*The following supplement accompanies the article*

# Plasticity of foot muscle and cardiac thermal limits in the limpet *Lottia limatula* from locations with differing temperatures

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## SUPPLEMENTAL METHODS

Likelihood-ratio tests comparing the goodness-of-fit between the null model and alternative model were used to further evaluate the hypothesis that limpets of higher $CT_{max}$ have reduced plasticy. Based on the assumption that limpet $CT_{max}$ responds to local temperatures with plasticity, the null linear regression model included site and acclimation as the only two explanatory variables predicting $CT_{max}$. The alternative model included the interaction between acclimation and site as an additional explanatory variable. If the alternative hypothesis significantly fitted the observations better, coefficient estimates of the interaction between acclimation and site were compared to see how plasticity differed among sites. Null and alternative hypothesis models' goodness-of-fit was also compared using AICc.

## SUPPLEMENTAL RESULTS

### $CT_{max}$ of foot muscle

Similar to the foot muscle $CT_{max}$ multi-model inference models, the hypothesis model testing also did not support the second hypothesis that limpets of higher foot muscle $CT_{max}$ have lower plasticity. The alternative hypothesis which included the interactions (between acclimation and site) showed statistically significant evidence of better explaining our data (Table S1A, Likelihood Ratio-test: $\chi^2_{2,7}=6.62$, $p=0.03$). However, a further look into the alternative hypothesis estimates reveal that no site and acclimation interactions had a strong effect on foot muscle $CT_{max}$ (Table S2A). The $\Delta$AICc between the null and alternative hypothesis was also only <2, which suggested that the null and alternative hypotheses was considered comparable to each other (Table S1A).

### ABT

Similar to the ABT multi-model inference models, the hypothesis model testing also contradicted the second hypothesis for ABT. The alternative hypothesis which included the interactions (between acclimation and site) showed statistically significant evidence of better explaining our

data (Table S1B, Likelihood Ratio-test: $\chi^2_{6,9}$=14.07, p<0.05). The ΔAICc was also >2, which corroborated that the alternative model was a better one. A closer look at interactions between acclimation and site revealed 17˚C Acclimation × Tomales and 21˚C Acclimation × Tomales had statistically significant positive effects on ABT, which suggests Tomales Bay limpets had the highest ABT plasticity (Table S2B).

FLT

Similar to the FLT multi-model inference models, the hypothesis model testing also directly contradicted our second hypothesis for FLT. The alternative hypothesis which included the interactions (between acclimation and site) showed statistically significant evidence of better explaining our data (Table S1C, Likelihood Ratio-test: $\chi^2_{6,9}$=13.18, p<0.05). The ΔAICc was also >2, which corroborated that the alternative model was a better one. 17˚C Acc × Tomales and 17˚C Acc × SF Bay had strong significant positive effects on FLT, which suggests that Tomales Bay and San Francisco Bay limpets had higher FLT plasticity than Elkhorn Slough limpets (Table S2C).

SUPPLEMENTAL TABLES

Table S1. Likelihood-ratio test results between null and hypothesis models for our second hypothesis for all three three metrics of $CT_{max}$.

| Model | AICc df | ΔAICc | Chi-sq | p-value |
|---|---|---|---|---|
| **(A) Foot Muscle $CT_{max}$** | | | | |
| Null | 5 | 0 | | |
| Alternative | 7 | -1.24 | 6.62 | <0.05 |
| **(B) ABT** | | | | |
| Null | 6 | 0 | | |
| Alternative | 10 | -6.28 | 14.70 | <0.05 |
| **(C) FLT** | | | | |
| Null | 6 | 0 | | |
| Alternative | 9 | -4.76 | 13.18 | <0.05 |

Table S2. Results of alternative hypothesis models of the likelihood-ratio tests for the plasticity hypothesis. Statistically significant variables are **bolded**.

| Variable | Value | Standard Error | t-value | p-value |
|---|---|---|---|---|
| **(A) Foot Muscle $CT_{max}$** | | | | |
| Intercept | 35.21 | 0.66 | 53.7 | <0.001 |
| Tomales | 0.67 | 0.88 | 0.78 | 0.45 |
| 17˚C Acc | -0.22 | 0.93 | -0.24 | 0.81 |
| 21˚C Acc | 1.85 | 1.00 | 1.85 | 0.07 |
| 17˚C Acc ×Tomales | 2.00 | 1.28 | 1.56 | 0.12 |
| 21˚C Acc ×Tomales | -1.36 | 1.33 | -1.02 | 0.31 |
| **(B) ABT** | | | | |
| Intercept | 30.49 | 0.82 | 37.05 | <0.001 |
| **SF Bay** | **4.47** | **1.13** | **3.96** | **<0.001** |
| **Tomales** | **4.57** | **1.13** | **4.04** | **<0.001** |
| 17˚C Acc | -3.40 | 1.42 | -2.39 | 0.02 |
| 21˚C Acc | 0.98 | 1.40 | 0.70 | 0.49 |
| **17˚C Acc ×Tomales** | **3.77** | **1.82** | **2.07** | **0.04** |
| 17˚C Acc ×SF Bay | 3.37 | 1.85 | 1.83 | 0.07 |
| **21˚C Acc ×Tomales** | **5.83** | **2.09** | **2.80** | **0.01** |
| **(C) FLT** | | | | |
| Intercept | 34.30 | 0.76 | 44.87 | <0.001 |
| SF Bay | 3.46 | 1.05 | 3.30 | <0.01 |
| **Tomales** | **3.10** | **1.05** | **2.95** | **<0.01** |
| **17˚C Acc** | **-4.24** | **1.32** | **-3.20** | **<0.01** |
| 21˚C Acc | -0.36 | 1.30 | -0.28 | 0.78 |
| **17˚C Acc ×SF Bay** | **4.52** | **1.69** | **2.67** | **0.01** |
| **17˚C Acc ×Tomales** | **4.25** | **1.71** | **2.48** | **0.02** |
| 21˚C Acc ×Tomales | 3.63 | 1.94 | 1.87 | 0.07 |

Table S3. Full averaged-models results of ABT and FLT with a subset of limpets with dry body weight between 1.0-2.0g. A) ABT: 13˚C acclimation, Elkhorn Slough site, and one ABT as the intercept; B) FLT: 13˚C acclimation, Elkhorn Slough site, one ABT as the intercept. Statistically significant predictor variable estimates are **bolded**.

| Variable | Estimate | Standard Error | z-value | p-value |
|---|---|---|---|---|
| **(A) ABT** | | | | |
| Intercept | 29.79 | 1.07 | 26.21 | <0.001 |
| **SF Bay** | **4.70** | **1.35** | **3.27** | **<0.01** |
| **Tomales** | **4.62** | **1.90** | **2.28** | **0.02** |
| 17˚C Acc | -0.50 | 1.05 | 0.46 | 0.64 |
| 21˚C Acc | -1.06 | 2.16 | 0.48 | 0.63 |
| ABT Number | 0.14 | 0.61 | 0.23 | 0.81 |
| **(B) FLT** | | | | |
| Intercept | 32.55 | 1.14 | 26.99 | <0.001 |
| **SF Bay** | **5.13** | **1.51** | **3.22** | **0.001** |
| **Tomales** | **6.17** | **1.89** | **3.06** | **0.002** |
| 17˚C Acc | -0.65 | 1.19 | 0.53 | 0.59 |
| 21˚C Acc | -1.67 | 2.79 | 0.58 | 0.55 |
| ABT number | 0.06 | 0.50 | 0.12 | 0.90 |

SUPPLEMENTAL FIGURES

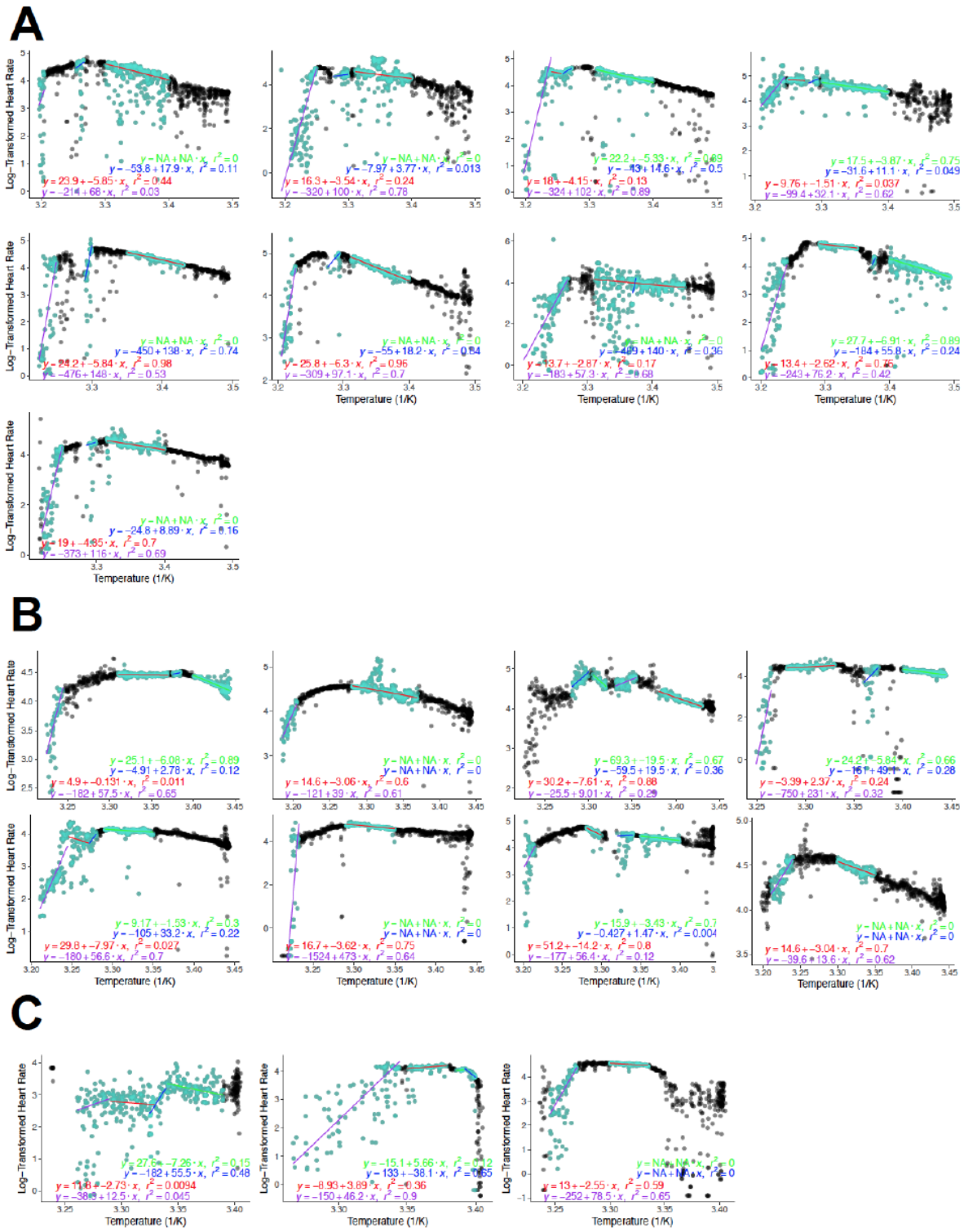


Figure S1. For San Francisco Bay limpets, ABT were similar between 13˚C (A) and 17˚C (B) acclimation, but dropped in the 21˚C acclimation (C). $CT_{max}$ was calculated by the intersection

of the two regressions lines before and after a break in the response of heartbeat to increasing temperature. Blue dots represent the heart rate points used to draw the regressions lines. Equations of these regression lines are displayed on the bottom of each graph. Final break points were calculated with the red and purple equations. Limpets with two heartbeat breaks had their first break points calculated with green and blue equations. In a few cases, it was sufficient to calculate first break points with red and blue equations.

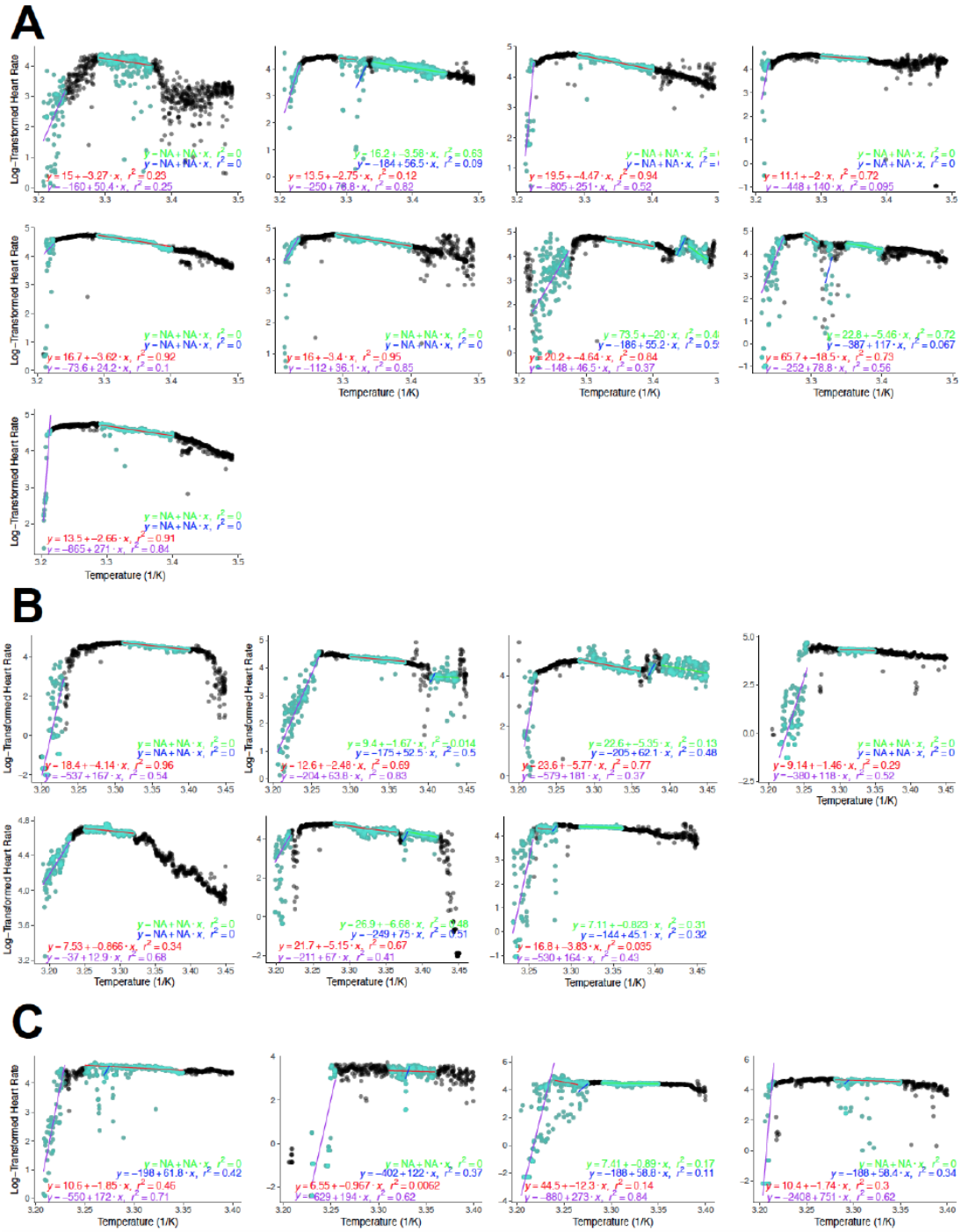


Figure S2. For Tomales Bay limpets, ABT remained consistent across 13˚C (A), 17˚C (B), and 21˚C (C) acclimation treatments.

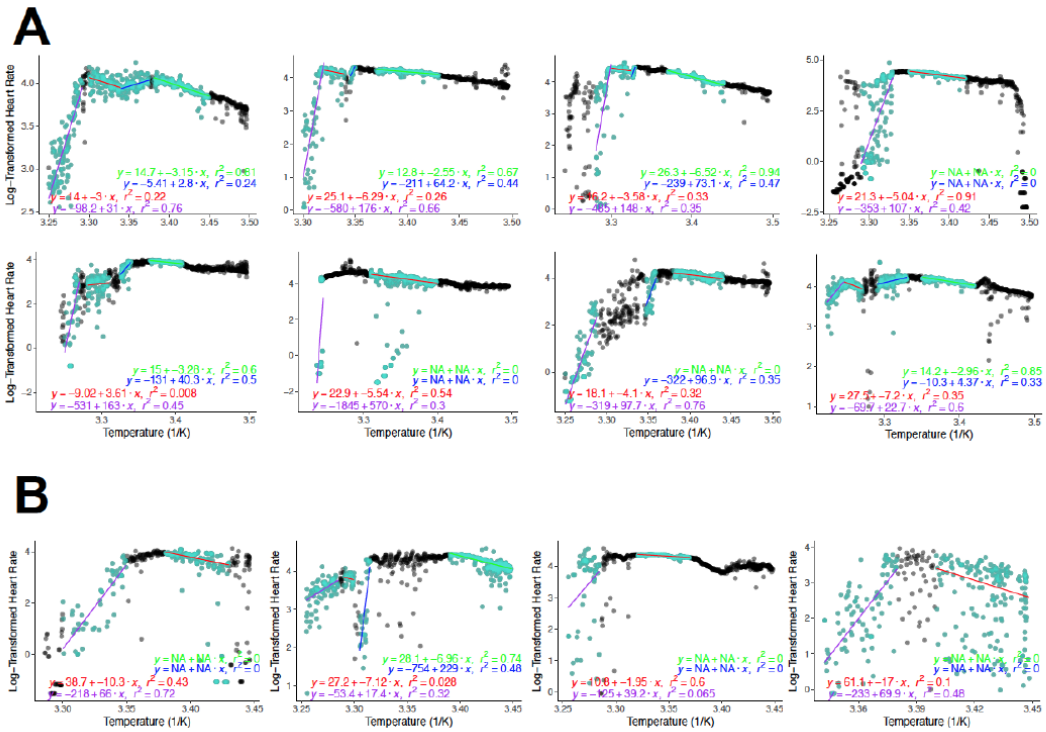


Figure S3. From 13˚C acclimation treatment (A) to 17˚C (B), Elkhorn Slough limpets ABT dropped and increased in variance. ABT data for 21˚C-acclimated Elkhorn Slough limpets was not sampled because of high mortality.

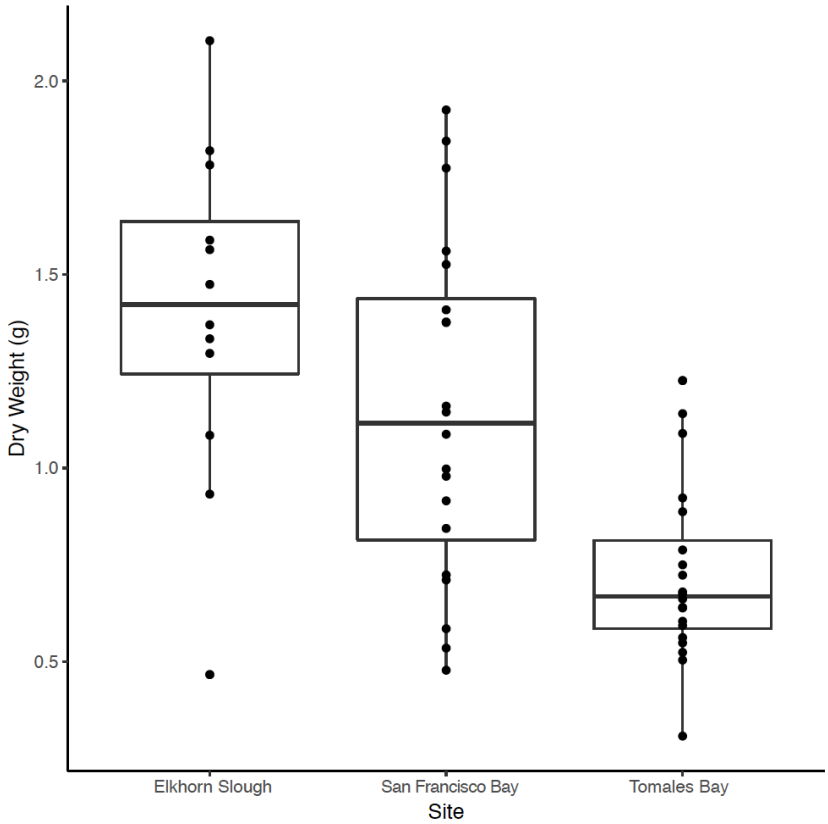


Figure S4. Boxplots of *Lottia limatula* dry weight and site show that limpets collected from Tomales Bay were smaller than the ones collected from Elkhorn Slough and San Francisco Bay.

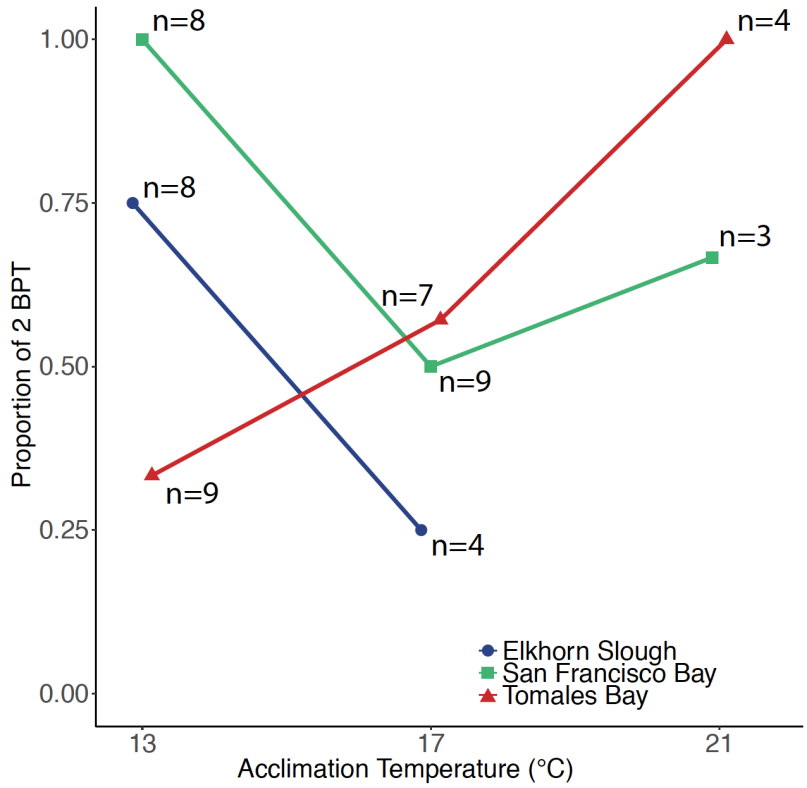


Figure S5. No relationship found in the proportion of L. limatula with 2 break point temperatures (BPTs) within acclimation and site groups.