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RESEARCH ARTICLE

Plasticity in parental effects confers rapid larval thermal tolerance in the estuarine anemone *Nematostella vectensis*

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ABSTRACT

Parental effects can prepare offspring for different environments and facilitate survival across generations. We exposed parental populations of the estuarine anemone, *Nematostella vectensis*, from Massachusetts to elevated temperatures and quantified larval mortality across a temperature gradient. We found that parental exposure to elevated temperatures resulted in a consistent increase in larval thermal tolerance, as measured by the temperature at which 50% of larvae die (LT50), with a mean increase in LT50 of 0.3°C. Larvae from subsequent spawns returned to baseline thermal thresholds when parents were returned to normal temperatures, indicating plasticity in these parental effects. Histological analyses of gametogenesis in females suggested that these dynamic shifts in larval thermal tolerance may be facilitated by maternal effects in non-overlapping gametic cohorts. We also compared larvae from North Carolina (a genetically distinct population with higher baseline thermal tolerance) and Massachusetts parents, and observed that larvae from heat-exposed Massachusetts parents had thermal thresholds comparable to those of larvae from unexposed North Carolina parents. North Carolina parents also increased larval thermal tolerance under the same high-temperature regime, suggesting that plasticity in parental effects is an inherent trait for *N. vectensis*. Overall, we find that larval thermal tolerance in *N. vectensis* shows a strong genetic basis and can be modulated by parental effects. Further understanding of the mechanisms behind these shifts can elucidate the fate of thermally sensitive ectotherms in a rapidly changing thermal environment.

KEY WORDS: Acclimation, Cnidaria, LT50, Maternal effects, Paternal effects, Thermal limits

INTRODUCTION

Parental effects encompass a range of mechanisms that can better prepare offspring for the conditions they may experience. These effects are often informed by the parental environment, especially if the environment the offspring will experience will resemble that of the parents (Jensen et al., 2014; Lacey, 1998; Qvasnstöm and Price, 2001; Putnam et al., 2020). Anticipatory parental effects allow parents to enhance the phenotypic plasticity of offspring to better match their future environment (Burgess and Marshall, 2014). Parental effects may influence offspring throughout their lifetimes, as, for instance, occurs in the water flea *Daphnia*, which will develop a helmeted anti-predation phenotype if parents were exposed to predators (Harris et al., 2012). Alternatively, effects may be short lived, mainly influencing early life stages, often through mechanisms such as maternal loading of RNA transcripts; increased energetic reserves (e.g. lipids) in seeds, embryos and larvae; or modification of the gestational environment, in order to enhance offspring survival or allow for faster acclimatization to environmental conditions (see reviews in Marshall and Uller, 2007; Uller, 2008). In an era of rapid climate change, swift phenotypic modifications facilitated by parental effects may become indispensable for species survival (Galloway and Etterson, 2007).

The ability of populations to respond to increasing temperatures will play a crucial role in determining the distribution and persistence of species as global temperatures rise (Logan et al., 2014). In the context of global climate change, phenotypic plasticity – the ability to modulate physiology, morphology, behavior or other phenotypes under different environments – has emerged as a rapid avenue for organisms to survive environmental change, in comparison to the slower route of selection upon the existing genetic variation and eventual adaptation (Aitken and Whitlock, 2013; Donelson et al., 2018; Reusch, 2014; Torda et al., 2017). Plasticity in the form of parental effects or transgenerational plasticity – usually epigenetic or other semi-heritable changes across generations – is thus being heralded as a potential safety net for vulnerable species as it allows plasticity to have intergenerational influence (e.g. Jensen et al., 2014; Putnam and Gates, 2015; Schunter et al., 2018; Putnam et al., 2020).

The impact of parental effects will depend on how reliably parental environments predict the conditions offspring will experience (Burgess and Marshall, 2014; Marshall and Uller, 2007; Uller et al., 2013), as well as the ability of parents to modify those effects based on changing conditions (i.e. plasticity). The degree to which plasticity can benefit organisms will depend on species- and environment-specific interactions (see Via and Lande, 1985; Reed et al., 2011; Kelly, 2019). Environments that are highly variable, especially at short time scales, can often promote plasticity over adaptation for a specific environmental optima (Bonamour et al., 2019; Chevin and Lande, 2015). In other words, there can be selection for plasticity in place of selection for higher thermal optima, for instance. In the context of temperature, the timing and duration of thermal variation relative to reproductive cycles, as well as the persistence of parental effects across future larval cohorts may facilitate the progression from acclimatization to adaptive processes, especially for thermally sensitive organisms (Putnam and Gates, 2015; Seebacher et al., 2015). Organisms that have both short and multiple reproductive cycles across their lifetime represent

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List of abbreviations

ASW	artificial seawater
CS	citrate synthase
HSP70	heat shock protein 70
LT50	lethal temperature 50, the temperature causing 50% mortality in a population
MA	Massachusetts
MD	Maryland
MnSOD2	manganese superoxide dismutase 2
NC	North Carolina
PSU	practical salinity units (equivalent to g kg^{-1})
STHS	short-term heat stress; referring to parental population that underwent the heat stress regime described in the Materials and Methods

interesting case studies for investigating the role and effectiveness of parental effects in modulating offspring fitness across environmental conditions and ecologically relevant timescales.

Nematostella vectensis Stephenson 1935 is a highly tractable experimental organism for studying development and ecophysiology (Darling et al., 2005). This estuarine anemone lives burrowed in the sediment of coastal salt marshes along the eastern and western coasts of North America and parts of the United Kingdom (Reitzel et al., 2008). Populations show strong genetic divergence, even across short geographic distances, suggesting limited gene flow or strong adaptation (Reitzel et al., 2008). *Nematostella vectensis* is able to fully regenerate from a small body fragment, a process that facilitates asexual reproduction and recovery from injury (Stefanik et al., 2013), and that can be used to generate clonal lineages by bisecting adults (Reitzel et al., 2007). Populations of *N. vectensis* can be easily maintained in laboratory conditions, and reproductive cycles can be reliably induced in 2- to 3-week intervals (Fritzenwanker and Technau, 2002; Stefanik et al., 2013). Females eject egg bundles that are fertilized externally by sperm released by males, facilitating controlled crosses (Hand and Uhlinger, 1992). Fertilized embryos develop into swimming planula larvae within 48 h and metamorphose into a primary polyp 7–10 days later (Darling et al., 2005). Although reproductive timing in the wild has not been studied, laboratory animals can be spawned year round. Given their wide geographic distribution and highly variable habitats, parental effects could serve as a fast and effective way to modulate the thermal limits of larvae during spawning cycles.

As *N. vectensis* inhabit shallow salt marsh pools, they experience substantial daily (as high as 20°C) and seasonal thermal variation (as much as 40°C between winter lows and summer highs) (Reitzel et al., 2013; Sachkova et al., 2020; Tarrant et al., 2019). Populations across latitudes also show different thermal tolerance thresholds during larval, juvenile and adult stages (Reitzel et al., 2013). For example, the temperature at which 50% of individuals die (LT50) varies by nearly 2°C between juveniles [~10 days post-fertilization (dpf)] from Massachusetts and those from South Carolina (Reitzel et al., 2013). Southern populations also show faster growth and higher survivorship at warmer temperatures, suggesting some level of adaptation to temperatures across latitudes (Reitzel et al., 2013). However, the degree of plasticity in these thermal thresholds or how these may be influenced by parental effects in early larvae is not known.

Here, we leveraged the thermal range of *N. vectensis* and exposed parents to an increasing temperature regime during gamete maturation to quantify temperature's influence on parental effects (both maternal and paternal), larval thermal tolerance, as well as adult heat tolerance. We tested whether parental effects persist through subsequent spawning events and explored possible causes for induction of larval thermal tolerance. We further compared the impact of parental effects on larval thermal tolerance to differences between genetically distinct *N. vectensis* populations from Massachusetts (MA) and North Carolina (NC). Our work examines how plasticity in parental effects, via mechanisms such as epigenetic mechanisms or transcript loading may alter thermal physiology to determine thermal thresholds across life-history stages, and the consistency of these patterns across geographically and genetically distinct populations.

MATERIALS AND METHODS

Animal collection and husbandry

Laboratory populations of *N. vectensis* were originally collected from the Great Sippewissett Marsh, MA (41.59°N, −70.63°W) and Fort Fisher, NC (33.95°N, −77.93°W) (thermal experiments). Populations were kept in glass containers under a 12 h:12 h, light: dark cycle, in filtered natural seawater diluted with deionized water to 15 practical salinity units (PSU; equivalent to g kg^{-1}). Water changes were conducted every 2 weeks and animals were fed freshly hatched brine shrimp larvae four to five times per week. Animals from MA were maintained at the Woods Hole Oceanographic Institution. NC animals were reared under comparable conditions at the University of North Carolina at Charlotte, until being transferred to MA 6 months before experiments (N=8 individuals). All animals had been acclimated to laboratory conditions for at least 2 years. Animals used for thermal experiments were placed under constant darkness at least 2 weeks prior to the start of experiments to reduce any confounding effects associated with variability in light levels across treatments (all constant dark).

Animals used for gametogenesis analyses were collected from Rhode River, Maryland (MD) (38.87°N, 76.54°W) and provided by Mark Martindale (University of Florida) and Craig Magie (Quinnipiac University). Anemones were cultured in 12 PSU artificial seawater (ASW) and acclimated to laboratory conditions for more than 5 years (Ikmi and Gibson, 2010). Female anemones were maintained at 18°C with ambient light, and fed once per month with *Artemia* nauplii until a week before the induction of spawning and daily until the day before spawn induction. The slight differences in husbandry practices between MA, NC and MD populations simply reflect variations in husbandry protocols between different laboratories. Similarly, the spawning cues used for histological quantification of gametogenesis (MD) and physiological assays (MA and NC) represent minor variations in laboratory-specific protocols that are described in subsequent sections.

Histological quantification of gametogenesis in females

One day before the induction of spawning, five females representing the ‘before spawn’ timepoint were anesthetized and fixed in 7% $MgCl_2$ in ASW (w/v) and 4% paraformaldehyde in ASW (v/v) at room temperature for 1 h. After 1 h, the aboral end was opened and fresh fixative was added for overnight fixation at 4°C. Remaining females were induced to spawn, as described below (‘Spawning for physiological assays’ section), except that the morning after the overnight light exposure the females were cold shocked by replacing room temperature culturing ASW with 18°C ASW and placed under ambient light conditions. Five females that were observed releasing egg bundles were anesthetized and fixed for the ‘after spawn’ timepoint as described above.

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After fixation, samples were washed in PBST (phosphate-buffered saline with 0.2% Triton X-100, v/v), five times (10 min each) at room temperature. Samples were then incubated in 1:5000 diluted SYBR™ Green I (S7567, Thermo Fisher Scientific, Waltham, MA, USA) and 1:1000 diluted SiR-Actin (CY-SC001, Cytoskeleton, Denver, CO, USA) in PBST at 4°C overnight, then washed three times (10 min each). Female mesenteries were then dissected out and immersed in modified Scale A2 (4 mol l^{-1} urea and 80% glycerol in PBS, v/v), and imaged using an Sp5 confocal microscope (Leica Microsystems, Buffalo Grove, IL, USA) with 4 µm per *z*-stack step to quantify the size and number of oocytes before and after spawning.

The area and density of oocytes from five anemones per timepoint (three mesentery views per anemone, 30 total views; total 496 oocytes before spawn and 469 oocytes after spawn) were manually circled and quantified using ImageJ/FIJI imaging software (Rueden et al., 2017; Schindelin et al., 2012). The density of oocytes within mesenteries was measured semi-automatically by FIJI macro, in which maximum-intensity projected images of F-actin channel (mesenteries) were filtered by Gaussian blur (σ=8), thresholded with the same value to automatically select oocyte tissue and manually curated to measure the area. Oocyte data were normally distributed (Shapiro–Wilk test, P=0.1513); therefore, an unpaired, two-tailed, *t*-test was used to quantify differences in oocyte area before and after spawn (N=5 anemones).

Establishment of clonal lineages and genotype-controlled parental populations

To control for genetic variability between parents, experiments were conducted with clonal parental populations whenever possible (see individual experimental descriptions below). To create clonal lineages, individuals were repeatedly bisected across the body column, and allowed to regenerate completely until lineages reached 20–40 individuals. A total of 53 lineages were created. Multiple individuals from each lineage were combined to incorporate genetic diversity into genotype-controlled parental populations (i.e. the same number of individuals per lineage were present in each population). Genotype-controlled (but diverse) populations, as opposed to single-sex clonal lineages, were used for experiments, owing to time constraints in generating clonal lineages and growing them to maturity in the numbers required for the desired larval outputs. In addition, mixed populations more closely simulate wild populations, which would contain more than a single genotype. A detailed enumeration of lineages/individuals in each MA experimental population is provided in Table S1. NC animals were bisected to obtain a total of 35 female individuals (N=3 lineages) and 60 male individuals (N=5 lineages).

Heat stress regime

We examined *in situ* temperature data from loggers deployed at both our MA and NC anemone source sites and previously published in Sachkova et al. (2020). Overall summer temperatures in NC are warmer than in MA (Fig. S1A), with mean daily summer (June to September) temperatures significantly higher in NC than in MA, as expected (P<0.01, two-sample *t*-test, N=122 days; Fig. S1B). However, the daily temperature range was comparable between the two sites (P=0.16, two-sample *t*-test, N=122 days; Fig. S1C). Using two Precision™ Dual Chamber 188 water baths (Thermo Fisher Scientific), animals were transitioned from 20°C to 33°C, at a rate of ∼3°C day^{-1}, held at 33°C for 4 days, simulating mean daytime (09:00–21:00 h) temperatures (32.76°C) during a midsummer week in Woods Hole, MA (Fig. 1A), and then returned to 20°C at the same rate, prior to spawning. The range of temperatures experienced during the treatment (13°C) is similar to the average daily temperature range that *N. vectensis* would experience in the field, both in MA and NC (Fig. S1C). HOBO™ Tidbit loggers (Onset Computer Corporation, Onset, MA, USA) were used to track treatment temperatures every 30 min during experimental incubations. Parental populations exposed to the heat stress regime are hereafter called short-term heat stress (STHS) parents. Water changes were conducted every other day for both STHS and control animals. Each bowl was fed the same ration of brine shrimp nauplii daily (0.2 g). Control animals were kept at 20°C in a low-temperature incubator (Thermo Fisher Scientific), humidified to prevent evaporation. All animals were kept under constant dark conditions for at least 2 weeks prior to and throughout the heat stress regime.

Spawning for physiological assays

To clear gametes and promote gametogenesis during experimental conditions, parents were induced to spawn 2 weeks prior to the start of each experimental incubation. Parental populations were again induced to spawn on the day following the end of the heat stress regime. Spawning was induced following the protocols detailed in Fritzenwanker and Technau (2002). Briefly, *N. vectensis* were individually fed mussel gonad tissue. After 5–6 h, their water was changed and anemones were placed under a bright full-spectrum light for 12 h overnight at room temperature. The following morning a half-water change was made, and the anemones were placed in the dark at 20°C. Induction of spawning for histological assays varied slightly from these steps and is described above.

Bowls were then checked for egg bundles every 30 min. For mixed male–female bowls, egg bundles were kept in the bowls for at least 30 min after release. For crosses between differently treated males and females, egg bundles released by females were transferred to male bowls to allow fertilization for at least 30 min. Fertilized egg bundles were then distributed across six-well culture plates for development in 15 PSU water in the 20°C incubator, in the dark. All gametes were incubated at the same temperature to minimize differences in larval thermal tolerance caused by developmental plasticity or effects of temperature on developmental timing, allowing us to isolate parental effects as the cause of thermal tolerance differences. The word cohort is used throughout the paper to refer to larvae resulting from a single parental treatment/parental population combination (e.g. larvae from parental population G1 with both parents exposed to STHS would be one larval cohort).

MA parental effects experiment

To investigate whether parental heat exposure could increase larval thermal tolerance, we compared larvae from STHS parents with those from control parents (Fig. 1B). Nine paired, genotype-controlled parental populations derived from the MA stock were used in this experiment. For six of the nine trials, parental populations were genetically identical across treatments, and for three of the nine trials, parental populations were genetically similar across treatments (i.e. there was at least one unique lineage within a treatment; see Table S1). Parents were subjected to temperature regimes, cued to spawn, and larval thermal tolerance was assessed as described below. For each larval cohort, 144–192 larvae were assessed (N=3–4 replicate larval heat stress trials).

To test for the persistence of parental effects following STHS, three (genetically identical) parental population pairs were placed in the 20°C incubator after the initial STHS exposure and re-spawned after 2 weeks along with their paired control populations to account for any effects on larval quality induced by repeated spawning.

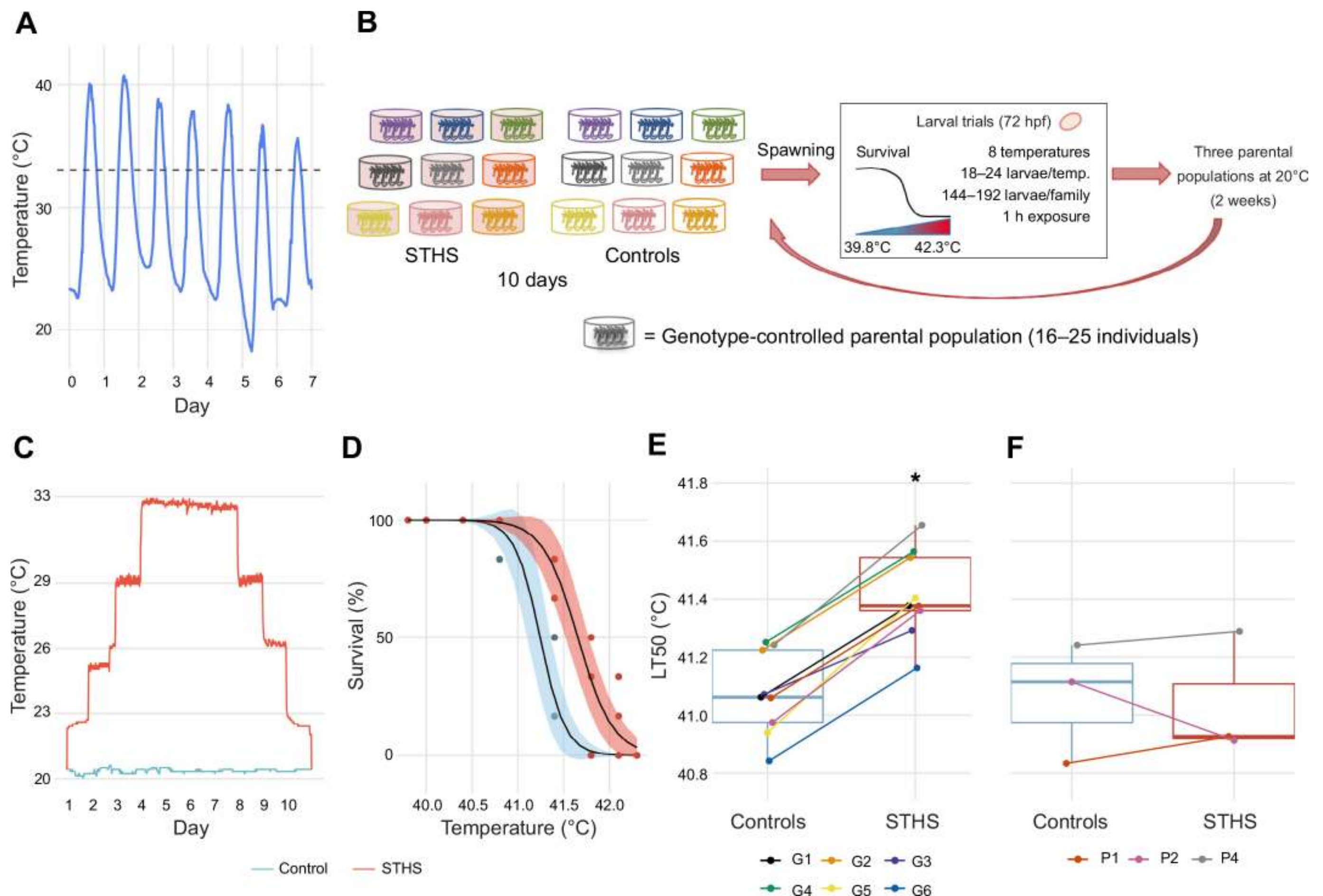

Fig. 1. ***Nematostella vectensis*** **larval thermal tolerance increases when parents experience heat stress during gametogenesis.** (A) *In situ* temperatures in Sippewissett Marsh, Woods Hole, Massachusetts (MA) over 1 week (15–22 July 2016; data from Sachkova et al. (2020). Dashed gray line denotes maximum short-term heat stress (STHS) treatment temperature (33°C). (B) Experimental design for MA parental effects experiment. See Table S1 for population breakdown. (C) Temperature regimes experienced by parents during the 10 days prior to spawning, logged every 30 min. (D) Example survivorship curves of larvae from control (blue) and STHS (red) parents when exposed to acute temperature stress. Ribbon shows 95% confidence interval for logistic survivorship model. Curves shown for larval cohort G6; curves for all the cohorts tested are shown in Fig. S2. (E) Temperature at which 50% of larvae die (LT50). Colors correspond to genotype-controlled families. Mean shift in LT50 is 0.34°C (*P=2.25×10^{-7}, two-tailed, paired *t*-test, N=9). (F) Larval LT50 for N=3 parental pairs that were re-spawned ∼2 weeks after the first experimental spawn (shown in E). Two-tailed, paired *t*-test for differences in LT50 between parental treatments were non-significant (P=0.57), indicating that parental effects subside during subsequent spawns. hpf, hours post-fertilization. The boxplots in E and F show the median and interquartile range (IQR). Whiskers show the range of the data (up to 1.5x the IQR).

MA maternal/paternal effects experiment

To investigate whether parental effects were predominantly paternal or maternal, six of the nine parental populations described above included additional female/male-only parental cohorts to enable reciprocal crosses of STHS and control males/females (Fig. 2A; Table S1). Fertilization between STHS×control males/females was achieved by transferring eggs from female-only bowls into their opposite treatment male-only bowls. Female bowls were checked for the presence of new egg bundles every 30 min, for 5 h. For each larval cohort, 144 larvae were assessed (N=3 replicate larval heat stress trials). In one population (G2), the STHS female×control male cross failed to produce viable larvae, and the STHS male×control female cross yielded only 96 viable larvae (N=2 replicate larval heat stress trials). For population G4, the STHS female×control male cross yielded only 48 viable larvae (N=1 larval heat stress trial).

Genetic versus parental effects experiment (MA versus NC)

Given the previously established differences in thermal thresholds in *Nematostella* from geographically and genetically distinct populations (Reitzel et al., 2013), we wanted to compare these differences (putatively from local adaptation) to shifts in larval thermal tolerance resulting from parental effects. To do so, we compared offspring from MA parents with those from NC parents. Owing to the small number of founder NC individuals (8), we generated clones of all genotypes to create a NC self-breeding population and a population to reciprocally cross with MA animals. Each parental combination had 14 female individuals (3 genotypes) and 24 male individuals (5 genotypes).

All four parental groups (MA, NC, MA female×NC male, NC female×MA male) were maintained under control conditions, spawned and used to measure control larval thermal tolerance. After 3 weeks, the same parents were subjected to the STHS regime and re-spawned to measure larval thermal tolerance after parental heat exposure (Fig. 3A). For this experiment, a temporal control was used, owing to the limited number of NC genotypes and the limits of generating sufficient clones within a reasonable time frame to have separate control and STHS populations. We should note that, during our subsequent spawning of MA

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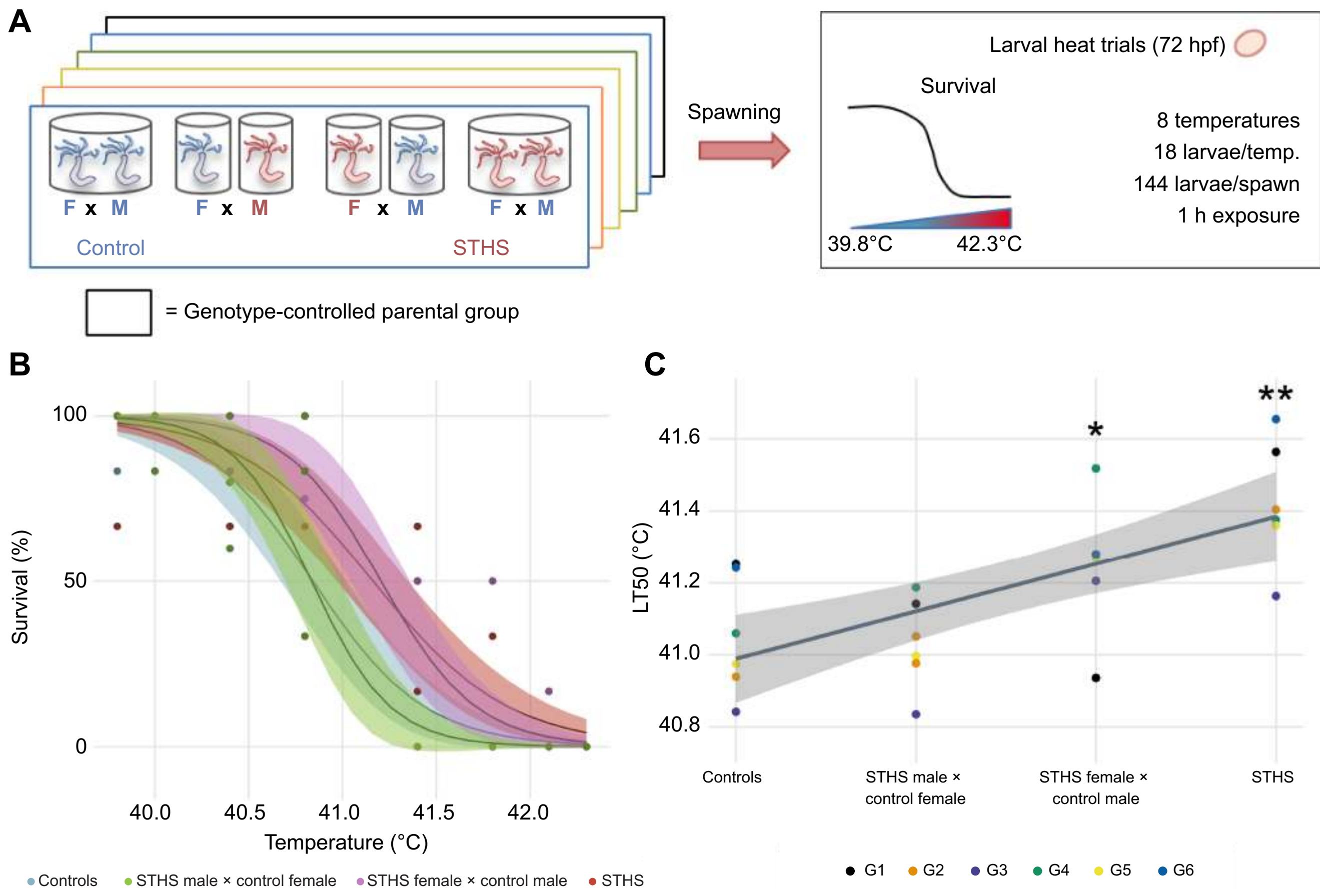

Fig. 2. Increase in *N. vectensis* larval thermal tolerance is not attributable to exclusively maternal or paternal effects. (A) Experimental design. Colored boxes represent genotype-controlled parental populations. See Table S1 for complete details. F, female; M, male. (B) Example survivorship curves of larvae from different parental treatments when exposed to acute temperature stress. Ribbon shows 95% confidence interval for logistic survivorship model. Curve shown for cohort G3; curves for all cohorts tested are shown in Fig. S3. (C) LT50 for larvae from each cohort (*N*=6) and parental treatment. Linear model showed a significant increase in larval LT50 in larvae with an STHS mother or both STHS parents, but not an STHS father only, relative to controls. **P*<0.05, ***P*<0.001.

individuals to test for the persistence of parental effects, there were no noticeable differences in the thermal tolerance of larvae from control parents from sequential spawns (Fig. 1F). As such, using a temporal control for this experiment is unlikely to have introduced any additional effects. Fertilization of MA×NC hybrids was conducted in the same manner described above for the maternal/paternal effects experiment. For each larval cohort, 192–288 larvae were assessed for thermal tolerance (*N*=4–6 replicate larval heat stress trials).

Larval thermal tolerance assays

To determine the larval temperature gradient, a series of preliminary trials was conducted with larvae from mixed MA parents under control conditions using the temperature gradient from Reitzel et al. (2013) as a starting point (37–43°C). Minimum temperatures were shifted warmer and the range of the gradient was adjusted to produce smaller temperature steps around the mid temperature to allow us to better capture small shifts in LT50 values that might be missed by larger temperature steps.

At 72 h post-fertilization (hpf), eggs that had developed into swimming planula larvae were individually pipetted into 0.2 ml PCR strip tubes (USA Scientific, Ocala, FL, USA) with 200 µl of 15 PSU water (as per Reitzel et al., 2013), and exposed to a temperature between 39.8°C and 42.3°C for 1 h using a C1000 PCR thermocycler (Bio-Rad, Hercules, CA, USA), with two 48-well heating plates. The temperature protocol was as follows: (1) 1 min at 25°C; (2) 4 min at 30°C; (3) 4 min at 38°C; (4) 1 h at the treatment temperature: 39.8, 40, 40.4, 40.8, 41.4, 41.8, 42.1 or 42.3°C; (5) 4 min at 38°C; (6) 4 min at 30°C; and (7) infinite hold at 22°C. Each run consisted of six eight-well strip tubes (48 wells total) per trial replicate per larval cohort. Replicate trials for larvae from treatment and control parents were always exposed simultaneously (one set per heating plate). The assignment of STHS and control larvae to one of the two heating plates within each run was alternated to minimize any possible variations between the thermocycler's heating plates.

Following the thermal exposure, larvae were maintained in the same PCR tubes and returned to the 20°C incubator, in the dark. Mortality was scored 48 h after each trial by examining larvae under a dissecting scope. By this time, dead larvae had begun to disintegrate and appeared as fuzzy clumps. To ensure that the small volume (0.2 ml) would not independently impact larval survival, we maintained larvae (larvae that were never experimented on as well as larvae from experiments) in tubes for over 2 weeks. We saw no mortality in larvae that had not been exposed to heat stress. Mortality in experimental larvae was exclusively observed only for those subjected to the higher ends of the temperature range. All surviving larvae also metamorphosed into polyps, suggesting that the effects of maintaining larvae in 0.2 ml tubes until scoring were negligible, if any. In any case, effects would have been consistent

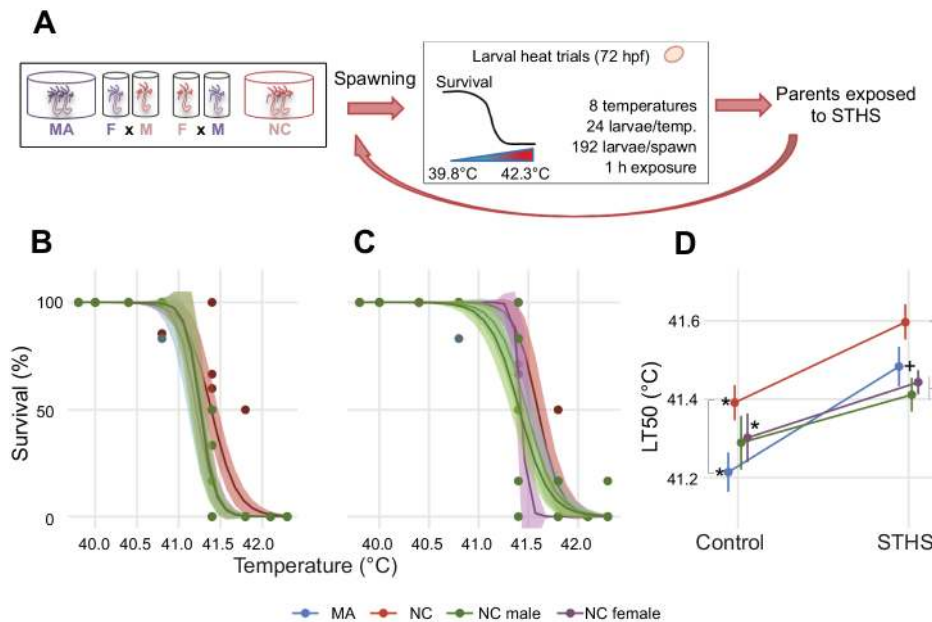

Fig. 3. In experimental *N. vectensis*, MA parental effects confer thermal tolerance equivalent to that of native NC larvae. (A) Experimental design. Animals from North Carolina (NC) are shown in red, animals from Massachusetts (MA) are in blue. Note that unlike for MA-only experiments (Figs 1 and 2), controlling for parental genotypes was achieved temporally instead of through the use of paired populations. The same groups of parents were spawned under control conditions and then after the STHS treatment. F, female; M, male. (B,C) Survival curves of larvae from control parents (B) and after parents underwent STHS ramp (C). (D) LT50 for each larval cohort and cross. Asterisks denote cohorts for which there was a significant (P<0.05, likelihood ratio test) increase in larval LT50 following STHS exposure of parents. Brackets denote cohort pairs that had significantly different LT50 within the parental treatment condition. Cross denotes insignificant difference between MA larvae from STHS parents and NC larvae from control parents. LT50 of larvae from STHS MA parents is comparable to that of larvae from NC parents from both control (P=0.17, likelihood ratio test) and STHS conditions (P=0.09, likelihood ratio test).

across larval cohorts, which would not have influenced our interpretations.

Statistical analysis of larval survival

For each larval cohort, replicate trials were used to form logistic regression models (LL.2) using the dose-response curve, 'drc' package (Ritz et al., 2015) in R (https://www.r-project.org/), to generate survival curves and calculate the dose (temperature) at which 50% of the larvae died (LT50). In other words, replicate larval trials were used to generate more precise estimates of LT50 values per larval cohort. Within each parental population, survival curves between different cohorts were simultaneously inferred (as larvae from genetically identical parents cannot be considered independent), and standard errors (of the LT50 estimate) were corrected for simultaneous inference using the 'glht()' function from the 'multcomp' package in R. These LT50 values were then compared between larvae from STHS parents and control parents as described below.

MA parental effects experiment (including persistence)

The LT50 estimates from models for control and STHS parents were compared using paired *t*-tests to account for variation in thermal tolerance between larvae from the same parental populations (N=9 parental populations). We conducted paired *t*-tests on just the LT50 values, as this was the main parameter of interest and allowed us to account for the effect of parental genotype on effect size, and follows the analyses conducted in Reitzel et al. (2013), facilitating comparisons with prior work on *N. vectensis*. In addition, paired *t*-tests eliminate the need for multiple comparisons as would have occurred if we tested each pair of treatment curves against each other. For the MA versus NC experiment (below), the latter approach was taken as there was only one set of parental populations examined.

MA maternal/paternal effects experiment

The LT50 estimates were analyzed using a general linear model with parental treatment as a fixed effect and parental population as a random effect using the 'lmer()' function from the 'lme4' package in R (Bates et al., 2015) (N=6 parental populations).

Genetic versus parental effects (MA versus NC) experiment

For each pair of STHS and control curves (e.g. NC control, NC STHS) we tested whether response curves were significantly different by comparing a model in which both treatments were estimated to have the same LT50 and one in which LT50 estimate could vary by treatment, using likelihood ratio tests through the 'anova()' function in R to compare the two model outputs. This method was used because there were a limited number of unique NC genotypes; therefore, it would have been impossible to obtain independent replication at the level of parental groups between STHS and control treatments. In this instance, the replication is at the level of the number of larval trials conducted for each parental cross and treatment combination (N=4–6 replicates).

Quantitative polymerase chain reaction (qPCR) assays of larval gene expression

To test whether larvae from STHS parents exhibited differential expression of genes commonly involved in stress response pathways, we measured expression of three genes: Heat shock protein 70 (*HSP70*), Manganese superoxide dismutase 2 (*MnSOD2*) and Citrate synthase (*CS*). Four pairs of genetically identical parental populations were either subjected to the STHS or control thermal regimes (Fig. 4A; Table S1). Larvae were allowed to develop as described above.

At 72 hpf, two to five replicate pools of 200–300 swimming planula larvae from each parental population and treatment were

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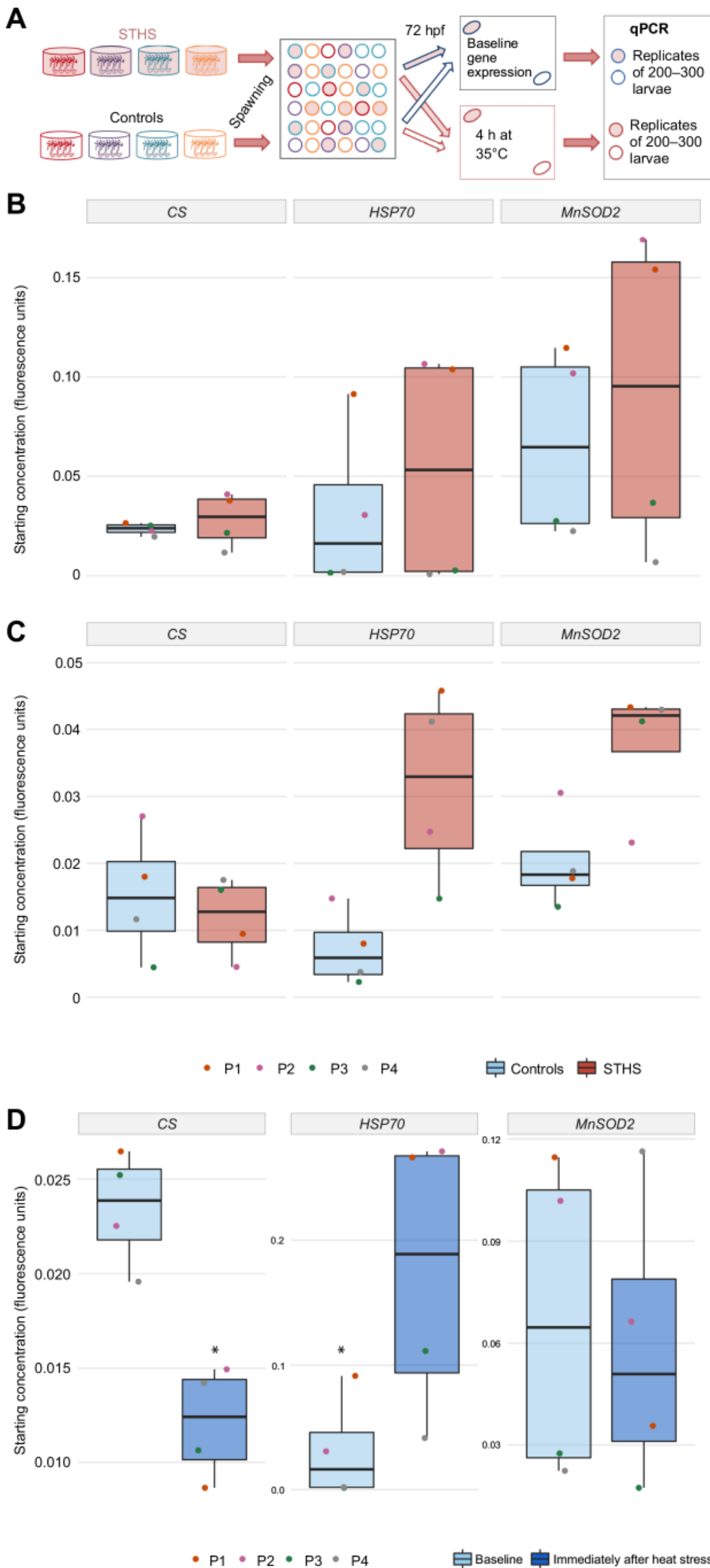

Fig. 4. *Nematostella vectensis* larval gene expression under baseline and heat stress conditions. (A) Experimental design. Colored bowls represent genotype-controlled parental populations. Filled shapes indicate STHS parents and their larvae. (B) Constitutive gene expression at baseline in 72 hpf larvae from four control (blue) and STHS (red) clonal parental populations. Colored points correspond to the larval cohort. (C) Inducible gene expression patterns 18 h after the acute heat shock treatment (bottom trajectory in A). Larvae from STHS parents have a slightly stronger induction of *HSP70* and *MnSOD2* than *CS* following heat stress (P=0.052, P=0.12, respectively, two-tailed, paired t-tests, N=4). (D) Larvae from control parents show decreased expression of *CS* and increased expression of *HSP70* (*P=0.04, *P=0.03, respectively, paired t-test, N=4) immediately after heat stress. In B-D, expression is shown as the starting concentration after normalizing to expression of housekeeping genes and correcting for cross-plate variability (see Materials and Methods).

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pipetted into 1.5 ml microcentrifuge tubes (Fig. 4A; Table S2) for RNA extraction ('baseline' timepoint). Additional larvae were subjected to a 4-h heat shock at 35°C using a Fisherbrand™ Isotemp heating plate (Thermo Fisher Scientific). Larvae were sampled for gene expression immediately after the heat shock ('immediate') (parental population G2 only) and 18 h following the end of the heat shock ('post') (Table S2). Differences in gene expression at different timepoints were tested using paired *t*-tests.

All larvae were immediately processed for RNA extraction using the phenol-chloroform based Aurum™ Total RNA Fat and Fibrous Tissue Kit (Bio-Rad) with on-column DNase treatment. RNA yields were assessed using a NanoDrop One™ spectrophotometer (Thermo Fisher Scientific), giving a mean yield of 44.9 ng μl^{-1} and a range of 12.8–132.2 ng μl^{-1} in a 15 µl total elution volume. For synthesis of complementary DNA (cDNA), we used 200 ng RNA per sample and the iScript™ DNA Synthesis kit (Bio-Rad) and C1000 PCR thermocycler (Bio-Rad) with the following protocol: (1) 5 min at 25°C; (2) 20 min at 46°C; (3) 1 h at 95°C; and (4) 4°C hold.

Primer sequences for *Actin*, *18S*, *L10*, *HSP70* and *MnSOD2* were obtained from Tarrant et al. (2014) and Helm et al. (2018), as these were previously used in *N. vectensis*. The gene sequence for *CS* was determined by searching the *N. vectensis* genome on the Joint Genome Institute database (https://mycocosm.jgi.doe.gov/Nemve1/Nemve1.home.html) and selecting the sequence annotated as 'eukaryotic-type Citrate Synthase'. The full sequence was then submitted to the Primer3 web portal to generate the best primer sequence. *Actin*, *18S* and *L10* were used as reference genes. Gene accession numbers and primer sequences are listed in Table S3.

For each gene, expression was measured in two 96-well plates with two technical replicates per sample, and two across-plate replicated samples, using iTaq™ universal Syber Green Supermix (Bio-Rad) and a CFX96™ thermocycler (Bio-Rad) with the following protocol: (1) 1 min at 95°C; (2) 40 cycles of amplification (15 s at 95°C, 25 s at 60°C); and (3) a final melt curve from 65°C to 95°C with a 0.5°C increase every 5 s. Raw, uncorrected fluorescence values were used to estimate the starting template concentration using LinRegPCR (Ramakers et al., 2003). Cross-plate variation was corrected using Factor_qPCR (Ruijter et al., 2015). To obtain final expression values, the expression of each gene was normalized by dividing the gene's estimated concentration by the geometric mean of the references.

Fertilized oocyte size measurements

To test whether the experimental conditions affected egg size and potential energetic provisioning, we examined egg bundles from all spawning females of four genetically identical parental population pairs from the MA parental effects experiment. We photographed eggs using a Zeiss™ Axio Cam 1Cc1 camera and imaging software (Carl Zeiss AG, Oberkochen, Germany). A stage micrometer was photographed under the equivalent magnification for scale. The diameters of 10 eggs per bundle were measured using ImageJ software (N=230, N=260, N=160 and N=220 eggs per control cohort and N=130, N=240, N=210 and N=200 eggs per STHS cohort, respectively). Differences in mean diameters between STHS and control females for each population pair were tested using paired *t*-tests (N=4 female populations).

Adult heat stress survival assays

To test whether thermal preconditioning resulted in priming of adult anemones to a subsequent heat shock, we exposed 80 mixed-genotype, MA adult individuals from the general laboratory populations to the STHS ramp described above and maintained another 80 individuals at 20°C. The day after the end of the STHS ramp, all anemones were subjected to a 6-h heat shock at 36°C and then returned to 20°C. Mortality was assessed 48 h after the acute heat shock. Adults that had ejected mesenteries, were decomposing or were unresponsive to touch were scored as dead. Differences in the proportions of surviving anemones by treatment were tested using a one-tailed, Fisher's exact test in R for lower survival in control (non-preconditioned) anemones.

RESULTS

Oocyte sizes before and after spawning

We examined the timing of gamete maturation to guide the development of protocols during which gametes would mature under different thermal regimes. Immature oocytes were retained after spawning, with a significant shift towards smaller oocytes following spawning (~45% decrease in size, P<0.001, two-tailed, two-sample *t*-test, N=5 females pre- and post-spawn; Fig. 5B). Oocyte density was higher after spawning, suggesting the release of larger, mature oocytes and subsequent contraction of the mesenteries following spawning (mean: 21.8 oocytes mm^{-2} before and 27.3 oocytes mm^{-2} after spawning). These data suggest selective spawning of mature oocytes, with retention of smaller immature oocytes that can continuously develop between spawns. Thus, our protocol for physiological assays included a 'clearing spawn' prior to heat ramp exposure to focus the assays on larvae produced from gametes that matured under experimental conditions.

MA parental effects

Across the nine genotype-controlled parental population pairs, we found a significant (P<0.001, one-tailed, paired *t*-test, N=9) increase in LT50 (mean ΔLT50: 0.34°C, range: 0.22–0.46°C) in larvae from STHS parents (Fig. 1E). However, once STHS parents were returned to 20°C for 2 weeks, larvae from the three parental populations that were re-spawned after 2 weeks at 20°C no longer showed higher thermal tolerance (P=0.57, one-tailed, paired *t*-test, N=3; Fig. 1F).

MA maternal versus paternal effects

Linear model results indicated that maternal effects conferred a significant (P<0.05) increase in larval thermal tolerance (mean ΔLT50: 0.18°C, s.d.: 0.087, N=5), whereas paternal effects induced no difference in larval LT50 compared with that of controls (mean ΔLT50: −0.001°C, s.d.: 0.084, N=6). Interestingly, when both parents were subjected to STHS, larvae showed the largest increase in LT50 (mean ΔLT50: 0.369°C, s.d.: 0.082, P<0.01, N=6), indicating there are either synergistic effects to having both parents exposed to heat stress or negative, epistatic effects when only one of the parents is exposed.

Genetic effects: NC and MA crosses

As expected from previous studies, we found that NC purebred larvae had higher larval LT50 than MA purebred larvae under control conditions (ΔLT50: 0.18°C, P<0.01, likelihood ratio test, N=6; Fig. 3D). Hybrid larvae showed intermediate phenotypes, between those of MA purebred and NC purebred larvae (N=6; Fig. 3B,C). Exposure of MA parents to STHS, however, resulted in larvae that had statistically indistinguishable LT50 values from those of purebred larvae from NC controls (P=0.17, likelihood ratio test, N=4-6; Fig. 3D) and NC STHS larvae (P=0.09, likelihood ratio test, N=4; Fig. 3D).

NC purebred larvae from STHS parents also showed a significant increase in larval LT50 compared with that of the controls (ΔLT50:

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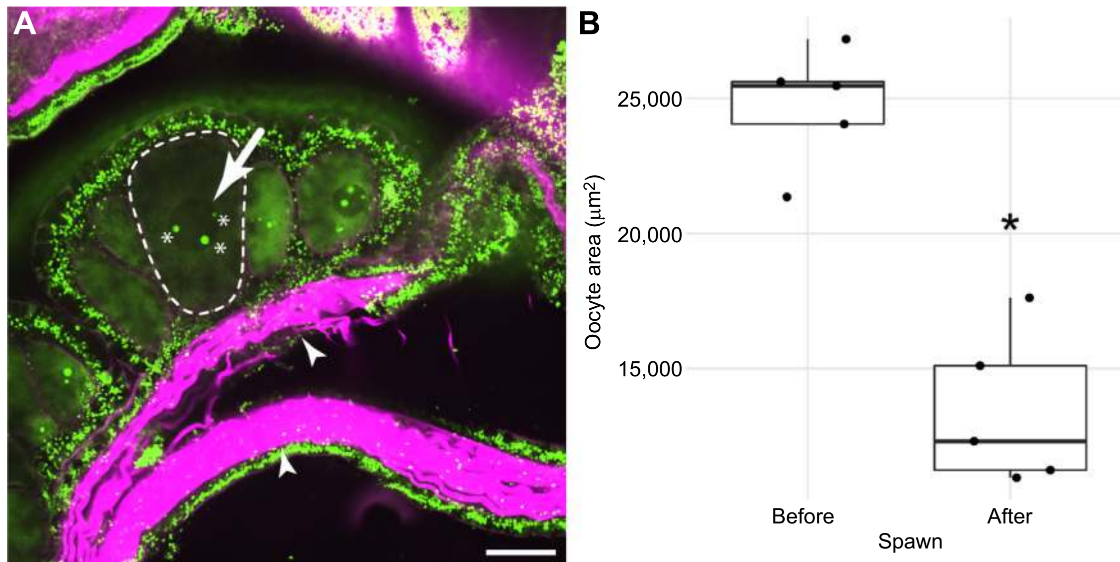

Fig. 5. *Nematostella vectensis* females retain immature oocytes after spawning. (A) Single focal plane confocal microscopy image of mesenteries containing oocytes. Oocytes (dashed outline) are distinguished from the surrounding tissue by size, the enlarged nuclei (arrow) and nucleoli (asterisks). The retractor muscle fibers (arrowheads) of the mesenteries are enriched with F-actin (magenta). Nuclei are labeled by SYBR™ Green I (green). Scale bar: 100 µm. (B) Mean oocyte area per animal before and after spawn (*N*=5 anemones). After spawn, the oocytes remaining in gonads are significantly smaller (**P*<0.001, two-tailed, two-sample *t*-test).

0.2°C, *P*=0.001, likelihood ratio test, *N*=4-6; Fig. 3D). Hybrid larvae with an NC mother and MA father showed a significant, although smaller, increase following STHS (ΔLT50: 0.17°C, *P*<0.01, likelihood ratio test, *N*=6; Fig. 3D). Hybrids from an MA mother and NC father showed slightly higher LT50 following STHS, but this difference was not statistically significant (ΔLT50: 0.13°C, likelihood ratio test, *P*=0.09, *N*=6; Fig. 3D).

Larval gene expression

HSP70 and *MnSOD2* were chosen based on previous work on the response of *N. vectensis* to a variety of stressors, such as oxidative stress, ultraviolet and pollutants (Helm et al., 2018; Tarrant et al., 2014). For instance, *HSP70* expression is quickly upregulated after 6 h of heat stress and remains elevated 24 h after recovery (Helm et al., 2018). *MnSOD2* levels increase most after recovery from heat stress, but respond quickly under ultraviolet stress (Helm et al., 2018; Tarrant et al., 2014). *CS* was chosen because activity is used as a proxy for mitochondrial density and aerobic capacity, which can play a role in thermal physiology and would be expected to increase under prolonged thermal stress (Gibbin et al., 2017; Hawkins and Warner, 2017).

Spawning success across parental cohorts used for this experiment was uneven; therefore, biological replication depth across different parental groups and larval treatments varied (Table S2). Statistical tests were conducted with replication at the level of the parental population (*N*=4). Overall, gene expression levels did not differ between larvae from STHS and control parents at baseline conditions (*P*=0.29 for *CS*, *P*=0.31 for *HSP70*, *P*=0.25 for *MnSOD2*, two-tailed, paired *t*-tests; Fig. 4B). Eighteen hours after the acute larval heat stress, larvae from STHS parents showed higher expression of *HSP70* and *MnSOD2* (*P*=0.052, *P*=0.12, respectively, paired *t*-test; Fig. 4C), although these differences were not significant. Levels of *CS* in larvae from STHS and control parents showed no discernible difference (*P*=0.68, two-tailed, paired *t*-test; Fig. 4C).

Gene expression was also measured immediately after the larval heat stress for larvae from control parental populations (*N*=4). In larvae from control parents, *HSP70* expression was significantly higher immediately after heat stress relative to baseline (*P*=0.03, two-tailed, paired *t*-test, *N*=4; Fig. 4D), whereas expression of *CS* was significantly lower (*P*=0.04, two-tailed, paired *t*-test, *N*=4; Fig. 4D). Gene expression results across all families and timepoints are shown in Fig. S4.

Fertilized oocyte sizes

We did not find any differences in fertilized oocyte diameter between control and STHS mothers (*P*=0.28, two-tailed, paired *t*-test, *N*=4; Fig. S5).

Pre-conditioning of adult anemones to heat stress

We found a significant difference (*P*=0.03, Fisher's exact test) in survival following an acute, 6-h heat shock at 36°C between control and pre-conditioned (STHS) adult anemones (*N*=80 anemones per treatment). On average, there was 80% survival in control anemones and 92% survival in STHS anemones, with an odds ratio of 0.38, indicating that anemones exposed to the STHS regime are less likely to die from an acute heat shock, as would be expected if thermal exposure induced physiological priming in *N. vectensis.*

DISCUSSION

We used *N. vectensis* as a powerful study system to measure the strength and plasticity of parental effects as well as explore the mechanisms promoting shifts in larval thermal tolerance across distinct populations. We first explored parental effects in MA populations. For our experiments, we created genetically controlled parental populations, which were replicated across parental treatments. Although the overall genetic composition of the parental populations across treatments was the same, one may wonder whether the contributions of individual clonal lineages to the gamete pool may have varied among treatments, potentially resulting in clonal effects across parental treatments. However, the consistency in the relative LT50 values across larval cohorts (e.g. the ranking of LT50s of larvae from the same parental populations was consistent across control and STHS parental treatments) and the

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fidelity in the reaction norm slope (mean: 0.34°C; s.d.: ±0.07) strongly suggest that the increase in larvae thermal tolerance is a result of parental effects and unlikely to stem from differential fecundity among more thermally tolerant parental clonal lines. In line with our observations, parental effects have also been shown to promote offspring thermal tolerance in polychaetes (Massamba-N'Siala et al., 2014), fruit flies (Lockwood et al., 2017), copepods (Vehmaa et al., 2012), corals (Putnam and Gates, 2015; Putnam et al., 2016), sticklebacks (Shama et al., 2016) and damselfish (Donelson et al., 2012), among many others.

Despite the increasing popularity of studies in parental effects and transgenerational plasticity, many studies only test the phenotypes of one cohort of offspring. For organisms with multiple reproductive cycles, it is important to also test the persistence of such effects across subsequent breeding periods. For instance, in polychaetes, the impact of maternal effects depends on when, during oogenesis, the mother experiences a particular environment, with exposure early in oogenesis providing stronger protective parental effects under variable environmental conditions (Massamba-N'Siala et al., 2014). In our case, we find that once STHS parents are returned to control temperatures, larvae from the parents' next spawn return to baseline levels of thermal tolerance (Fig. 1F), indicating reversible (plastic) parental effects in *N. vectensis*. The continuous gametogenic cycle in *N. vectensis* likely facilitates this process by maintaining a constant pool of immature oocytes that can be modified by cues from the environment in which they directly develop (Fig. 5). Given the anemone's naturally fluctuating environment, this plasticity in parental effects combined with continual gametogenesis could be more beneficial than adjusting irreversibly in any one direction (Beaman et al., 2016). Our results underscore the need to test for reversibility or persistence across breeding periods, as well as to monitor gametogenesis, in order to better understand how such mechanisms may help (or fail to help) organisms keep pace with global climate change.

It is also important to determine what degree of parental exposure or stress yields beneficial results for offspring. In MA, *N. vectensis* experience a wide diel (∼15°C during summer months) and seasonal range in temperatures (∼40°C between winter and summer temperatures) (Tarrant et al., 2019). Our experiments used laboratory-acclimated animals, which have not been exposed to diel temperature fluctuations for several years. Parental exposure to diel temperature fluctuations prior to spawning could potentially lead to different results, as parents would experience high temperatures every day, albeit for a shorter duration. In such a case, parental anemones may consistently produce larvae with higher thermal limits, instead of constantly attempting to modulate parental effects. Given the vast seasonal temperature variation in their natural habitat, it is also possible that, under field conditions, parental effects may more closely track seasonal variation instead of the inter-week differences tested here. Nevertheless, our results clearly show that *N. vectensis* is capable of such modulation. Future studies that examine temperature regimes that more closely match the short- and long-term variability in field temperatures, as well as compare different timescales of parental exposure to such variability, would provide new insights.

We found that parental effects in *N. vectensis* were consistent across populations, as both NC and MA parents conferred similar increases in thermal tolerance to their larvae, signifying that such effects are not unique to MA populations, and may, instead, be an inherent characteristic of *N. vectensis* that suits its naturally variable estuarine habitat. The ecological relevance of these increases is underscored by the fact that the thermal threshold of larvae from STHS MA parents was comparable to that of larvae from control NC parents (Fig. 3D) – a genetically distinct and isolated population that is likely locally adapted to warmer temperatures (Reitzel et al., 2013). The shallower slope in the reaction norm of hybrid larvae, however, suggests that epistatic effects may arise when combining distinct populations and that differences in larval thermal tolerance between sites have a strong genetic basis.

Uncovering the mechanisms behind parental effects can also improve understanding of organismal physiology under changing conditions. Preliminary experiments (data not shown) showed a substantially smaller increase in thermal tolerance for juveniles (7 dpf) from STHS parents, suggesting that gamete provisioning mechanisms (such as increased lipid or antioxidant content, transcript loading or early epigenome influences) may be more likely than effects that last further into development (e.g. induction of developmental plasticity), as the former would enhance fitness of early larval stages but wane as larvae grow. The timing of gametogenesis in relation to stress exposure may also play an important role, as has been shown for polychaetes (Massamba-N'Siala et al., 2014). During the development of germ cells, there are critical windows in which the epigenome is more readily influenced by environmental cues (Bale, 2014; Xavier et al., 2019). During our trials, we spawned animals prior to the start of the experimental heat ramps, which facilitates clearance of mature oocytes (Fig. 5B). This would imply that the experimental larvae were primarily derived from oocytes that completed later stages of maturation in the experimental conditions. As such, STHS parents may have been able to modify gamete epigenomes in a manner that could facilitate larval thermal tolerance. The timing of epigenetic programming in *N. vectensis*, however, is not known. Furthermore, we cannot be certain that all mature gametes were released during the pre-spawn. It is possible, therefore, that some of the effects described here arose from a combination of direct parental effects and environmental effects on developing gametes, as the timing of gametogenesis could not be entirely constrained to the experimental period, despite our best efforts [see Torda et al. (2017) and Byrne et al. (2020) for a detailed discussion of such considerations].

We investigated two possible mechanisms for increased larval tolerance: modulation of egg size and larval gene expression. Larger eggs and larvae could be expected to be more sensitive to thermal stress associated with oxygen-limitation (Martin et al., 2017). We found that eggs from STHS and control parents did not differ in size (Fig. S5). Anecdotally, egg masses from STHS mothers sometimes had fewer eggs, but this pattern was inconsistent and not rigorously quantified. A potential trade-off between egg number and egg size, overall fecundity or other provisioning (e.g. lipid content) could be investigated in future studies. Such a trade-off may be responsible for the range of egg diameters seen in STHS egg clutches (Fig. S5).

As a second possible mechanism, modulation of gene expression might enhance larval thermal tolerance. In *Drosophila*, for instance, loading of maternal transcripts for a heat shock protein into eggs enhanced embryonic thermal tolerance (Lockwood et al., 2017). Comparison of egg and sperm transcriptomes in coral also suggest that early development is largely governed by parentally derived transcripts (Van Etten et al., 2020). Larvae from STHS parents showed higher levels of *HSP70* and *MnSOD2* expression 18 h after heat stress, suggesting that sustained expression could be influencing tolerance (Fig. 4C). However, larvae from control parents showed a prompt return to baseline (pre-stress) levels of gene expression after showing differences in expression immediately after heat stress (Fig. S4). This pattern, termed

'transcriptomic resilience', has been linked to stress tolerance in other species such as seagrasses and corals (Franssen et al., 2011; Thomas et al., 2019). As such, it is surprising that transcriptomic resilience in larval gene expression patterns for *N. vectensis* is not coupled with higher thermal tolerance (i.e. it was larvae from control parents that showed this pattern, not larvae from STHS parents). If gene expression is contributing to larval tolerance, then it is possible that the dynamics of expression (e.g. timing and magnitude) are more important than either factor alone. We saw no differences in expression of *CS* under baseline conditions or after recovery from heat stress between larvae from control or STHS parents, suggesting that mitochondrial density was not substantially different between larvae from different parental treatments (Fig. 4C). Interestingly, we did see a decrease in *CS* expression when comparing larvae from control parents at baseline and immediately after heat stress, which could suggest mitochondrial fusion (and decreasing density) under elevated temperatures (Fig. S4). Previous studies on mitochondrial fission and fusion rates in vertebrate neural cells, estimate fusion rates of 0.023 fusions min^{-1} (Cagalinec et al., 2013). If similar rates can occur in *N. vectensis*, then around four to six fusions might be possible over the span of 4 h, which could impact expression levels. However, it is also possible that heat stress may decouple *CS* expression from mitochondrial function, especially in developing larvae, so these results should be interpreted with caution. Owing to limitations in sampling size, our study may have missed other dynamic expression patterns or lacked the power to detect substantial differences in expression across timepoints. Examining gene expression in larvae under heat stress is doubly challenging as temperature can accelerate developmental processes that also prompt widespread changes in gene expression (Politis et al. 2017). Future transcriptomics studies tracking global gene expression through RNA sequencing or similar methods, and which compare individual larvae, could reveal more complex patterns and identify candidate genes involved in increasing larval thermal tolerance. In particular, examining gene expression patterns in larvae from single parent crosses or a split-brood design may provide higher power to detect such differences.

We found that exposure of mothers alone to the STHS regime leads to an increase in larval LT50, whereas exposure of fathers alone does not result in any detectable shift in larval thermal tolerance (Fig. 2C). Maternal effects alone, however, account for only about half of the LT50 increase seen when both parents are exposed to the STHS regime, suggesting that having both parents under the same conditions confers additional benefits (Fig. 2C). The mechanisms responsible for this synergy are unknown, but theoretical, antagonistic epistasis between maternal effects and offspring genotypes have been described (Wolf, 2000). Although examples of maternal effects abound, evidence for direct paternal effects is only beginning to emerge (Crean and Bonduriansky, 2014; Soubry, 2015). Paternal influences on zygotic phenotypes through transfer of cytosolic compounds to the fertilized egg, or through compounds in the seminal fluids, have been described in humans (Kumar et al., 2013), trout (Danzmann et al., 1999), mice (Rassoulzadegan et al., 2006) and insects (Avila et al., 2011). Potential influences of maternal effects on the efficacy of paternal effects have also been conceptually explored (Crean and Bonduriansky, 2014), and have been shown for paternally inherited quantitative trait loci that influence thermal tolerance in rainbow trout (Danzmann et al., 1999). In addition to transcript loading, the parental epigenome may influence early offspring performance or gene expression (Bale, 2014; Xavier et al., 2019), although this influence may be short lived as the epigenome is often reset in early development in most animals and plants studied (Feng et al., 2010). It is possible that male *N. vectensis* are providing larvae with additional resources or epigenetic differences, but these are insufficient when decoupled from maternal contributions. Combined with the elevated expression of *HSP70* and *MnSOD2* in larvae from STHS parents following heat stress, our results suggest that RNA loading or epigenetics may contribute to larval thermal performance and that these effects could be stronger when both parents are exposed to the same STHS regime.

Exposure of *N. vectensis* to elevated temperatures also substantially increased the survival of adult anemones to acute heat shock, suggesting that *N. vectensis* can also modulate adult physiology to match thermal conditions. Priming effects such as these have previously been reported for cnidarians, including corals (Gibbin et al., 2018; Putnam and Gates, 2015) and anemones (Hawkins and Warner, 2017), and across other taxa such as sculpins (Todgham et al., 2005). A study of adult thermal acclimation to a range of temperatures (6–33°C) suggests that *N. vectensis* rapidly adjusts respiration rates and later increases metabolic capacity (activity of mitochondrial enzymes) when exposed to different temperatures (D. Brinkley, H.E.R. and A.M.T., unpublished data), a response that mirrors that found in polychaetes under thermal acclimation (Gibbin et al., 2018). Studies that focus on mitochondrial physiology in adults and larvae may help to identify the mechanisms responsible for the rapid acclimation of *N. vectensis* to different thermal regimes.

Although plasticity, both of adult physiology and parental effects on their offspring, can enhance short-term survival, there may also be trade-offs. For instance, heritable transgenerational changes that increase offspring's aerobic scope have been described in spiny damselfish (Ryu et al., 2018), but F2 generation fish maintained at warmer temperatures were unable to breed, suggesting a strong trade-off between thermal performance and reproduction (Veilleux et al., 2018). Such trade-offs should be explored in future studies in order to fully characterize the potential of parental effects to promote species persistence under changing climate scenarios. We find that parental effects on thermal tolerance in *N. vectensis* are substantial, but quickly reversible (subsequent cohorts lose protection), suggesting that *N. vectensis* responds quickly to its current environment and may take advantage of parental effects without long-term trade-offs. Studies that follow multiple generations of *N. vectensis* through parental heat exposure and track offspring growth and eventual reproduction could elucidate any potential trade-offs associated with increased thermal tolerance early in life. A parental strategy that favors short-term gamete provisioning over longer-term epigenetic changes may be better suited to the highly variable, yet broadly predictable (seasonal and tidal), environment of *N. vectensis*.

Studies of *N. vectensis* reproduction in the field are scarce. Only one study, to our knowledge, describes gravid gonads in field-collected individuals from Nova Scotia, and only during August and September (Frank and Bleakney, 1976). A handful of other studies suggest that reproduction is mainly asexual under natural conditions, given the high levels of clonality observed in field-collected anemones (Eckelbarger et al., 2008; Hand and Uhlinger, 1994; Reitzel et al., 2008). Dedicated studies of the reproductive cycle of *N. vectensis* in the field, including the effects of natural thermal variation on reproduction and larval phenotypes, would offer greater understanding of its parental strategies.

Overall, *N. vectensis* offers a robust organismal system in which to study thermal responses, owing to its wide thermal range, easy husbandry, fast development, ease of spawning, ability to generate

clonal lineages and well-developed genomic resources. Here, we show that *N. vectensis* is capable of quickly modulating parental effects to increase larval thermal tolerance. Although maternal exposure can result in significant shifts, exposure of both parents to different environments results in a larger increase in larval thermal tolerance. In a northern population (MA), shifts resulting from parental effects result in larval thermal limits that are comparable to those of a southern, more thermally tolerant, population (NC). These patterns point to both a genetic and plastic basis for thermal tolerance in *N. vectensis*. Given our rapidly changing global thermal environment, studies that aim to uncover the mechanisms responsible for these rapid shifts in thermal performance can provide insights into the sensitivity, acclimation and adaptation potential of vulnerable species such as marine ectotherms.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: H.E.R., A.M.T.; Methodology: H.E.R., C.-Y.C., A.M.T.; Formal analysis: H.E.R., C.-Y.C.; Investigation: H.E.R.; Resources: M.C.G.; Writing - original draft: H.E.R.; Writing - review & editing: H.E.R., C.-Y.C., M.C.G., A.M.T.; Visualization: H.E.R., C.-Y.C.; Supervision: A.M.T.; Project administration: M.C.G., A.M.T.; Funding acquisition: M.C.G., A.M.T., H.E.R.

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Data availability
Raw data and code for all statistical analyses and figure generation are available at GitHub: https://github.com/hrivera28/Nematostella–ParentalEffects/ and https://github.com/Penguinayee/Nematostella_ParentalEffects. Original data underlying this manuscript can be accessed from the Stowers Original Data Repository at http://www.stowers.org/research/publications/libpb-1600

Supplementary information
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