Thermal performance curves reveal shifts in optima, limits, and breadth in early life

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Summary statement: We experimentally isolate and compare thermal performance curves in early life, giving new insights into changes in thermal sensitivity with developmental changes in size, complexity, and exposure to thermal challenge.

Abstract

Understanding thermal performance at life stages that limit persistence is necessary to predict responses to climate change, especially for ectotherms whose fitness (survival and reproduction) depends on environmental temperature. Ectotherms often undergo stage-specific changes in size, complexity, and duration that are predicted to modify thermal performance. Yet performance is mostly explored for adults, while performance at earlier stages that typically limit persistence remains poorly understood. Here, we experimentally isolate thermal performance curves at fertilization, embryo development, and larval development in an aquatic ectotherm whose early planktonic stages (gametes, embryos, and larvae) govern adult abundances and dynamics. Unlike previous studies based on short-term exposures, responses with unclear links to fitness, or proxies in lieu of explicit curve descriptors (thermal optima, limits, and breadth), we measure performance as successful completion of each stage after exposure throughout, and at temperatures that explicitly capture curve descriptors at all stages. Formal comparisons of descriptors using a combination of generalized linear mixed modelling and parametric bootstrapping reveal important differences among life stages. Thermal performance differs significantly from fertilization to embryo development (with thermal optimum declining by ~2 °C, thermal limits shifting inwards by ~8–10 °C, and thermal breadth narrowing by ~10 °C), while performance declines independently of temperature thereafter. Our comparisons show that thermal performance at one life stage can misrepresent performance at others, and point to gains in complexity during embryogenesis, rather than subsequent gains in size or duration of exposure, as a key driver of thermal sensitivity in early life.

Introduction

Predicting responses to climate change is a key goal of ecology, evolution, and conservation. Ectotherms are highly sensitive to such change, since environmental temperature regulates not only their body temperature, but virtually all critical functions they need to perform ecologically-relevant activities and ultimately maintain fitness — that is, survival and reproduction (Deutsch et al., 2008; Diffenbaugh et al., 2017; Treasure and Chown, 2019). How ectotherms respond to future climates, in particular, will depend on their capacity to survive and reproduce under projected increases in average and extreme temperatures (Seebacher et al., 2015; Sinclair et al., 2016). Understanding this capacity requires understanding thermal performance and sensitivity (the degree to which performance responds to temperature; Angilletta, 2009) at life stages that limit population viability.

Most ectotherms have complex life cycles, progressing through multiple stages that differ in size, complexity, or duration. In theory, those differences can alter thermal performance, and make thermal challenges more intense or prolonged at some stages than others (Huey et al., 2012; Pörtner et al., 2017; Rezende et al., 2014). In practice, most predictions about thermal performance are based on adult performance, when earlier stages can differ in thermal sensitivity (Bowler and Terblanche, 2008) and even be more sensitive (Pandori and Sorte, 2019; Truebano et al., 2018). Mounting studies document thermal performance at early life stages (e.g., Austin and Moehring, 2019; Byrne, 2011; Collin and Chan, 2016; Karelitz et al., 2017; Radchuk et al., 2013), but are often limited for logistic reasons to responses with unclear links to fitness, responses to subsets of temperatures (typically an ambient control and one or more treatments based on climate projections) that fail to assess the full performance range, or responses to short-term exposures that fail to capture exposure durations in nature (but see Amarasekare and Sifuentes, 2012). Even data on thermal sensitivity in insects — arguably the best-studied group — remain surprisingly limited in these respects (Kingsolver and Buckley, 2020). Consequently, we still have an incomplete understanding of thermal performance for life stages that may be weak links in life cycles, or how descriptors of performance that explicitly capture thermal sensitivity (e.g., thermal optima, limits, and breadth) differ among stages.

The mechanisms that limit thermal performance in ectotherms are actively debated, but are likely to reflect thermal limits on aerobic metabolism, and hence on energy budgets to support maintenance (including synthesis and regulation of stress-response proteins),

growth, and reproduction (Schulte, 2015; Sinclair et al., 2016). Theory argues that those limits depend on size, since, all else being equal, larger cell or body sizes have lower surface-to-volume ratios that limit the uptake of resources from the external environment and their delivery to metabolizing cells (Gillooly et al., 2001; Pörtner et al., 2017). Yet, empirical evidence that thermal performance scales with size remains equivocal, with some studies supporting the idea (Di Santo and Lobel, 2017; Leiva et al., 2019; Verble-Pearson et al., 2015; Woods, 1999) and others contradicting it (Baudier and O'Donnell, 2018; Pincheira-Donoso et al., 2008; Ribeiro et al., 2012). Such mixed results question how well arguments based cellular or even subcellular processes predict organismal performance (Schulte, 2015), and infer that the latter depends on additional factors.

Thermal performance may also depend on organismal complexity (Pörtner, 2001). Gains in complexity (e.g., from coordination of single cells to that of multiple organ systems) are expected to increase metabolic demands, and to therefore come at the cost of greater thermal sensitivity. Comparative analyses uphold this expectation, revealing greater sensitivity for whole-organism functions than for cells and molecules (Rezende and Bozinovic, 2019), and for animals and plants than for unicellular organisms (Storch et al., 2014). Organisms also gain in complexity during early life, as gametes fuse to form zygotes, as zygotes develop into multicellular embryos, and as embryonic cells differentiate into tissues and organs that support whole-organism functions. These gains in complexity are also said to drive ontogenetic changes in thermal sensitivity (Pörtner et al., 2017), potentially complicated by ontogenetic changes in stress-response proteins. Such proteins are often not expressed in late gametogenesis or early embryogenesis, leaving those stages exposed to stress unless buffered by proteins from parents (Feder and Hofmann, 1999; Goldstone et al., 2006; Hamdoun and Epel, 2007). Again, however, empirical evidence of thermal sensitivity in relation to developmental complexity remains equivocal. Sensitivity generally increases with progression through life stages in insects (Bowler and Terblanche, 2008), but marine invertebrates show the opposite pattern (Pandori and Sorte, 2019). For fishes, sensitivity is especially high in eggs and embryos that gain resources from parents or passive diffusion from the environment, then relaxes as stages develop organs that allow functional independence, before rising again as adults grow and reproduce (Dahlke et al., 2017; Pörtner and Farrell, 2008). Thus, current evidence suggests that some gains in complexity, at least, may make organisms less thermally sensitive rather than more so.

Last, thermal performance can depend not only on the intensity of thermal stress, but also on the duration of exposure (Huey et al., 2012; Kingsolver and Woods, 2016; Truebano et al., 2018). This is in principle because performance at most, if not all, levels of organization is measured by biological rate processes (e.g., resources produced or consumed per unit time, survival from one age to the next) that are time-dependent (Kingsolver and Woods, 2016; Schulte et al., 2011). Theory and data duly show that effects of thermal stress on performance follow a typical dose-response relationship (i.e., the higher the stress, the less time that organisms can withstand it), which holds across cold and heat stress due to declines in physiological rates at lower temperatures and in metabolic efficiency or homeostasis at higher ones (Rezende et al., 2014; Schulte, 2015). Consequently, thermal sensitivity is expected to increase with exposure duration, highlighting the need to focus on durations that are relevant to organisms in nature (Hoffmann et al., 2013; Huey et al., 2012). Understanding thermal performance at early life stages thus entails exposures of sufficient duration to assess the capacity to complete each stage, and thereby continue the life cycle.

Thermal performance curves (Fig. 1) describe how changes in temperature affect ectotherm performance (often expressed as individual rates, like growth and development) or fitness (Gilchrist, 1995; Sinclair et al., 2016). Their precise shape can depend on the trait in question, with curves for rates tending to be skewed and curves for survivorship tending to be more symmetrical (Kingsolver et al., 2011; van der Have, 2002). Nonetheless, thermodynamic effects on physiological rates make performance rise from zero at its lower thermal limit (CT_{min}) to a peak (P_{max}) at the thermal optimum (T_{opt}), before loss of metabolic efficiency or damage to proteins and membranes at higher temperatures make it fall again to zero at its upper thermal limit (CT_{max}). These curve descriptors are used to derive thermal breadth (T_{br}, the range where performance is at least 50% of P_{max}), and are increasingly combined with climate data to predict extinction risk (Pinsky et al., 2019; Sinclair et al., 2016). While many studies have by necessity focused on proxies for thermal performance curves, including thermal limits or optima in isolation, or performance at temperatures not informed by known limits or optima (Huey et al., 2012; Kellermann et al., 2019), estimates of full curves remain far rarer (Rezende and Bozinovic, 2019). Thus, it is still unclear whether curve descriptors respond in unison to temperature (as per classic hypotheses like 'hotter-is-better', arguing positive associations between P_{max} and T_{opt}, and 'jack-of-all-temperatures',

arguing tradeoffs between P_{max} and thermal breadth; Huey & Kingsolver 1989; Angilletta 2009) , or to other factors including progression through life stages.

Here, we estimate and compare full thermal performance curves for early life stages of an aquatic ectotherm — the externally-fertilizing tubeworm, *Galeolaria caespitosa*. Like most aquatic ectotherms, *Galeolaria* has planktonic gametes, embryos, and larvae that are dispersed by currents, undergo the key processes of fertilization (considered to initiate the life cycle here) and development in habitats that lack refugia from ambient thermal conditions, and are major bottlenecks for population persistence under thermal stress (Byrne, 2011; Pinsky et al., 2019; Walsh et al., 2019). These stages progress through changes in size, complexity, and duration of exposure to thermal challenge, presenting unique scope to assess the impacts on thermal performance, and whether performance curves vary in shape during ontogeny. Using a split-cohort experimental design to standardize genetic and environmental backgrounds across stages, we expose replicate cohorts at each stage to temperatures spanning the full performance range (i.e., from lower to upper limits marked by complete mortality), and maintain exposures until mortality or successful completion of stages. We then estimate and compare stage-specific thermal performance curves using a combination of generalized linear mixed modelling and parametric bootstrapping of curve descriptors. Our analyses reveal important shifts in thermal performance curves in early life.

Materials and methods

Study species and sampling. *Galeolaria caespitosa* (Lamark 1818), henceforth *Galeolaria,* is a calcareous tubeworm native to rocky shores of southeastern Australia, where it acts as an ecosystem engineer by forming dense colonies of adult tubes that provide habitat and reduce abiotic stress for associated communities (Wright and Gribben, 2017). Sessile adults breed year-round by releasing sperm and eggs into the sea, where they must fuse for fertilization (Chirgwin et al., 2020; Monro and Marshall, 2015). In ~2 hours, zygotes start dividing into multicellular embryos, which complete development into independently-functioning larvae ~24 hours later with little gain in size. Larvae start to grow as they feed in the plankton, where development continues for ~2–3 more weeks and ends with rapid changes in size, morphology, and behavior that signal onset of metamorphosis (readiness to settle and recruit into adult populations; Marsden & Anderson 1981). These early life stages are bottlenecks for population persistence under thermal stress (Byrne, 2011; Walsh et al.,

2019), but complete descriptions of thermal performance curves (including optima, limits, and breadth), and formal comparisons of curve descriptors, are still lacking for them.

We sampled adult *Galeolaria* between April and October 2018 (excluding much of July and August) from Brighton, Port Phillip Bay, Victoria, where water temperatures range from 9 °C in winter to 25 °C in summer (Chirgwin et al., 2020). Adults were transferred in insulated aquaria to seawater tanks in the laboratory, held for 2 hours at the temperature on the sampling date, then adjusted to the annual mean water temperature of 16.5-17 °C (Chirgwin et al., 2017). Since gametogenesis is continuous and gametes can ripen in under two weeks, adults were acclimatized to this temperature for 14 days to reduce any effects of differences among sampling dates before collecting gametes for experiments.

Measuring thermal performance at early life stages. For each stage (fertilization, embryo development, and larval development), we assayed thermal performance as successful completion of the stage at each of a wide range of temperatures (2, 5, 9, 13, 17, 21, 25, 29, 32, 35 and 37 °C) chosen to capture lower and upper thermal limits (CT_{min} and CT_{max}). Since *Galeolaria* occurs in a global marine hotspot that has warmed much faster than the global average rate in recent decades, and is projected to increase ~2-5 °C by the century's end (Hobday and Lough, 2011; Hobday and Pecl, 2014), these temperatures span the conditions now experienced by our study population and regional projections for the coming decades.

Performance was assayed in replicate vials of filtered, pasteurized seawater (loosely capped to allow oxygen flow) suspended upright in water baths. Baths were maintained at designated temperatures (± 0.1 °C) using controlled immersion heaters (Grant Optima TX150) for those ≥ 13 °C and a refrigerated circulator (Julabo FP50) for cooler ones. Four replicates were done for all three stages at all 11 temperatures except 2 °C, which had three replicates per stage (129 vials in total, with success scored for 50 eggs, embryos, or larvae in each). Replicates were done in an incomplete block design, with temperatures assigned haphazardly to blocks and unreplicated within them. Each block comprised gametes, embryos, and larvae from the same cohort of parents used in one assay of fertilization, one assay of embryo development, and one assay of larval development at 2–4 different temperatures per block (see methods below). Hence, within blocks, all stages were assayed concurrently using different subsets of material from the same parents, under identical conditions aside from the manipulation of temperature. There were 12 blocks in total.

Gamete collection and general fertilization protocol. To collect gametes for experiments, each mature adult was extracted from its tube into a dish with 1-2 mL of fresh 0.22 µL filtered seawater to spawn. We checked the quality of gametes (shape and appearance of eggs, and motility of sperm) under a microscope, and used them within an hour of spawning (viability does not decline in this time; E. Chirgwin, unpublished data). To minimize male-female compatibility effects on fertilization and development (Chirgwin et al., 2017; Marshall and Evans, 2005), we pooled fifteen females' eggs and fifteen males' sperm for each block. Pooled eggs were diluted to a final concentration of ~50 eggs mL^{-1} and pooled sperm were kept at ~10^7 sperm mL^{-1} before dilution to a final concentration of ~5 x 10^5 sperm mL^{-1} at fertilization (which optimized fertilization success in pilot work). These gamete concentrations fall within the continuum present in the field and yield fertilization rates comparable to field estimates (Hollows et al., 2007).

Egg and sperm solutions were given 30 minutes to adjust separately to their designated temperatures before combining them in the same vial to initiate fertilization. After a designated contact time (see fertilization assays below), vial contents were rinsed through 0.25µm Nitex mesh with fresh seawater to remove excess sperm. This general fertilization protocol was used in all performance assays.

Thermal performance at fertilization. First, we measured thermal performance in terms of fertilization success. We did so after different sperm-egg contact times (5, 10, 15, 30, and 60 minutes), since temperature can increase or decrease sperm-egg collision rates via effects on water viscosity and sperm swimming speeds (Chirgwin et al., 2020; Kupriyanova and Havenhand, 2005). Different contact times can offset those effects by giving gametes more or less opportunity to collide, allowing more thorough examination of thermal performance at this stage. At each temperature, fertilization was initiated by adding 0.5 mL of sperm solution to a vial with 9.5 mL of egg solution. After the designated contact time, vial contents were rinsed to remove sperm and re-suspended in fresh seawater. Two hours later, they were preserved with Lugol's solution to stop development. From each vial, 50 randomly-selected eggs were examined under a compound microscope and scored as fertilized if they had a distinct fertilization envelope (including a raised cone at the site of sperm fusion) or begun to cleave, or as unfertilized if they had not. Since the envelope and raised cone form

several minutes after fertilization in this species (Marshall and Bolton, 2007), two hours was sufficient to reliably detect fertilization (if present) at even the coldest test temperatures.

***Thermal performance at embryo development*.** Next, we measured thermal performance in terms of successful completion of embryo development. To isolate the effects of temperature at this life stage, all material used in assays was fertilized at 17 °C (following the general protocol above) and with 60 minutes of gamete contact time (which maximized fertilization success in assays above). Two hours after fertilization, fifty embryos at the 1- to 2-cell stage were allocated to each of two vials per temperature, ensuring that all embryos were exposed to designated temperatures at a similar point in development. Embryos were scored for successful completion of embryogenesis at 24 hours and 48 hours post-fertilization. Embryos that had died, or not yet developed into actively-swimming larvae by 48 hours, were scored as unsuccessful. Based on pilot work, embryos that do not complete development by this time never do so.

Thermal performance at larval development. Last, we measured thermal performance in terms of successful completion of larval development. To isolate the effects of temperature at this life stage, all material used in assays was fertilized at 17 °C (as above) and completed embryo development at 17 °C. Twenty-four hours after fertilization (the time taken to complete embryogenesis at 17 °C), 50 actively-swimming larvae were randomly selected and allocated to each of 30 vials per temperature. Larvae were fed a mix of microalgae *ad libitum* (~1 x 10^4 cells mL^{-1} every second day) and one vial of larvae was sampled destructively each day to check survival and for changes signaling completion of development (Marsden and Anderson, 1981). Sampling continued for 2–4 weeks depending on temperature (Table S1), and ended when a vial was sampled in which larvae had either all died or successfully completed development. Successful completion was scored based on normal onset of metamorphosis into the sessile form (Marsden and Anderson, 1981).

Modelling thermal performance curves. We fitted thermal performance curves to life stages using a binomial mixed-effects regression model with Laplace approximation (where 1 meant successful completion of a stage and 0 meant lack of success). We chose this approach because other methods for modelling curves (e.g., available in the *rTPC* package)

do not, to our knowledge, accommodate binary data or random effects. Performance was modelled as a cubic function of temperature (which improved model fit relative to a quadratic function; X^2 = 10.51, d.f. = 3, P = 0.02) using orthogonal polynomials. Life stage, and its interactions with linear, quadratic, and cubic effects of temperature, were modelled as additional fixed effects (interactions were removed where doing so did not lower model fit). Blocks and vials within blocks were initially modelled as nested random effects, which were both estimated as 0. Block effects were removed from the model since the nesting meant they were adequately modelled by vial effects (e.g., Kruuk and Hadfield, 2007), while vial effects stayed in the model to maintain correct data structure. Since fertilization success was assayed at different gamete contact times, we chose a single time for analysis (60 minutes; see results below) based on an equivalent regression model fitted to fertilization data, with time replacing life stage. Model selection was done by comparing fits of nested models using likelihood-ratio X^2 tests, and fixed effects in selected models were tested for significance using Wald X^2 tests (Bolker et al., 2009). Where fixed effects were significant, *post-hoc* contrasts of estimated means or regression coefficients were done using *z* tests corrected for multiplicity. Overdispersion did not affect model fitting. Modelling was conducted in RStudio 1.2.1335 using R 3.5.3 (R Core Team 2019) and its packages, *lme4* (Bates et al., 2015), *car* (Fox and Weisberg, 2019), and *emmeans* (Lenth et al., 2020).

Estimates and confidence intervals of curve descriptors. For each life stage, we extracted standard descriptors of thermal performance curves (Fig. 1) from the final model fit. Thermal optimum (T_{opt}) was calculated as the temperature of peak performance (P_{max}). Critical thermal limits (CT_{min} and CT_{max}) were calculated as lower and upper temperatures at which performance was 5% of its peak, following Kellermann *et al.* (2019). This approach differs to classical measures based on acute performance limits, but was done to accommodate our binary data (see Kellermann *et al.* 2019 for details) and gave similar results to limits marked by complete mortality here. Thermal breadth (T_{br}) was calculated as the temperature range at which performance was at or above 50% of its peak (following Sinclair *et al.* 2016). This breadth calculation gave near-identical results to one calculated at or above 80% of peak performance, and also to thermal tolerance (CT_{max}-CT_{min}), so only the former is presented here. Next, to compare descriptors among life stages, we used parametric bootstrapping (implemented in the R package *boot* v.1.3-23; Canty & Ripley 2019) to generate a mean

estimate and 95% confidence interval for each descriptor based on 10,000 bootstrap replicates. We considered descriptors to differ significantly among stages if their 95% confidence intervals did not overlap.

Results

Modelling thermal performance curves. The best-fitting binomial regression model gave a good overall fit to the observed data (despite slightly underestimating fertilization success at 21 °C; Fig. 2), and detected significant differences in thermal performance curves among life stages (Table 1). Curves for all stages were largely symmetrical, but stages differed in overall performance (main effect of life stage in Table 1), implying differences in curve height (Fig. 2), and also differed in the linear and quadratic effects of temperature on performance (life stage x temperature interactions in Table 1), implying differences in curve skew, position, or breadth (Fig. 2). Based on *post-hoc* contrasts of regression coefficients, both interactions relate to differing effects of temperature on performance from fertilization to embryo development (linear coefficients: $|z| = 3.94$, $P < 0.01$; quadratic coefficients: $|z| = 8.16$, $P < 0.01$), but not from embryo development to larval development (linear coefficients: $|z| = 0.78$, $P = 0.71$; quadratic coefficients: $|z| = 1.40$, $P = 0.33$). We further interpreted differences among life stages by estimating standard descriptors of thermal performance curves (and their 95% confidence intervals) from the model fit.

The predicted probability of fertilization depended on gamete contact time ($X^2 = 149.57$, d.f. = 4, $P < 0.01$; Fig. 3), increasing significantly from 5 to 30 minutes of contact ($z = 9.09$, $P < 0.01$), but not thereafter ($z = 1.95$, $P = 0.29$) — likely because fertilizations became saturated at the gamete densities used, or else gamete viability started to decline. Contact time affected fertilization success independently of temperature (interaction $X^2 = 10.23$, d.f. = 12, $P = 0.60$), leaving thermal performance at fertilization unchanged except for curve height (Fig. 3). Based on this analysis, we fitted our main model to fertilization data at 60 minutes (Fig. 2, Table 1), but data at 30 minutes gave near-identical results.

Estimates and confidence intervals of curve descriptors. Inspection of estimates and confidence intervals for curve descriptors showed that peak performance (P_{max}) remained similar across fertilization and embryo development, but declined significantly at larval development (Fig. 4A). Other differences among curves were driven by differing thermal

performance at fertilization compared to later life stages. Specifically, the thermal optimum (T_{opt}) was ~2–2.5 °C warmer at fertilization than at later stages (Fig. 4B). Lower and upper thermal limits (CT_{min} and CT_{max}) were also more extreme at fertilization (Fig. 4C–D), equating to a ~9 °C decline in thermal breadth (T_{br}) from this stage to later stages (Fig. 4E). Descriptors other than P_{max} remained similar across embryo development and larval development: hence, those stages differed in peak performance (illustrating the main effect of stage in Table 1), but not in their response to temperature.

Discussion

Understanding thermal performance at life stages that limit persistence is necessary to predict population responses to climate change. This is especially vital for ectotherms, whose ability to survive and reproduce depends critically on environmental temperature (Seebacher et al., 2015; Sinclair et al., 2016), and whose complex life cycles often involve stage-specific changes in size, complexity, and duration that may alter thermal sensitivity (Bowler and Terblanche, 2008; Kingsolver and Buckley, 2020; Pörtner et al., 2017). We estimated and compared full thermal performance curves at fertilization, embryo development, and larval development for *Galeolaria*, an aquatic ectotherm with planktonic early stages (gametes, embryos, and larvae) considered to limit population persistence under thermal stress in species with similar biology (Byrne, 2011; Pinsky et al., 2019; Walsh et al., 2019). This includes most aquatic species (Blumer, 1979; Monro and Marshall, 2015). Our comparisons reveal shifts in thermal optimum, limits, and breadth that point to gains in complexity as a key driver of thermal sensitivity, exacerbated by a decline in peak performance (survival) at the stage with longest duration. Fertilization is surprisingly robust to thermal extremes, and may even fare better in future climates or current warming events, while larval development is the main bottleneck for persistence in early life.

Based on the effects of size and temperature on metabolism (Gillooly et al., 2001; Pörtner et al., 2017), larger size is often expected to increase thermal sensitivity and thereby narrow the thermal limits of performance (Leiva et al., 2019). However, empirical tests of this idea in the context of development show little consensus, with larger life stages proving more thermally sensitive in some cases (Lear et al., 2019; Winne and Keck, 2005; Zani et al., 2005), but not others (Klockmann et al., 2017; Pincebourde and Casas, 2015). We found little evidence that size determines thermal sensitivity in *Galeolaria*, since thermal limits

narrowed with progression from fertilization to embryo development, despite little gain in size during this time, but not with further progression to larval development, despite a near-fivefold gain in size during this time (Marsden and Anderson, 1981). While the gain in size did coincide with a decline in peak performance (P_{max}), this decline without shifts in thermal limits or optimum infers that larvae have higher mortality than embryos regardless of temperature, as predicted for planktonic stages with longer duration (Marshall and Morgan, 2011; Rumrill, 1990). Larvae may even be less thermally sensitive than embryos, since performance at the latter stage declines more rapidly with displacement from the thermal optimum. Exploring thermal performance for different size classes per stage could help to clarify the relationship between size and thermal sensitivity in early life.

Thermal sensitivity in *Galeolaria* clearly depends on developmental changes other than size, with fertilization (taking in gamete interactions, fusion, and reactivation of the dormant metabolism of eggs to initiate cell division; Epel, 1977) proving much less sensitive than embryo and larval development. First, all life stages have thermal optima that exceed the annual mean temperature at our field site (Chirgwin et al., 2018), but only the optimum at fertilization tracks local conditions projected for the end of the century and seen during recent extreme warming events (Mills et al., 2013; Oliver et al., 2017). Second, thermal breadth nearly halves from fertilization to embryo development, identifying embryogenesis as a critical threshold in thermal sensitivity. The gain in sensitivity at this stage does not relax with further development of larvae, contrasting with results for externally-fertilizing fishes (Pörtner and Farrell, 2008; Pörtner and Peck, 2010) and other marine invertebrates exposed to short-term thermal challenge (Pandori and Sorte, 2019; but see Byrne 2011, 2012;). Hence, for *Galeolaria* at least, fertility prior to embryogenesis is less sensitive to temperature than recently suggested (Walsh et al., 2019); but, once embryogenesis is reached, ecologically-relevant deviations from the thermal optimum have greater impacts on viability that become compounded by attrition in later development.

The decline in thermal breadth from fertilization to embryo development argues that thermal sensitivity may respond to gains in complexity during embryogenesis. Cell division, entailing strict control of the cell cycle and demanding energy to do so, may be foremost among those gains, based on evidence that temperature inactivation of cell-cycle proteins ultimately sets the thermal limits of viability by slowing division rates and blocking development (Angilletta, 2009; Begasse et al., 2015; van der Have, 2002). Fertilization, in

contrast, entails contact between terminally-differentiated gametes whose cell cycles are arrested, until fusion reactivates egg metabolism and cell division begins (Epel, 1977). Waterborne gametes may also be rich in stress-response proteins supplied by parents before release, which could buffer fertilization against stress but dissipate as embryos start to divide (and embryos seem not to express such proteins until later development because they potentially inhibit early divisions; Byrne, 2011; Feder and Hofmann, 1999; Hamdoun and Epel, 2007). Such properties of gametes may help explain why fertilization is thermally robust, and why thermal sensitivity increases as embryos develop. Note too that in *Galeolaria*, as in many ectotherms, embryos undergo cell division and differentiation without growth, before all three processes become closely coordinated in larvae (van der Have, 2002; van der Have and de Jong, 1996). That thermal sensitivity changes with progression through embryo development, but not larval development, thus points to a key dependence on complexity in terms of cell division, but not growth, in early life.

Cumulative effects of thermal stress on metabolism and resource depletion are expected to see thermal sensitivity increase (i.e., optimal temperatures decline, or thermal limits become narrower) with longer exposure (Kingsolver and Woods, 2016; Rezende et al., 2014). Two aspects of our results go against this expectation. First, longer exposure of gametes to stress did not alter thermal sensitivity at fertilization — instead, longer contact between gametes increased fertility regardless of temperature, at least until fertilization rates were maximized or gametes lost viability. By considering the full thermal tolerance range, our results extend past work detailing the effects of gamete contact time on external fertilization, including in *Galeolaria* (Kupriyanova, 2006; Levitan et al., 1991). Nonetheless, fertilization is also sensitive to sperm density and eggs encounter sperm at varying densities in nature (Hollows et al., 2007), so future work should test how this factor plays into the dynamics seen here. Second, while longer exposure of embryos to stress (24–48 hours *versus* 90 minutes for gametes) could have contributed to the decline in thermal optimum and narrowing of thermal limits from fertilization to embryo development, longer exposure of larvae (2–4 weeks) had no such effects at larval development. Instead, larvae had lower survival than embryos regardless of temperature (which affected both stages similarly), attesting more to a longer attrition period than a relationship between duration of thermal challenge and thermal sensitivity. A recent synthesis also detected no effect of exposure duration on thermal sensitivity throughout life in marine invertebrates (Pandori and Sorte,

2019). One possibility is that the common practice of feeding larvae *ad libitum* during exposure, as done here, mitigates thermal stress at this stage (Byrne, 2012; Schulte, 2015; Sinclair et al., 2016). Another is that the expected relationship emerges when survival is expressed as a rate (per unit time; e.g., Rezende et al., 2014), rather than the cumulative measure used here. Both possibilities warrant further testing.

We estimated thermal performance curves after standardizing thermal history at prior stages (including parents), as is also common practice (Sinclair et al., 2016). Yet, thermal history within and across generations may alter curves in ways that currently evade prediction and may be adverse or beneficial, although evidence for the latter remains limited (Donelson et al., 2018; Sgrò et al., 2016). In *Galeolaria* alone, parental exposure to near-future temperatures lowers fertilization success when exposure at fertilization does not (Guillaume et al., 2016), but also enhances early larval survival at the same temperature (Chirgwin et al., 2018). Our results add further complexity to this picture by showing that near-future temperatures are potentially less stressful (i.e., nearer thermal optima) than ambient controls, emphasizing first, the limits to inferring responses as adverse or beneficial without explicit reference to thermal optima (Huey et al., 2012), and second, the need to better explore how thermal performance curves respond to thermal history at relevant life stages (Angilletta, 2009; Sinclair et al., 2016). The thermal histories of parents as gametes develop are a particular priority, with recent speculation that meiosis is a more thermally-sensitive process than mitosis (Walsh et al., 2019) raising the possibility of fertilization being less robust than reported here (see also Byrne, 2011) if modified substantially by temperature at gametogenesis.

Overall, our work reinforces that thermal performance at one life stage can misrepresent other stages, and gives new insights into correlates of thermal sensitivity in ectotherms by analyzing how thermal performance curves for *Galeolaria* respond to gains in size, complexity, and duration of exposure in early life. Gains in complexity during embryo development may be a decisive factor in thermal sensitivity, but further experiments are needed to clarify the roles of size and exposure that, to our knowledge, have not yet been done. The dispersive stages tested here govern the distribution and abundance of countless aquatic ectotherms, and may therefore govern their vulnerability to climate change (Byrne and Przeslawski, 2013; Munday et al., 2013). Since vulnerability combines sensitivity and exposure to change (Williams et al., 2008), our results may ultimately point to larvae as the

bottleneck for *Galeolaria*'s persistence owing to thermal sensitivity emerging in embryos combining with attrition throughout the larval stage. Likewise, since vulnerability depends further on adaptive capacity (Munday et al., 2013; Williams et al., 2008), our results also emphasize that complex life cycles like *Galeolaria*'s can offer multiple paths to adaptation — in particular, vulnerability may ease with plastic or genetic changes that increase larval survival (and evidence supports genetic potential to do so; Chirgwin et al., 2018; Chirgwin et al., 2015), or decrease thermal sensitivity at embryo development. More work on adaptive capacity is needed, and should be combined with thermal performance curves to better predict population responses to the current and near-future thermal challenges they face.

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Figures

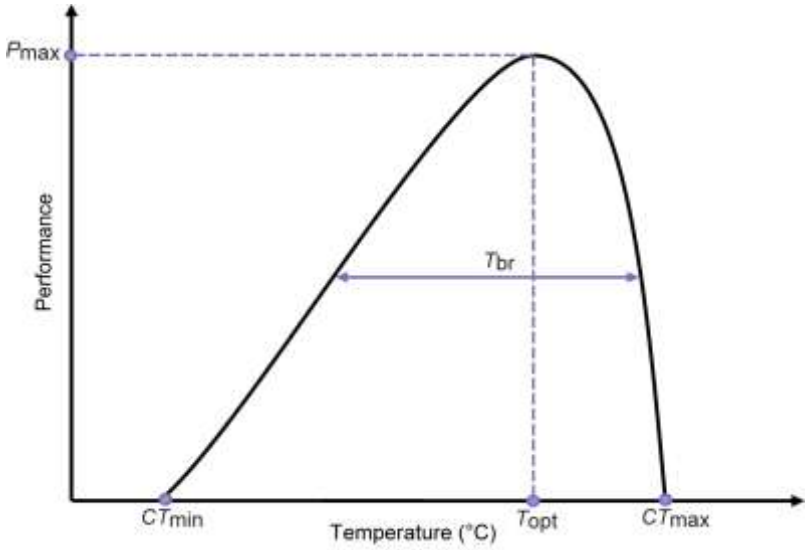

Fig. 1. Theoretical thermal performance curve. Peak performance (P_{max}) at the thermal optimum (T_{opt}), the upper and lower thermal limits (CT_{min} and CT_{max}) where performance drops to zero, and the thermal breadth (T_{br}) where performance remains at or above 50% of its peak value.

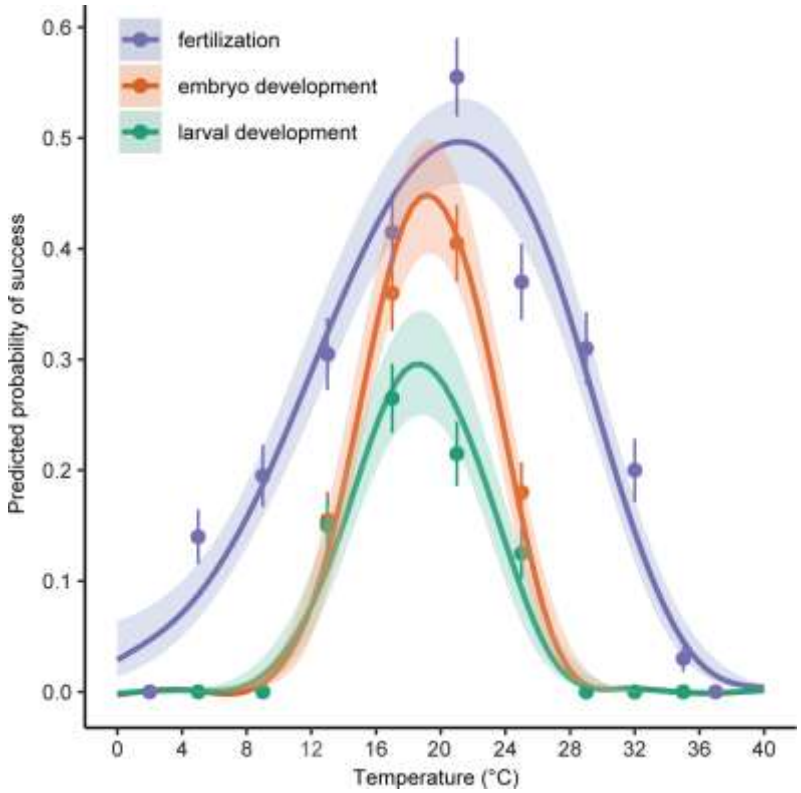

Fig. 2. Thermal performance curves showing the predicted probabilities of successfully completing early life stages (fertilization, embryo development, and larval development). Points are observed success (mean proportion ± 1 s.e.m.) at each temperature. Curves are predicted from a binomial mixed-effects regression of success on temperature, with shaded areas indicating 95% confidence intervals of curve predictions. There were *n*=4 replicates (with success scored for 50 eggs, embryos, or larvae in each) per stage per temperature except 2 °C, which had *n*=3 replicates per stage. See details in Materials and Methods.

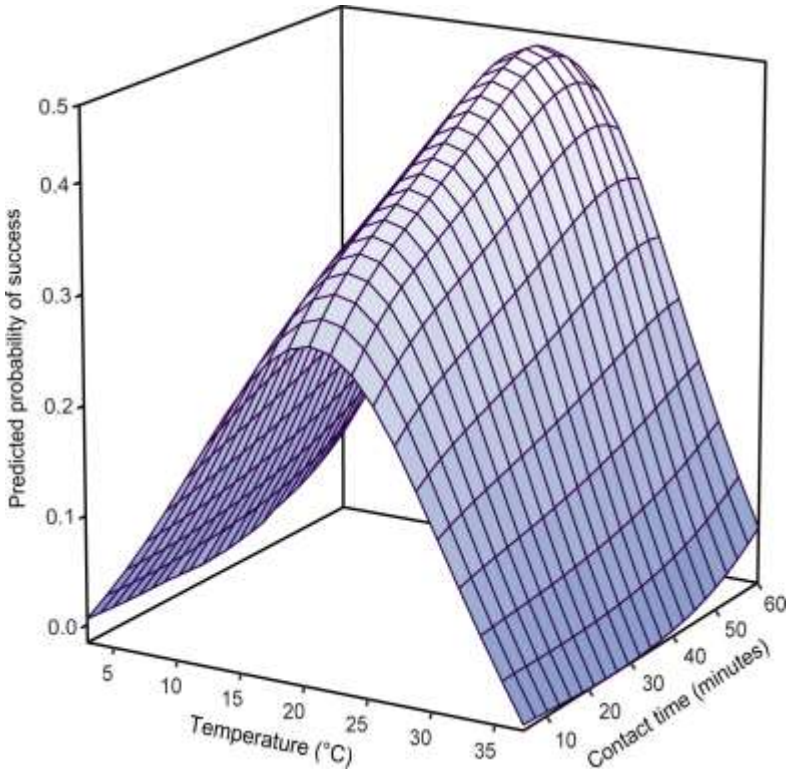

Fig. 3. Effect of gamete contact time on thermal performance at fertilization. Longer contact increased fertilization success (curve height, or P_{max}), but did not interact with temperature to affect other descriptors of performance (interaction X^2 = 10.23, d.f. = 12, P = 0.60). The surface is predicted from a binomial mixed-effects regression of success on temperature. There were n=4 replicates (with success scored for 50 eggs in each) per contact time per temperature except 2 °C, which had n=3 replicates per contact time. See details in Materials and Methods.

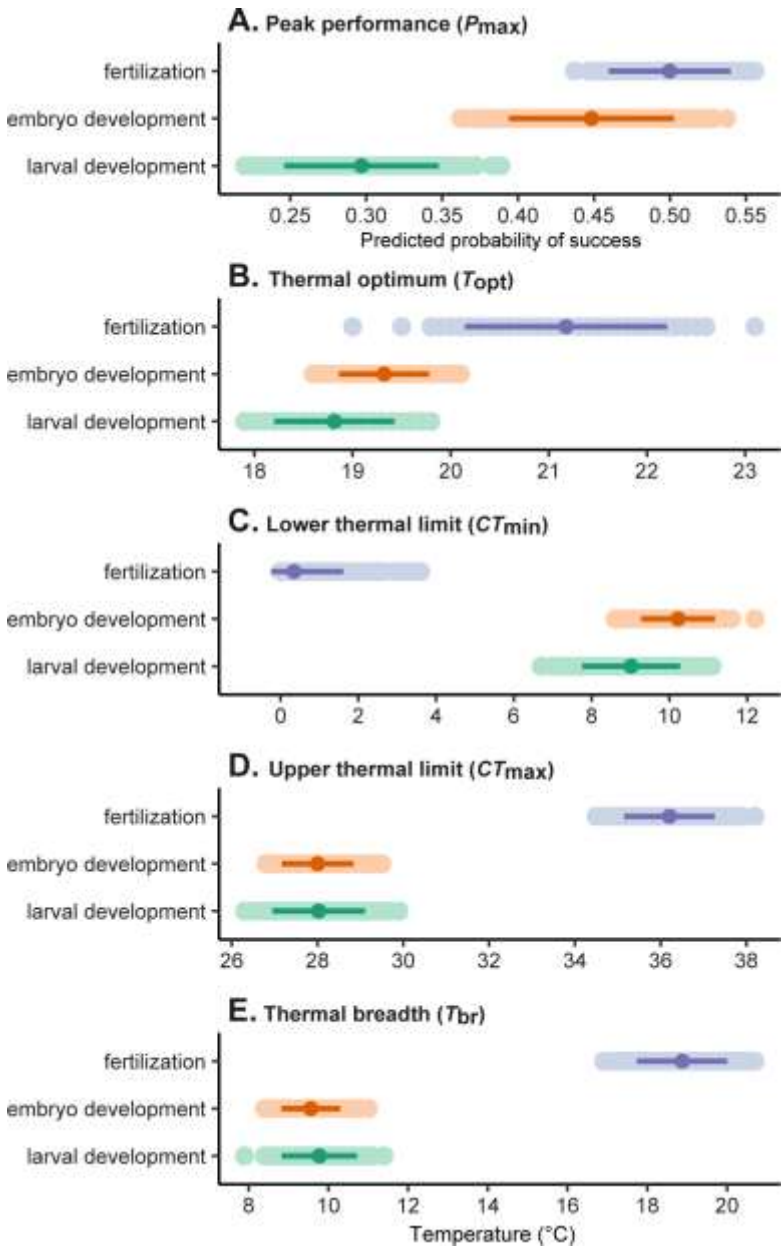

Fig. 4. Peak performance (P_{max}), thermal optima (T_{opt}), thermal limits (CT_{min}, CT_{max}), and thermal breadth (T_{br}) for thermal performance curves fitted to early life stages (fertilization, embryo development, and larval development). Darker points are mean estimates and 95% confidence intervals for curve descriptors based on 10,000 bootstrap replicates (lighter points). See bootstrapping details in Materials and Methods.

Table

Table 1. Linear, quadratic, and cubic effects of temperature on successful completion of early life stages (fertilization, embryo development, and larval development). Estimates are from a binomial mixed-effects regression model fitted using orthogonal polynomials (see details in Materials and Methods). The interaction of life stage with the cubic effect of temperature was non-significant (X^2 = 1.16, d.f. = 2, P = 0.56) and removed from the model.

Fixed effects	X^2	d.f.	P
Life stage	167.51	2	<0.001
Temperature (linear effect)	37.61	1	<0.001
Temperature (quadratic effect)	265.70	1	<0.001
Temperature (cubic effect)	9.60	1	<0.01
Life stage x Temperature (linear effect)	29.77	2	<0.001
Life stage x Temperature (quadratic effect)	102.87	2	<0.001

Table S1. Development time of larvae (mean days ± s.e.m.) at different test temperatures.

Larvae did not complete development at test temperatures below 13°C and above 25 °C.

Temperature (°C)	Development time (mean days ± s.e.m.)
13	22.8 ± 0.6
17	16.8 ± 0.5
21	15.3 ± 0.5
25	13.0 ± 0.7

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