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ESTIMATING THERMAL TOLERANCE IN SELECTIVELY-BRED NEW ZEALAND GREENSHELL MUSSELS (*PERNA CANALICULUS*)

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SUMMARY

New Zealand's aquaculture industry is under growing pressure from recurring marine heatwaves, that threaten the productivity and sustainability of marine farms, emphasizing the need for targeted genetic solutions. This study focuses on the Greenshell™ mussel (*Perna canaliculus*) (GSM), a key aquaculture species in New Zealand (NZ) that has been selectively bred since 2002. This well-established family-based breeding programme provides an opportunity to breed for thermal tolerance and improve survival and growth during periods of elevated temperature for this farmed species. We defined thermal tolerance as the number of days to death (DTD) at 26°C. This study explores the appropriate model for estimating the heritability for thermal tolerance in GSM. A total of 850 mussels produced in 2021 and 2022 were evaluated, consisting of 55 families from 49 sires and 43 dams. The estimated heritability was moderate (0.48±0.10), suggesting that if thermal tolerance is included in a selection index, there is adequate genetic variation for thermal tolerance in this mussel to allow genetic improvement in this trait.

INTRODUCTION

The GSM is NZ's most valuable aquaculture export (www.aquaculture.org.nz). Although selective breeding programs and hatchery production methods are well established for this species (Camara and Symonds *et al*. 2014) most of the industry relies on wild caught seed 'spat' (Jeffs *et al.* 2018). However, with increasing sea water temperatures, this wild spat supply is becoming scarce (Wu *et al*. 2024) and the need for more consistent and sustainable sources, including hatchery-reared spat, is growing. Seawater temperatures between 16 – 19°C are ideal for the GSM (Venter *et al*. 2023) while Delorme *et al*. (2024) reported that sea surface temperatures are surpassing 24°C in many areas of the North Island. Selective breeding has the potential to develop mussels that are more resilient to increasing sea water temperatures.

Survival during heat stress is becoming an economically important trait in shellfish, with genetic parameters being published for oysters (Jiang *et al*. 2023; Chi *et al*. 2024) and abalone (Liu *et al*. 2022). For GSM, recent studies have showed that thermal tolerance is influenced by genetics (Ericson *et al.* 2023) and the interaction between genetics and age (Delorme *et al.* 2024), but there are no reports on the heritability of survival during heat stress in GSM. Therefore, this study aims to explore the genetic potential of selecting for thermal tolerance in the GSM.

MATERIALS AND METHODS

Data. We evaluated a total of 850 mussels for thermal tolerance: 55 families with 13-16 individuals/family. These mussels are part of a commercial breeding program with 22 years of selection and come from the 2021 and 2022 cohorts. The 55 families consist of 54 full-sib families, some of which are also half-sib families (n=14), selected for commercial production traits.

Trial design. On 12th February 2024, we collected adult mussels from the farms in the Marlborough Sounds, NZ and transported to the Cawthron Aquaculture Park (CAP). We cleaned all mussels of any biofouling, engraved them with an ID and divided them (2 mussels/family, 114 mussels/tank) into eight 100 L tanks of flowing seawater (2.5 - 4 L min^{-1} tank^{-1}). We maintained the

temperature at 20°C for the first eight days, then increased by 1°C per day until reaching 26°C, which exceeds the chronic thermal maximum for this species (Delorme *et al.* 2024). The mussels were not fed during the experiment. We kept the mussels at 26°C and checked individual survival twice daily (dead = shell gaping).

Statistical analysis. We defined thermal tolerance as the number of days to death (DTD) at 26°C. By day 29 only 1 mussel remained, which we censored for the analysis. We performed all statistics using R 4.3.3 software. We obtained descriptive statistics using the 'psych' package and performed the genetic models using the Restricted Maximum Likelihood procedure in ASReml-R v4 (Gilmour *et al*. 2009). We estimated the variance components and genetic parameters for DTD and shell length using a bivariate animal which is represented as;

$$\begin{bmatrix}\mathbf{y}_i\\ \mathbf{y}_j\end{bmatrix} = \begin{bmatrix}\mathbf{X}_i & 0\\ 0 & \mathbf{X}_j\end{bmatrix}\begin{bmatrix}\mathbf{b}_i\\ \mathbf{b}_j\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_i & 0\\ 0 & \mathbf{Z}_j\end{bmatrix}\begin{bmatrix}\boldsymbol{\alpha}_i\\ \boldsymbol{\alpha}_j\end{bmatrix} + \begin{bmatrix}\mathbf{W}_i\\ 0\end{bmatrix}\boldsymbol{c} + \begin{bmatrix}\mathbf{e}_i\\ \mathbf{e}_j\end{bmatrix}$$

where for traits i (length) and j (DTD), $\begin{bmatrix}\boldsymbol{y}_i\\ \boldsymbol{y}_j\end{bmatrix}$ is a vector of phenotypes, $\begin{bmatrix}\mathrm{b}_i\\ \mathrm{b}_j\end{bmatrix}$ is a vector of fixed effects of cohort and tank, $\begin{bmatrix}\boldsymbol{\alpha}_i\\ \boldsymbol{\alpha}_j\end{bmatrix}$ is a vector of random animal genetic effects, **c** is a vector of the common environmental (CE) effects for trait i and $\begin{bmatrix}\boldsymbol{e}_i\\ \boldsymbol{e}_j\end{bmatrix}$ is a vector of random residuals. The **X**, **Z** and **W** are design matrices for the corresponding fixed and random effects for traits i and j. It was assumed that genetic effects were distributed as $\sim N\left(\begin{bmatrix}0\\0\end{bmatrix}, A\otimes\begin{bmatrix}\sigma^2_{a_i} & \sigma_{a_{ij}}\\ \sigma_{a_{ji}} & \sigma^2_{a_j}\end{bmatrix}\right)$, where $\begin{bmatrix}\sigma^2_{a_i} & \sigma_{a_{ij}}\\ \sigma_{a_{ji}} & \sigma^2_{a_j}\end{bmatrix}$ is the additive genetic variance-covariance structure, the CE effects were distributed as $\sim\mathrm{N}\left(\begin{bmatrix}0\\0\end{bmatrix}, I\otimes\begin{bmatrix}\sigma^2_{c_i} & 0\\ 0 & 0\end{bmatrix}\right)$, where $\begin{bmatrix}\sigma^2_{c_i} & 0\\ 0 & 0\end{bmatrix}$ is the CE variance-covariance structure and the residual effects were distributed as $\sim\mathrm{N}\left(\begin{bmatrix}0\\0\end{bmatrix}, I\otimes\begin{bmatrix}\sigma^2_{e_i} & \sigma_{e_{ij}}\\ \sigma_{e_{ji}} & \sigma^2_{e_j}\end{bmatrix}\right)$, where $\begin{bmatrix}\sigma^2_{e_i} & \sigma_{e_{ij}}\\ \sigma_{e_{ji}} & \sigma^2_{e_j}\end{bmatrix}$ is the residual variance-covariance structure, where **A** is the additive genetic relationship matrix and **I** are identity matrices. To determine inclusion in the models, the significance of fixed and CE effects were tested using Wald-F statistics, likelihood ratio test (LRT test) and the Bayesian information criterion (BIC) (Lynch and Walsh 1998). Narrow-sense heritability for each trait was calculated as $h^2_i = \frac{\sigma^2_{\alpha_i}}{\sigma^2_{p_i}}$, where $\sigma^2_{\alpha_i}$ are the additive genetic variance and $\sigma^2_{p_i}$ is the total phenotypic variance which was $(\sigma^2_{\alpha_i} + \sigma^2_{c_i} + \sigma^2_{e_i})$ for length and $(\sigma^2_{\alpha_i} + \sigma^2_{e_i})$ for DTD. The effect of the CE (c^2_i) was calculated as $c^2_i = \frac{\sigma^2_{c_i}}{\sigma^2_{p_i}}$ for length. The Z-score was used to assess whether the h^2 was significantly different from zero.

RESULTS AND DISCUSSION

Descriptive statistics for DTD and shell length are in Table 1. The average survival of GSM when exposed to water temperatures of 26°C was 15±4 days and the average mussel shell length was 84.5±15 mm.

In a commercial breeding program, juvenile mussels are reared in the same full-sib family tank until they are mature enough to be placed on single-family rope droppers in the sea, where they complete their growth through to harvest size (~1-2 years). If statistical models don't account for this process (fit the CE effect), h^2 are likely to be overestimated (Alcapan *et al*. 2007). However, when the CE is included as a random effect along with the individual animal, depending on the data structure, this can cause confounding effects and lead to model convergence issues, or underestimate the genetic component of the trait. Results from the LRT test showed that the CE had a significant

effect on shell length but not on DTD. This variation captured by fitting CE in the model is evident for length with the c^2 of 0.20±0.15.

Table 1. Descriptive statistics of days to death (DTD) and length (mm) of Greenshell™ mussels in NZ

Trait	N	Mean	SD[1]	Min	Max	CV[2]
Length	850	84.46	15.14	51	166	18
DTD	850	15.38	3.99	6	29	26

[1]SD = standard deviation, [2]CV = coefficient of variation.

The estimated h^2 for DTD was 0.48±0.10 which is significant and at the upper range of h^2 estimates for physiological resilience to stress in other shellfish/aquatic species. For example, thermal tolerance h^2 have been estimated for *C. gigas* (0.16-0.20±0.03-0.04 (Chi *et al*. 2024)), hybrid oysters (*Crassostrea gigas* ♀ x *C. angulata* ♂) which ranged from 0.19-0.27±0.03-0.05 (Jiang *et al*. 2023), from 0.35-0.42±0.03-0.06 for abalone (Liu *et al*. 2022), and from 0.41-0.48±0.06-0.07 in rainbow trout (Perry *et al*. 2005). Although common environmental effects were not significant for DTD, the h^2 observed in this study may be partially the result of overestimation of additive genetic variance via common rearing of full-sib families.

The r_g between DTD and shell length was moderate and negative (-0.49±0.27) suggesting that faster growing mussels may be less resilient to thermal stress. However, this relationship might be confounded by size because faster growing mussels were larger at the time of testing. This observation aligns with findings from Delorme *et al.* (2024) where larger mussels from the same family were less tolerant to heat stress, indicating a potential size-related vulnerability to thermal conditions. Other studies have found the r_g between thermal tolerance and growth traits tend to be low or weak in oysters, ranging from -0.28 to +0.24 in *C. gigas* (Chi *et al*. 2024), and 0.016 in hybrid oysters (Jiang *et al*. 2023). Similarly, low to no correlations between thermal tolerance and growth traits have been observed in finfish. For example, Perry *et al*. (2005) reported genetic correlation of upper thermal tolerance and body weight in rainbow trout ranging from -0.28 to +0.04.

Table 2. Estimates of additive genetic variance (σ_a^2), common full-sib variance (σ_c^2), residual variance (σ_e^2), heritability (h^2), common full-sib environmental effect (c^2), genetic (r_g) and phenotypic (r_p) correlations for days to death (DTD) and shell length of Greenshell™ mussels in New Zealand

Trait	Model	σ_a^2	σ_c^2	σ_e^2	h^2 ± SE[1]	± SE[1]	r_p ±SE[1]	r_g ±SE[1]
Length	Full	.78	6.91	31.18	0.41 ± 0.34	!0 ± 0.15	0.19 ± 0.16	-0.49 ± 0.27
DTD	Full	.87	-	7.43	0.48* ± 0.10	-		

[1]SE = standard error. *Statistically significant

CONCLUSIONS

The moderate heritability of DTD suggests that there is adequate genetic variation to achieve genetic improvement for thermal tolerance in GSM in NZ. The common full-sib rearing environment does not significantly influence thermal tolerance but there does seem to be a moderate negative correlation between thermal tolerance and shell length, which is influenced by the common full-sib environment. The estimated genetic parameters in this study provide evidence for the inclusion of DTD into a selection index.

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