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The ontogeny of tolerance curves: Habitat quality versus acclimation in a stressful environment

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Summary

1. Stressful environments affect life-history components of fitness through (i) instantaneous detrimental effects, (ii) historical (carry-over) effects, and (iii) history-by-environment interactions, including acclimation effects. The relative contributions of these different responses to environmental stress are likely to change along life, but such ontogenic perspective is often overlooked in studies of tolerance curves, precluding a better understanding of the causes of costs of acclimation, and more generally of fitness in temporally fine-grained environments.
2. We performed an experiment in the brine shrimp *Artemia* to disentangle these different contributions to environmental tolerance, and investigate how they unfold along life. We placed individuals from three clones of *A. parthenogenetica* over a range of salinities during a week, before transferring them to a (possibly) different salinity for the rest of their lives. We monitored individual survival at repeated intervals throughout life, instead of measuring survival or performance at a given point in time, as commonly done in acclimation experiments. We then designed a modified survival analysis model to estimate phase-specific hazard rates, accounting for the fact that individuals may share the same treatment for only part of their lives.
3. Our approach allowed us to distinguish effects of salinity on (i) instantaneous mortality in each phase (habitat quality effects), (ii) mortality later in life (history effects), and (iii) their interaction. We showed clear effects of early salinity on late survival, and interactions between effects of past and current environments on survival. Importantly, analysis of the ontogenetic dynamics of the tolerance curve reveals that acclimation affects different parts of the curve at different ages.
4. Adopting a dynamical view of the ontogeny of tolerance curve should prove useful for understanding niche limits in temporally changing environments, where the full sequence of environments experienced by an individual determines its overall environmental tolerance, and how it changes throughout life.

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Data accessibility

All data associated with this study are available at the Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.vb6k6> (Nougé *et al.* 2016)

Keywords

Environmental tolerance curve; Adaptive acclimation; phenotypic plasticity; Habitat quality; Changing environment; Hazard rate; Niche limits

Introduction

Individuals placed in a sub-lethal stressful environment for a short time may be better able to tolerate environmental stress later in life. Such adaptive acclimation (the terms hardening or hormesis are also used for shorter exposures to somewhat stronger stress, Hoffmann, Sorensen & Loeschcke 2003; Gems & Partridge 2008) has received considerable attention from ecologists (reviewed for temperature by Hoffmann 1995; Hoffmann, Sorensen & Loeschcke 2003; Angilletta 2009), notably in the context of climate change (Calosi, Bilton & Spicer 2008; Palumbi *et al.* 2014). Acclimation is thought to result from adaptive phenotypic plasticity of traits influencing fitness, and there is indeed a clear conceptual connection between phenotypic plasticity of traits under stabilizing selection and environmental tolerance curves representing fitness against the environment (Chevin, Lande & Mace 2010; Chevin *et al.* 2013; Lande 2014). Accordingly, a hallmark of the adaptive acclimation hypothesis is that individuals acclimated in a given environment should have higher fitness in that environment than in others – and than individuals acclimated in other environments – (e.g. Leroi, Bennett & Lenski 1994), because they produce a phenotype through phenotypic plasticity that matches the requirements set by this environment (Via & Lande 1985). This prediction implicitly assumes that the environment did not change much between development and selection in the evolutionary history of a species (Moran 1992; Gavrillets & Scheiner 1993; Scheiner 1993; Tufto 2000; Lande 2009).

Somewhat surprisingly, early tests of the adaptive acclimation hypothesis often found that one of the acclimation environments causes individuals to later perform best across all environments, rather than only in that environment (Leroi, Bennett & Lenski 1994; Bennett & Lenski 1997; Gibert, Huey & Gilchrist 2001; Wilson & Franklin 2002; Woods & Harrison 2002). This puzzling discrepancy between results and expectations has been interpreted and explained in three main ways: (i) these studies constitute outright rejections of the beneficial acclimation hypothesis (Leroi, Bennett & Lenski 1994; Bennett & Lenski 1997); (ii) the discrepancy comes from a wrong or incomplete definition for acclimation in these experiments, with respect to either the time scale at which acclimation is thought to occur (Wilson & Franklin 2002), or the geographic scale at which it is likely to evolve (Woods & Harrison 2002); or (iii) these experiments illustrate the importance of costs of acclimation, which are not included in simple predictions (Hoffmann 1995; Hoffmann, Sorensen & Loeschcke 2003). However, acclimation patterns are expected to be altered by costs only if these costs are environment-specific, and not simply broad costs of having the machinery to acclimate, as described in the literature on costs of phenotypic plasticity (Dewitt, Sih & Wilson 1998).

Probably the most unavoidable environment-specific cost of acclimation is that, in order to acclimate to a stressful environment, individuals have to spend time in that environment,

which is likely to depress their fitness – at least during exposure. Such reduction in fitness occurs whenever stress can be attributed (at least partly) to low-quality habitats that are inherently unfavorable and reduce demographic performance by affecting vital rates (survival, fecundity) for all genotypes of a species. This includes habitats that are physically challenging (drought for plants), or offers few resources, unstable conditions, or intense antagonistic interactions (competition, predation or parasitism). In this context, there is a clear trade-off between benefits and costs of acclimation. Indeed, if an individual spends its early life in a stressful environment, it may cope better with stress as an adult because of adaptively plastic developmental or physiological responses, but will also probably incur a direct cost just from spending time in a harsh environment.

Importantly, there is a crucial temporal aspect to this trade-off between cost of stress exposure and benefit of acclimation. If the environment acts as a cue for phenotypic plasticity (e.g., Moran 1992; Gavrillets & Scheiner 1993; Scheiner 1993; Tufto 2000; Lande 2009; Reed *et al.* 2010), then spending more time in a stressful environment may allow integrating environmental information over a longer duration, thus producing a phenotype that is likely to better match the selective pressure, and ultimately increase fitness later in life in this stressful environment. On the other hand, if environmental stress partly results from low habitat quality, then the time spent in a stressful environment reduces overall fitness, through the cumulative effect of stress exposure. As an example, think of the situation where a stressful environment only becomes common late in life. In this scenario, higher lifetime fitness should be reached by spending most time before that age in a benign environment, rather than acclimating for long in the stressful environment and paying the cost of long exposure to stress.

A further temporal issue is that costs of stress exposure may remain even after exposure has stopped, if stress has resulted in poor condition that persisted throughout life. Importantly, the relative contributions from these different components of stress response are likely to change along life. This implies that, whenever the environment varies on a time scale lower than individual lifespan (i.e., is temporally fine-grained), environmental tolerance curves should not be seen as static properties of genotypes and individuals, but rather as dynamic features, with ontogenic trajectories that integrate the full sequence of environments encountered along individual life. However, such a dynamic view of the ontogeny of tolerance curves remains little explored empirically (but see Kingsolver & Woods 2016 for a mechanistic model of heat tolerance with a time component).

Our aim is here to show how a dynamic perspective on the ontogeny of tolerance curves allows efficiently partitioning the different components of stress responses discussed above, yielding a better understanding of how these responses unfold along life. Experimentally and statistically disentangling these effects requires an experiment where:

- (i) the environment is varied along life, by transferring individuals from a range of acclimation environments to a range of final environments;
- (ii) individual life-histories are monitored through time at each stage of the experiment, rather than measuring fitness (or performance as a surrogate) only at a given point in life, as commonly done in studies of tolerance curves;

- (iii) environmental effects on fitness components are estimated as rates per-unit-time, and integrated over duration of exposure, also allowing for current rates to depend on past environments.

To illustrate how this can be performed in practice, we conducted an experiment on salinity tolerance in the brine shrimp *Artemia*, an extremophile invertebrate adapted to thrive across very broad salinity gradients. We then designed a modified survival-analysis model to analyze this experiment. This has allowed us to partition the direct effects of environmental quality on survival (environmental effect, E) from the impact of past environments on future survival (individual history effect, H) and their interaction (HxE effect). In combination, these effects yield a clearer picture of the ontogenic dynamics of tolerance curves throughout life.

Methods

Artemia clonal lineage and nauplii acquisition

Brine shrimps of the genus *Artemia* are halophile branchiopods that are found in the wild over a broad range of high salinities, and can be reared in the laboratory from near seawater (around 35g/L) to around 250g/L (salt saturation occurs at 359g/L) (Browne, Sorgeloos & Trotman 1991). Salinity tolerance in brine shrimps has been a topic of intense research (e.g. Cole & Brown 1967; Vanhaecke, Siddall & Sorgeloos 1984; Browne, Davis & Sallee 1988; Lenz & Browne 1991; Triantaphyllidis *et al.* 1995 ; Browne & Wanigasekera 2000; Castro-Mejía *et al.* 2009; Gajardo & Beardmore 2012; Nougué *et al.* 2015a), both because they are good model extremophile organisms, and because of their use in aquaculture (Browne, Sorgeloos & Trotman 1991). Most of these studies found that the optimal salinity is around 80-100g/L. Survival is possible at lower salinities, but often compromised by the presence of fish predators at low salinities *in natura* (Browne, Sorgeloos & Trotman 1991). In addition, *Artemia* also suffer at very low salinities because of the low tolerance of their gut microbiota to those salinities (Nougué *et al.* 2015a).

Artemia parthenogenetica clonal lineages can easily be bred in the laboratory through parthenogenetic reproduction (Baxeavanis, Kappas & Abatzopoulos 2006). Diploid parthenogenetic females have high fecundity (up to c.a. 100 individuals per brood) and monoclonal lines can be reared in the laboratory for several generations under homogeneous environmental conditions while keeping a single genotype (Nougué *et al.* 2015b). Three monoclonal lines of *A. parthenogenetica* were used in our experiments, in order to determine whether experimental results were consistent and robust across different genetic lineages. They were isolated from single field-sampled female, and maintained in the laboratory for around five generations before the start of the experiment. One line was isolated from the La Mata population in Spain (hereafter LM7) and the other two originated from the Aigues-Mortes saltern population in France (hereafter PAM7 and PAM10). Each line was propagated at 80g/L NaCl with algae for food (*Dunaliella salina*) for the last two generations before the experiment, to control for maternal effects induced by the environment (i.e., transgenerational plasticity, e.g. Salinas & Munch 2012). Culture medium was made by diluting field-collected concentrated brine (280 g/L NaCl) from Aigues-Mortes saltern with osmosed water.

Ten adult females from PAM7 and PAM10 and seven females from LM7 were put in individual 500 mL cups in a 80g/L salt solution and fed every two days with 5 mL of an algae and yeast mix (75% of a 500 000 cells/mL algae solution + 25% of a 1 000 cells/mL yeast solution). For two weeks, we monitored the cups every day for newly hatched nauplii (nauplii hatched during weekends were not used in the experiment). These were immediately placed in individual tubes to run the experiment. After two weeks, we had a total of 1826 nauplii (744 LM7; 748 PAM7; 333 PAM10) divided in 9 cohorts.

Experimental set-up

The experimental protocol included two phases (P_1 and P_2), separated by a transfer of nauplii across salinities. During P_1 , nauplii were individually placed in 50mL tubes with a filter bottom, and evenly dispatched into water tanks at three salinities, with 6 tanks per salinity, evenly distributed between two experimental rooms at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. We used $S_1 = 40, 80$ and 120 g/L, because preliminary work and published literature (Triantaphyllidis *et al.* 1995; Saygı 2004) indicated that this range was likely to cause variation in survival among treatments, while still allowing for enough surviving individuals in all treatments to be transferred to the next phase. After 7 days (the duration of P_1) individual tubes of each clonal lineage from each salinity were randomly dispatched into tanks with four salinities ($S_2 = 40, 80, 120$ and 180 g/L) for the second phase P_2 , which lasted at least four weeks and up to seven weeks, depending on the individual (the experiment was stopped on the same day for all individuals, but individuals entered the experiment at different dates, depending on their hatching day). During these two phases, individuals were fed every day with 1 mL of the same algae and yeast mix as adult females.

Survival was checked twice a week in both P_1 and P_2 , rather than only at the end of the experiment. The timing of death of each individual was recorded to occur in a (possibly open) time interval, resulting in interval- and right-censored data. The duration of the experiment ensured that we followed a substantial portion of the lifetime of most individuals, as can be seen on Figure 1. (More generally the lifespan of *Artemia parthenogenetica* may range from a few weeks to a few months, depending on the conditions of food, temperature, etc).

Modified survival analysis

We developed a modified survival-analysis model for data stemming from acclimation experiments like the one we performed, where an individual spends different parts of its life in different environments, and survival is followed throughout. A key aspect of the model is that mortality risk is estimated in both phases, but survival in the second phase is conditional on survival in the first phase, accounting for the fact that individuals may share the same treatment for only part of their lives. This allows us to split overall survival at each time into instantaneous effects of environmental quality (or direct stress exposure), and “individual memory” effects of S_1 on survival in P_2 .

We used data on individual timing of death (time when last seen alive), in combination with the sequence of salinities, in order to estimate the likelihood of life-history parameters in different phases and environments. The probability that a particular death event occurred in a

given time interval $[t_1, t_2]$ was computed as $S(t_1) - S(t_2)$, where $S(t)$ is the survivorship function, the proportion of all nauplii entering the beginning of phase 1 that survived up to time t into the experiment. The probability that a particular death event occurred after a given time t_1 (right-censored data) was computed as $S(t_1)$. The likelihood of different models was computed by specifying a parametric function for $S(t)$, following standard survival analysis. Defining the hazard function $\mu(t)$ that gives the instantaneous *per capita* mortality risk at time t , we have $S(t) = \exp(-\int_0^t \mu(x)dx)$. We used a Weibull hazard function that allows instantaneous mortality risk to change with age, $\mu_{a,\lambda}(t) = \lambda a (\lambda t)^{a-1}$, where the parameter a determines the shape of the function while λ determines the mean hazard (chapter 25 in Crawley 2012). This function was reparametrized using the transformation $\lambda = \Gamma[1 + 1/a]/L$ (where $\Gamma(\cdot)$ is Euler's gamma function), such that all individuals with a given hazard function $\mu_{a,L}(t)$ have life expectancy L , regardless of the value of parameter a . The associated cumulative hazard function is thus $M_{a,L}(t) = \int_0^t \mu_{a,L}(x)dx = \left(\frac{\Gamma[1 + 1/a]t}{L}\right)^a$.

This ordinary survival analysis was modified to incorporate multiple phases, with transition effects between them. First, phase effects were modelled by explicitly considering piecewise survival curves, which kept track for any given individual of the actual environments it experienced in phases 1 and 2. For instance, individuals exposed to the same environment in phase 1 had the same survival curve in this phase, but possibly different curves later in phase 2 depending on their treatment. The influence of this transition *per se* was modelled by incorporating *delay* and *transfer* effects. For the delay effect, we introduced a parameter δ measuring the temporal lag before the hazard rate starts to depend on the next environment (S_2 in our experiment). Indeed, vital rates might be expected to remain unchanged for some time after the occurrence of the environmental change, for instance because of a lag in the physiological response to the new environment. To implement this, we simply let the hazard rate in the new environment still be determined for some time δ by the previous environment the individual experienced. We also allowed hazard rates to be briefly modified because of the transfer itself (manipulation of individuals, immediate stress effect). We introduced a parameter c measuring this cost of transfer, which increased hazard by a quantity c for a brief duration τ following transfer to S_2 . Given the time scale of observations (every other day), we considered that a period of $\tau = 3$ days would be adequate to capture this brief transfer effect.

Overall, we thus have, denoting as T_1 the duration of phase 1,

$$-\ln S(t) = \begin{cases} M_{a_1, L_1}(t) + c U_{\tau}(t - T_1) & \text{if } t \leq T_1 + \delta \\ M_{a_1, L_1}(T_1 + \delta) + M_{a_2, L_2}(t - T_1 - \delta) + c U_{\tau}(t - T_1) & \text{if } t > T_1 + \delta \end{cases}$$

$$\text{with } U_{\tau}(x) = \begin{cases} 0 & \text{if } x \leq 0 \\ x & \text{if } 0 < x \leq \tau \\ \tau & \text{if } x > \tau \end{cases}$$

Note that in this analysis, hazard rates can vary with age for two reasons. First, hazard rates are Weibull within phases, which allows for some flexibility in their age-dependence. Second, hazard functions are fitted separately in P_1 and P_2 even for individuals that stay at the same salinity, which can also capture some age variation in hazard rates. A diagram schematizing the structure of this survival analysis is represented in Figure 2.

With this overall structure of the survival analysis, we investigated how the parameters changed with the specific combination of salinities in P_1 and P_2 . We allowed the expected lifetime parameter L to depend on several factors and their interactions. We investigated these effects using a log-link function, to ensure positive expected lifetime estimates and a well-defined likelihood. These factors (all treated as fixed effects) were the clone, and the salinity in each phase (S_1 , S_2 , and their interaction). Current salinity is interpreted as a direct environmental effects (E), while past salinity contributes to history or history x environment effects (H / H x E, see Results for more detail). We assumed that phase transition effects c and δ could not vary with salinity, although they could vary with clones. The Weibull age-dependence parameter a was also allowed to potentially vary between P_1 and P_2 , and between clones, but not with salinity. All computations and parameter estimation were implemented in Mathematica 10. We used the Akaike information criterion (hereafter AIC, Akaike 1974) to compare models with different effects of salinity and clone on parameters of the survival curve across phases.

Results

Figure 1 shows the empirical survival curves for different salinities in phases 1 and 2, pooling all clones together (similar curves for each clones are provided as Supplementary Figure S1). The estimated curves from the best model (specified below) are also represented in dashed lines. It is immediately apparent from visual inspection of this data that mortality is higher at 180g/L in phase 2, but less so when the salinity experienced in phase 1 was 120g/L. Another clear feature of these curves is that their slope changes between phases, but seems to do so with a delay, changing at about day 10 while the transfer between phases takes place at day 7. Other patterns are less straightforward to visualize directly, or may be concealed by interactions with clone effects for instance, so we rely on a more formal statistical analysis based on modified survival models. This allows us to partition environmental (E) and history (H) effects on survival, as well as their interaction (H x E).

Model comparison of the survival analysis

We performed an extensive model comparison to detect effects of salinity in each phase (S_1 and S_2), clones, and their interactions, on instantaneous hazard rates in each phase, which determine the dynamics of tolerance curves along life. The results of this model comparison are shown in Table 1. As it is neither practically feasible nor particularly meaningful to compare all possible models when multiple parameters are estimated, we instead focused on a set of biologically meaningful models, as advocated for model selection by Burnham and Anderson (2003). We started by simple models without interactions between phases, to investigate the effect of current salinity on survival as would be estimated ignoring history effects; we then turned to models allowing for a general form of interaction between phases;

lastly, we investigated simplifications for these interactions that correspond to biologically meaningful scenarios.

How does current salinity affect survival ignoring historical effects?—Our first set of models included no history effects, such that survival in each phase only depends on the salinity in this phase and the clone. There was strong evidence for an overall difference in survival between the two phases: the model that constrained the parameters a and L of the hazard function (conditioned on salinity and clone) to be the same in both phases was strongly rejected, with an AIC higher by about 200 than models that did not have this constraint (Model 1 versus Models 2-3). Among models where the life expectancy does change among phases, a model where L_2 is equal to L_1 multiplied by the same factor at all salinities (Model 2) was slightly favored (AIC = 2.09) over a model that allowed L_1 and L_2 to be fully independent (model 3). This indicates that the effect of current salinity of survival is consistent across phases.

Do present and past salinity interact, and does this depend on the clone?—We then considered models with $S_1 \times S_2$ interactions affecting the hazard rate in phase 2, implying an effect of individual history on survival. We started with the most general form for this interaction, whereby a different parameter was fit for each combination of S_1 and S_2 (models 4 to 11, “Full $S_1 \times S_2$ interaction” in Table 1). All these models had much lower AIC than models with no interaction, strongly supporting the occurrence of historical effects. The best models also included a delayed effect of S_1 onto survival in P_2 : removing the delay dramatically increased AIC by 266.76 (Model 5 vs 4) to 355.46 (Model 7 vs 6). In contrast, there was no support for a transfer cost in our data, as removing this effect essentially did not improve likelihood, thus increasing AIC by 2 (Model 4 versus 6). Therefore, unless otherwise stated, all the remaining models (including those above with no $S_1 \times S_2$ interactions) include a delayed effect of S_2 , but no transfer cost.

The effect of salinity on survival across the two phases of the experiment differed between clones, especially distinguishing Aigues-Mortes PAM clones from the La Mata LM7 clone (Figure 3 and Supplementary Figure S1). In order to investigate whether the interaction of past and present salinity was dependent on the genotype, we built models that include clone $\times$ salinity interactions. The most general form of this interaction allows each clone $\times S_1$ combination in P_1 , and each clone $\times S_1 \times S_2$ combination in P_2 , to have its own L parameter in the hazard function. The corresponding model was well supported by our data, decreasing the AIC by 24.93 (Model 8 vs 6). Also allowing the clone to interact with the delay and the shape of the Weibull hazard function further improved AIC by 38.49 (Model 9 vs 8). This led to a very complex model – with 54 parameters –, making the interpretation of the results challenging. However, a substantial reduction in AIC (by more than 20 points) was reached through a key simplification: allowing the clone to interact *only* with the salinity in phase 1, *not* with the $S_1 \times S_2$ interaction (Model 10 in Table 1). That this model was strongly supported implies that individual history effects on survival were consistent across genotypes, and therefore robust. Putting back in the model a transfer cost that interacts with the clone did not improve AIC (model 11, AIC = 3.96).

Do effects of past salinity on survival conform to simple biological

interpretations?—The left panel in Figure 3 represents the estimated effect of salinity on instantaneous contributions to survival, in the best model. The dashed and dotted lines show survival in the first phase against the corresponding salinity S_1 , for the three clones. All clones survive best at 120g/L, and the two clones from Aigues-Mortes (PAM7 and PAM10) survive consistently better than the one from La Mata (LM7). The continuous lines show survival in phase 2 against the “late” salinity S_2 , for different values of the “early” salinity S_1 . This shows that the way survival depends on salinity in phase 2 strongly depends on the salinity experienced in phase 1. Furthermore, the differences between the three curves (corresponding to different S_1) appear to be strongest at the highest late salinity, $S_2 = 180\text{g/L}$. This pattern prompted us to test more specific hypotheses about the interaction between effects of past and current salinity on survival (models 12 to 18 in Table 1). We started by a model with the same features as the best model above, but where $S_1 \times S_2$ interactions only exist for $S_2 = 180\text{g/L}$. In other words, we fitted only one survival parameter at each $S_2 < 180\text{g/L}$, while at $S_2 = 180\text{g/L}$ we fitted a different survival parameter for each value of S_1 . However, this model was not supported by the data, having higher AIC by 9.36 (Model 12 in Table 1). Restricting interactions to extreme salinities in phase 2 ($S_2 = 40$ and 180g/L) was slightly better, but still performed less well than the best model (Model 13, AIC = 4.36).

We then tested a more specific biological hypothesis related to the beneficial acclimation hypothesis (model 14), where a different survival term in phase 2 was fitted for each value of $|S_2 - S_1|$, consistent with the hypothesis that fitness is higher for individuals that stay in similar environments in phases 1 and 2 (see Introduction). We also considered an alternative model (model 15) where the interaction between past and present salinity depends on their (signed) difference $S_2 - S_1$, rather than the absolute distance. This latter model has lower AIC (by about 2 units) than the model of beneficial acclimation, suggesting that it is not only the *absolute* change in salinity between phases that influences survival, but also the *direction* of this change: survival is generally reduced when moving to a higher salinity, regardless of what this salinity is (Figure 3, right panel). However this effect was necessarily confounded with S_2 to some extent here, as the largest differences in salinity were found for $S_2 = 180\text{g/L}$. Furthermore, both these models had larger AIC than the best model with full interaction (AIC = 23.79 and 21.71, respectively). To test whether their AIC could be decreased by reducing the number of parameters, we investigated a parametric model for the $S_1 \times S_2$ interaction, in order to capture the relationship between survival and the difference in salinity between phases that can be visualized on the right panel of Figure 3. However, a model with a polynomial (quadratic) relationship between survival effects and $S_2 - S_1$ did not improve AIC (model 16, AIC = 121.65). Our flexible approach allows considering a large series of other possible biological hypotheses describing historical effects, such as threshold effects whereby the interaction only matters beyond some degree of environmental change between phases. Although they are not particularly useful in the specific case study investigated here, they could turn out to be much more relevant in other applications.

Finally, we considered models with pure history effects, without history-by-environment interactions. In these models, either the $S_1 \times S_2$ interaction (model 17), or all survival in

phase 2 (model 18), were solely determined by the salinity in phase 1, and not at all by salinity in phase 2. These models were strongly rejected by the data (higher AIC by 87.13 and 340.74, respectively), indicating that the effect of S_1 on survival in phase 2 cannot be boiled down to a long-lasting effect of S_1 that is consistent across S_2 , as would occur for instance when survival all along life is mostly determined by habitat quality experienced as a juvenile (long-lasting effect of stress).

Ontogenic dynamics of the tolerance curve

Our method is based on estimating instantaneous mortality risks over different phases where an organism experiences different environments. The estimated model can then be used to compute the cumulative effect of mortality risk integrated over time. This predicts the proportion of survivors at different points in time, thus revealing how the environmental tolerance curve changes during lifetime in response to the sequence of environments encountered by an individual. Figure 4 illustrates these dynamics of the tolerance curve in our experiment.

The left panel in Figure 4 shows the survival probability in P_2 of those individuals that did survive through P_1 – i.e., the *conditional* tolerance curve – for different points in times during P_2 . The shape of the tolerance curve, and its dependence on the salinity S_1 in P_1 , both change along life. One day into P_2 (day 8 of the experiment), the reaction norms are essentially flat, because differences in survival between treatments have yet to accumulate. But by day 9, substantial differences in survival between salinities can already be seen, and at day 15, all tolerance curves show markedly reduced survivorship at 180g/L. More importantly, differences between individual histories (i.e., earlier salinities S_1) have largely accumulated at $S_2 = 180\text{g/L}$ by day 15, distinguishing $S_1 = 120\text{g/L}$ from other salinities. However by day 50, these differences are less marked at $S_2 = 180\text{g/L}$, since the proportion of survivors has decreased for the treatment $S_1 = 120\text{g/L}$, while it has obviously remained near 0 for other treatments. In contrast at the lowest salinity $S_2 = 40\text{g/L}$, differences between individual histories are not much marked until day 50, where the treatment $S_1 = 40\text{g/L}$ has a substantially reduced proportion of survivors, in the actual data and the model.

The right panel in Figure 4 shows the *unconditional* tolerance curve, where the proportion of survivors in P_2 is computed relative to the beginning of the experiment, rather than to the beginning of P_2 . For these unconditional curves, the differences between effects of individual histories are more marked initially than in the conditional curve (compare left and right panel at day 8), because the cumulative effect of mortality in S_1 carries over to the next phase, yielding fewer individuals in environments where mortality risk has previously been higher. In other words, these curves include the cost of acclimation caused by exposure to stress. But since tolerance curves integrate hazard rates over time, they later become more and more dominated by mortality in the second phase – which lasts longer –, and thus resemble the unconditional tolerance curves (compare left and right panel at days 15 and especially 50). However we stress that this dominance of the second phase depends on our particular experimental design, and in general the contributions of different phases to the overall tolerance curve depends on their relative durations, in combination with their relative hazard rates.

It is important to note that studies of environmental tolerance usually focus on the unconditional tolerance curve (left panel in Figure 4), while studies of acclimation sometimes focus on the conditional curve (right panel in Figure 4), tracking the survival of those individuals that did survive prior exposure to stress. But in general, richer information is contained in the full survival model.

Discussion

Contrary to the common practice in studies investigating the effect of acclimation on tolerance curves (e.g. Leroi, Bennett & Lenski 1994; Bennett & Lenski 1997; Gibert, Huey & Gilchrist 2001; Deere & Chown 2006; Cooper, Czarnoleski & Angilletta 2010), we did not measure fitness components (or more often, performance as a surrogate, Arnold 1983) *at one point in life*, but instead we monitored a direct component of fitness (survival) repeatedly throughout lifetime. We then analyzed this experiment using a modified survival analysis, where two phases have different hazard rates (instantaneous mortality risks), with different dependencies on the treatment (past and current salinity). This enabled us not only to quantify the cost of acclimation caused by exposure to stress during P_1 (Hoffmann 1995; Hoffmann, Sorensen & Loeschcke 2003), but also to detect a memory effect of past environment on current survival (H effect) and general history-by-environment (HxE) interactions, of which beneficial acclimation is just one particular case. This approach was also able to detect more temporally refined effects that are currently little investigated, such as a transient influence of the historical environment, causing the new environment to only start affecting survival after a delay. We could also rule out putative transfer costs caused by manipulation upon phase transition.

We found that all three genotypes we investigated generally survived better at 120g/L in both phases (Figure 1), indicating that this represents a habitat of higher quality than 40, 80, or 180g/L for this species. We also detected strong historical effects, whereby survival in phase 2 (P_2) depends on the environment in phase 1 (P_1). Importantly, this effect was not homogeneous across environments in P_2 , as attested by the $S_1 \times S_2$ effect in the best model (Table 1), which corresponds to a history-by-environment (HxE) effect. More precisely, we found that the instantaneous mortality risk in P_2 is reduced for individuals that developed at 120g/L in P_1 , relative to those that developed at 40 or 80g/L, but that this effect is mostly seen at $S_2 = 180\text{g/L}$. In a system similar to ours, Lee et al. (2003) also showed that salinity tolerance in the copepod *Eurytemora affinis* was affected by the salinity experienced as a juvenile, but that the acclimation environment had contrasting effects on low- versus high-salinity tolerance.

In accordance with previous studies (Leroi, Bennett & Lenski 1994; Bennett & Lenski 1997; Gibert, Huey & Gilchrist 2001; Wilson & Franklin 2002; Woods & Harrison 2002), our results did not conform to the simplest version of the adaptive acclimation, since individuals acclimated at 120g/L survived better at most salinities in P_2 , rather than just at 120g/L (Fig. 3, left). Interestingly, this salinity is also the salinity where individuals survived best in P_1 , suggesting that part of the results can be attributed to long-lasting effects of habitat quality encountered during P_1 , which constitute an environment-specific cost of acclimation. Furthermore, survival in P_2 was less well predicted by the absolute distance between past

and present salinities, $|S_1 - S_2|$ than by their signed difference $S_1 - S_2$ (Table 1). This implies that survival is reduced when brine shrimps are moved to a higher salinity than they encountered before, although this is difficult to distinguish in our experiment from reduced survival at the highest salinity $S_2 = 180\text{g/L}$. Kingsolver et al. (2015) also found asymmetric effects of acclimation to temperature on growth in the tobacco hornworm (*Manduca sexta*), where it causes increased performance at higher but not lower temperatures.

In our model system, this pattern of asymmetric acclimation could result from different biological mechanisms, which connect more or less directly to salinity (for instance oxygen concentration covaries with salinity, and oxygen deprivation has been shown to have long lasting effects on fitness in brine shrimps Spicer & El-Gamal 1999). Probably the most significant of these mechanisms, which has received considerable attention, is salt excretion. The total activity of salt-excreting ionic pumps (Na-K ATP-ase) has been shown to increase with increasing salinity in *Artemia* (Holliday, Roye & Roer 1990). This increased activity is caused by increased expression of genes coding for these proteins (Jorgensen & Amat 2008), but, importantly there is a lag of several days between beginning of exposure to higher salinity and increased protein production (Holliday, Roye & Roer 1990). Because of this lag, if an insufficient number of pumps is present on membranes upon transfer to higher salinity, mortality could be largely increased, due to a mismatch between osmotic machinery and physiological requirements (Croghan 1958). Furthermore, the number of salt-excreting pumps is likely to be higher for individuals that have been exposed to high salinity for longer. But of course producing, maintaining, and running these protein pumps also requires energy and probably involves trade-offs that are likely to impact other life history traits (e.g. reproductive investment, resistance to pathogens, etc), and ultimately fitness. The overall ontogenetic variation in life histories therefore extends beyond survival, and more work is needed to integrate different fitness components (e.g. Shaw *et al.* 2008), so as to obtain a complete view of the dynamic of tolerance curves.

Nevertheless, our approach based on survival analysis is an important step in that direction, as it can reveal the ontogenetic nature of environmental tolerance curves and, perhaps more importantly, of their dependence on treatments (such as historical environments in our case). Survival plotted against the environment has to start flat at 1, and eventually become flat at 0 after all individuals have died. But in between, there may be different dynamics in different parts of the tolerance curve, and the effects of treatments (here, the individual history of past environments) may also act at different points in time (illustrated in Figure 4 for our experiment). Therefore, the timing of the observation can have profound consequences on the shape of the observed tolerance curve. For instance, Kingsolver et al (2015) recently showed that the shape of the thermal tolerance curve of the tobacco hornworm strongly depends on the duration of exposure and age at measurement. In our experiment, our analysis allowed us to distinguish the contributions of survival during the acclimation phase P_1 and survival post-acclimation to the overall tolerance curve at different points in life (Figure 4). More generally, because any treatment (including acclimation) is likely to affect the shape of a tolerance curve differently at different points in time, 'static' measurements of this tolerance curve would lead to different interpretations depending on the time when these curves are measured. More fundamentally, beyond laboratory experiments, a better understanding and quantification of ontogenetic changes in tolerance curves should be

useful for understanding and predicting niche limits in temporally changing environments (Holt 2003; Holt 2009).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Literature Cited

- Akaike H. A new look at the statistical model identification. *Automatic Control, IEEE Transactions on*. 1974; 19:716–723.
- Angilletta, M. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press; Oxford, UK: 2009.
- Arnold SJ. Morphology, performance and fitness. *Integrative and Comparative Biology*. 1983; 23:347–361.
- Baxeianis AD, Kappas I, Abatzopoulos TJ. Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. *Molecular Phylogenetics and Evolution*. 2006; 40:724–738. [PubMed: 16753307]
- Bennett AF, Lenski RE. Evolutionary adaptation to temperature .6. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution*. 1997; 51:36–44. [PubMed: 28568801]
- Browne RA, Davis LE, Sallee SE. Effects of temperature and relative fitness of sexual and asexual brine shrimp *Artemia*. *Journal of Experimental Marine Biology and Ecology*. 1988; 124:1–20.
- Browne, RA., Sorgeloos, P., Trotman, CNA. *Artemia Biology*. CRC Press; Boca Raton, FL: 1991.
- Browne RA, Wanigasekera G. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. *Journal of Experimental Marine Biology and Ecology*. 2000; 244:29–44.
- Burnham, KP., Anderson, DR. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media; 2003.
- Calosi P, Bilton DT, Spicer JJ. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol Lett*. 2008; 4:99–102. [PubMed: 17986429]
- Castro-Mejía J, Castro-Barrera T, Hernández-Hernández LH, Arredondo-Figueroa JL, Castro-Mejía G, de Lara-Andrade R. Effects of salinity on growth and survival in five *Artemia franciscana* (Anostraca: Artemiidae) populations from Mexico Pacific Coast. *International Journal of Tropical Biology and Conservation*. 2009; 59
- Chevin LM, Gallet R, Gomulkiewicz R, Holt RD, Fellous S. Phenotypic plasticity in evolutionary rescue experiments. *Philos Trans R Soc Lond B Biol Sci*. 2013; 368:20120089. [PubMed: 23209170]
- Chevin LM, Lande R, Mace GM. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology*. 2010; 8:e1000357. [PubMed: 20463950]
- Cole GA, Brown RJ. The chemistry of *Artemia* habitats. *Ecology*. 1967:858–861.
- Cooper BS, Czarnoleski M, Angilletta MJ Jr. Acclimation of thermal physiology in natural populations of *Drosophila melanogaster* : a test of an optimality model. *J Evol Biol*. 2010; 23:2346–2355. [PubMed: 20825540]
- Crawley, MJ. *The R book*. John Wiley & Sons; 2012.
- Croghan PC. The osmotic and ionic regulation of *Artemia salina* (L.). *J exp Biol*. 1958; 35:219–233.
- Deere JA, Chown SL. Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *Am Nat*. 2006; 168:630–644. [PubMed: 17080362]

- Dewitt TJ, Sih A, Wilson DS. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*. 1998; 13:77–81. [PubMed: 21238209]
- Gajardo GM, Beardmore JA. The brine shrimp *Artemia*: adapted to critical life conditions. *Frontiers in physiology*. 2012; 3
- Gavrilets S, Scheiner SM. The genetics of phenotypic plasticity. 5. Evolution of reaction norm shape. *Journal of Evolutionary Biology*. 1993; 6:31–48.
- Gems D, Partridge L. Stress-response hormesis and aging: “that which does not kill us makes us stronger”. *Cell Metab*. 2008; 7:200–203. [PubMed: 18316025]
- Gibert P, Huey RB, Gilchrist GW. Locomotor performance of *Drosophila melanogaster*: Interactions among developmental and adult temperatures, age, and geography. *Evolution*. 2001; 55:205–209. [PubMed: 11263741]
- Hoffmann AA. Acclimation: increasing survival at a cost. *Trends in Ecology and Evolution*. 1995; 10:1–2.
- Hoffmann AA, Sorensen JG, Loeschcke V. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology*. 2003; 28:175–216.
- Holliday CW, Roye DB, Roer RD. Salinity-induced changes in branchial Na/K ATPase activity and transepithelial potential difference in the brine shrimp *Artemia salina*. *J exp Biol*. 1990; 151:279–296.
- Holt R. On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research*. 2003; 5:159–178.
- Holt RD. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc Natl Acad Sci U S A*. 2009; 106:19659–19665. [PubMed: 19903876]
- Jorgensen PL, Amat F. Regulation and function of lysine-substituted Na, K pumps in salt adaptation of *Artemia franciscana*. *Journal of Membrane Biology*. 2008; 221:39–49. [PubMed: 18074167]
- Kingsolver JG, Higgins JK, Augustine KE. Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. *J Exp Biol*. 2015; 218:2218–2225. [PubMed: 25987738]
- Kingsolver JG, Woods HA. Beyond Thermal Performance Curves: Modeling Time-Dependent Effects of Thermal Stress on Ectotherm Growth Rates. *American Naturalist*. 2016; 187:283–294.
- Lande R. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*. 2009; 22:1435–1446. [PubMed: 19467134]
- Lande R. Evolution of phenotypic plasticity and environmental tolerance of a labile quantitative character in a fluctuating environment. *Journal of Evolutionary Biology*. 2014; 27:866–875. [PubMed: 24724972]
- Lee CE, Petersen CH. Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. *Physiol Biochem Zool*. 2003; 76:296–301. [PubMed: 12905115]
- Lenz PH, Browne RA. Ecology of *Artemia*. *Artemia Biology*. 1991; 10:237–253.
- Leroi AM, Bennett AF, Lenski RE. Temperature-Acclimation and Competitive Fitness - an Experimental Test of the Beneficial Acclimation Assumption. *Proc Natl Acad Sci U S A*. 1994; 91:1917–1921. [PubMed: 8127906]
- Moran NA. The evolutionary maintenance of alternative phenotypes. *American Naturalist*. 1992; 139:971–989.
- Nougué O, Gallet R, Chevin LM, Lenormand T. Niche Limits of Symbiotic Gut Microbiota Constrain the Salinity Tolerance of Brine Shrimp. *American Naturalist*. 2015a; 86
- Nougué O, Rode NO, Zahab R, Segard A, Chevin LM, Haag CR, Lenormand T. Automixis in *Artemia*: solving a century-old problem. *Journal of Evolutionary Biology*. 2015b soumis.
- Nougué O, Svendsen N, Zahab R, Lenormand T, Chevin LM. Data from: The ontogeny of tolerance curves: Habitat quality versus acclimation in a stressful environment. *Dryad Digital Repository*. 2016; doi: 10.5061/dryad.vb6k6
- Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA. Mechanisms of reef coral resistance to future climate change. *Science*. 2014; 344:895–898. [PubMed: 24762535]

- Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT. Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society Series B-Biological Sciences*. 2010; 277:3391–3400.
- Salinas S, Munch SB. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol Lett*. 2012; 15:159–163. [PubMed: 22188553]
- Saygı YB. Characterization of parthenogenetic *Artemia* populations from Çamaltı (Izmir, Turkey) and Kalloni (Lesbos, Greece): Survival, growth, maturation, biometrics, fatty acid profiles and hatching characteristics. *Hydrobiologia*. 2004; 527:227–239.
- Scheiner SM. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*. 1993; 24:35–68.
- Shaw RG, Geyer CJ, Wagenius S, Hangelbroek HH, Etterson JR. Unifying life-history analyses for inference of fitness and population growth. *Am Nat*. 2008; 172:E35–47. [PubMed: 18500940]
- Spicer JJ, El-Gamal MM. Hypoxia accelerates the development of respiratory regulation in brine shrimp - but at a cost. *J Exp Biol*. 1999; 202(Pt 24):3637–3646. [PubMed: 10574741]
- Triantaphyllidis GV, Pouloupoulou K, Abatzopoulos TJ, Pérez CAP, Sorgeloos P. International study on *Artemia* XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristics of a bisexual and a parthenogenetic population of *Artemia*. *Hydrobiologia*. 1995; 302:215–227.
- Tufto J. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *American Naturalist*. 2000; 156:121–130.
- Vanhaecke P, Siddall SE, Sorgeloos P. International study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin. *Journal of Experimental Marine Biology and Ecology*. 1984; 80:259–275.
- Via S, Lande R. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*. 1985; 39:505–522. [PubMed: 28561964]
- Wilson RS, Franklin CE. Testing the beneficial acclimation hypothesis. *Trends in Ecology & Evolution*. 2002; 17:66–70.
- Woods HA, Harrison JF. Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution*. 2002; 56:1863–1866. [PubMed: 12389732]

Figure 1. Survivorship curves.

The proportion of individuals that are still alive at different times into the experiment are represented for all salinities in phase 2 (warmer colors denote higher S_2) and in phase 1 (one panel per S_1). Full lines represent actual survivorships averaged among clones, and dashed lines represent fits from the best model in Table 1, using a Weibull hazard function. Vertical arrows indicate the time of transfer between phases.

Figure 2. Schematic diagram of the survival model.

The hazard rate (instantaneous mortality risk) as modelled in the analysis of our experiment is represented along lifetime. For simplicity, we display a hazard that does not change with age within phase, corresponding to $a = 1$ in the Weibull model, or more simply an exponential survival model. The transition between phases includes a delay δ during which survival in phase 2 still depends on the environment in phase 1, and a transfer cost c for a duration τ . Mortality risk is represented in the colour of the phase on which it depends, and the effects of salinity in each phase (S_1 and S_2) and their interaction are represented as curved arrows with the same colors.

Figure 3. Effects of salinities in phases 1 and 2 on survival.

The logarithm of the parameter L of the Weibull hazard function that determines life expectancy (inversely proportional to an instantaneous mortality risk), as estimated in our best model, is represented as a function of salinity. The left panel represents survival in phase 1 (dotted and dashed lines) and in phase 2 (continuous lines). Note that effects are expressed on a log-link, so they should be interpreted as (log) multiplicative factors on life expectancy L . For phase 1, each line corresponds to a clone (dashed: LM7; dot-dashed: PAM10; dotted: PAM7). For phase 2, there is no clone effect in the best model, but there are historical effects: each line corresponds to a salinity in the first phase (light gray: $S_1 = 40$; dark gray: $S_1 = 80$; black: $S_1 = 120$ g/L). The right panel shows the longevity parameter in phase 2 as a function of the difference in salinity between phases, $S_2 - S_1$. The continuous line shows the best fit by a third-order polynomial.

Figure 4. Ontogeny of tolerance curves.

The tolerance curve in response to salinity in phase 2 are shown for different salinities in phase 1 (light gray: $S_1 = 40$ g/L, dark gray: $S_1 = 80$ g/L, black: $S_1 = 120$ g/L). The dashed lines show predictions from the best model in Table 1. The tolerance curves on the left are conditioned on surviving to the start of phase 2, meaning that the proportion of surviving individuals is set to 1 at the beginning of phase 2, while those on the right are unconditional,

such that the proportion of survivors at the beginning of phase 2 matches that at the end of phase 1.

Table 1
Model selection for the survival analysis.

Each model is described by a label number (#), a description (model specification), its maximum log-likelihood (LogLik), number of parameters (K), Akaike information criterion (AIC), AIC difference with the best model (ΔAIC), and Akaike weight (w) (values were rounded to 0.01 precision for presentation). All models include salinity effects in both phases, *i.e.* we did not address models constraining survival to be constant across salinities. P_1 and P_2 are phases 1 and 2 of the experiment, S_1 and S_2 are the corresponding salinities, and shape is the parameter of the Weibull distribution that determines how the hazard rate changes with age within phase. See main text for further explanations.

#	Model specification	LogLik	K	AIC	ΔAIC	w
No $S_1 \times S_2$ interaction, delayed S_2 effect						
1	Same hazard in P_1 and P_2	-4482.94	9	8983.88	445.66	0
2	Same ratio of hazards between P_1 and P_2	-4384.05	10	8788.11	249.89	0
3	Independent hazards in P_1 and P_2	-4383.1	12	8790.20	251.98	0
Full $S_1 \times S_2$ interaction						
4	Delay & transfer cost	-4290.97	21	8623.93	85.71	0
5	Transfer cost & no delay	-4425.35	20	8890.70	352.47	0
6	Delay & no transfer cost	-4290.97	20	8621.93	83.71	0
7	No delay & no transfer cost	-4469.70	19	8977.40	439.17	0
8	Clone x ($S_1, S_1 \times S_2$) interaction, delay	-4250.50	48	8597.00	58.78	0
9	Clone x ($S_1, S_1 \times S_2$, delay, shape) interact.	-4225.26	54	8558.52	20.29	0
10	Simplified model (SM): Clone x (S_1 , delay, shape) interaction	-4239.11	30	8538.22	0	0.79
11	SM + Clone x Transfer cost	-4238.09	33	8542.18	3.96	0.11
Specific $S_1 \times S_2$ interaction in SM						
12	Only at 180 g/L	-4249.79	24	8547.58	9.36	0.01
13	Only at extreme S_2	-4245.29	26	8542.58	4.36	0.09
14	Depends on absolute salinity difference	-4254.01	27	8562.01	23.79	0
15	Depends on signed salinity difference	-4250.96	29	8559.93	21.71	0
16	Explicit function of salinity difference	-4305.94	24	8659.87	121.65	0
17	Depends only on S_1	-4287.68	25	8625.36	87.13	0
18	All survival in P_2 depends only on S_1	-4418.48	21	8878.96	340.74	0