

Previous exposure mediates the response of eelgrass to future warming via clonal transgenerational plasticity

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Abstract. Mortality and shifts in species distributions are among the most obvious consequences of extreme climatic events. However, the sublethal effects of an extreme event can have persistent impacts throughout an individual's lifetime and into future generations via withingeneration and transgenerational phenotypic plasticity. These changes can either confer resilience or increase susceptibility to subsequent stressful events, with impacts on population, community, and potentially ecosystem processes. Here, we show how a simulated extreme warming event causes persistent changes in the morphology and growth of a foundation species (eelgrass, Zostera marina) across multiple clonal generations and multiple years. The effect of previous parental exposure to warming increased aboveground biomass, shoot length, and aboveground-belowground biomass ratios while also greatly decreasing leaf growth rates. Long-term increases in aboveground-belowground biomass ratios could indicate an adaptive clonal transgenerational response to warmer climates that reduces the burden of increased respiration in belowground biomass. These transgenerational responses were likely decoupled from clonal parent provisioning as rhizome size of clonal offspring was standardized at planting and rhizome starch reserves were not impacted by warming treatments. Future investigations into potential epigenetic mechanisms underpinning such clonal transgenerational plasticity will be necessary to understand the resilience of asexual foundation species to repeated extreme climatic events.

Key words: Zostera marina; extreme warming event; marine heatwave; carry-over effects; delayed effects; stress memory; clonal transgenerational plasticity.

INTRODUCTION

Extreme climatic events can have ratchet-like impacts on ecosystems (Wethey et al. 2011), and can prominently shape physiological, ecological, and evolutionary processes in those systems (Gutschick and BassiriRad 2003). This is particularly true when extreme climatic events impact foundation species (Wernberg et al. 2012, Thomson et al. 2015, Alatalo et al. 2016). Extreme climatic events (such as floods, drought, and heatwaves) are defined as falling outside of the 90th percentile of current average climatic variability and are predicted to occur more frequently under future climate scenarios (Stocker et al. 2013, Smale et al. 2019). Predicting foundation species' response to global climate change improves when extreme climate events are incorporated into models of future scenarios (Sanginés de Cárcer et al. 2018). Accomplishing this requires understanding

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the nature and mechanisms of the legacy of specific extreme events (Byrnes et al. 2011, Seidl et al. 2014, Fabina et al. 2015, Wu et al. 2018), as exposure to repeated extreme events both within an individual's life-time and across generations could either confer resilience or result in compounded stress (Walter et al. 2013).

The legacy of extreme events can be detected through changes in individual phenotype. Within-generation phenotypic plasticity (WGP) is defined as the ability of a genotype to produce distinct phenotypes when exposed to different environmental conditions (Pigliucci 2005), encompassing both developmental plasticity (irreversible trait variation resulting from environmental exposure during development) and phenotypic flexibility (continuous but reversible trait variation in response to environmental fluctuation; Piersma and Drent 2003). Both permanent and labile forms of WGP could play a large role in the response of individuals to extreme events, with important consequences for population persistence (Gienapp et al. 2008, Munday et al. 2013, Chevin and Hoffmann 2017). Long-term phenotypic change after a disturbance (such as an extreme event) that alters an individual's response to future stress occurs across a broad range of ecological contexts. This phenomenon is

captured by several related terms. *Carry-over* or *delayed effects* occur when environment in one life-history stage or season alters phenotypic response to future situations (Sorensen et al. 2009, Hettinger et al. 2012, O'Connor et al. 2014). Similarly, *stress memory* is commonly used in the plant sciences to describe plant hardening to environmental stress (Bruce et al. 2007, Walter et al. 2013, Brotherton and Joyce 2015). The ecological phenomena described by these terms all emphasize that the previous experience of individuals contributes to within-generation phenotypic variation within species, and that understanding the duration of this legacy is required to predict response to future events.

Although these phenomena emphasize persistence of stress effects within a generation, the legacy of an extreme event can also impact the phenotype of future generations (Donelson et al. 2018). This transgenerational plasticity (TGP) occurs when environments experienced by either parent prior to fertilization result in the modification of the phenotype of the offspring, with maternal effects being a specific type of TGP (Salinas and Munch 2012). Questions remain about what conditions increase the likelihood of adaptive TGP effects, with meta-analyses revealing positive and negative TGP effects of varying strength across varying taxa (Uller et al. 2013, Yin et al. 2019). Positive effects of TGP likely become more important under conditions with sufficient spatial or temporal variability to limit local genetic adaptation, yet are sufficiently predictable to allow parental exposure to match offspring experience (Salinas and Munch 2012, Walsh et al. 2016, Donelson et al. 2018). Examples of adaptive TGP are increasing in number (Parker et al. 2011, Donelson et al. 2012, Miller et al. 2012, Salinas and Munch 2012, Shama et al. 2014, Thor and Dupont 2015, Herman and Sultan 2016, Walter et al. 2016), highlighting the need to understand how both TGP and WGP could interactively impact a species' ability to cope with environmental change (Luquet and Tariel 2016).

Phenotypic response of clonal organisms to environmental conditions can also be passed on to asexually produced offspring, similar to TGP in sexually reproducing species (Schwaegerle et al. 2000, Latzel and Klimešová 2010, Gonzalez et al. 2016, Münzbergová and Hadincová 2017). Many habitat-forming plant species (e.g., some trees and shrubs, and many forbs and graminoids) propagate vegetatively by sprouting clonal offspring along rhizomes, forming a series of ramets, which can later become physically separated from one another as belowground connections break (see review of clonality in Jackson et al. 1985). After extreme events, the persistence and function of ecosystems based on clonal foundation species will depend on how the phenotypes of clonal offspring are influenced by the parent clone's experience. Additionally, as the return interval for these events may be shorter than the duration between sexual generations, TGP might play a particularly important role in fine-tuning organismal traits and performance in species that primarily reproduce clonally (Dodd and Douhovnikoff 2016).

Here, we use the marine foundation species, eelgrass (Zostera marina), as a model system to investigate the potential role of clonal TGP in plants coping with repeated thermal stress. Eelgrass inhabits sheltered coastlines ranging throughout warm temperate to boreal seas in the northern hemisphere (Olsen et al. 2004), forming intertidal and shallow subtidal meadows that provide ecosystem services such as supporting valuable fisheries (Hughes et al. 2009) and carbon sequestration (Röhr et al. 2018). Over the last 15 yr extreme warming events have impacted the primary and secondary production of eelgrass and other tropical seagrass ecosystems in Europe (Reusch et al. 2005, Marbà and Duarte 2010), Australia (Thomson et al. 2015), and the northern Pacific (Reynolds et al. 2016, Ha and Williams 2018). Eelgrass can propagate by sprouting clonal side-shoots along a network of rhizome, which regularly separate from the parent ramet during periods of winter senescence (Vermaat and Verhagen 1996). Thus, clonal offspring side shoots can be physically separated from parent ramet and continue to grow within the meadow. In this study, we test how parental exposure to an extreme warming event alters the phenotype of first- and second-generation clonal offspring and how this change in phenotype mediates offspring response to a second extreme warming event in the following growing season. Specifically, we experimentally replicate the extreme warming events of 2014 and 2015 in the Northern Pacific called the "Blob" (Gentemann et al. 2017, Sanford et al. 2019), to assess the effect of repeated warming on eelgrass morphology and different metrics of productivity.

METHODS

Field collection and mesocosm preparation

During August 2016, we collected 200 eelgrass (Zostera marina) ramets from an intertidal eelgrass meadow near Westside Park, Bodega Harbor, California (38°19.192' N, 123°03.189' W). Bodega Harbor is located within the middle of the geographic range of eelgrass along the west coast of North America (Williams and Heck 2001). At Westside Park, the eelgrass meadow maintains an average yearly shoot density of approximately 350 shoots m^{-2} , an average canopy height of approximately 1.5 m, and supports a diverse community of invertebrates (Best and Stachowicz 2014, Ha and Williams 2018). We collected plants approximately 5 m seaward from the upper edge of the eelgrass meadow. Individual ramets were collected along two 100 m transects that were placed 1 m apart and run parallel to the tidal gradient at a tidal elevation of approximately -0.25 m, a depth at which plants are exposed to air for only a few hours per month during the lowest tides. Ramets were collected every meter along these two transects. This collection design was implemented to minimize the effect of tidal height on ramet phenotype and ensure that shoots were collected from unique genotypes. Previous work at this field site demonstrates that collecting ramets at 1-m intervals will rarely resample the same genotype (Reynolds et al. 2017, Abbott et al. 2018).

We kept ramets in outdoor flow-through seawater tanks for less than 48 h before processing and planting. We standardized ramets to one terminal shoot (hereafter termed the "parent" or "F₀" shoot; see definitions of clonal generations) with a rhizome length of 3 cm before planting in plastic flowerpots (8.89 cm³) filled with sieved and homogenized sediment from the plant collection site. We placed the planted F₀ shoots within an array of 20 mesocosms at the Bodega Marine Laboratory: 10 shoots were placed in a single mesocosm (60 cm long \times 30 cm wide \times 60 cm deep, a volume of 113 L). The flow rate into each mesocosm was approximately 60 L/h; water was sand filtered to 30 microns.

Definitions for clonal generations: F_0 , F_1 , and F_2

We define a clonal generation as all vegetatively produced clonal shoots (F_1) connected to an original parent shoot (F_0) through the rhizome. Once this rhizomal connection is broken, the now-independent F_1 shoot is a terminal parent shoot and all new vegetatively produced clonal shoots would be considered a new clonal generation (F_2). Thus, wild-collected parent shoots (F_0) produced numerous clonal offspring (F_1) shoots, and together comprised the whole parent (F_0) ramet (see Fig. 1B). Once an F_1 shoot was broken off of the F_0 rhizome, we considered the clonal shoots vegetatively produced by this separated F_1 shoot to be a new generation of rhizomally connected offspring (F_2) shoots. We define the whole offspring (F_1) ramet as the interconnected F_1 and F_2 shoots (see Fig. 1C).

Parent (F_0) shoot exposure to warming event: Long-term within-generation plasticity

We allowed F₀ shoots to acclimate to ambient mesocosm conditions for 17 d. After the initial acclimation period, half of the 20 mesocosms were warmed for 45 d (September–October 2016) to $15.4 \pm 1.13^{\circ}$ C, and the control tank temperature remained at 13.7 ± 0.96 °C (mean \pm SD). Our warm temperature treatment falls within the range of high-temperature anomalies (14.9-17.4°C) experienced in Bodega Bay during the 2014 and 2015 marine extreme warming events and is above the 90th percentile for Bodega Bay's long-term average summer temperatures of approximately 13°C (Gentemann et al. 2017, Sanford et al. 2019). We did not measure immediate or direct effects of this initial warming event on F_0 shoots, as previous work found the effect of warming to be delayed (Reynolds et al. 2016), saving our replicates to increase our power for the TGP experiment; see Transgenerational Plasticity Experiment. We randomly selected half of the F₀ subreplicates to quantify the long-term effects of warming on growth and biomass of the F_0 shoot, as well as the number and biomass of clonal offspring (F_1) shoots produced (end of March through the beginning of April 2017; see Fig. 1A for timeline). The whole ramet was divided into the originally planted F_0 shoot, F_1 shoots, and belowground biomass (rhizomes + roots) and each were dried separately at 60°C for several weeks before weighing. We kept all belowground material together because we could not unambiguously assign belowground tissue to F_0 vs. F_1 generation shoots. We recorded ramet survival within treatments at the end of the warming event (October 2016) and 6 months later during early spring (April 2017). The remaining subreplicates were kept in mesocosm to continue to propagate and grow clonal offspring (F_1) shoots for another 4 months for use during our TGP experiment.

Transgenerational plasticity experiment: F_1 exposure to warming event

During August 2017 we collected two clonal offspring shoots (F_1) from each of the remaining whole parent (F_0) ramets; these F_1 shoots were not present during the first experimental warming event. Eelgrass propagates shoots one at a time with about one clonal shoot produced per month (Short and Duarte 2001). We selected the two largest F_1 shoots on each ramet, which were likely older F1 shoots, but shoots in each pair developed at different times (weeks to months) after the first warming event. We standardized the rhizomes of the F₁ shoots to 3 cm and planted in plastic flowerpots (8.89 cm³) filled with sieved and homogenized sediment from Westside Park. We then returned the planted F_1 shoots to the mesocosms. Each pair of F1 shoots collected from a parent was split across temperature treatments: we assigned one shoot to receive the same temperature treatment as its parent and assigned the other shoot to the remaining (novel) temperature treatment. Thus, our experimental design was a fully factorial split-plot design with both parent exposures represented within each mesocosm and a F₁ shoot from each parent represented within each temperature treatment (see Fig. 1A).

We allowed F_1 shoots to recover from transplant and acclimate to ambient mesocosm conditions for 44 d. After the acclimation period, we increased the temperature in the warmed treatment for 40 d (October-November 2017). This second warming treatment also mimicked recent marine heatwaves (warmed tank temperatures averaged 16.66 ± 1.07 °C, while the ambient treatment averaged 13.48 ± 0.90 °C). To capture the delayed effects of warming (Reynolds et al. 2016), we measured the response of clonal offspring after a 1month recovery period at ambient temperatures. On the F_1 shoot (terminal shoot), we used the "hole punch" method (see Dennison 1987) to determine the leaf growth rates on all leaves, and we also measured shoot length. We calculated leaf relative growth rate (RGR) as (leaf growth rate/leaf length) \times 100. We counted the number of second-generation clonal offspring produced (F₂ shoots). We divided above- and belowground tissues,



FIG. 1. Experimental design. (A) Schematic showing the application of treatments to a single warmed (light red) and control (light blue) tank. Ten parent (F_0) shoots were placed in each control (light blue pots) or warmed (dark red pots) tank. The first warming event (dark red background denotes warming) lasted until October 2016. Half of the whole F_0 ramets were harvested 6 months later to determine response of F_0 to warming, including production of F_1 offspring. The remaining F_0 shoots produced first-generation clonal offspring (F_1) shoots until July 2017, after which time a pair of F_1 shoots from each parent ramet were harvested and replanted. For each pair of F_1 shoots, one was kept in the same environment as the parent and the second was transplanted to the remaining treatment (i.e., control or warmed) for the secondary warming event during September 2017. The whole F_1 ramet (i.e., planted F_1 shoots + newly produced F_2 shoots) was harvested 1 month after the warming treatment ended. (B) A representative diagram of the whole F_0 oramet (i.e., F_1 shoots) 6 months after the first warming event. (C) A representative diagram of the whole F_1 offspring ramet (i.e., F_1 shoots) 1 month after the second warming event. [Color figure can be viewed at wileyonlinelibrary.com]

and aboveground tissues were further separated into the planted terminal shoot (F_1) and new clonal offspring shoots (F_2) . We then dried each tissue type separately at 60°C for several weeks before weighing dried biomass.

Nonstructural carbohydrate analysis

As an indicator of plant energy stores, we measured nonstructural carbohydrate content of the rhizomes of both the whole F_0 and whole F_1 ramets using a modified method from Alcoverro et al. (1999). First, dried rhizomes were ground to a fine powder. We then extracted sucrose from 25 mg of ground tissue in 96% ethanol at 80°C for 15 min, and we repeated this ethanol extraction three times for each sample. Starch was extracted from remaining pellet by dissolving it in 0.1 N NaOH for 24 h at room temperature. Extracted starch concentrations were determined with a spectrophotometer using an anthrone assay with sucrose as a standard.

Data analysis

Long-term WGP response of parent (F_0) shoots to the initial warming event was analyzed using linear mixed-

effects models and generalized linear mixed-effects models. For most response variables, we used linear mixed-effect models including a fixed effect of temperature treatment and a random effect of mesocosm (to account for nonindependence of subreplicates within each mesocosm). Because the number of F_1 shoots produced (clonal propagation) consists of count data, we used a generalized linear mixed-effects model in order to specify a Poisson error distribution. This model also included a fixed effect of temperature treatment as well as a random effect of mesocosm.

To assess the TGP and WGP response of clonal offspring (F_1) to parent (F_0) shoot prior exposure to warming and F1 exposure to warming, we used linear mixedeffects models and generalized linear mixed-effects models. We used linear mixed-effects models for the following response variables: F1 shoot dry biomass, F1 shoot length, F1 relative leaf growth rate, total F2 shoot dry biomass, F₂ average shoot size (per shoot biomass), F₂ provisions (starch availability per shoot), total aboveground dry mass (whole F₁ ramet), total belowground biomass (whole F₁ ramet), aboveground-belowground biomass ratio, and total rhizome starch. These models included fixed effects of F1 temperature treatment and F₀ temperature treatment as well as a random effect of F_0 temperature treatment nested within mesocosm (to account for nonindependence in our split-plot design). We tested for an interaction between the fixed effects by comparing models with and without an interaction using AIC, retaining the interaction term only when it improved model fit (see Results). Total F₂ shoots dry biomass, total belowground biomass, and rhizome starch were all strongly positively skewed and these data were log-transformed prior to analysis to allow convergence of the linear mixed-effects model. We used a generalized linear mixed-effects model to analyze number of F₂ shoots produced, as above. This model also included fixed effects of F1 temperature treatment and F0 temperature treatment as well as a random effect of F₀ temperature treatment nested within mesocosm.

The residuals of all linear models were checked for normality using a Shapiro-Wilk test. All data analyses were conducted in R Version 3.5.1 (R Foundation for Statistical Computing, 2018), in the R-packages "lme4" (version 1.1-21) and "stats" (version 3.6.0). R script and data files are in public repository at GitHub (see *Data Availability*).

RESULTS

Parent (F_0) long-term within-generation plasticity to warming

Six months after warming, we were able to detect slight long-term WGP effects in parent (F_0) ramets that suggest morphological trade-offs in response to temperature treatment. Wild-collected parent (F_0) shoots that had previously experienced warming had 14% less biomass than control F_0 shoots (Fig. 2A, Table 1). In contrast, exposure to warming increased biomass of F₁ offspring shoots by 37% (Fig. 2B, Table 1). This increase was driven by the production of three more F_1 shoots by warmed parents relative to parents grown at ambient temperature (Fig. 2C, Table 1), indicating a shift from maintaining the F₀ shoot towards vegetative production of new clonal offspring shoots. Average F₁ shoot size and F₁ provisions (starch available on a pershoot basis) did not differ across treatments (Fig. 2D, 2E, Table 1). Whole ramet belowground biomass increased by approximately 25% in response to warming (Appendix S1: Fig. S1B; Table 1), likely a reflection of additional F_1 shoot production (Fig. 2C). Whole parent ramet aboveground biomass (Appendix S1: Fig. S1A; Table 1), aboveground-belowground biomass ratio (Appendix S1: Fig. S1C; Table 1), and total rhizome starch did not differ by temperature treatment (Appendix S1: Fig. S1D; Table 1).

Clonal offspring (F_1) within-generation and transgenerational plasticity to warming

Response of clonal offspring (F1) to a second warming event depended on parent (F_0) shoot prior exposure to warming, revealing independent effects of both WGP and TGP, and an interaction between WGP × TGP. As observed for parent (F_0) shoots response to the first warming event, the second warming event reduced the biomass of the parent F_1 shoot (Fig. 3A, Table 1). Despite having less biomass, parent F₁ shoot length was 12% greater in the warmed than ambient treatments (a WGP effect only; Fig. 3B, Table 2). The within-generation response of F_1 leaf relative growth rate (RGR) was modified by the transgenerational effect of parent shoot environment (a WGP \times TGP interaction), where F₁ RGR was reduced by 50% in response to warming only if parent was also exposed to warming. If the parent shoot was naïve to warming, F₁ RGR did not change in response to warming. Together this caused a strong negative TGP effect in the F_1 warming treatment, with RGR of F1 shoots from warmed parents reduced by approximately 30% compared to F₁ shoots from naive parents (Fig. 3C, Table 2).

We assessed WGP and TGP changes to F1 clonal reproductive effort by measuring F2 biomass, shoot number, size, and provisioning. Biomass of F2 shoots depended on both F₁ environment and previous exposure of their grandparent shoot (F_0) to warming. F_1 warming reduced total biomass of F_2 shoots by 33% (a WGP effect), which was counteracted by F₀ exposure to warming that increased biomass of F_2 shoots by 25% (a TGP effect; Fig. 4A, Table 2). This increase in F_2 biomass for plants whose grandparents (F_0) were warmed was driven by both the production of one additional F2 shoot (when F1 is also warmed; Fig. 4B) or by larger F2 shoots (when F1 is in ambient conditions; Fig. 4C). Overall, F1 warming decreased the number of F₂ offspring produced (Fig. 4B, Table 2). Neither F_1 environment nor F_0 environment impacted F2 starch provisioning (Fig. 4D, Table 2)



FIG. 2. Parent (F_0) whole ramet response measured 6 months after exposure to first warming event (mean ± 1 SE). (A) Parent (F_0) shoot dry biomass, (B) total dry biomass of all clonal offspring (F_1) shoots, (C) total number of F_1 shoots produced, (D) average size of F_1 shoots, and (E) F_1 shoot provisioning in terms of starch availability on a per-shoot basis. See Table 1 for model estimates and *P* values. N = 41-42. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1. The results of linear and generalized linear mixed-effects models examining the long-term response of parents (F_0) and whole F_0 ramets to the first warming event, measured 6 months after the return to ambient temperatures.

Response variable	Estimate	SE	<i>t</i> -value	z-value	Р	Figure
F_0 biomass (g)	-0.102	0.071	-1.439	NA	0.15	2A
F_1 biomass (g)	0.35	0.239	1.462	NA	0.144	2B
F_1 size (g/shoot)	0.003	0.021	0.137	NA	0.891	2D
F ₁ provision (g starch/shoot)	0.300	0.999	0.300	NA	0.764	2E
Total above (g dry mass)	0.21	0.248	0.849	NA	0.396	Appendix S1: Fig. S1A
Total below (g dry mass)	0.409	0.257	1.588	NA	0.112	Appendix S1: S1B
Above:below	-0.132	0.131	-1.012	NA	0.312	Appendix S1: S1C
Total starch (g)	12.159	12.714	0.956	NA	0.339	Appendix S1: S1D
Number of F ₁ shoots	0.334	0.217	NA	1.541	0.123	2C

Note.: Total observations = 68.

WGP and TGP effects also impacted F_1 morphology on the level of the whole F_1 ramet. Total aboveground biomass $(F_1 + F_2)$ increased as a result of F_0 exposure to warming (a TGP effect), and F_1 warming reduced aboveground biomass (a WGP effect; Fig. 5A, Table 2). F_0 warming did not affect total belowground biomass, but F_1 warming reduced belowground biomass by approximately 25% (Fig. 5B, Table 2). Consequently, aboveground–belowground biomass ratios were elevated by 20% in response to F_0 exposure to warming (a strong TGP effect) but were unaffected by F_1 environment (Fig. 5C, Table 2). Rhizome starch reserves were not altered by F_0 exposure or F_1 warming treatment (Fig. 5D, Table 2). Most of the whole parent (F_0) and clonal offspring (F_1) ramets survived the experiment and the frequency of mortality did not vary among treatments (see Appendix S1: Table S1). This suggests that our results were not driven by differential selection on thermal tolerance among treatments.

DISCUSSION

Our results demonstrate that parent (F_0) shoot exposure to an extreme warming event alters the phenotype of clonal offspring (F_1) in eelgrass (*Z. marina*), favoring clonal offspring shoot production over maintenance of





FIG. 3. First-generation clonal offspring (F_1) response to an extreme warming event when parent (F_0) shoots were grown under control conditions (solid line) or exposed to a warming event 1 yr prior (dashed line) (mean \pm 1 SE), as measured by (A) shoot dry biomass, (B) shoot length, and (C) leaf relative growth rate (RGR). Transgenerational and within-generation phenotypic plasticity (TGP and WGP) effects below a threshold of P = 0.1 are depicted with a gray arrow and asterisks, respectively. See Table 2 for details on model estimates and P values. N = 29-37. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. The results of linear and generalized mixed-effects models examining the response of clonal offspring (F_1) and whole F_1 ramets to the second warming event (F_1 environment [env.]) and parent's previous exposure to warming (F_0 env.).

Response variable	Fixed effects	Estimate	SE	<i>t</i> -value	z-value	Р	Figure
F ₁ biomass (g)	F ₁ env.	-0.09	0.065	-1.386	NA	0.166	3A
	F ₀ env.	0.077	0.055	-1.384	NA	0.166	
F ₁ length (cm)	F_1 env.	12.112	6.216	1.949	NA	0.051	3B
	F ₀ env.	7.729	5.559	1.390	NA	0.164	
F_1 relative growth rate (%/d)	$F_1 env. \times F_0 env.$	-1.457	0.615	-2.371	NA	0.017	3C
	F ₁ env.	0.729	0.447	1.629	NA	0.009	
	F ₀ env.	-0.535	0.598	-0.894	NA	0.886	
Total F ₂ biomass (g)†	F ₁ env.	-0.759	-0.284	-2.676	NA	0.007	4A
	F ₀ env.	0.420	0.198	2.122	NA	0.033	
F ₂ size (g/shoot)	F ₁ env.	-0.019	0.012	-1.622	NA	0.105	4C
	F ₀ env.	0.016	0.008	1.930	NA	0.054	
F ₂ provision (mg starch/shoot)	F ₁ env.	0.179	0.579	0.302	NA	0.763	4D
	F ₀ env.	0.050	0.379	0.132	NA	0.895	
Total above (g)	F ₁ env.	-0.220	0.110	-1.963	NA	0.049	5A
	F ₀ env.	0.180	0.067	2.69	NA	0.007	
Total below (g)†	F ₁ env.	-0.311	0.174	-1.784	NA	0.074	5B
	F ₀ env.	0.047	0.091	0.521	NA	0.602	
Above:below	F ₁ env.	-0.081	0.175	-0.461	NA	0.645	5C
	F ₀ env.	0.251	0.132	1.896	NA	0.058	
Total starch (mg)†	F ₁ env.	-1.869	2.276	-0.821	NA	0.412	5D
	F_0 env.	1.312	1.943	0.675	NA	0.499	
Number of F ₂ shoots	F ₁ env.	-0.223	0.097	NA	-2.287	0.022	4B
	F ₀ env.	0.139	0.106	NA	1.320	0.187	

Notes.: As per methods, interaction terms are only presented if model comparison with AIC indicated that the interaction term improved model fit. Total observations = 133.

[†]Data were log-transformed to allow for model convergence.

parent shoot and increasing aboveground–belowground biomass ratios. Whether the F_1 transgenerational changes in phenotype could be adaptive under ocean warming scenarios is difficult to interpret, as many of these fitness-linked traits responded in opposite directions, indicating the potential for complex trade-offs among traits. For example, under warmed conditions F_1 shoots with parents that previously experienced warming had severely reduced leaf relative growth rates compared to all other treatments (Fig. 3C, Table 2), yet maintained greater production of clonal offspring (F_2 shoots; Fig. 4A, B, Table 2). Overall, warming appears to permanently reduce the biomass of the parent shoot that directly experiences thermal stress, a trend observed 1 month after warming in F_1 response (Fig. 3A, Table 2) and also 6 months after warming in F_0 response (Fig. 2A, Table 1). One month after warming, warmed F_1 shoots produced slightly fewer F_2 offspring



FIG. 4. First-generation clonal offspring (F₁) investment in clonal reproduction (F₂) in response to an extreme warming event when parent shoots (F₀) were grown under control conditions (solid line) or exposed to a warming event 1 yr prior (dashed line; mean \pm 1 SE), as measured by (A) total dry biomass of all F₂ shoots, (B) number of F₂ shoots produced, (C) average size of F₂ shoots, and (D) F₂ shoot provisioning in terms of starch availability on a per-shoot basis. Transgenerational and within-generation phenotypic plasticity (TGP and WGP) effects below a threshold of P = 0.1 are depicted with gray arrows and asterisks, respectively. See Table 2 for details on model estimates and *P* values. N = 29-37. [Color figure can be viewed at wileyonlinelibrary.com]

(1.1 or 0.76 fewer when F_0 exposure was control or warmed; Fig. 4B, Table 2). In contrast, 6 months after warming this trend was reversed and the effect size was much greater with warmed F_0 shoots producing three additional clonal offspring (F_1) compared to control F_0 shoots (Fig. 2C, Table 1). Together these results indicate a long-term shift in allocation to greater clonal reproduction over maintenance of parent shoot, a change in ramet phenotype that can take months to become detectable. This TGP impact of F_0 warming on ramet phenotype caused a long-term increase in aboveground biomass in whole F_1 ramets (Fig. 5A, Table 2); contributing to an increase in aboveground–belowground biomass ratios (Fig. 5C, Table 2).

Aboveground-belowground biomass ratios in eelgrass increase by 0.207 for every 1°C increase in temperature (Clausen et al. 2014; Fig. 5C), a change in morphology thought to reduce respiratory burden of nonphotosynthetic tissues (Vermaat and Verhagen 1996). Thus, the change in aboveground-belowground ratios detected here is potentially an adaptive response to a warmer environment, where transitioning from leaf growth to production of new shoots increases relative aboveground biomass (i.e., area of photosynthetic tissue) reducing the burden of belowground biomass (i.e., nonphotosynthetic tissue). These clonal offspring with greater aboveground-belowground biomass ratios responded to F1 warming exactly as clonal offspring with parents naïve to warming that retained smaller aboveground-belowground biomass ratios (i.e., there was no WGP \times TGP interaction and biomass was equally reduced by F1 warming under both F0 exposures, Fig. 5A, Table 2). However, it is worth noting that warmed F1 individuals with warmed parents achieved about the same aboveground biomass as naïve plants at control temperatures, suggesting that the F₀ morphological responses to warming may have allowed the maintenance of biomass production under novel environments. Although our results reveal clonal TGP effects in eelgrass, further investigations on long-term survival, growth, and reproduction would be needed to understand its adaptive significance fully. Such TGP effects where offspring of one parent always perform better despite current environment is termed a "silver spoon" effect (Uller et al. 2013), but is usually attributed to enhanced parent provisioning when parent environment is relatively less stressful (which is not the case here).

Epigenetic reprogramming (e.g., DNA methylation, histone modification, small interfering RNA) and



FIG. 5. Whole F_1 ramet (i.e., $F_1 + F_2$) response to an extreme warming event when parent shoots (F_0) had been grown under control conditions (solid line) or exposed to an extreme warming event 1 yr prior (dashed line; mean ± 1 SE), as measured by (A) total ramet aboveground dry biomass (Tot. above), (B) total ramet belowground biomass (Tot. below), (C) the aboveground–belowground biomass ratios (Above:below), and (D) total amount of starch contained within the rhizome. Transgenerational and within-generation phenotypic plasticity (TGP and WGP) effects below a threshold of P = 0.1 are depicted with gray arrows and asterisks, respectively. See Table 2 for details on model estimates and P values. N = 29-37. [Color figure can be viewed at wileyonlinelibrary.com]

maternal effects (e.g., transmission of provisioning molecules or hormones) are likely mechanisms behind clonal TGP (Verhoeven and Preite 2014, Herman and Sultan 2016, Yin et al. 2019). In sexually reproducing plants, maternal effects (or memory) were originally attributed to seed provisioning, and similar mechanisms could operate here because of physical connections among parents and clonal offspring through rhizomes. However, our results indicate a mechanism beyond parental provisioning as (1) rhizomes of first-generation clonal offspring (F_1) were all standardized to 3 cm before F_1 offspring were planted for the TGP experiment, (2) rhizome starch provisioning of F1 shoots did not differ across parental exposure treatments when F_1 shoots were still attached to the F_0 shoot (Fig. 2E). Similarly, Schwaegerle et al. (2000) found clonal transgenerational plasticity in a plant's response to nutrient and light stress was partially independent of the clonal offspring's tiller size at planting. To date, evidence for environmentally directed DNA methylation (in contrast to untargeted DNA methylation) as a mechanism of clonal TGP is indirect (Verhoeven and Preite 2014). For example, phenotypic and epigenetic variation is correlated with home-site environmental variation in clonal poplar cuttings (Vanden Broeck et al. 2018) and alligator weed (*A. philoxeroides*; Shi et al. 2019). Common stress-garden experiments applying demethylation treatments demonstrate that demethylation can inhibit clonal TGP, but also alter performance of control plants, making interpretation of these results difficult (Gonzalez et al. 2016, Münzbergová et al. 2019). Future work on eelgrass epigenetic variation across environmental contexts could greatly enhance our understanding of potential mechanisms driving the clonal TGP described in our results.

Clonal TGP in plants is likely widespread (Latzel and Klimešová 2010) and could be a means for rapid adaptive response to climate change (Donelson et al. 2012, Münzbergová and Hadincová 2017). Clonal TGP in response to drought (or reductions in soil moisture) occurs across diverse plant groups including trees (*Populus*; Raj et al. 2011), herbs (*Trifolium repens*; Gonzalez et al. 2016), and grasses (*Festuca rubra*; Münzbergová et al. 2019). Clonal TGP also occurs in response to nutrient addition in an Arctic sedge (*Eriphorum vaginatum*; Schwaegerle et al. 2000), and a highly invasive herb (*Alternanthera philoxeroides*; Dong et al. 2018). Our results appear to be the first to demonstrate clonal TGP in response to temperature in a plant. Given the sensitivity of eelgrass genotypes to modest temperature changes (e.g., Reynolds et al. 2016, DuBois et al. 2019), and the small-scale spatial-temporal patchiness in temperature within these estuarine systems (DuBois, *unpublished data*), there is the potential that clonal TGP allows for fine-tuning of eelgrass to varying thermal regimes. As eelgrass can produce many clonal generations per sexual generation (Reusch et al. 1999), clonal transgenerational plasticity could also provide a mechanism for more rapid response to frequent extreme warming events compared to the much longer time span it could take for genetic adaptation to occur in this species.

Persistent shifts in morphology of a foundation species could drastically impact the value of ecosystem services by altering ecosystem function, resilience, and community processes. Seagrass meadows provide economically important ecosystem services such as sequestering carbon (Röhr et al. 2018) and supporting fisheries (Hughes et al. 2009, Tuya et al. 2014). The 14-month increase in aboveground biomass reported here (Fig. 5A, Table 2) would alter the habitat quality utilized by invertebrate epifaunal communities and key fisheries species (Sirota and Hovel 2006). The 1-month decrease in belowground biomass (Fig. 5B, Table 2) as well as the long-term reduction in leaf growth rates (therefore reducing production of leaf detritus; Fig. 3C) could alter sediment carbon sequestration and carbon cycling (Arias-Ortiz et al. 2018). In terms of ecosystem resilience to multiple environmental stressors, long-term changes in phenotype to greater aboveground-belowground biomass ratios (Fig. 5C, Table 2) could cause individuals to be more vulnerable to light limitation experienced during periods of low water quality or during the winter (Vermaat and Verhagen 1996, Govers et al. 2015), and increased storm energy (Duarte 2002). Finally, the severe reduction in leaf growth rate (Fig. 3C, Table 2) could make individuals more vulnerable to epiphytes, compounding the negative impact of leaf shading by epiphytes under eutrophic conditions (Howard and Short 1986).

Our results highlight how an extreme event can impact the morphology of a clonally reproducing foundation species beyond the lifespan of the individual shoot that initially experienced warming, and that the clonal offspring response can only be understood in the context of both WGP and TGP effects. The potential for TGP to be an adaptive response depends on how well parent environmental history predicts offspring environment, which in turn is dependent on the degree of environmental variability and time lag between generations (Auge et al. 2017). In cases where TGP and directional selection reinforce one another, phenotypic optimums could be achieved more quickly (Auge et al. 2017). Mechanisms underlying clonal TGP are in need of further investigation but could be driven by epigenetic processes, providing an additional layer of diversity and increasing the adaptive potential in clonal organisms (Dodd and Douhovnikoff 2016). This interplay between epigenetic and genetic adaptation could be especially important in eelgrass, given that eelgrass has limited dispersal and demonstrates strong population structure (Ruckelshaus 1996, Olsen et al. 2004, Kamel et al. 2012), yet can have many clonal generations between sexual generations (Reusch et al. 1999). Better predictions of species and ecosystem-level resilience to future climate scenarios will need to incorporate the legacy of prior exposure to extreme events as well as consider how the legacy of parent exposure persists into future generations in both sexually and asexually reproducing species.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/ 10.1002/ecy.3169/suppinfo

DATA AVAILABILITY

R script and data files are available on Zenodo. https://doi.org/10.5281/zenodo.3373228