Vol. 16: 43–57, 2024
https://doi.org/10.3354/aei00472

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published February 15

Paternal heat exposure affects progeny larval development in green-lipped mussels *Perna canaliculus*

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ABSTRACT: The green-lipped mussel *Perna canaliculus* is critically important to the New Zealand aquaculture industry. However, the rise in marine heatwave (MHW) events poses an emerging threat to this industry through summer mortality events. This study investigated the potential for paternally mediated transgenerational plasticity to improve offspring performance under heat stress. We simulated a week-long MHW event, exposing male *P. canaliculus* broodstock to elevated (22°C) or ambient (17.5°C) temperatures immediately prior to spawning, and evaluated the effects of paternal heat exposure on successful development, size and acute thermal tolerance of their larvae that were also reared under ambient or elevated (20°C) temperatures through to completion of the lecithotrophic trochophore stage. Elevated paternal and larval temperatures both increased incidence of abnormal development, reducing larval yield, while initial D-veliger shell length was predominantly influenced by developmental temperature, with longer shells formed at 20°C. Veligers from heat-exposed fathers raised under 20°C showed a small, but significant, elevation in lethal tolerance 50 (LT_{50}), the temperature at which 50% of the larvae are predicted to die, when exposed to an additional 1 h heat-shock. These results indicate that paternal heat exposure over a relatively short period can influence offspring performance in this species. The paternal exposure investigated showed limited positive effects on offspring thermal tolerance, which may be outweighed by the negative impact on larval development. As MHWs are forecasted to continue accelerating, understanding transgenerational effects of heat stress will be critical for maintaining high-quality hatchery yields through broodstock selection and may inform wild population forecasting models.

KEY WORDS: *Perna canaliculus* · Marine heatwaves · Paternal effects · Aquaculture · Transgenerational effects · New Zealand

1. INTRODUCTION

Marine heatwave (MHW) events are increasing in frequency, intensity and duration with global climate change (Frölicher et al. 2018, Oliver et al. 2018, 2021). MHW events have doubled since 1982, and predictions indicate that MHWs may become annual events or permanent in regions such as New Zealand by the end of the century (IPCC 2021, Behrens et al. 2022). Accelerating MHW events have devastating consequences for biodiversity and the corresponding essential ecosystem services, ultimately impacting fisheries (Caputi et al. 2016, Smale et al. 2019, Cheung & Frölicher 2020, Smith et al. 2021, 2023). As the human population continues to grow under these conditions, aquaculture is surpassing wild catch as a method of providing protein (Gentry et al. 2017, Martin 2017). However, like wild stocks, farmed species are susceptible to many of the same anthropogenic stressors that plague wild fisheries.

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Publisher: Inter-Research · www.int-res.com

New Zealand's aquaculture industry has been rapidly expanding to meet growing seafood demand (Alfaro et al. 2014, Stenton-Dozey et al. 2021). However, the threat of MHWs necessitates innovation in order to meet this need. In recent years, New Zealand has begun to explore the expansion of marine shellfish aquaculture further offshore to avoid stressors such as ocean acidification and warming that are often most exacerbated near the coast (Heasman et al. 2020). Since 1981, the Tasman Sea region has warmed at a linear rate of 0.2–0.3°C per decade (Sutton & Bowen 2019). In summer 2017–2018, sea surface temperature (SST) anomalies in the Tasman Sea reached +2.5 to +3.7°C in the hottest eastern region, marking a more widespread and intense, though shorter, event than the MHW just 2 yr earlier in 2015–2016, which lasted for nearly a year (Perkins-Kirkpatrick et al. 2019, Salinger et al. 2019, Oliver et al. 2021).

Over the past decade, summer MHWs off the coast of New Zealand have been associated with large-scale die-offs of *Perna canaliculus* (Gmelin 1791), the green-lipped mussel (Thomsen et al. 2019, Li et al. 2020). The New Zealand green-lipped mussel (commercially 'Greenshell^{TM}') has immense cultural and economic significance as an endemic aquaculture species that makes up the majority of New Zealand's aquaculture harvest, accounting for 71% of annual aquaculture exports and over $381 million NZD in revenue (Stenton-Dozey et al. 2021). Given the importance of this species, in this study we sought to explore the effects of MHW temperatures on *P. canaliculus* broodstock through the performance of their larvae under different environmental temperatures during development.

Aquaculture provides a unique opportunity to manipulate the biological or environmental features of a system to confer greater resilience (Gavery & Roberts 2017). Heat stress events of 2 h or less can induce persistent heat tolerance in adult *Mytilus californianus* and even resistance to *Vibrio* infection in adult *Perna viridis* mussels (Aleng et al. 2015, Moyen et al. 2020). One tractable mechanism to explore adaptive capacity in aquaculture species is that of parental effects or 'transgenerational plasticity' (TGP), where parental experiences influence their offspring's performance. TGP studies in molluscs, including the mussel *Mytilus edulis*, have focused primarily on ocean acidification. Parental exposure to increased CO_2 levels has shown ameliorating effects on offspring response to CO_2 at different life stages (Kong et al. 2019, Hawkins et al. 2023) and adaptive capacity has been documented in naturally variable environments (Thomsen et al. 2017). Comparatively few studies have investigated transgenerational effects of ocean warming in molluscs, and those few which have observed negative responses (Kessel & Phillips 2018, Hawkins et al. 2023). Both positive and negative carryover effects of temperature have been observed along intertidal gradients (Waite & Sorte 2022, Wang et al. 2023).

Paternal effects are thought to be primarily epigenetic (Munday 2014, Eirin-Lopez & Putnam 2019). Mechanistically, epigenetic modifications may drive phenotypic plasticity through heritable changes in gene expression, a potentially important means for rapid adaptation to changing environments that could be leveraged in aquaculture (Gavery & Roberts 2017, Eirin-Lopez & Putnam 2019, Wang et al. 2023). Paternal effects are largely understudied compared to maternal effects (Crean & Bonduriansky 2014); however, research has shown that paternal effects, mediated through sperm, can significantly influence offspring performance both positively (Jensen et al. 2014, Lane et al. 2015, Gasparini et al. 2018, Leach & Hofmann 2023) and negatively (Gasparini et al. 2018, Leach et al. 2021), and can sometimes have a larger impact than maternal effects (Guillaume et al. 2016). In aquaculture, unlike natural populations, it is possible to control the parental experiences of the 2 sexes separately, by conditioning them differently or selecting males and females from different source areas. This raises the question whether, in a hatchery, it might be possible to induce positive transgenerational effects solely by manipulating the paternal environment without any risk of harming the nutritional provisioning to the eggs by keeping the mothers in unstressful conditions.

Our goal in this study was to examine the role of paternal effects in altering progeny thermal tolerance in the context of MHWs. Though hatchery programs in New Zealand have rapidly expanded in recent years, the majority of the industry still relies on wild spat collected largely from a single area of coastline, Ninety Mile Beach on the North Island of New Zealand. Another important source of wild-caught spat comes from Golden Bay in the South Island, allowing for extended mussel harvesting periods (Alfaro et al. 2011). In the South Island, green-lipped mussels display a bimodal spawning pattern in the austral spring and fall; therefore, a period of peak *P. canaliculus* gametogenesis aligns with summer MHW events (Buchanan 2001, Alfaro et al. 2011). For hatchery production, broodstock are often collected from coastal mussel farms and brought into controlled facilities only shortly before spawning. Therefore, both hatchery-derived and wild-collected spat supply and quality may be affected by MHWs via transgenerational

effects. In a hatchery setting, it is critical to consider how the environmental history of each parental line impacts offspring performance with regard to stressors, and whether practices should adjust to take advantage of, or counteract, any potential consequences. The focus of this investigation was to isolate the transgenerational effects of the paternal line in order to consider the advantages or risks to the aquaculture industry of paternal heat exposure. To accomplish this, we simulated a MHW event in the lab, exposed male *P. canaliculus* broodstock to elevated or ambient temperatures for 1 wk immediately prior to spawning, and assessed the effects of paternal heat exposure on the successful development, size and acute thermal tolerance of the mussel larvae.

2. MATERIALS AND METHODS

2.1. Experimental design

In March 2019, mature *Perna canaliculus* were transferred from a long-line farm in Pelorus Sound (Marlborough, New Zealand) to a nearby broodstock management facility at the Cawthron Aquaculture Park (Nelson, New Zealand), where they received ambient seawater pumped from Tasman Bay, enriched with elevated natural phytoplankton levels and tracking with SST (Ragg et al. 2010). Each individual was induced to spawn (see below) to establish sex and then allowed to restore gonads for 9 mo before the next spawning period. In November 2019, immediately prior to spawning, randomly selected adult males were conditioned in one of 2 treatment tanks: in one tank they experienced a 1 wk thermal challenge at ~22°C (considered heat exposed: 'H'); the other was held at a constant ~17.5°C (considered naïve 'N') (Fig. 1, Table 1). N temperature was selected based on current ambient temperatures in November. H temperature was selected to simulate MHW conditions, as it is above average maximum summer SST for the region, but within the range experienced in the South Island during recent MHW events (Thomsen et al. 2019). Females (n = 8) were all held at 17.5°C to isolate the effects of paternal temperature exposure. Pools of eggs from all females combined were fertilized by individual males from each treatment. Upon fertilization, embryos sired from each father (n = 5 per adult acclimation treatment) were divided into 2 controlled larval temperature treatments of ~17.5°C (ambient: 'A') or 20°C (warm: 'W') (Fig. 1, Table 1). This warm temperature was more commonly experienced than the paternal heat exposure and was recorded for several weeks during recent MHWs in the Marlborough Sounds region (Broekhuizen et al. 2021). These larval temperatures yielded 4 treatment groups (Fig. 1): (1) HW, those from heat-exposed fathers (H) raised under warm temperatures (W); (2) HA, those from heat-exposed (H) fathers raised under ambient (A) temperatures; (3) NW, those from naïve (N) fathers exposed to warm (W) temperatures; and (4) NA, those from naïve (N) fathers exposed to ambient (A) temperatures. Larvae were raised to the early D-veliger larval stage in static cultures before being assessed for shell size, abnormality and acute thermal tolerance.

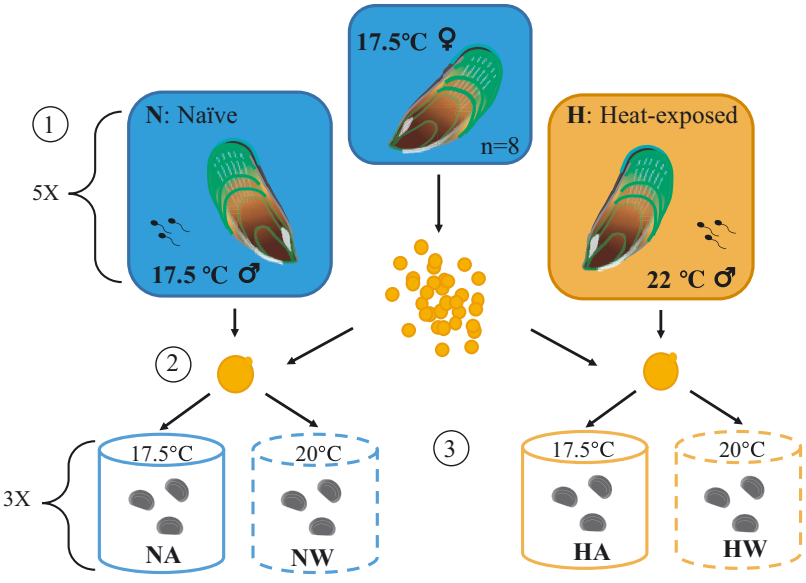

Fig. 1. Experimental design. (1) Male *Perna canaliculus* mussels were held at 17.5°C (naïve, N) or 22°C (heat-exposed, H) for 1 wk prior to spawning. (2) The conditioned fathers (n = 5 per treatment) were individually crossed to pooled eggs from 17.5°C mothers. (3) Fertilized eggs from each of the 10 crosses were then divided into 17.5°C ambient (A) or 20°C warm (W) treatments to develop until the veliger larval stage (n = 3 larval culture replicates per combination of father and developmental temperature). There are 4 final combinations of paternal and larval treatment: HW, larvae from heat-exposed fathers (H) raised under warm temperatures (W); HA, larvae from heat-exposed (H) fathers raised under ambient (A) temperatures; NW, larvae from naïve (N) fathers exposed to warm (W) temperatures; and NA, larvae from naïve (N) fathers exposed to ambient (A) temperatures

Table 1. Temperatures (±SD) for adult conditioning and larval development of *Perna canaliculus* measured every 5 min using Water Temperature Pro v2 Data Loggers (Onset Hobo™)

	Treatment	Temperature (°C)
Adult	Heat exposed (H)	22.04 ± 0.68
	Naïve (N)	17.51 ± 0.05
Larval	Warm (W)	19.78 ± 0.29
	Ambient (A)	17.63 ± 0.29

2.2. Adult mussel exposure and spawning

During acclimation, the mussels were held in flow-through aquaria and supplied with mono-cultured algae (*Tisochrysis lutea* and *Chaetoceros calcitrans*, 1:1 cell ratio, ~40 cells μl^{-1}). Temperature was recorded at 10 min intervals with *in situ* loggers (Water Temperature Pro v2 Data Loggers, Onset Hobo™) and verified by daily spot checks. On 13 November 2019, mussels were spawned by 'thermal cycling', following industry practices with cycles of 12 and 26°C (Gale et al. 2016). When sperm and eggs had successfully been collected from all groups, sperm concentrations were calculated from 5 males from each acclimation temperature (17.5 and 22°C) using a hemocytometer on an inverted microscope. Egg concentrations were calculated from 8 females, all from 17.5°C, by counting three 20 µl aliquots of eggs per female using an inverted microscope so that equal amounts of eggs could be used from each female. To ensure reliability, the average count from the 3 aliquots was used to determine the egg concentration. The coefficient of variation (CV) for the 3 egg counts was less than 10% for all females. Eggs were visually checked for quality, indicated by uniformity and sphericity, during counting.

2.3. Fertilization and larval rearing

Ten egg pools were made by gently combining eggs from all 8 females in approximately equal numbers to help ensure genetic diversity of the offspring. Each egg pool was then fertilized with the sperm of one father from either the 17.5°C (N) or 22°C (H) exposure treatment. Fertilizations were conducted in seawater pre-incubated with 4 mM EDTA, and sperm was added at a concentration of 200 sperm per egg with 1000 eggs ml^{-1} seawater (Gale et al. 2016, McDougall et al. 2020). After gently mixing the fertilization pools for 10–15 min, the formation of polar bodies was confirmed by checking aliquots of a few egg pools under the microscope. Fertilized eggs were loaded at a density of 50 embryos ml^{-1} into 3 replicate 400 ml plastic beakers, and 6 replicate 4 ml tissue culture dish (TCD) wells per father per treatment filled with EDTA-enriched seawater incubated to ~17.5°C (A) or 20°C (W) (Table 1). The 400 ml cultures were aerated periodically by hand; TCD cultures were not manually aerated due to their small volume. At the veliger stage, embryos from the 400 ml static cultures were used for the thermal tolerance trial, and embryos in the TCDs were used to assess first veliger shell (prodissoconch I) size and incidence of malformation.

2.4. Larval sampling

Embryogenesis took place in EDTA-enriched seawater at the respective treatment temperatures until the prodissoconch I was formed, approximately 38 h post-fertilization (hpf) for the 20°C treatment and 41 hpf for the 17.5°C treatment. As the larvae from the 20°C larval treatment developed more quickly, sampling of treatments was staggered in time, with visual assessment and thermal tolerance trials conducted first on the larvae reared at 20°C and then approximately 3 h later for the larvae reared at 17.5°C, when they had developed complete prodissoconch I shells. When the D-hinge veliger stage was reached in the TCDs for each treatment, the larvae were fixed by the addition of 200 µl of 10% formalin and stored at 4°C until they were scored and measured, within 4 d of sampling. Within a given larval temperature, TCDs were fixed within 10 min of one another, 400 ml cultures were sampled within 1 h, and the thermal tolerance trial began within 1.5 h after sampling had begun.

2.5. Thermal tolerance trial

To evaluate the effects of paternal heat exposure and developmental temperature on thermal tolerance, larvae from each treatment were subjected to an acute heat shock within a thermal gradient for 1 h, allowed to recover at ambient temperature overnight, and then scored as alive or dead. To prepare larvae for the acute heat shock trial, when the D-hinge veliger stage was reached in the 400 ml culture vessels, larvae were passed through a 43 µm mesh to concentrate them and remove degrading tissue. The concentrated larvae were suspended in a uniform volume of 3 ml of seawater to approximately standardize concentra-

tions. Scintillation vials (20 ml) of EDTA-enriched seawater were equilibrated to each of 8 temperatures across a temperature gradient from 28 to 38°C. An aluminum heat block with water baths set to different temperatures plumbed to either end was used to create a stable temperature gradient across each row. A volumetrically equal number of concentrated veliger larvae from all replicates from each father/treatment combination were pooled together, yielding 2 treatment pools for the 20°C reared larvae: HW and NW. Thus, the lethal temperature 50 trial (LT_{50}, the predicted temperature at which 50% of the larvae from a given treatment die) was conducted at the scale of the treatment group, not the individual father. Larvae from each pool were gently mixed to achieve a uniform distribution, and then 1000 larvae were pipetted into 16 corresponding treatment vials across the temperature gradient, with each treatment having 2 replicate vials at the same column on the heat block. Control larvae were held in ambient scintillation vials at ~18.3°C during the 1 h challenge period. Vials were capped, and larvae were incubated at their temperatures for 1 h. The temperature of each vial was measured and recorded using a thermocouple at the start and end of the trial. Vials were then removed from the block and allowed to return to ambient temperature. After 24 h, the vials were gently swirled to create a uniform suspension of individuals. A ~1 ml aliquot per vial was viewed through an inverted microscope and the first 100 larvae seen were scored as either alive or dead. Larvae were scored based on the signs of movement, even cilial movement, regardless of normality. When the 17°C larvae (HA and NA treatments) reached the veliger stage, the pooling, incubation and scoring process was repeated.

A generalized linear mixed-effects model was used to statistically assess differences in thermal tolerance among treatments, including vial temperature, paternal temperature treatment, larval temperature treatment and their interaction as fixed effects, and replicate as a random effect using the lmerTest (Bates et al. 2009, Kuznetsova et al. 2017) and lme4 packages (Bates et al. 2009) in R (version 3.5.1). A Wald chi-square test was used to assess the significance of each factor. An α level of 0.05 was used for all statistical tests. LT_{50} for each treatment was calculated using logistic regression with the MASS package (Venables & Ripley 2013) in R.

2.6. TCD assessments, normality and larval body size

After preservation in 0.5% formalin, larvae in the 6 TCD wells per father per treatment were scored for development and normality (120 cultures). Eggs and larvae were identified to be in one of 5 categories: (1) egg, (2) 'splatter', (3) pre-veliger, (4) abnormal D-hinge veliger and (5) D-hinge veliger. Every individual in each well was scored (n = ~180 per well). Previous trials (N.L.C.R. unpubl. obs.) suggested that early embryos tended to destabilize in the fixative, resulting in a 'splatter' of unconsolidated cells. The 'pre-veliger' and 'splatter' categories were later collapsed into a single class representing arrested development. Individuals were categorized as 'abnormal D-hinge larvae' if they had reached the veliger stage but displayed shell or tissue deformities such as a concave, convex or warped hinge shell, or protruding tissue. Veliger size was measured as the longest length of the shell parallel to the hinge (n = 35 individuals measured per well; cellSens™ image analysis software, Olympus). Statistical comparisons of incidence of normal development were conducted using a generalized linear mixed-effects model considering paternal treatment, larval treatment and their interaction as fixed effects, and father and assessor (the person who scored the sample) as random effects. The model for each stage was fitted using a logit link function. A Wald chi-square test was used to assess the significance of each factor on the proportion of larvae determined to be at a given stage. A generalized linear mixed-effects model was also used to compare shell size between treatments with paternal temperature treatment, larval temperature treatment and their interaction as fixed effects, and father as a random effect, followed by a Wald chi-square test to assess the significance of each factor. Statistical tests were run in R using the lmerTest and lme4 packages. Post-hoc tests to compare individual treatments were conducted in the lsmeans package (Lenth 2013) using the Tukey method to adjust for multiple comparisons.

3. RESULTS

3.1. Larval development: normality

A higher proportion of individuals developed normally in the 17.5°C ambient (A) larval treatment as compared with the 20°C warm (W) larval treatment (Fig. 2). Within each larval treatment, larvae from 22°C heat-exposed (H) fathers had a higher incidence of developmental abnormality than those from 17.5°C-acclimated naïve (N) fathers. Larvae from naïve fathers raised in ambient conditions (NA) had the highest proportion of normal development (87.86 ±

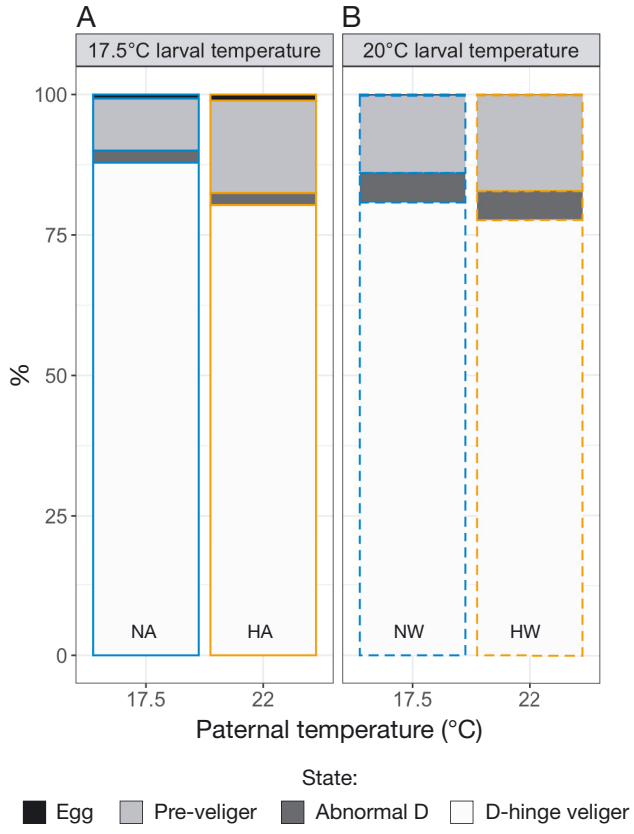

Fig. 2. Effect of paternal (heat-exposed [H, orange] or naïve [N, blue]) and larval treatment (A: ambient [A, solid line]; B: warm [W, dashed line]) on larval development and normality of *Perna canaliculus*. Stacked bars display the average percentage of embryos from each treatment that were found in each state of development and normality calculated from 6 replicate wells per 5 fathers per treatment (N = 30 per treatment). Percentage of embryos in a given state is shown by internal bar shading. Percent eggs (black) is too low to be visible in some treatments; values are shown in Table 2

3.20%, ±SD), while larvae from heat-exposed fathers reared under warm conditions (HW) had the lowest proportion of normal development (77.62 ± 8.75%). Larvae from naïve fathers raised in warm larval temperatures (NW) and those from heat-exposed fathers raised under ambient conditions (HA) exhibited intermediate levels of normal development (80.78 ± 5.64 and 80.32 ± 9.70%, respectively). There was a significant difference in the proportion of normal veligers in the NA treatment group compared to all other groups, and a significant difference between the proportion of normal veligers developed from heat-exposed fathers depending on the larval treatment they were reared in (HW relative to HA). Paternal (Wald chi-squared test: χ^2 = 28.57, df = 1, p <<< 0.001) and larval treatment (χ^2 = 92.99, df = 1, p <<< 0.001), as well as their interaction (χ^2 = 27.35, df = 1, p <<< 0.001) explained a significant amount of the variation in percentage of normal larvae.

When considering the proportion of larvae that successfully reached veliger stage, regardless of normality and relative to those stalled at an earlier stage, there was a significant effect of paternal (χ^2 = 18.80, df = 1, p <<< 0.001) and larval treatment (χ^2 = 28.89, df = 1, p <<< 0.001), as well as their interaction (χ^2 = 15.10, df = 1, p < 0.001). A higher percentage (90 ± 2.59%) of embryos from naïve fathers raised in ambient conditions (NA) reached veliger stage than from any other treatment group (86.03 ± 4.88% of NW, 82.48 ± 9.37% of HA, 82.78 ± 6.58% of HW). The proportion of veliger larvae that displayed abnormalities from each treatment group varied significantly by developmental temperature (χ^2 = 83.96, df = 1, p < 0.001), but not by paternal temperature (χ^2 = 2.63, df = 1, p = 0.105) or their interaction (χ^2 = 2.98, df = 1, p = 0.085). Veligers from the warm developmental treatments (NW and HW) had a relatively higher frequency of abnormalities (6.11 ± 3.57 and 6.43 ± 4.54% respectively) compared to those from the ambient developmental treatments (2.38 ± 1.84% for NA and 2.66 ± 2.67% HA). Finally, more eggs remained undeveloped in the 17.5°C ambient larval treatment than in the 20°C warm larval treatment (χ^2 = 11.75, df = 1, p < 0.001) (Table 2). Paternal temperature did not have a significant effect on the number of eggs remaining (χ^2 = 1.44, df = 1, p = 0.23).

3.2. Larval development: shell size

Length of the veliger shell was significantly influenced by larval developmental temperature (Wald chi-squared test: χ^2 = 269.31, df = 1, p <<< 0.001), as larvae developing in 20°C were generally longer (89.96 ± 2.37 µm for NW and 90.33 ± 2.49 µm for HW) (Fig. 3A) than those developing in 17.5°C (88.36 ± 2.10 µm for NA, 88.98 ± 2.11 µm for HA) (Fig. 3B). Larvae from heat-exposed fathers were on average 1.34 µm longer when raised under warm conditions compared to ambient, while larvae from naïve fathers were on average 1.60 µm longer when raised under the same conditions. Veligers from heat-exposed fathers tended to be longer than their naïve-sired counterparts (χ^2 = 494.82, df = 1, p = 0.026), and the interaction between paternal and larval treatment was not significant (χ^2 = 364.6, df = 1, p = 0.056). In post-hoc comparisons using the Tukey method, only those treatments with different developmental temperatures were significantly different (p < 0.05)

(Fig. 3). Under ambient developmental conditions veligers from heat-exposed (H) fathers were on average 0.62 µm longer than veligers from naïve (N) fathers, while under warm developmental conditions, larvae from heat-exposed fathers were on average 0.37 µm longer than those from naïve fathers. These results were consistent with a preliminary trial that manipulated temperature during early development of *P. canaliculus*, in which our results showed that veliger shell lengths increased with temperature across a range from 17 to 21°C (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/q016p043_supp.pdf).

There was higher variation in veliger shell size among individual larvae from the 20°C larval treatments, NW and HW (CV: 2.63 and 2.76%, respectively), compared to the 17.5°C ambient larval treatments, NA and HA (CV: 2.38 and 2.37%, respectively). Although the size ranges of larvae from each sire within a treatment largely overlapped, there was slightly more variation in average size of a veliger from different heat-exposed sires relative to naïve sires, whose larvae displayed a more constrained average size (Fig. 3, inner boxplot). The mean veliger size from each heat-exposed father had a variance of 0.32 µm in the ambient larval treatment and 0.36 µm in the warm larval treatment, while the mean veliger size from each naïve father had a variance of 0.16 and 0.15 µm in the ambient and warm larval treatments, respectively.

3.3. Larval performance: thermal tolerance

In the thermal tolerance trial, paternal temperature (Wald chi-squared test: $\chi^2 = 5.90$, df = 1, p = 0.015), larval temperature ($\chi^2 = 5.11$, df = 1, p = 0.024) and the interaction between paternal and larval temperature ($\chi^2 = 4.92$, df = 1, p = 0.027) explained a significant amount of the variation in the

Table 2. Mean ± SD percentage of embryos from each treatment that were found in each state of development and normality. Different letters among treatments represent statistically significant differences for a particular state of development ($p < 0.05$) after a Tukey multiple comparisons test. Treatments are: NA, larvae from naïve (N) fathers raised in ambient (A) larval temperatures; NW, larvae from naïve (N) fathers raised in warm (W) larval temperatures; HA, larvae from heat-exposed (H) fathers raised in ambient (A) larval temperatures; and HW, larvae from heat-exposed (H) fathers raised in warm (W) larval temperatures

Treatment	D-hinge veliger (%)	Abnormal D-hinge veliger (%)	Pre-veliger (%)	Egg (%)
NA	87.86 ± 3.20^{a}	2.14 ± 1.58^{a}	9.27 ± 2.55^{a}	0.73 ± 0.66^{a}
NW	80.78 ± 5.64^{b}	5.25 ± 3.10^{b}	13.75 ± 4.91^{b}	0.23 ± 0.38^{b}
HA	80.32 ± 9.70^{b,c}	2.16 ± 2.24^{a}	16.42 ± 8.94^{b}	1.10 ± 1.13^{a}
HW	77.62 ± 8.75^{c}	5.16 ± 3.50^{b}	17.04 ± 6.61^{b}	0.18 ± 0.30^{b}

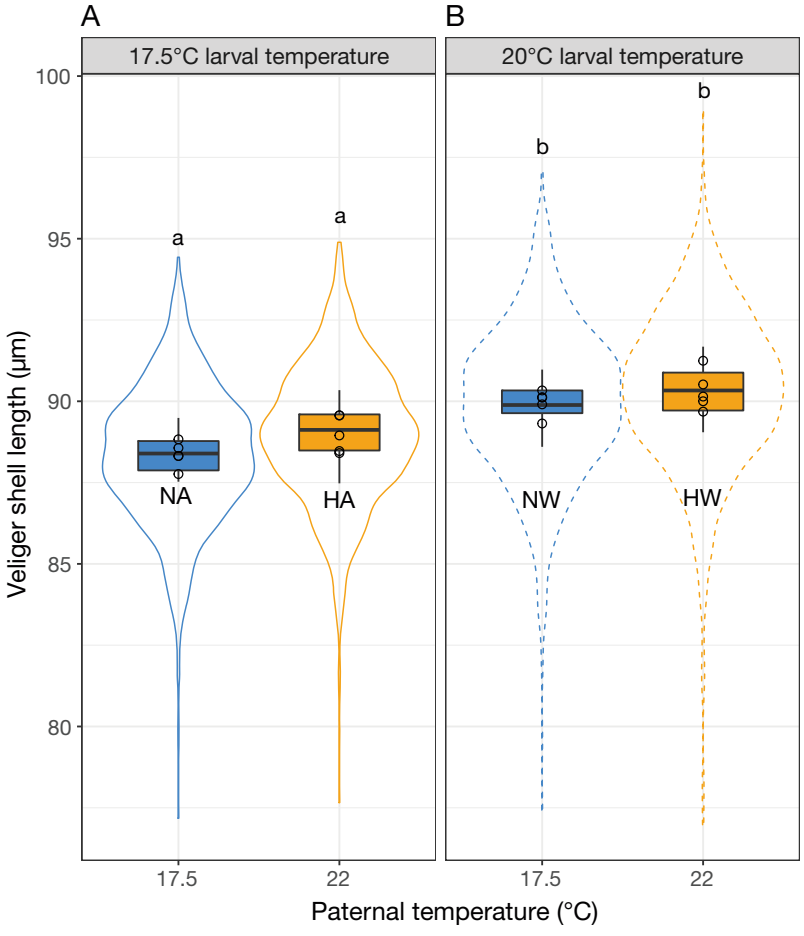

Fig. 3. Effect of paternal (heat-exposed [H, orange] or naïve [N, blue]) and larval treatment (A: ambient [A, solid line]; B: warm [W, dashed line]) on first D-hinge veliger shell length (µm) of *Perna canaliculus*. Inner boxplots display the distribution of average veliger size for each of 6 technical replicates per 5 fathers per treatment (N = 30 per treatment). The outer violin plot shows the variation between each individual larva in a treatment (N = 1050 per treatment). Different letters (a,b) among treatments represent statistically significant differences ($p < 0.05$) after a Tukey multiple comparisons test. Open circles show the average veliger length for larvae from each of the 5 fathers per treatment

binomial survival data. Yet, the LT_{50} for each treatment group was tightly constrained around 36°C (Fig. 4). The LT_{50} was highest for larvae from heat-exposed fathers raised under the warm developmental treatment (LT_{50} HW = 36.27 ± 0.05°C). The LT_{50} values for all other treatment groups were slightly lower (LT_{50} NA = 36.16 ± 0.05°C, NW = 36.12 ± 0.05°C, HA = 36.15 ± 0.05°C).

4. DISCUSSION

4.1. Summary of results

We investigated the effects of male *Perna canaliculus* broodstock heat exposure prior to spawning on larval development and performance under ambient and warm developmental temperatures. The salient findings were: (1) elevated paternal and developmental temperatures both hindered larval development, (2) larvae that reached veliger stage grew larger on average under warm developmental temperatures and (3) veligers from heat-exposed fathers raised under warm developmental temperatures had the highest LT_{50}, though LT_{50} was high and very similar for all treatment groups. Overall, the persistent increase in MHWs in New Zealand may have detrimental effects on green-lipped mussel aquaculture, as elevated temperatures negatively affect larval development in the wild, and heat-exposed fathers produce decreased larval yields in hatcheries.

4.2. Context of acclimation temperatures

MHWs are an ongoing and escalating threat in New Zealand. The temperatures used to induce thermal stress in this experiment, 22°C for the fathers and 20°C for the offspring (Table 1), are within the range currently possible in the South

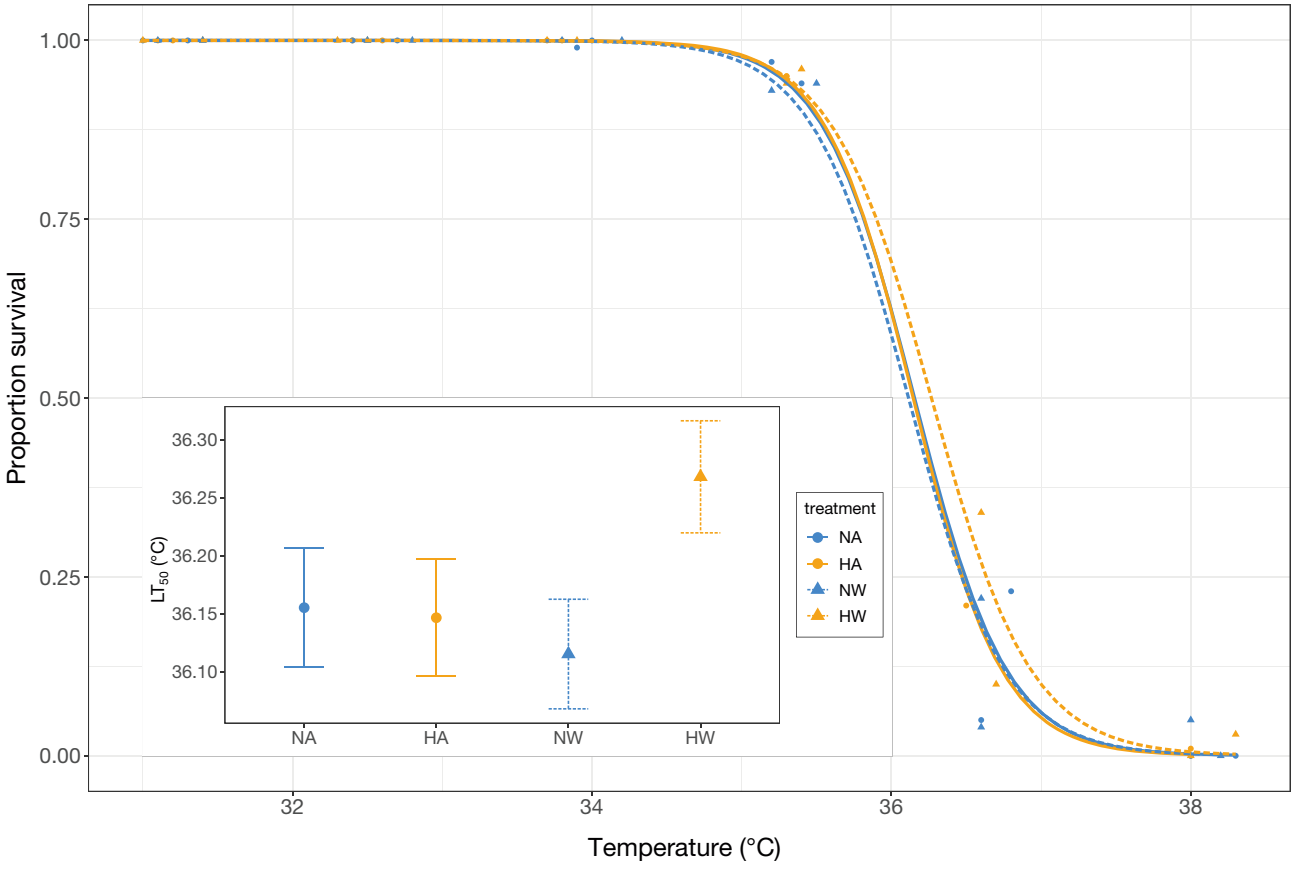

Fig. 4. Effect of paternal (heat-exposed [H, orange] or naïve [N, blue]) and larval treatment (ambient [A] or warm [W]) on veliger thermal tolerance of *Perna canaliculus*, measured as proportion survival of 100 larvae scored per vial after 1 h acute heat shock and recovery. Two replicate vials were measured across a gradient of acute heat shock temperatures. Temperatures below 31°C have been removed for ease of visualization. Inset displays the calculated LT_{50} for each treatment, error bars represent means ± SE

Island, where the Marlborough Sounds encompass the largest mussel growing area in New Zealand (Heasman et al. 2020). The higher paternal exposure temperature simulated an acute 1 wk MHW event, around the upper limit of temperatures currently experienced during MHWs in the region. Large numbers of larvae are needed for successful spat recruitment, so small changes in developmental success can be significant ecologically and commercially. As green-lipped mussels tend to have a bimodal spawning behavior in the South Island, the alignment of these MHWs with gametogenesis is likely concentrated heavily around the spawning period in late summer to early fall (Buchanan 2001) (Fig. 5). Based on the results of this study, this temporal alignment could have strong ecological consequences. North Island populations typically show a broader single spawning period from June to December as temperatures are increasing from their lowest point, which may indicate that parental exposure to positive thermal anomalies prior to spawning is less likely for these populations (Alfaro et al. 2001).

4.3. Effects of the larval developmental environment

Our results showed that the warm (W) developmental environment slightly but significantly increased the size of newly formed veliger larvae (Fig. 3). There was also higher variance in size among the larvae in the warm developmental treatment. As this study only measured the first veliger stage, it is unclear whether the observed increase in size with temperature would persist throughout development, resulting in larger spat at recruitment. Growth rate in bivalve mollusc larval development from veliger to spat is positively correlated with developmental temperature, though temperature variation also interacts with other factors of the larval environment such as salinity, food availability and pCO_2 (Rico-Villa et al. 2009, Lazo & Pita 2012). There can be a trade-off between growth rate and size at metamorphosis, with high temperature driving faster development but smaller sizes at settlement (Pechenik et al. 1990, Galley et al. 2010). If the size trends do continue throughout development, faster growth under warmer conditions could alter the length of the free-living larval

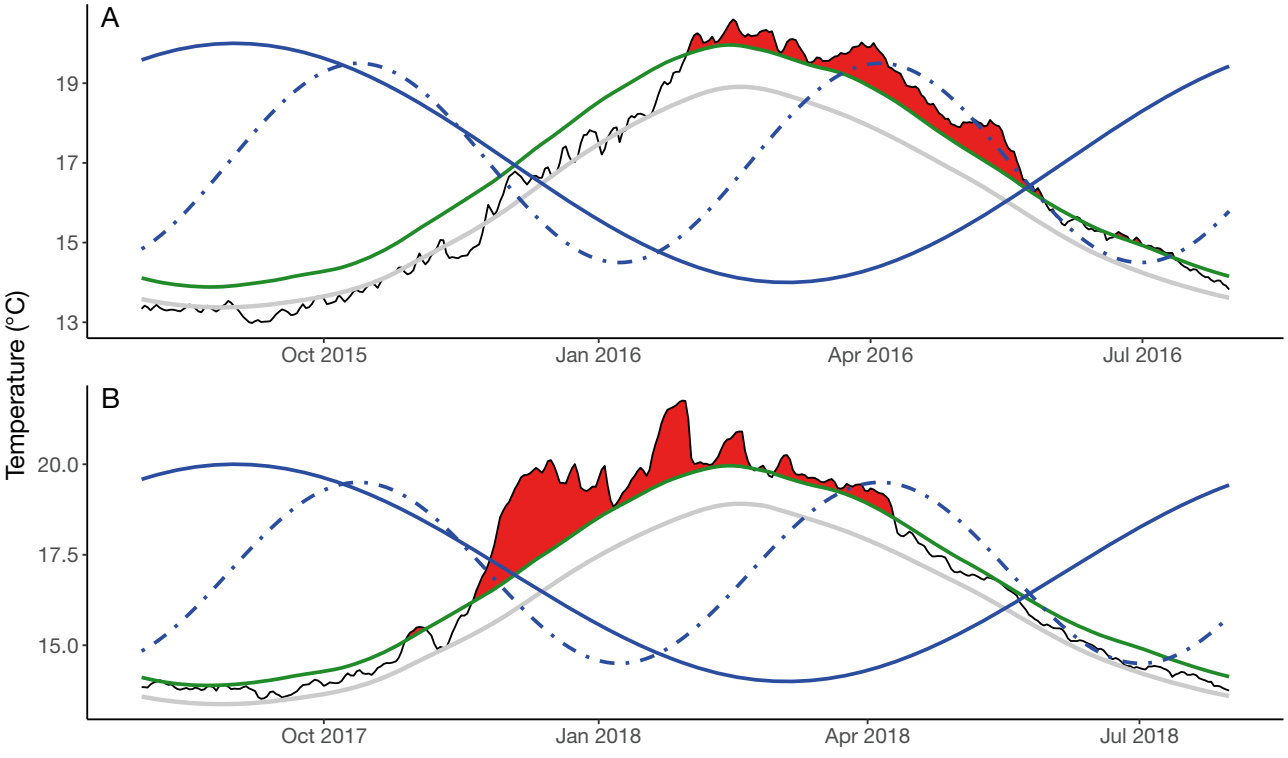

Fig. 5. Alignment of the general annual spawning cycle for *Perna canaliculus* populations on the North Island (solid blue line) and South Island (dashed blue line) of New Zealand with 2 recent marine heatwave (MHW) events (Alfaro et al. 2011). Shown are examples of representative MHW events (red shading) impacting the Tasman Sea in (A) 2015–2016 and (B) 2017–2018 visualized in heatwaveR (v 0.4.5), where temperature (black line) persists above the 90th percentile threshold (green) above the seasonal climatology (grey) for at least 5 d (Schlegel & Smit 2018) using NOAA 1/4° Daily Optimum Interpolation Sea Surface Temperature (OISST) data (Huang et al. 2021) for the region between −45 and −35° latitude and 165 and 175° longitude

stage and the distribution of new recruits, affecting population connectivity and commercially important spat catching sites.

The effects of warm water temperature on incidence of abnormal development and thermal tolerance were more complicated and mediated by the paternal treatment. Fewer larvae from the warm developmental treatment made it successfully to the veliger stage or formed a normal veliger shell (Fig. 2). For those larvae from naïve (N) fathers, the larval temperature had a significant effect on both metrics. However, for larvae from heat-exposed (H) fathers, the warm developmental temperature only had a negative effect on the number of normal veligers formed, but if malformed individuals were also considered, the total number of larvae reaching veliger stage held constant. The number of normal veligers is likely a more important distinction, as the abnormal veligers are unlikely to survive through the larval bottleneck, especially in the wild (Helm et al. 2004, Ragg et al. 2019). Importantly, the environmental conditions experienced during embryogenesis in *P. canaliculus* can have latent effects, with subtle differences in the size and morphology of the first D-veliger stage subsequently amplifying into extended growth, survival and recruitment differences (Ragg et al. 2019). Therefore, the abnormally formed veliger larvae associated with warmer temperature in this experiment are likely to achieve lower levels of recruitment. Under warm developmental temperatures, the normally developed larvae may develop a little more quickly and grow to a slightly larger size, which could help to offset the survival costs of higher abnormality at the population level.

Some species, such as the Sydney rock oyster *Saccostrea glomerata*, display an optimum temperature for larval performance in terms of fertilization, survival and normal development, with decreasing performance on either side of this optimum (Parker et al. 2009). Though the optimum for *P. canaliculus* cannot be determined from the current experimental design, negative impacts of high temperature on development may set in below 20°C. Interestingly, more individuals remained at the egg stage without developing further under the ambient (A) larval developmental treatment, regardless of paternal temperature. As fertilization was carried out under identical conditions following robust protocols for this species (Adams et al. 2009), it is unlikely that the differences reflected variability in fertilization success. However, it is possible that this may have been an artifact of warmer temperatures in the warm larval development treatment driving rapid decomposition of unfertilized eggs into a state not clearly identifiable as an egg, resulting in these individuals being categorized with the other 'pre-veliger' embryos.

4.4. Effects of the paternal acclimation environment

Paternal heat exposure was associated with a slight (non-significant) increase in veliger size (Fig. 3). However, the effects on normal development were largely negative, with a lower proportion of larvae from heat-exposed fathers successfully reaching veliger stage (Fig. 2). As size was only assessed in larvae that successfully reached veliger, this trend towards larger veliger larvae from heat-exposed fathers is more likely a selective artifact ('survivor bias') than a positive transgenerational effect. Because fewer veligers successfully developed from these groups, those that did reach this stage might be those with the most advantageous genetic makeup, resulting in a skewed sample.

Within the ambient larval treatment, a lower proportion of larvae from heat-exposed fathers reached veliger stage or formed a normal veliger shell. These same trends were evident within the warm developmental treatment, but were non-significant. The fact that these negative effects of paternal acclimation environment were seen when the offspring were raised under ambient temperature means that they could be a significant factor driving yields, even in a hatchery setting, where rearing temperature can be controlled. In addition, there was more variability in survival to veliger and normal development from heat-exposed fathers than naïve fathers (Table 2, Fig. S2). This observation accentuates the need to consider the interaction between underlying genetics and transgenerational effects. This same increase in variation between larvae from different heat-exposed fathers in contrast to different naïve fathers can also be seen in the veliger size data (Fig. 3, inner boxplots).

The presence of negative paternal effects is consistent with some other studies. Leach et al. (2021) saw that for the purple urchin *Strongylocentrotus purpuratus*, sperm from males acclimated to high temperatures had reduced fertilization success, despite the sperm being resilient to high temperatures during the fertilization process itself. In experiments manipulating the thermal environment of the marine tubeworm *Galeolaria caespitosa*, fertilization success was largely driven by paternal environment, with males from warm temperatures showing the lowest fertilization success (Guillaume et al. 2016). Larvae from warm-

acclimated fathers also had higher rates of arrested larval development, similar to our results. However, other studies have detected positive transgenerational effects to stressors including salinity and pH in other species of tubeworm (Jensen et al. 2014, Lane et al. 2015) and temperature in crickets (Gasparini et al. 2018). It is important to note that there was not an exact match between paternal and larval elevated temperature in the present study (the paternal heat exposure was hotter than the warm larval temperature) and results may have been varied if the temperatures had been identical or if a different phenology of exposure was employed.

4.5. Caveats of LT_{50} and disagreement with previous studies

The combination of elevated paternal and larval temperatures correlated with the highest larval LT_{50}, 36.27 ± 0.05°C, a mean increase of 0.11–0.15°C (Fig. 4). As the larvae from different fathers within a treatment were pooled for this assay, our ability to discern differences between offspring of different fathers is restricted. The LT_{50} values observed in this study were much higher than the LT_{50} of *P. canaliculus* larvae in the literature, which was previously found to be a more ecologically relevant 32.9–33.9°C (Dunphy et al. 2013). The substantial discrepancy to the prior LT_{50} with that seen in this study is likely driven by methodological differences. In Dunphy et al. (2013), stationary larvae were considered dead if they displayed uncontrolled gaping, gut extrusion through valves, or a loss of defined internal structures, whereas in the present study larvae were only scored as dead if they exhibited no movement, even of cilia. In a study of *Mytilus californianus* larvae that used similar death metrics to the present study (no movement or dull opaque shells), LT_{50} reached up to 35.17°C, indicating that methodology may explain the discrepancies (Waite & Sorte 2022). Dunphy et al. (2013) found no latitudinal gradient along the South Island in LT_{50} of F2 veligers. Larval LT_{50} for these populations may be resistant to large changes driven by acclimation or local adaptation, consistent with the small increase we observed with combined paternal and larval heat exposure. Based on either study, this early life stage exhibits a large thermal safety margin with regard to short-term exposure to extreme temperatures. Sub-lethal chronic stress may be a better predictor of future performance and distributions of species and populations, as species with similar upper thermal limits can have different stress responses which influence their ecological interactions. This assertion was put forth by Dunphy et al. (2013) with respect to *P. canaliculus* larvae and Tagliarolo & McQuaid (2015) with respect to adult mussels *Perna perna* and *Mytilus galloprovincialis*. Sub-lethal stress at early stages may be particularly impactful because the challenges of predation and finding suitable settlement habitat contribute to a larval survival bottleneck for many marine invertebrates. Adult *P. canaliculus* have a lower LT_{50} than other marine mussel species in New Zealand; if this tracks with sub-lethal stress as well, *P. canaliculus* may be more vulnerable than other, less commercially valuable species (Sorte et al. 2019).

In this study, for the 3 phenotypic metrics assessed (successful development, LT_{50} and size), negative, positive and non-significant paternal effects were observed. This accentuates the need for further studies into the mechanisms driving paternal effects and careful consideration when interpreting results in order to determine which phenotypic differences may be most ecologically significant or industry-relevant.

4.6. Implications for wild spat

The New Zealand aquaculture industry relies on both hatchery-derived and wild-caught *P. canaliculus* spat, so we must consider the implications of these results for both supplies in the future. Wild recruitment, particularly for the summer/fall spawning period, will likely be significantly influenced by MHWs. Hayden (1995) found that the supply of early larvae and settlers is the primary driver of the number of wild *P. canaliculus* recruits in the Marlborough Sounds 8 wk after settlement. A decrease in larval supply, either by adult mortality (Li et al. 2020) or arrested larval development due to paternal effects or larval environment, could drastically reduce spat supply.

As MHW events become more prevalent, it will be critical to track their imprint on wild spat recruitment over time. Spat forecasting models have shown a positive correlation between settlement and temperature 1 mo prior at some South Island sites, specifically when coupled with other environmental factors. However, they have not examined the effects of MHW events explicitly (Atalah & Forrest 2019). For Ninety Mile Beach, daily and seasonal water temperatures are not known to influence the timing or the scale of spatfall events, and the natural gametogenesis and spawning cycle for the North Island falls

within the winter/spring months. Thus, North Island populations are less likely to be affected by MHWs in the same way as the South Island populations, where both gametogenesis and spawning can overlap with MHWs (Fig. 5) (Alfaro et al. 2010).

4.7. Implications for hatchery aquaculture

In a hatchery setting, temperatures through early development can more easily be controlled. However, the results of this study indicate that breeders should carefully consider the thermal conditions of their broodstock immediately prior to spawning to support the desired results. In the present study, paternal heat exposures as short as 1 wk had negative impacts on the successful early development of larvae. Adjusting for this may involve bringing broodstock into a controlled cooler hatchery setting earlier during a MHW prior to spawning. Further studies would be required to see whether this type of recovery period would have the desired effect, and the duration of recovery required.

If broodstock are to be brought in and spawned immediately, natural thermal variation provides the ability to select broodstock from more desirable thermal environments, across sites or depth within one site depending on what is available to the operation in question. Temperatures in the Marlborough Sounds can vary significantly over short scales, across space and depth. In peak summer, the temperature varied by up to 4.59°C across the depth range of a mussel rope (Fig. S3). Between the spring and fall, 9 sites throughout the Marlborough Sounds varied by a maximum of 5.14°C and an average of 2.17 ± 0.81°C at any given time at the same depth (N. J. Delorme & P. M. South unpubl. data). Knowledge of local thermal variation and biological understanding of within- and across-generation carryover effects from thermal exposure can be coupled with recent advances in MHW forecasting in order to expand options for farms to maintain yields under extreme conditions (Holbrook et al. 2020, Jacox et al. 2022).

4.8. Necessary future work

The results of this study are compelling in that they indicate paternal effects induced by a relatively short thermal exposure, of the order possible in a MHW scenario. However, further studies on paternal effects and how they interact with factors such as maternal effects, underlying genetics and different time scales are needed to fully clarify how parental exposure to MHWs will affect *P. canaliculus* and how the aquaculture industry should adapt.

In the wild, both sexes will generally experience a MHW simultaneously. Therefore, it is critical to consider transgenerational effects through each parental line and their interaction. Previous work indicates that transgenerational effects can vary within the same species when transmitted through the maternal or paternal lineage (Guillaume et al. 2016, Venkataraman et al. 2019). It is possible that the time scale of exposure may also influence the 2 parental lines differently, given the offset in the length of gametogenesis for each sex in most species.

The parental capacity to mobilize a transgenerational acclimatory response can also be dependent upon their underlying genetics, influencing both the magnitude and direction of effects (Parker et al. 2012, 2015, Goncalves et al. 2016). Previous population genetic studies of *P. canaliculus* have found a distinct break between the North and South Island populations at the Cook Strait correlated with SST (Apte et al. 2003, Wei et al. 2013). The combination of this natural genetic variation in wild populations along with industry-selective breeding programs could strongly influence the effects of parental conditioning in this species.

Furthermore, it is important to consider the timing, duration and predictability of environmental stressors when considering transgenerational effects (Burgess & Marshall 2014). Paternal effects have been observed to vary between stable and variable environments (Guillaume et al. 2016), based on the condition the parents experienced immediately prior to reproduction (Jensen et al. 2014), and timing of exposure throughout life history (Gasparini et al. 2018). In the case of the green-lipped mussel, effects may manifest differently between the spring and summer/fall spawning periods as the match between paternal experience and larval environment could be drastically different if a MHW precedes spawning or arises while larvae are in the water column. A week of simulated heatwave exposure is sufficient to bring about significant changes in bivalve gonad morphology (Vázquez et al. 2021) and influence subsequent embryogenesis (present study). Therefore, it is important to determine whether even more acute thermal challenges, including the industry-standard thermal cycling approach used to induce spawning in *P. canaliculus* in hatcheries (and in the current trial), may also influence offspring phenotype (Ragg et al. 2010, Gale et al. 2016).

5. CONCLUSIONS

Overall, our study raises a critical concern that paternal effects can influence offspring phenotype in *Perna canaliculus* and that negative consequences for larval development can be induced after just 1 wk of paternal heat exposure. In addition, elevated larval temperatures negatively affected larval developmental success. Therefore, for green-lipped mussels, the negative effects of MHWs will be felt not only within the generation of exposure but across generations through the paternal line. Wild populations will be affected at both the parental and larval stage by MHWs, with consequences for larval normality. Although the larval developmental environment can be controlled in a hatchery, this study shows that negative effects of MHWs can still impact hatchery larval yields through paternal carryover effects. Species-specific information regarding the transgenerational effects of heat stress will be invaluable for application population models, such as green-lipped mussel spat forecasting. However, for transgenerational effects to be incorporated effectively in projections, key knowledge gaps such as how effects vary with timing and duration of exposure must be addressed alongside efforts to accurately characterize larval dispersal patterns (Atalah et al. 2022).

Though New Zealand may suffer less in terms of average temperature increase relative to tropical regions, the last decade has shown that these acute heatwave events are likely to shape the future of aquaculture in the country. Warmer temperatures already have harsh effects both directly on organismal growth and performance, but also indirectly through food chain disruption and higher incidence of disease and toxins (Lake et al. 2018, KPMG 2020, Salinger et al. 2020). Aquaculture will be critical to meet our future global protein needs; however, the effects of climate change and MHWs will vary greatly based on the spatial extent, geographic region and species considered, and urgently require dedicated research and consideration (Weatherdon et al. 2016, Barange et al. 2018, Holbrook et al. 2020, Oyinlola et al. 2020). This study highlights the need to consider the transgenerational implications of MHW events when attempting to disentangle their consequences for the industry.

Acknowledgements. This work was supported by the Cawthron Shellfish Aquaculture Research Platform funded by the New Zealand Ministry for Business, Innovation and Employment (contract CAWX1801). The authors are grateful to the Cawthron Aquaculture Park team for their support, especially Joanna Copedo for generously sharing her knowledge and time. L.C.K. was supported by a National Science Foundation Graduate Research Fellowship during this research, and this research was further supported by an award from the Schmidt Family Foundation to L.C.K.

LITERATURE CITED

Adams SL, Tervit HR, McGowan LT, Smith JF, and others (2009) Towards cryopreservation of Greenshell™ mussel (*Perna canaliculus*) oocytes. Cryobiology 58:69–74

Aleng NA, Sung YY, MacRae TH, Abd Wahid ME (2015) Non-lethal heat shock of the Asian green mussel, *Perna viridis*, promotes Hsp70 synthesis, induces thermotolerance and protects against Vibrio infection. PLOS ONE 10:e0135603

Alfaro AC, Jeffs AG, Hooker SH (2001) Reproductive behavior of the green-lipped mussel, *Perna canaliculus*, in northern New Zealand. Bull Mar Sci 69:1095–1108

Alfaro AC, McArdle B, Jeffs AG (2010) Temporal patterns of arrival of beachcast green-lipped mussel (*Perna canaliculus*) spat harvested for aquaculture in New Zealand and its relationship with hydrodynamic and meteorological conditions. Aquaculture 302:208–218

Alfaro A, Jeffs A, Gardner J, Bollard Breen B, Wilkin J (2011) Green-lipped mussels in GLM 9. N Z Fish Assess Rep 48: 80

Alfaro AC, Jeffs AG, King N (2014) Enabling and driving aquaculture growth in New Zealand through innovation. N Z Mar Freshw Res 48:311–313

Apte S, Star B, Gardner JP (2003) A comparison of genetic diversity between cultured and wild populations, and a test for genetic introgression in the New Zealand greenshell mussel *Perna canaliculus* (Gmelin 1791). Aquaculture 219:193–220

Atalah J, Forrest BM (2019) Forecasting mussel settlement using historical data and boosted regression trees. Aquacult Environ Interact 11:625–638

Atalah J, South PM, Briscoe DK, Vennell R (2022) Inferring parental areas of juvenile mussels using hydrodynamic modelling. Aquaculture 555:738227

Barange M, Bahri T, Beveridge M, Cochrane KL, Funge-Smith S, Poulain F (2018) Impacts of climate change on fisheries and aquaculture. United Nations' Food Agric Org 12:628–635

Bates D, Maechler M, Bolker B, Walker S and others (2009) Package 'lme4'. http://lme4.r-forge.r-project.org

Behrens E, Rickard G, Rosier S, Williams J, Morgenstern O, Stone D (2022) Projections of future marine heatwaves for the oceans around New Zealand using New Zealand's Earth System Model. Front Clim 4:798287

Broekhuizen N, Plew DR, Pinkerton MH, Gall MG (2021) Sea temperature rise over the period 2002–2020 in Pelorus Sound, New Zealand — with possible implications for the aquaculture industry. NZ J Mar Freshw Res 55:46–64

Buchanan S (2001) Measuring reproductive condition in the Greenshell™ mussel *Perna canaliculus*. NZ J Mar Freshw Res 35:859–870

Burgess SC, Marshall DJ (2014) Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. Oikos 123:769–776

Caputi N, Kangas M, Denham A, Feng M, Pearce A, Hetzel Y, Chandrapavan A (2016) Management adaptation of

invertebrate fisheries to an extreme marine heatwave event at a global warming hot spot. Ecol Evol 6:3583–3593
Cheung WW, Frölicher TL (2020) Marine heatwaves exacerbate climate change impacts for fisheries in the northeast Pacific. Sci Rep 10:6678
Crean AJ, Bonduriansky R (2014) What is a paternal effect? Trends Ecol Evol 29:554–559
Dunphy BJ, Ragg NLC, Collings MG (2013) Latitudinal comparison of thermotolerance and HSP70 production in F2 larvae of the greenshell mussel (*Perna canaliculus*). J Exp Biol 216:1202–1209
Eirin-Lopez JM, Putnam HM (2019) Marine environmental epigenetics. Annu Rev Mar Sci 11:335–368
Frölicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. Nature 560:360
Gale SL, Burritt DJ, Adams SL (2016) The role of ethylenediaminetetraacetic acid in green-lipped mussel (*Perna canaliculus*) embryo development: a biochemical and morphological characterization. Aquaculture 463:22–27
Galley TH, Batista FM, Braithwaite R, King J, Beaumont AR (2010) Optimisation of larval culture of the mussel *Mytilus edulis* (L.). Aquacult Int 18:315–325
Gasparini C, Lu C, Dingemanse NJ, Tuni C (2018) Paternal effects in a terrestrial ectotherm are temperature dependent but no evidence for adaptive effects. Funct Ecol 32: 1011–1021
Gavery MR, Roberts SB (2017) Epigenetic considerations in aquaculture. PeerJ 5:e4147
Gentry RR, Froehlich HE, Grimm D, Kareiva P, and others (2017) Mapping the global potential for marine aquaculture. Nat Ecol Evol 1:1317–1324
Gmelin J (1791) Caroli a Linné, systema naturae. Tom. I. Pars VI. Beer, Lipsiae, p 3021–3909
Goncalves P, Anderson K, Thompson EL, Melwani A, Parker LM, Ross PM, Raftos DA (2016) Rapid transcriptional acclimation following transgenerational exposure of oysters to ocean acidification. Mol Ecol 25:4836–4849
Guillaume AS, Monro K, Marshall DJ (2016) Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? Funct Ecol 30: 1175–1184
Hawkins S, Todd P, Russell B, Lemasson A and others (2023) Surviving the anthropocene: the resilience of marine animals to climate change. Oceanogr Mar Biol Annu Rev 61: 35–80
Hayden BJ (1995) Factors affecting recruitment of farmed greenshell mussels, *Perna canaliculus* (Gmelin) 1791, in Marlborough Sounds. PhD thesis, University of Otago, Dunedin
Heasman KG, Scott N, Ericson JA, Taylor DI, Buck BH (2020) Extending New Zealand's marine shellfish aquaculture into exposed environments — adapting to modern anthropogenic challenges. Front Mar Sci 7:751
Helm MM, Bourne N, Lovatelli A (2004) Hatchery culture of bivalves: a practical manual. FAO Fish Tech Pap 471. FAO, Rome
Holbrook NJ, Sen Gupta A, Oliver EC, Hobday AJ and others (2020) Keeping pace with marine heatwaves. Nat Rev Earth Environ 1:482–493
Huang B, Liu C, Banzon V, Freeman E and others (2021) Improvements of the Daily Optimum Interpolation Sea Surface Temperature (DOISST) Version 2.1. J Clim 34: 2923–2939
IPCC (2021) Climate change 2021: the physical science basis. In: Masson-Delmotte V, Zhai P, Pirani A, Connors SL and others (eds) Contribution of Working Group I to the sixth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
Jacox MG, Alexander MA, Amaya D, Becker E and others (2022) Global seasonal forecasts of marine heatwaves. Nature 604:486–490
Jensen N, Allen RM, Marshall DJ (2014) Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. Funct Ecol 28:724–733
Kessel GM, Phillips NE (2018) Global change scenarios trigger carry-over effects across life stages and generations of the intertidal limpet, *Siphonaria australis*. PLOS ONE 13:e0194645
Kong H, Jiang X, Clements JC, Wang T and others (2019) Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. Mar Environ Res 145:73–80
KPMG (2020) Climate-related risk scenarios for the 2050s. Exploring plausible futures for aquaculture and fisheries in New Zealand. KPMG, New Zealand
Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. J Stat Software 82:1–26
Lake R, Bolton A, Brightwell G, Cookson A, Benschop J, Burgess S, Tait A (2018) Adapting to climate change: information for the New Zealand food system. Ministry for Primary Industries, Wellington
Lane A, Campanati C, Dupont S, Thiyagarajan V (2015) Transgenerational responses to low pH depend on parental gender in a calcifying tubeworm. Sci Rep 5:10847
Lazo CS, Pita IM (2012) Effect of temperature on survival, growth and development of *Mytilus galloprovincialis* larvae. Aquacult Res 43:1127–1133
Leach TS, BuyanUrt B, Hofmann GE (2021) Exploring impacts of marine heatwaves: paternal heat exposure diminishes fertilization success in the purple sea urchin (*Strongylocentrotus purpuratus*). Mar Biol 168:103
Leach TS, Hofmann GE (2023) Marine heatwave temperatures enhance larval performance but are meditated by paternal thermal history and inter-individual differences in the purple sea urchin, *Strongylocentrotus purpuratus*. Frontiers Physiol 14:1230590
Lenth RV (2013) lsmeans: least-squares means. R package version 1.06–05. https://CRAN.R-project.org/package=lsmeans
Li S, Alfaro AC, Nguyen TV, Young T, Lulijwa R (2020) An integrated omics approach to investigate summer mortality of New Zealand Greenshell™ mussels. Metabolomics 16:1–16
Martin C (2017) Not so many fish in the sea. Curr Biol 27: R439–R443
McDougall DR, Vignier J, Ragg NLC, Finnie B, Jeffs A, Adams S (2020) The value of EDTA treatment of hatchery water to rear Greenshell™ mussel (*Perna canaliculus*) larvae. Aquacult Int 28:1579–1592
Moyen NE, Crane RL, Somero GN, Denny MW (2020) A single heat-stress bout induces rapid and prolonged heat acclimation in the California mussel, *Mytilus californianus*. Proc Biol Sci 287:20202561
Munday PL (2014) Transgenerational acclimation of fishes to climate change and ocean acidification. F1000Prime Rep 6:99
Oliver EC, Donat MG, Burrows MT, Moore PJ and others (2018) Longer and more frequent marine heatwaves over the past century. Nat Commun 9:1324

Oliver EC, Benthuysen JA, Darmaraki S, Donat MG and others (2021) Marine heatwaves. Annu Rev Mar Sci 13: 313–342
Oyinlola MA, Reygondeau G, Wabnitz CC, Cheung WW (2020) Projecting global mariculture diversity under climate change. Glob Change Biol 26:2134–2148
Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). Glob Change Biol 15: 2123–2136
Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner HO (2012) Adult exposure influences offspring response to ocean acidification in oysters. Glob Change Biol 18:82–92
Parker LM, O'Connor WA, Raftos DA, Pörtner HO, Ross PM (2015) Persistence of positive carryover effects in the oyster, *Saccostrea glomerata*, following transgenerational exposure to ocean acidification. PLOS ONE 10: e0132276
Pechenik JA, Eyster LS, Widdows J, Bayne BL (1990) The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. J Exp Mar Biol Ecol 136: 47–64
Perkins-Kirkpatrick S, King A, Cougnon E, Grose M and others (2019) The role of natural variability and anthropogenic climate change in the 2017/18 Tasman Sea marine heatwave. Bull Am Meteorol Soc 100:105–110
Ragg NL, King N, Watts E, Morrish J (2010) Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. Aquaculture 306:270–280
Ragg NL, Gale SL, Le DV, Hawes NA and others (2019) The effects of aragonite saturation state on hatchery-reared larvae of the Greenshell mussel *Perna canaliculus*. J Shellfish Res 38:779–793
Rico-Villa B, Pouvreau S, Robert R (2009) Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture 287:395–401
Salinger MJ, Renwick J, Behrens E, Mullan AB and others (2019) The unprecedented coupled ocean-atmosphere summer heatwave in the New Zealand region 2017/18: drivers, mechanisms and impacts. Environ Res Lett 14: 044023
Salinger MJ, Diamond HJ, Behrens E, Fernandez D and others (2020) Unparalleled coupled ocean-atmosphere summer heatwaves in the New Zealand region: drivers, mechanisms and impacts. Clim Change 162: 485–506
Schlegel RW, Smit AJ (2018) heatwaveR: a central algorithm for the detection of heatwaves and cold-spells. J Open Source Softw 3:821
Smale DA, Wernberg T, Oliver EC, Thomsen M and others (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Chang 9:306–312
Smith KE, Burrows MT, Hobday AJ, Sen Gupta A and others (2021) Socioeconomic impacts of marine heatwaves: global issues and opportunities. Science 374:eabj3593
Smith KE, Burrows MT, Hobday AJ, King NG and others (2023) Biological impacts of marine heatwaves. Annu Rev Mar Sci 15:119–145
Sorte CJ, Bernatchez G, Mislan K, Pandori LL, Silbiger NJ, Wallingford PD (2019) Thermal tolerance limits as indicators of current and future intertidal zonation patterns in a diverse mussel guild. Mar Biol 166:1–13
Stenton-Dozey JM, Heath P, Ren JS, Zamora LN (2021) New Zealand aquaculture industry: research, opportunities and constraints for integrative multitrophic farming. NZ J Mar Freshw Res 55:265–285
Sutton PJH, Bowen M (2019) Ocean temperature change around New Zealand over the last 36 years. NZ J Mar Freshw Res 53:305–326
Tagliarolo M, McQuaid CD (2015) Sub-lethal and sub-specific temperature effects are better predictors of mussel distribution than thermal tolerance. Mar Ecol Prog Ser 535:145–159
Thomsen J, Stapp LS, Haynert K, Schade H and others (2017) Naturally acidified habitat selects for ocean acidification-tolerant mussels. Sci Adv 3:e1602411
Thomsen MS, Mondardini L, Alestra T, Gerrity S and others (2019) Local extinction of bull kelp (*Durvillaea* spp.) due to a marine heatwave. Front Mar Sci 6:84
Vázquez E, Woodin SA, Wethey DS, Peteiro LG, Olabarria C (2021) Reproduction under stress: acute effect of low salinities and heat waves on reproductive cycle of four ecologically and commercially important bivalves. Front Mar Sci 8:685282
Venables WN, Ripley BD (2013) Modern applied statistics with S-PLUS. Springer Science & Business Media, New York, NY
Venkataraman YR, Spencer LH, Roberts SB (2019) Larval response to parental low pH exposure in the Pacific oyster *Crassostrea gigas*. J Shellfish Res 38(3):743–750
Waite HR, Sorte CJB (2022) Negative carry-over effects on larval thermal tolerances across a natural thermal gradient. Ecology 103:e03565
Wang X, Cong R, Li A, Wang W, Zhang G, Li L (2023) Transgenerational effects of intertidal environment on physiological phenotypes and DNA methylation in Pacific oysters. Sci Total Environ 871:162112
Weatherdon LV, Magnan AK, Rogers AD, Sumaila UR, Cheung WW (2016) Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal tourism, and human health: an update. Front Mar Sci 3:48
Wei K, Wood AR, Gardner JP (2013) Seascape genetics of the New Zealand greenshell mussel: sea surface temperature explains macrogeographic scale genetic variation. Mar Ecol Prog Ser 477:107–121

Editorial responsibility: Vengatesen Thiyagarajan, Hong Kong SAR
Reviewed by: 3 anonymous referees

Submitted: December 16, 2022
Accepted: October 18, 2023
Proofs received from author(s): February 9, 2024