

1 **A series of unfortunate events:**

2 **Characterizing the contingent nature of physiological extremes using long-term environmental records**

3 W. Wesley Dowd^{1*} and Mark W. Denny²

4 ¹Washington State University, School of Biological Sciences, Pullman, WA 99164, USA

5 ²Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950, USA

6 *Corresponding author: wes.dowd@wsu.edu

7
8 School of Biological Sciences

9 Washington State University

10 P.O. Box 644236

11 Pullman, WA 99164-4236, USA

12 (office) 1-509-335-8122

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15 threshold

16 **ABSTRACT**

17 Accelerating shifts in global climate have focused the attention of ecologists and physiologists on
18 extreme environmental events. However, the dynamic process of physiological acclimatization
19 complicates study of these events' consequences. Depending on the range of plasticity and the
20 amplitude and speed of environmental variation, physiology can be either in tune with the surroundings
21 or dangerously out of synch. We implement a modified quantitative approach to identifying extreme

events in environmental records, proposing that organisms are stressed by deviations of the environment from the current level of acclimatization, rather than by the environment's absolute state. This approach facilitates an unambiguous null model for the consequences of environmental variation, identifying a unique subset of events as 'extremes.' Specifically, it allows one to examine how both the temporal extent (the acclimatization window) and type of an environmental signal affect the magnitude and timing of extreme environmental events. For example, if physiology responds to the moving average of past conditions, a longer acclimatization window generally results in greater imposed stress. If instead physiology responds to historical maxima, longer acclimatization windows reduce imposed stress, albeit perhaps at greater constitutive cost. This approach should be further informed and tested with empirical experiments addressing the history-dependent nature of acclimatization.

33 INTRODUCTION

34 In light of ongoing dramatic shifts in global climate, extreme environmental events have become a focus
35 for ecologists and environmental physiologists [1-9]. However, study of the biological consequences of
36 these events is hampered by complexities inherent in the dynamic interaction between organism and
37 environment. These complexities include physiological acclimatization, the process by which organisms
38 adjust their physiology to maintain function under prevailing environmental conditions. This
39 acclimatization takes time [e.g., 10]—in a temporally variable world, physiology is often chasing the
40 environment. Depending on the range of an organism's physiological plasticity (which can be small or
41 large), the environmental signal(s) to which an organism responds (which could incorporate many
42 aspects of temporal variation), and the amplitude and speed of environmental fluctuations (which vary
43 drastically among habitats), an organism's physiology can be well adjusted to potential stressors or
44 dangerously out of synch. Thus, our ability to predict extreme events and their consequences hinges not
45 only on our ability to predict changes in the physical environment but also on our understanding of the
46 complex temporal mechanics of acclimatization [6, 8].

47 At present, this understanding is woefully incomplete. For example, experimental studies of acclimation
48 (the laboratory analog of acclimatization) typically measure an organism's physiological response to a
49 step change to a new constant environment, such as the change in thermal tolerance caused by moving
50 from a constant low temperature to a constant higher temperature [11, 12]. Although such experiments
51 provide useful demonstrations of the degree of physiological plasticity, it is difficult to translate their
52 results to nature, where environments vary continuously. A next step toward disentangling the
53 interactions between environment and physiology might be to obtain paired time series of a pertinent
54 environmental factor and simultaneous physiological capacity; e.g., a year-long record of water
55 temperature and the co-occurring thermal limits of an aquatic organism. From these paired records,

time-series analysis [13] could identify pertinent aspects of thermal history that affect the acclimatization of thermal tolerance. For instance, an organism's current level of acclimatization might be correlated with the average daily maximum temperature encountered in the preceding three days. One could then predict how a shift in environmental variability would affect the likelihood of the organism exceeding its thermal limits. Furthermore, this knowledge would allow one to more efficiently address the physiological mechanisms underlying acclimatization (e.g., production of heat-shock proteins, shifts in membrane fluidity)[14] by focusing the design of future experiments. However, to our knowledge, there are few, if any, available time series pairing high-frequency measurements of physiological limits with environmental records. Existing physiological time series are typically restricted to either short durations or infrequent (~monthly) sampling [15, 16]. The reasons for this are largely logistical. For instance, measuring a species' thermal limits requires killing many organisms; a lengthy time series of such measurements in any single population would be impractical, if not unethical. The current paucity of physiological time series does not preclude theoretical exploration of how acclimatization could affect the magnitude and likelihood of stressful events. To that end, in this exploration we:

1. Identify several potential environmental signals to which physiology might acclimatize,
2. Use environmental time-series from representative habitats to analyze how the temporal extent of each environmental signal affects the magnitude and frequency of stressful events,
3. Discuss physiological studies that are needed to further clarify the relationship between environmental variation and physiological acclimatization.

We couch our discussion in the context of elevated body temperature and its relationship with organismal physiology, a choice driven by three considerations. (1) Concerns over global change give

issues of temperature variation unusual and immediate importance. (2) Unlike other environmental factors (e.g., salinity, pH, oxygen concentration), the biological effects of temperature change are nearly universal [17]; thus, any conclusions likely apply across taxa. (3) Long-term temperature records are readily available.

We make a simplifying assumption regarding the physiological consequences of environmental variation: that the stress placed on an organism scales, perhaps nonlinearly, with the difference between the current state of acclimatization (that is, what the organism's physiology 'expects') and what the environment imposes. This deviation-based index of environmental stress is both easily quantified and intuitive. For example, if, based on recent experience or endogenous rhythms, an organism has acclimatized to a winter temperature of 10°C, sudden imposition of 25°C (a 15°C deviation) could be stressful. For the same organism acclimatized in summer to 20°C, imposition of 25°C (a mere 5°C deviation) would likely be benign.

We acknowledge that this simple, deviation-based characterization of stress does not consider all potential aspects of the complex physiology involved in organism/environment interactions. For example, instead of (or in addition to) the magnitude of deviation, the acute rate of environmental change during a stressful event might be important [18, 19]. It is also possible that the same magnitude of deviation might have different consequences in different seasons. These aspects could be integrated in the future, but here we concentrate solely on the magnitude of deviation.

This idea of extremes as local 'anomalies' or 'pulses' against a background that is itself dynamic has recently received attention [3, 20, 21]. However, this approach is far from universal, and studies often do not account for acclimatization. For example, even recent studies of extreme temperatures address only the highest values within one season [e.g., 9]. We suggest that the deviation-based method

101 represents an improved, unambiguous null model for the degree of physiological stress experienced by
102 organisms inhabiting realistically dynamic environments.

103 **Environmental signals for acclimatization**

104 Our first task is to identify aspects of the environment ('signals') to which physiology might respond to
105 maintain organismal performance. Here, we evaluate four of many possible indices that plausibly could
106 serve as environmental signals in the context of thermal physiology:

- 107 (1) the *average temperature*, the moving, weighted average of all temperatures encountered
108 over some recent time interval: the *acclimatization window*;
- 109 (2) the *average daily maximum (or minimum) temperature*, the moving, weighted average of
110 daily maxima (or minima) over the window;
- 111 (3) the *absolute maximum (or minimum) temperature*, the single highest (or lowest) value
112 encountered over the window;
- 113 (4) the *historic periodic temperature*, the temperature 'expected' by an organism's physiology
114 for predictable, periodic fluctuations based on information from previous experience or
115 inherited from prior generations. For example, if endogenous rhythms tell an organism that air
116 temperature fluctuates sinusoidally with a 365-day period, it can prepare its physiology
117 accordingly. (Note that this mechanism provides a means to remove the lag between
118 environmental change and acclimatization intrinsic to the first three indices.)

119

120 It is possible that different traits respond to different environmental signals [22], or that other indices
121 (and combinations of indices) could inform physiology, but these four provide a broad set of heuristic
122 examples.

123 Here, we model the adjustment of physiology to prevailing environmental conditions via acclimatization
124 as a continuous process; we assume that physiology is constantly updated based on the most recent
125 index value. For example, if the signal is the average temperature in a 7-day acclimatization window, we
126 assume that, at any given time, the organism is as adjusted as its physiology allows to the previous 7
127 days. Note that this assumption does not imply that physiology is necessarily adjusted to the current
128 temperature. For instance, during a period of continuous temperature increase, physiology will be
129 acclimatized to a temperature cooler than that the organism currently experiences.

130 To calculate the average-temperature index, we use a moving, locally-weighted smoothing algorithm (a
131 modified LOWESS regression) [23] (Figure 1). Given a time-series of environmental temperature data
132 (e.g., hourly air temperature), a weighted linear regression is fitted to the data within an acclimatization
133 window of n temperatures preceding and including the current time point. [By contrast, the standard
134 LOWESS approach uses a window extending in both directions from the current point.] The weight given
135 to each temperature in the window declines for temperatures farther in the past, thus implementing an
136 implicit assumption that physiology is most influenced by the most recent conditions (we return to this
137 assumption in the Discussion). The value of the weighted regression at the current time is then taken as
138 the expected (acclimatized) temperature. This retrospective calculation can be repeated for all points in
139 the time-series except for the initial $n-1$ points.

140 Indices 2 and 3 are then estimated for the same acclimatization window. To calculate the average daily
141 maximum temperature index, we reapply the locally weighted algorithm using only the daily maxima in
142 the acclimatization window preceding, but not including, the present day (today's maximum is not yet
143 known). For the absolute-maximum temperature index, we simply record the highest temperature in
144 the acclimatization window preceding, but not including, the present.

To estimate the historic periodic temperature index, we average temperatures at each given time in a year across all years of the environmental time series. For example, for a 20-year time series, we average all 20 measurements of the temperature at 8:40 am on March 23 to generate the index for that date and time in any year. This procedure captures both circadian and annual temperature fluctuations, but it smooths over tidal fluctuations, whose lunar period aligns with neither the solar calendar nor the 24-h clock.

The acclimatization window—which incorporates both the interval over which the environment is monitored and the time allowed for physiological adjustment—could vary from extremely short cycles [e.g., tidal cycles, 24, 25] to diel oscillations [26] to annual cycles or perhaps even longer periods [27]. Our analyses address this potential diversity by repeating the calculations above using acclimatization windows from 4 hours to 1 year. To calculate temperature deviations for a given signal index and acclimatization window, we subtract the time series of expected/acclimatized temperatures from the corresponding time series of measured values. These results allow us to dissect the effect of acclimatization-window duration on the resulting distribution of temperature deviations, and thereby on the proposed degree of physiological stress. The acclimatization window acts as a low-pass filter; the longer the window, the less sensitive a signal is to high-frequency thermal variation and the rate of temperature change. As a result, the magnitude of deviations depends on both acclimatization-window length (the strength of the low-pass filter) and frequency-dependent variation in the thermal environment.

Definitions: ‘extreme’ versus ‘threshold-exceeding’

Given a time series of thermal deviations, our next task is to identify the subset of deviations that are potentially of physiological, ecological, and evolutionary interest. However, we must first deal with a

semantic issue. Typically, these events are referred to as ‘extreme,’ and we have used this term informally above. However, dictionary definitions of ‘extreme’ encapsulate two distinct ideas:

1. *An event is extreme if it is both rare and far from the average.* This statistical definition refers to the low-probability events in a distribution’s tails. For example, one might reasonably propose that, to be extreme, a high-temperature event must fall within the highest x% of all measurements, where x is small [e.g., 9].
2. *Alternatively, an event is extreme if it exceeds prescribed bounds.* In a biological context, this refers to events that exceed some functional threshold (e.g., the critical thermal maximum) and, therefore, have deleterious consequences. For example, Gutschick and BassiriRad [4] defined extremes as events that exceed an organism’s acclimatory capacity.

The same concepts apply to values in the lower tail of a distribution or values that fall below some lower critical threshold (e.g., temperatures below the freezing point). Biologists have struggled to reconcile these two components—statistical rarity and biological consequences—into utilitarian definitions of an extreme event [1, 4, 28-30].

To avoid confusion, henceforth we refer to events that satisfy Definition 1 as *extreme*, and those that exceed a functional threshold—and, therefore, satisfy Definition 2—as *threshold-exceeding*. The distinction is useful because a given event need not satisfy both definitions: events that are rare may not have functional consequences, while events that exceed a given threshold might not be rare. For example, limited plasticity of upper thermal-tolerance thresholds, observed in many ectotherms [12], implies absolute constraints on physiological limits. Consequently, the warmest temperatures in a particularly warm spell of a warm year could exceed thermal tolerance thresholds without falling in the uppermost percentiles of deviations from expected. The distinct terms *threshold-exceeding* and *extreme* allow clear discussion of such circumstances.

In the analyses below, we focus on the delineation of extreme and threshold-exceeding events from environmental datasets. Using Definition 1, we (arbitrarily) define extreme events as those lying in the top 5% of a distribution, whether that distribution is the raw distribution of temperatures (raw extremes) or the distribution of deviations (deviation extremes); the Supplement includes results for other percentiles. In some scenarios physiologists have identified seasonal or otherwise appropriate absolute-temperature thresholds [e.g., 10, 31], but we are unaware of comparable deviation-based biological threshold estimates. To identify threshold-exceeding deviations from acclimatized temperature using Definition 2, we explore the consequences of setting a range of threshold values. We systematically examine patterns of extreme and threshold-exceeding events when the percentile/threshold, the environmental signal, and the length of the acclimatization window change.

Temperature datasets

To illustrate the resulting patterns, we employ three representative time series, with a focus on environments for which it is reasonable to assume that operative body temperatures of organisms follow recorded environmental patterns (for details of each record, see Supplemental Table 1):

1. A 29-year record of air temperature at an arctic field site, recorded hourly [32];
2. An 18-year record of mid-latitude desert soil temperature, recorded hourly at a depth of 20 cm [33];
3. A 7-year record of near-shore tropical ocean temperature, recorded every half hour [34].

With few exceptions [e.g., some corals; 35] aquatic ectotherms' body temperatures reliably mirror the temperature of the surrounding liquid medium [36]. Similarly, body temperatures of small, soil-dwelling organisms likely equal habitat temperature. By contrast, above-ground terrestrial ectotherms' body temperatures can be heavily influenced by other biophysical factors (e.g., solar irradiance, evaporation),

physiological processes (e.g., heat generation), body size [37], and/or behavioral thermoregulation [12, 38], leading to large differences between body and air temperatures [36]. Therefore, our record of arctic air temperature approximates that of a hypothetical small ectotherm in a shaded habitat.

The distributions of raw habitat temperatures vary in shape across these datasets (Figure 2, insets); none is normally distributed as is often modeled [39]. The tropical-sea distribution is left-skewed, the arctic-air distribution is relatively flat topped, and the desert-soil distribution is bimodal.

RESULTS

Expectations matter: The magnitude of extremes and frequency of threshold-exceeding events depend on the environmental signal and length of the acclimatization window

The average magnitude of extreme deviations is sensitive to acclimatization-window length, but the pattern of sensitivity differs dramatically depending on the acclimatization signal. For the average-temperature and average-daily-maximum temperature signals, longer acclimatization windows generate smoother, less variable time-series of physiological expectations. Consequently, the magnitude of deviations tends to increase as the window lengthens, particularly in the desert-soil and arctic-air datasets (Figure 3A for top 5% of deviations; Supplemental Figure 1A,B,F,G for a range of percentiles). In other words, if organisms in these habitats adjust in response to a moving average, longer acclimatization windows result in greater imposed stress. Physiological lags, most likely acting in concert with excessive energetic costs, prohibit near-instantaneous acclimatization [40]. Nonetheless, if organisms in these habitats respond to some moving average of environmental conditions, they should minimize the length of the acclimatization window; otherwise, they risk being physiologically under-prepared for the next extreme elevated temperature. This pattern is, however, not universal. In the tropical-seawater dataset, the mean magnitude of extreme deviations is maximal at an intermediate

acclimatization window and is much less sensitive overall to changes in the acclimatization window (Fig. 3A; Supplemental Figure 1K,L).

In contrast to moving-average signals, if the organism adjusts its physiology in response to the absolute-maximum-temperature signal, longer acclimatization windows result in extremes with *decreasing* mean deviations for all three datasets (Figure 3B for top 5% of deviations; Supplemental Figure 1D,I,N for a range of percentiles). The longer the acclimatization window, the greater the likelihood that one of the few highest values in the overall distribution will be encountered. Consequently, for long acclimatization windows nearly all experienced temperatures are lower than the temperature to which the organism is acclimatized, and the average of extreme deviations becomes increasingly negative (Figure 3B). Thus, organisms that acclimatize to long-term absolute maximum temperatures are physiologically adjusted to higher temperatures than they are likely to soon encounter. This conservative strategy might backfire if costs of thermal defenses are high [41], and over-preparation could influence life-history tradeoffs or related biological phenomena [42]. However, it is not uncommon to find organisms with median thermal tolerances above mean annual maximum temperatures [e.g., 41, 43], perhaps suggesting that costs of thermal defenses are not always substantial.

Endogenous programming of responses to periodic temperature changes does not necessarily decrease the magnitude of extreme deviations. For short acclimatization windows (less than approximately 25-30 days), in the desert-soil and arctic-air datasets mean extreme deviations from the historic periodic signal (dashed lines in Figure 3A) are *larger* than those from the average-temperature signal. However, this pattern reverses at longer acclimatization windows. In other words, historic knowledge would reduce the intensity of extremes encountered in these two habitats only if the only other option were to respond to a relatively long-term moving average. Over timescales of a few weeks, historic knowledge of periodicity is—somewhat counterintuitively—not beneficial. The picture is again different for tropical-

ocean temperatures, for which deviations from moving average signals are always less than those from the historic periodic signal. This is likely due to variation in this dataset being driven more by episodic upwelling of cold water and by tidal rhythms than by diel or annual oscillations (Supplemental Figure 2). In sum, the utility of knowledge of environmental periodicity depends on the predictability with which the environment fluctuates.

In order to integrate temporal acclimatization with Definition 2 for threshold-exceeding events, we selected a range of deviation magnitudes that might serve as a biological threshold in each dataset. For a given threshold deviation and window length, acclimatizing to the average daily maximum temperature always results in fewer threshold-exceeding events than acclimatizing to the average temperature index (Figure 4A; Supplemental Figure 3A,B,E,F,I,J). While the frequency of threshold-exceeding events rises monotonically in the arctic air and desert soil datasets for these two indices, as before the tropical-seawater dataset is unique. It exhibits local maxima in the frequency of threshold-exceeding events at an intermediate window length (Supplemental Figure 3I,J). Using the absolute-maximum-temperature index, the longer the acclimatization window the fewer events cross a given threshold deviation (Figure 4B; Supplemental Figure 3C,G,K; Supplemental Figure 4C,G,K). Knowledge of historic periodic temperature reduces the number of threshold-exceeding events per year relative to the moving average indices for the arctic-air and desert-soil datasets, but only for long acclimatization windows (Figure 4A; Supplemental Figure 3); such historic knowledge is never advantageous relative to any length window for tropical-sea temperatures (Supplemental Figure 3L). As above for the magnitude of deviation extremes, the historic periodic temperature acclimatization strategy is advantageous in some habitats only if organisms are otherwise restricted to lengthy acclimatization windows.

The temperatures of threshold-exceeding events and extremes need not fall in the tails of a raw distribution

Many temperatures that would be considered mundane in the distribution of raw values nonetheless represent substantial deviations from what an organism expects based on its thermal history (Figure 2B,D,F). For example, only temperatures above 14.3°C fall in the top 5% of the raw temperature distribution for the arctic-air dataset, whereas a temperature as low as -33.3°C (only the 6th percentile of the raw data) qualifies as a warm deviation extreme with a 7-d acclimatization window. This context-dependency of an extreme represents perhaps the greatest advantage of defining such events based on deviations from the expected, while also presenting a formidable challenge in identifying the appropriate length of acclimatization window for any given organism (see below).

There is negligible overlap between measurements identified as deviation extremes and those that would be considered raw extremes (see red points in Figure 2). This degree of overlap increases as the acclimatization window increases in length, but it rarely exceeds 50% even with windows of up to one year (Supplemental Figure 5). At the limit, with exceedingly long windows, deviation extremes will converge on raw extremes. However, such long acclimatization windows are likely to be rare in nature; many organisms live less than a year, and a litany of studies across many taxa have documented short-term plasticity of thermal physiology [reviewed elsewhere, e.g., 12, 39].

DISCUSSION

Challenges for the context-dependent approach

One advantage of this context-dependent, quantitative approach is that it makes concrete, testable predictions. Some of these are counterintuitive. For example, it will now be incumbent upon physiologists who study overwintering to ascertain whether sudden deviation to a warmer temperature,

302 still well below the freezing point, can induce the same sorts of cellular perturbations (e.g., of
303 membrane or enzyme structure) that accompany 'canonical' heat stress [14].

304 There are at least three challenges that must be overcome to take full advantage of this context-
305 dependent approach to extreme and threshold-exceeding events. First, further empirical work is needed
306 to delineate the signals organisms use to set environmental expectations. Progress in this respect has
307 been hampered by physiologists' experimental designs, which only rarely have included realistic
308 temporal patterns of environmental variation [e.g., 44, 45]. The optimal signal likely varies considerably
309 among habitats (in our analyses tropical seawater consistently exhibits unique patterns) and among
310 organisms, depending at least in part on the relative contributions of physiology (acclimatization
311 capacity) and behavior (ability to modulate environmental exposure) [12]. Although beyond the scope of
312 this contribution, a synthetic analysis of the timescales of acclimatization in organisms from a variety of
313 habitats exhibiting different spectral qualities of temperature variation would be very informative. In
314 such an analysis, the suite of possible signals identified here could function as null models, yielding
315 testable predictions regarding the magnitude and frequency of stressful events under alternative
316 acclimatization strategies.

317 A second potential challenge involves the length and sampling frequency of the environmental time
318 series itself. If stressful events occur randomly in time, as they often do [7], the longer the time over
319 which the distribution of events is measured, the more extreme are the raw values encountered [a
320 fundamental conclusion of Extreme Value Theory; 46]. Thus, even in the absence of climate warming,
321 the highest temperatures encountered in a year typically are lower than the highest temperatures
322 encountered in a decade. This issue poses a potential problem to the statistical delineation of
323 extremes—the distribution of raw extreme values varies with the length of the environmental time
324 series. However, preliminary assessment of artificially shortened versions of the datasets analyzed here

indicates that the mean and standard deviation of the magnitude of deviation extremes are relatively insensitive to the length of the time series (Supplemental Figure 6). This observation warrants further attention to examine its generality. In addition, sampling frequency must be sufficiently high [47]; to capture the rate of acute change during individual extreme events requires at least hourly sampling.

A third challenge involves the under-explored role of carryover effects between repeated events that are separated by intervals of varying lengths. While much discussion in the climate-change biological literature focuses on changes in the frequency and intensity of extreme events in a warmer and more variable world [e.g., 2], these events are often treated as discrete, isolated incidents. However, as noted by Gutschik and BassiriRad [4], when environmental conditions exceed biological thresholds they impart a ‘legacy’ to an organism, modifying (at least temporarily) the manner in which it interacts with its environment. The duration of this legacy varies, and the underlying mechanisms might vary with duration [48]. At one end of the spectrum, hardening has effects that appear to dissipate after one or a few days [e.g., 49, 50]. Developmental plasticity, in which early experience irreversibly alters adult traits [51, 52], often takes considerably longer. Furthermore, while we may be tempted to conclude that what matters most for an organism is its experience during its lifetime, evolutionary processes, transgenerational effects of environmental experience, and possibly other mechanisms link organisms and environments across considerably longer time spans [14, 53, 54]. The simple approach we have taken here does not incorporate these carryover effects. For example, we implicitly—but unrealistically—assume that the physiological effect of a given magnitude of thermal deviation is the same before and after another threshold-exceeding or extreme event. Furthermore, for some organisms, the rate of acclimatization as temperatures rise is different from that as temperatures fall [11, 55]. These complexities demand greater empirical attention.

Given the likelihood of carryover effects [8], studies of threshold-exceeding and extreme environmental conditions must consider their temporal relationship to each other. In this regard, it is imperative not only to quantify (or forecast) the magnitude of extreme or threshold-exceeding events, but also to quantify distributions of the intensity of extremes (duration x magnitude) and inter-event intervals. These metrics are perhaps the most relevant to forecasting the biological consequences of climate change [2, 56]. For example, warm extreme and threshold-exceeding events tend to occur in clusters (heat waves). Heat waves already show signs of increasing frequency, which may have profound biological effects [21, 57-59].

Along with a focus on the temporal distribution of extreme events must come experimental designs that mimic patterns that currently – or soon will – occur in nature and repeated measurements of the state of biological systems experiencing those patterns [6, 60]. We believe that physiologists must redouble their efforts to understand the contingent nature of thresholds [61], including their relationship with developmental or life-history transitions. In each of these experimental scenarios, physiologists will need to quantify shifts in critical thresholds, metabolic or developmental rates, and other indicators of functional impairment. A greater emphasis on sublethal thresholds should also provide valuable insight [e.g., 62]. Finally, nonlinearities in physiological (or ecological) rate functions could have profound impacts on the likelihood of exceeding functional thresholds [63], particularly in the likely event that those thresholds vary through time and/or among individuals. Much difficult work remains to be done in reconciling the concepts of extreme and threshold-exceeding in the context of thermal biology in nature.

CONCLUSIONS

When discussing the biological consequences of environmental fluctuations, and particularly when expounding on likely impacts of global change, biologists often assume that the intensity and frequency

of extreme events will increase in the future. A modified approach to delineating extreme and/or threshold-exceeding events from environmental records reveals that incorporating the dynamic process of acclimatization can fundamentally change the subset of environmental conditions that should be the focus of environmental physiologists and ecologists. This approach recognizes and attempts to incorporate the potentially complex, context-dependent interactions between organisms and their variable environments. It also offers unambiguous, testable predictions of the magnitude and frequency of stressful events from long-term environmental records. We hope and expect that, ultimately, a more comprehensive, mechanistic synthesis will emerge from empirical study of biological systems facing these extra-ordinary circumstances.

Data accessibility

See Supplemental Table 1 for links to publicly available data records. Matlab code is available at https://github.com/wwdowd/Dowd_Denny_thermal_deviations.

Authors' contributions

W.W.D. and M.W.D. developed ideas, analyzed datasets, drafted the manuscript, and approved its publication.

Competing interests

We have no competing interests.

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- 394 1. Smith M. D. 2011 An ecological perspective on extreme climatic events: a synthetic definition
395 and framework to guide future research. *J Ecol* **99**, 656-663.
- 396 2. Jentsch A., Kreyling J. and Beierkuhnlein C. 2007 A new generation of climate-change
397 experiments: events, not trends. *Front Ecol Environ* **5**, 365-374.
- 398 3. Harris R. M. B., Beaumont L. J., Vance T. R., Tozer C. R., Remenyi T. A., Perkins-Kirkpatrick S. E.,
399 Mitchell P. J., Nicotra A. B., McGregor S., Andrew N. R., et al. 2018 Biological responses to the press and
400 pulse of climate trends and extreme events. *Nature Clim Change* **8**, 579-587.
- 401 4. Gutschick V. P. and BassiriRad H. 2003 Extreme events as shaping physiology, ecology, and
402 evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist*
403 **160**, 21-42.
- 404 5. Buckley L. B. and Huey R. B. 2016 Temperature extremes: geographic patterns, recent changes,
405 and implications for organismal vulnerabilities. *Global Change Biol* **22**, 3829-3842.
- 406 6. Dowd W. W., King F. A. and Denny M. W. 2015 Thermal variation, thermal extremes, and the
407 physiological performance of individuals. *J Exp Biol* **218**, 1956-1967.
- 408 7. Denny M. W., Hunt L. J. H., Miller L. P. and Harley C. D. G. 2009 On the prediction of extreme
409 ecological events. *Ecol Monogr* **79**, 397-421.
- 410 8. Williams C. M., Buckley L. B., Sheldon K. S., Vickers M., Pörtner H.-O., Dowd W. W., Gunderson
411 A. R., Marshall K. E. and Stillman J. H. 2016 Biological impacts of thermal extremes: Mechanisms and
412 costs of functional responses matter. *Integr Comp Biol* **56**, 73-84.
- 413 9. Kingsolver J. G. and Buckley L. B. 2017 Quantifying thermal extremes and biological variation to
414 predict evolutionary responses to changing climate. *Phil Trans Roy Soc B* **372**, 20160147.
- 415 10. Buckley B. A., Owen M. E. and Hofmann G. E. 2001 Adjusting the thermostat: the threshold
416 induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a
417 function of thermal history. *J Exp Biol* **204**, 3571-3579.
- 418 11. Healy T. M. and Schulte P. M. 2012 Factors affecting plasticity in whole-organism thermal
419 tolerance in common killifish (*Fundulus heteroclitus*). *J Comp Physiol B* **182**, 49-62.
- 420 12. Gunderson A. R. and Stillman J. H. 2015 Plasticity in thermal tolerance has limited potential to
421 buffer ectotherms from global warming. *Proc R Soc B* **282**, 20150401.
- 422 13. Diggle P. J. 1991 *Time series: a biostatistical introduction*. New York, Oxford University Press;
423 257 p.
- 424 14. Somero G. N., Lockwood B. L. and Tomanek L. 2017 *Biochemical Adaptation: Response to*
425 *Environmental Challenges from Life's Origins to the Anthropocene*. Sunderland, MA, Sinauer Associates,
426 Inc.; 572 p.
- 427 15. Bourget E. 1983 Seasonal variations of cold tolerance in intertidal mollusks and relation to
428 environmental conditions in the St. Lawrence Estuary. *Can J Zool* **61**, 1193-1201.
- 429 16. Udaka H. and Sinclair B. J. 2014 The overwintering biology of the acorn weevil, *Curculio*
430 *glandium* in southwestern Ontario. *J Thermal Biol* **44**, 103-109.
- 431 17. Dell A. I., Pawar S. and Savage V. M. 2011 Systematic variation in the temperature dependence
432 of physiological and ecological traits. *Proc Natl Acad Sci USA* **108**, 10591-10596.
- 433 18. Mitchell K. A. and Hoffmann A. A. 2010 Thermal ramping rate influences evolutionary potential
434 and species differences for upper thermal limits in *Drosophila*. *Func Ecol* **24**, 694-700.
- 435 19. Terblanche J. S., Hoffmann A. A., Mitchell K. A., Rako L., le Roux P. C. and Chown S. L. 2011
436 Ecologically relevant measures of tolerance to potentially lethal temperatures. *J Exp Biol* **214**, 3713-
437 3725.

20. Zscheischler J., Mahecha M. D., von Buttlar J., Harmeling S., Jung M., Rammig A., Randerson J. T., Schölkopf B., Seneviratne S. I., Tomelleri E., et al. 2014 A few extreme events dominate global interannual variability in gross primary production. *Environ Res Lett* **9**, 035001.

21. Hobday A. J., Oliver E. C. J., Gupta A. S., Benthuyzen J. A., Burrows M. T., Donat M. G., Holbrook N. J., Moore P. J., Thomsen M. S., Wernberg T., et al. 2018 Categorizing and naming MARINE HEATWAVES. *Oceanography* **31**, 162-173.

22. Reyer C. P. O., Leuzinger S., Rammig A., Wolf A., Bartholomeus R. P., Bonfante A., de Lorenzi F., Dury M., Gloning P., Abou Jaoudé R., et al. 2013 A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Global Change Biol* **19**, 75-89.

23. Cleveland W. S. and Devlin S. J. 1988 Locally weighted regression: An approach to regression analysis by local fitting. *J Am Stat Assoc* **83**, 596-610.

24. Williams E. and Somero G. 1996 Seasonal-, tidal-cycle- and microhabitat-related variation in membrane order of phospholipid vesicles from gills of the intertidal mussel *Mytilus californianus*. *J Exp Biol* **199**, 1587-1596.

25. Gracey A. Y., Chaney M. L., Boomhower J. P., Tyburczy W. R., Connor K. and Somero G. N. 2008 Rhythms of gene expression in a fluctuating intertidal environment. *Curr Biol* **18**, 1501-1507.

26. Overgaard J. and Sørensen J. G. 2008 Rapid thermal adaptation during field temperature variations in *Drosophila melanogaster*. *Cryobiology* **56**, 159-162.

27. Levins R. 1968 *Evolution in Changing Environments*. Princeton, New Jersey, USA, Princeton University Press.

28. Wingfield J. C., Pérez J. H., Krause J. S., Word K. R., González-Gómez P. L., Lisovski S. and Chmura H. E. 2017 How birds cope physiologically and behaviourally with extreme climatic events. *Phil Trans Roy Soc B* **372**, 20160140.

29. van de Pol M., Jenouvrier S., Cornelissen Johannes H. C. and Visser Marcel E. 2017 Behavioural, ecological and evolutionary responses to extreme climatic events: challenges and directions. *Phil Trans Roy Soc B* **372**, 20160134.

30. Bailey L. D. and van de Pol M. 2016 Tackling extremes: challenges for ecological and evolutionary research on extreme climatic events. *J Anim Ecol* **85**, 85-96.

31. Bulger A. J. and Tremaine S. C. 1985 Magnitude of seasonal effects on heat tolerance in *Fundulus heteroclitus*. *Physiol Zool* **58**, 197-204.

32. Environmental Data Center Team. Retrieved 2019. Meteorological monitoring program at Toolik, Alaska. Toolik Field Station, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775. http://toolik.alaska.edu/edc/abiotic_monitoring/data_query.php Supported by National Science Foundation grant 1623461. (

33. Jornada Basin Long-Term Ecological Research (LTER) project and the USDA-ARS Jornada Experimental Range. <https://jornada.nmsu.edu/lter/dataset/49696/view>. Funded by USDA and National Science Foundation grant DEB-1235828. (

34. Physical Monitoring Program, Smithsonian Tropical Research Institute. https://biogeodb.stri.si.edu/physical_monitoring/research/sst. (

35. Jimenez I. M., Kühl M., Larkum A. W. D. and Ralph P. J. 2008 Heat budget and thermal microenvironment of shallow-water corals: Do massive corals get warmer than branching corals? *Limnol Oceanogr* **53**, 1548-1561.

36. Denny M. W. 2016 *Ecological Mechanics: Principles of Life's Physical Interactions*. Princeton, NJ, USA, Princeton University Press; 503 p.

37. Stevenson R. D. 1985 Body size and limits to the daily range of body temperature in terrestrial ectotherms. *Am Nat* **125**, 102-117.

38. Kearney M., Shine R. and Porter W. P. 2009 The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proc Natl Acad Sci USA* **106**, 3835-3840.

486 39. Angilletta M. J., Jr. 2009 *Thermal Adaptation: A Theoretical and Empirical Synthesis*. New York,
487 Oxford University Press Inc.; 289 p.

488 40. Padilla D. K. and Adolph S. C. 1996 Plastic inducible morphologies are not always adaptive: the
489 importance of time delays in a stochastic environment. *Evol Ecol Res* **10**, 105-117.

490 41. Denny M. W. and Dowd W. W. 2012 Biophysics, environmental stochasticity, and the evolution
491 of thermal safety margins in intertidal limpets. *J Exp Biol* **215**, 934-947.

492 42. Flatt T. and Heyland A. 2011 *Mechanisms of Life History Evolution: The Genetics and Physiology*
493 *of Life History Traits and Trade-Offs*. (p. 512. New York, Oxford University Press, Inc.

494 43. Sgrò C. M., Overgaard J., Kristensen T. N., Mitchell K. A., Cockerell F. E. and Hoffmann A. A. 2010
495 A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in
496 populations of *Drosophila melanogaster* from eastern Australia. *J Evol Biol* **23**, 2484-2493.

497 44. Niehaus A. C., Angilletta M. J., Sears M. W., Franklin C. E. and Wilson R. S. 2012 Predicting the
498 physiological performance of ectotherms in fluctuating thermal environments. *J Exp Biol* **215**, 694-701.

499 45. Petavy G., David J. R., Gibert P. and Moreteau B. 2001 Viability and rate of development at
500 different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J*
501 *Thermal Biol* **26**, 29-39.

502 46. Coles S. 2001 *An Introduction to Statistical Modeling of Extreme Values*. London, Springer-
503 Verlag; 209 p.

504 47. Kearney M. R., Matzelle A. and Helmuth B. 2012 Biomechanics meets the ecological niche: the
505 importance of temporal data resolution. *J Exp Biol* **215**, 922-933.

506 48. Teets N. M. and Denlinger D. M. 2013 Physiological mechanisms of seasonal and rapid cold-
507 hardening in insects. *Physiological Entomology* **38**, 105-116.

508 49. Loeschcke V. and Hoffmann A. A. 2007 Consequences of heat hardening on a field fitness
509 component in *Drosophila* depend on environmental temperature. *Am Nat* **169**, 175-183.

510 50. Bilyk K. T., Evans C. W. and DeVries A. L. 2012 Heat hardening in Antarctic notothenioid fishes.
511 *Polar Biol* **35**, 1447-1451.

512 51. Cooper B. S., Tharp J. M., Jernberg I. I. and Angilletta M. J. 2012 Developmental plasticity of
513 thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster*. *J Thermal Biol*
514 **37**, 211-216.

515 52. Schaefer J. and Ryan A. 2006 Developmental plasticity in the thermal tolerance of zebrafish
516 *Danio rerio*. *J Fish Biol* **69**, 722-734.

517 53. Schiffer M., Hangartner S. and Hoffmann A. A. 2013 Assessing the relative importance of
518 environmental effects, carry-over effects and species differences in thermal stress resistance: a
519 comparison of *Drosophilids* across field and laboratory generations. *J Exp Biol* **216**, 3790-3798.

520 54. Salinas S. and Munch S. B. 2012 Thermal legacies: transgenerational effects of temperature on
521 growth in a vertebrate. *Ecol Lett* **15**, 159-163.

522 55. Cossins A. R., Friedlander M. J. and Prosser C. L. 1977 Correlations between behavioral
523 temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic
524 membranes. *J Comp Physiol* **120**, 109-121.

525 56. Rezende E. L., Castañeda L. E. and Santos M. 2014 Tolerance landscapes in thermal ecology.
526 *Func Ecol* **28**, 799-809.

527 57. Stillman J. H. 2019 Heat Waves, the New Normal: Summertime Temperature Extremes Will
528 Impact Animals, Ecosystems, and Human Communities. *Physiology* **34**, 86-100.

529 58. Diffenbaugh N. S. and Ashfaq M. 2010 Intensification of hot extremes in the United States.
530 *Geophys Res Lett* **37**, L15701.

531 59. McKechnie A. E. and Wolf B. O. 2010 Climate change increases the likelihood of catastrophic
532 avian mortality events during extreme heat waves. *Biol Letters* **6**, 253-256.

- 533 60. Thompson R. M., Beardall J., Beringer J., Grace M. and Sardina P. 2013 Means and extremes:
534 building variability into community-level climate change experiments. *Ecol Lett* **16**, 799-806.
- 535 61. Marshall K. E. and Sinclair B. J. 2014 The relative importance of number, duration and intensity
536 of cold stress events in determining survival and energetics of an overwintering insect. *Func Ecol* **29**,
537 357-366.
- 538 62. Zhang W., Zhao F., Hoffmann A. A. and Ma C.-S. 2013 A single hot event that does not affect
539 survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS ONE* **8**, e75923.
- 540 63. Buckley L. B. and Huey R. B. 2016 How extreme temperatures impact organisms and the
541 evolution of their thermal tolerance. *Integr Comp Biol* **56**, 98-109.

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Figure 1.

A modified approach to delineating stressful events in environmental records. Here, a hypothetical organism experiences a series of body temperatures (grey line; 1 week is depicted). Expected values at each time were generated using a retrospective LOWESS smoothing function on hourly data (average-temperature index in the text) with an acclimatization window of 7 days (dashed black line). Extremes can be defined as the largest deviations between the 'observed' and 'expected' conditions. Using this approach, the absolute warmest temperatures are not always extreme. For example, compare the length of the blue arrow (small deviation at high temperature) to that of the red arrow (large deviation at low temperature).

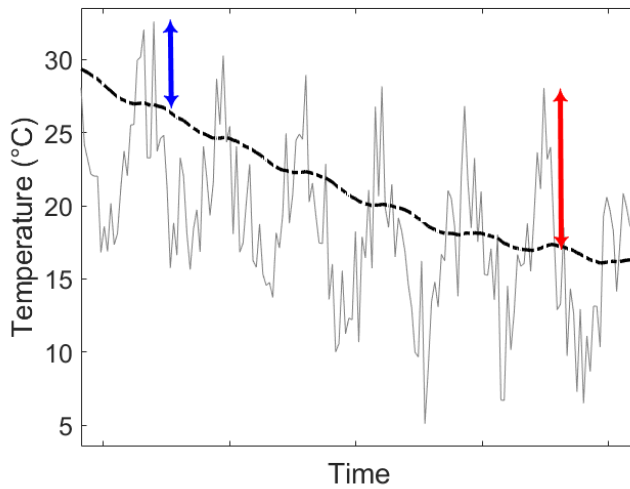
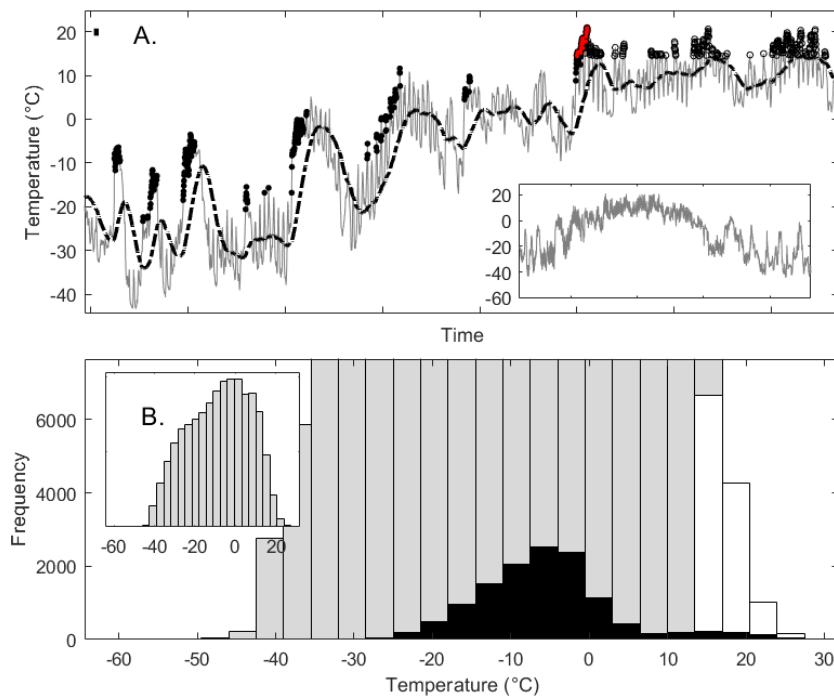
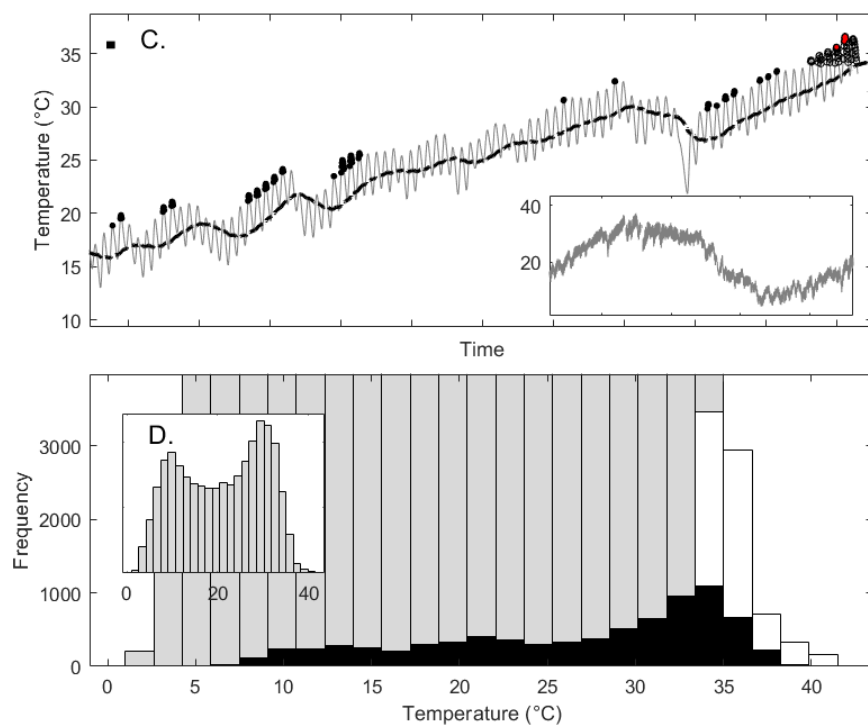


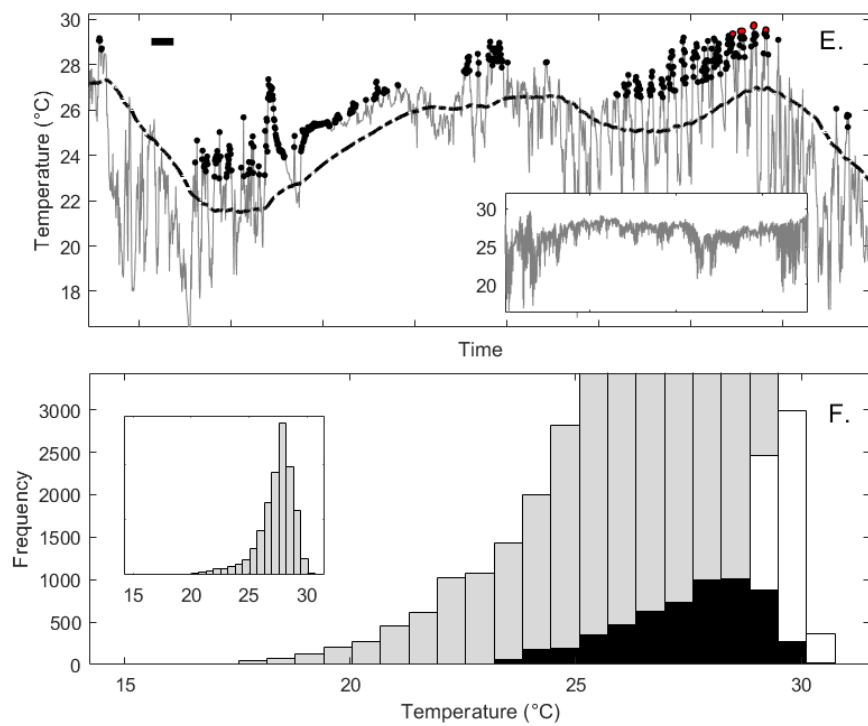
Figure 2.

The distribution of extreme deviations from expected/acclimatized temperature varies across habitat types, and deviations identified as extreme represent a surprisingly broad range of absolute temperatures. A) Temperature extremes for the arctic air dataset, recorded at 1-h intervals (thin grey line). Expected values for each time were generated using the average-temperature index (dashed black line) with an acclimatization window of 7 d. Black circles, deviation extremes (largest 5% of deviations from expected); open circles, raw extremes (largest 5% of raw values); red circles, extreme according to both criteria. The small, black scale bar in the upper left indicates one day. The inset shows one year of data from this location. B) Histograms illustrating the overall shape of the air temperature distribution (light grey background and inset) as well as the distributions of different varieties of extremes. White bars indicate raw extremes. Superimposed black bars indicate deviation extremes. C) and D) same as A) and B) but for mid-latitude desert soil at 1-h intervals. E) and F) same as A) and B) but for 0.5-h intervals in the tropical seawater dataset.





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Figure 3.

The magnitude of high-temperature deviation extremes varies among habitat types, and it is sensitive to the choice of acclimatization signal. A) The mean of the top 5% of deviations from expected values generated using the average temperature index for the three datasets. Deviations were calculated for acclimatization windows from 4 h to 1 yr. Horizontal, dashed lines indicate the mean extreme deviations using the historic periodic temperature index. B) The mean of deviation extremes generated using the absolute maximum temperature index, for acclimatization windows of 4 h to 1 yr. Note the difference in y-axis scales. Mean deviation extremes for other acclimatization signals and percentiles that could be used to define an extreme are found in Supplemental Figure 1.

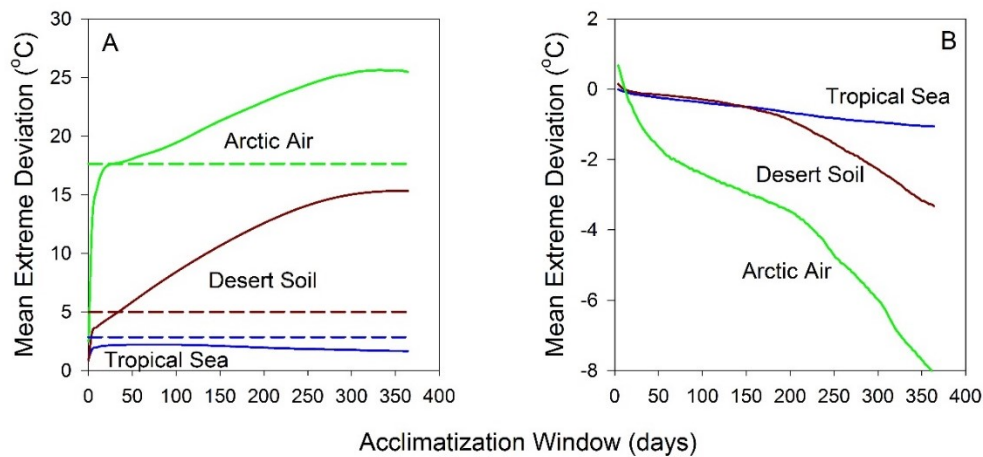


Figure 4.

The number of threshold-exceeding events per year in the arctic air dataset varies among acclimatization signals and as the acclimatization window increases in length. A) Events crossing a deviation-based threshold of 6.5, 8.5, or 10.5°C for the average temperature index (black) or the historic periodic temperature index (blue; independent of acclimatization window length). B) Events crossing a different set of thresholds for the absolute maximum temperature index (most deviations are negative for this index). Analogous plots for the desert soil and tropical seawater datasets are found in Supplemental Figures 3 and 4.

