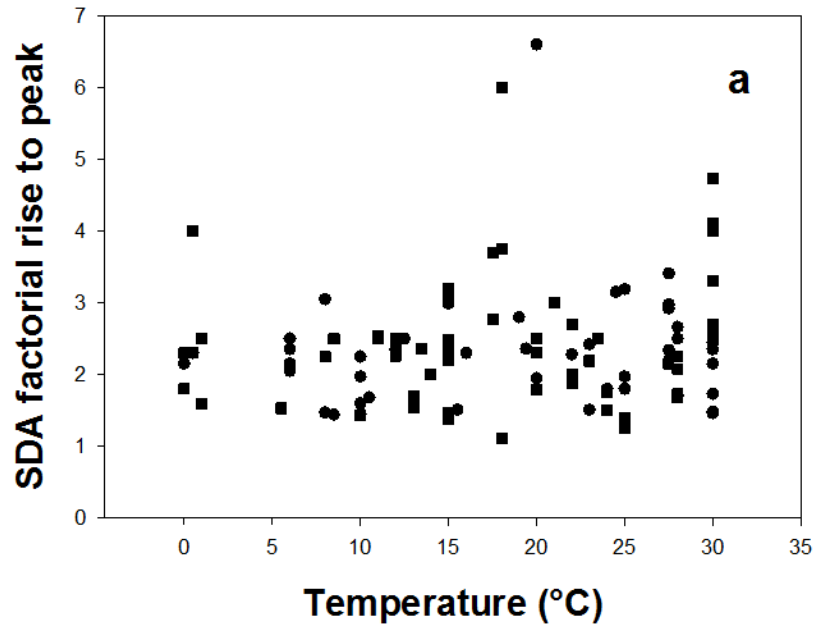
644 Figure 2a. Routine oxygen consumption (MO_2, cm^3 g dry tissue $mass^{-1}$ h^{-1}) at ambient
645 temperature for bivalve molluscs from tropical to polar latitudes. Each data point represents
646 a single species, and where there is more than 1 record in the literature the value plotted is
647 the mean of rate and temperature. 2b: An Arrhenius plot for Ln MO_2 for bivalve molluscs.
648 Symbols as in Fig 1b. The fitted line is the relationship for temperate and tropical species
649 ($LnMO_2 = 23.25 - 4.947(\frac{1000}{T})$; $r^2 = 0.29$, F = 28.9, 71 df, P<0.0001); the dotted line is the
650 extension of the relationship for temperate and tropical species to polar temperatures. Raw
651 data and sources are given in supplementary table 3.
652
653 Figure 3. SDA data for marine ectotherms at ambient temperature from the tropics to the
654 poles. 3a: SDA peak height (factorial peak (FP) rise in MO_2 over pre-feeding level). There is
655 no significant relationship with temperature ($FP= 0.232 + 0.009T$, $r^2=0.00$, $F_{1,110}=0.85$,
656 P=0.358, VIF= 1.00). 3b: SDA duration (days), which is the time taken from the initiation of a
657 rise in MO_2 following feeding to the return to pre-feeding levels. Each data point represents
658 a single species, and where there is more than 1 record in the literature the value plotted is
659 the mean of rate and temperature. 3c: An Arrhenius plot of SDA duration where the solid
660 line is the relationship for temperate and tropical species (Ln SDA (days) = -11.13
661 $+ 3.32(\frac{1000}{K})$; $r^2=0.107$, F=10.4, P<0.0001, 88 df); the dotted line is the extension of the
662 relationship for tropical and temperate species to polar temperatures. In all plots each data
663 point represents a single species, and where there is more than 1 record in the literature the
664 value plotted is the mean for duration and temperature. Closed symbols are for temperate
665 and tropical species living at mean temperatures above 5°C, open symbols are for polar

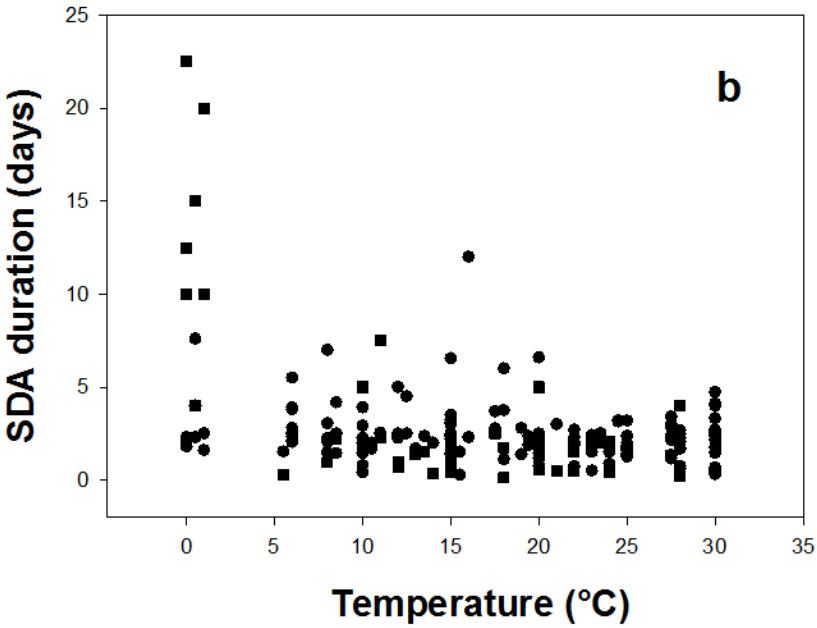
species living permanently near or below 0°C; ●= marine invertebrates; ■ = marine fish.

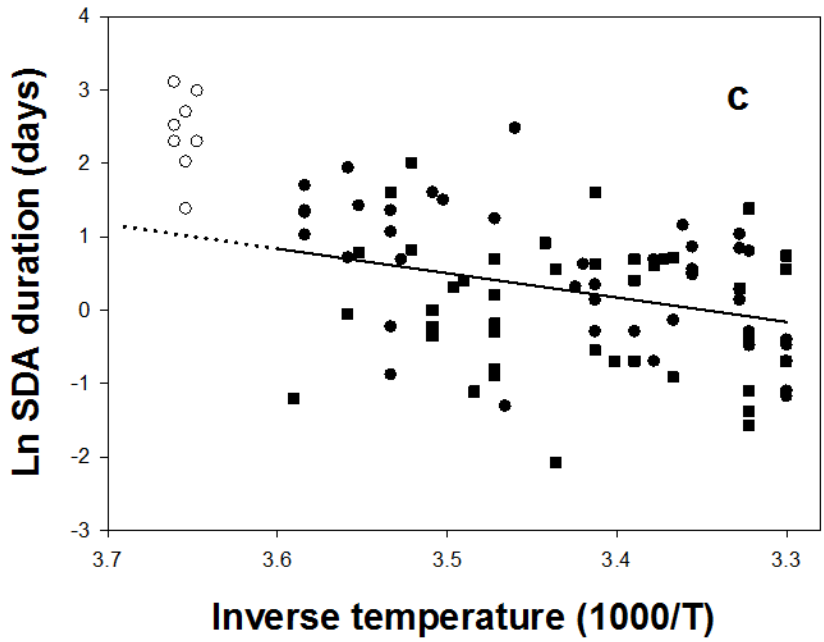
Raw data and sources are given in supplementary table 4.

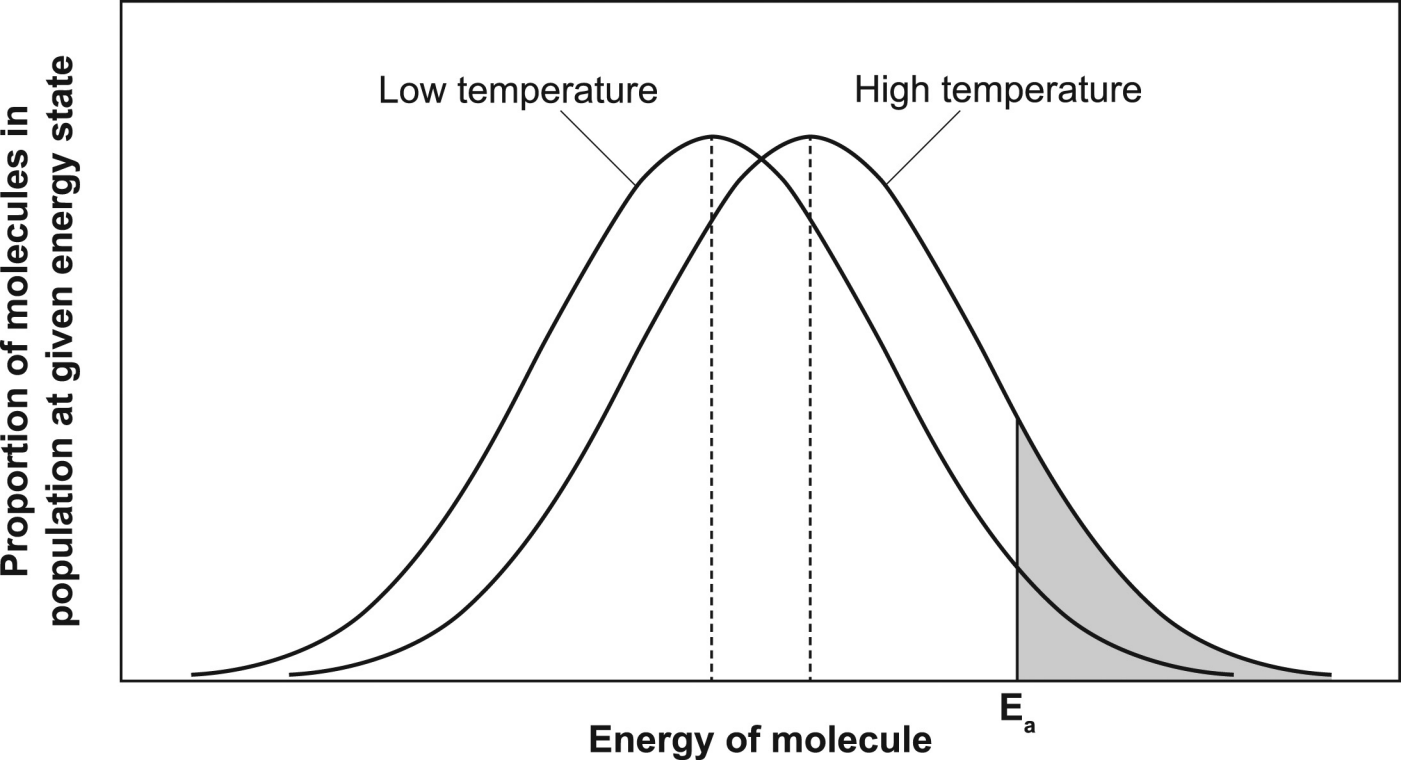

a
Brooding period (weeks)
Temperature(°C)
b
Ln Development rate (Ln(1/t))
Inverse temperature (1000/T)
c
von Bertalanffy K
Temperature (°C)
d
Ln von Bertalanffy K
Inverse temperature (1000/T)

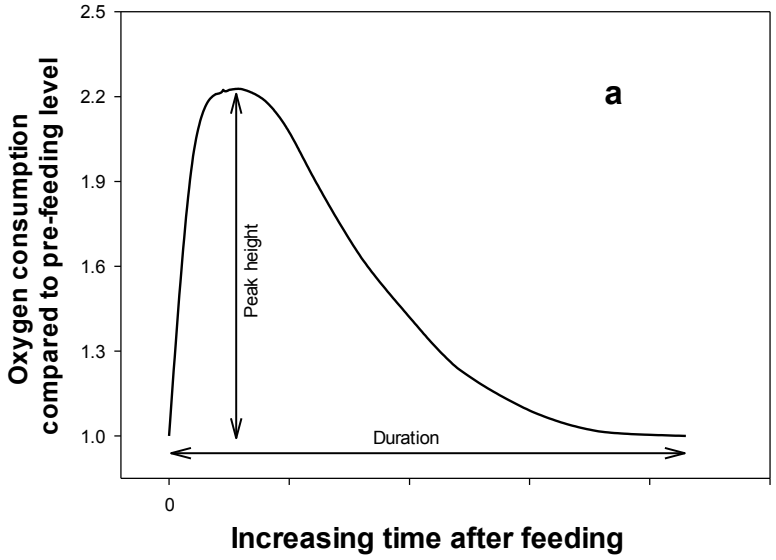

a
Oxygen consumption
(cm3 g dry mass-1 h-1)
Temperature (°C)
0
5
10
15
20
25
30
b
Ln Oxygen consumption
(cm3 g dry mass-1 h-1)
Inverse temperature (1000/T)
4
5
6
7
8
3.7
3.6
3.5
3.4
3.3

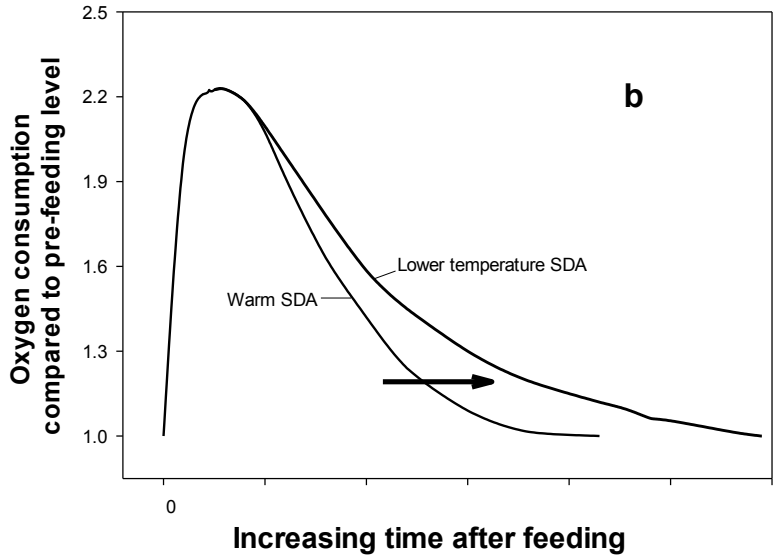

SDA factorial rise to peak
a
Temperature (°C)

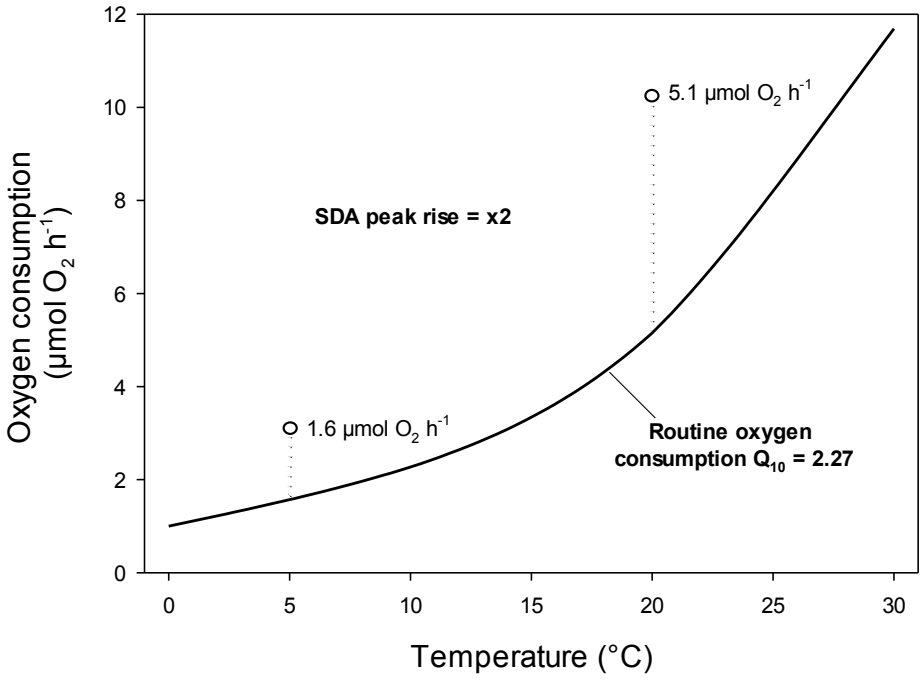

SDA duration (days)
b
Temperature (°C)

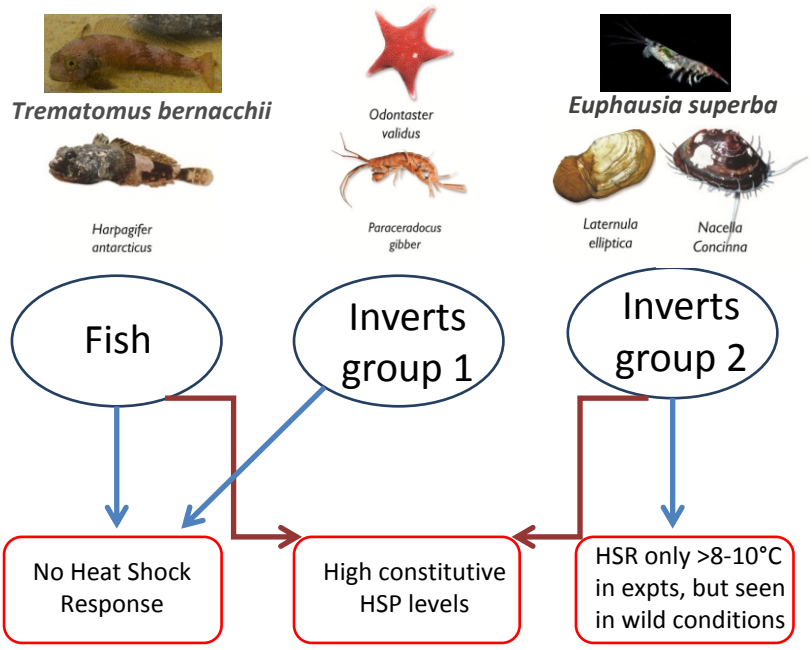

Ln SDA duration (days)
c
Inverse temperature (1000/T)


Low temperature
High temperature
Proportion of molecules in population at given energy state
E_a
Energy of molecule


a
Oxygen consumption compared to pre-feeding level
2.5
2.2
1.9
1.6
1.3
1.0
Peak height
Duration
0
Increasing time after feeding


b
Oxygen consumption compared to pre-feeding level
2.5
2.2
1.9
1.6
1.3
1.0
Lower temperature SDA
Warm SDA
0
Increasing time after feeding


Oxygen consumption (µmol O_2 h^{-1})
Temperature (°C)
5.1 µmol O_2 h^{-1}
1.6 µmol O_2 h^{-1}
SDA peak rise = x2
Routine oxygen consumption Q_{10} = 2.27
0
2
4
6
8
10
12
0
5
10
15
20
25
30


Trematomus bernacchii
Harpagifer antarcticus
Odontaster validus
Paraceradocus gibber
Euphausia superba
Laternula elliptica
Nacella Concinna
Fish
Inverts group 1
Inverts group 2
No Heat Shock Response
High constitutive HSP levels
HSR only >8-10°C in expts, but seen in wild conditions