1    **Research Article in *Biology Letters***

2

3    **Exploring thermal tolerance across time and space in a tropical bivalve, *Pinctada margaritifera***

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14    **Keywords** thermal tolerance, thermal limits, thermal death time, critical temperature, tropical

15    bivalve, global warming vulnerability, ontogeny

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17    **Number of words in abstract:** 200

18    **Number of words in main text:** 3317

19    **Number of references:** 63

20    **Number of figures:** 2

21    **Number of tables:** 1

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29 **Abstract**

30    Ectotherm's vulnerability to climate change is predicted to increase with temperature variations.

31    Still, translating laboratory observations of organisms' heat stress responses to the natural fluctuating

32    environment remains challenging. In this study, we used an integrative framework combining insights

33    from the TDT curves and physiological reaction norms, to precisely capture *Pinctada margaritifera*

34    stage -specific thermal tolerance. On a second hand, this study aimed to explore applicability of the

35    model of accumulation of thermal injury, by making in situ predictions at three contrasting sites across

36    French Polynesia. By working with two life stages (early planktonic vs. spat), our study revealed an

37    ontogenetic shift in lethal (CTmax) and sub-lethal (Tc) thermal limits, with higher vulnerability for

38    early larval stages. Cumulative injury calculations resulted in thermal failure (100% injury) for larvae

39    within 12 and 22 h, at the most 'extreme' site (Nuku Hiva; T°C > 38°C), and warm lagoon (Reao

40    Atoll), respectively. While substantial damages accumulated in spats, when exposed to consecutive

41    extreme tides (Nuku Hiva) for 8 days. Overall, our results revealed that *P. margarifiera* is living closer

42    to their upper thermal limits than previously estimated, and inhabit environments where important

43    reduction of settlement and heat stress are already occurring during warmest months.

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## 1. Introduction

Temperature is one of the most pervasive drivers of species eco-evolutionary dynamics. Not surprisingly, an extensive body of literature exists trying to answer the questions: (i) How do organisms perform across the range of temperatures they experience currently, and assuming climate-change scenario projections (e.g., Sinclair et al., 2016a)?, and (ii) what is the future of natural populations and ecosystems in the face of ongoing rapid climate change (e.g., Pigot et al., 2023) ?

Thermal performance curves (TPCs), which describe the performance of fitness-related traits as a function of body temperature (Gilchrist, 1995; Huey & Kingslover, 1989; Huey & Stevenson, 1979), are used extensively as an heuristic tool to predict ectothermic organism's responses to climate change (Angilletta, 2009; Little & Seebacher, 2021; Sinclair et al., 2016b). Indeed, this unimodal and asymmetric modelling curve provides information on how specific physiological systems react to temperature as well as critical thermal limits (e.g., the temperature at which performance is zero; CTmin and CTmax). Thermal tolerance limits in particular, have become a fundamental proxy to assess species vulnerability to climate change (e.g., Sunday et al., 2012). Importantly, however, the insights gathered from both TPCs and critical thermal limits can depend strongly on the methods used (Chown et al., 2009; Kellermann et al., 2019), thus complicating its application, particularly for fluctuating environments (Khelifa et al., 2019; Kingsolver et al., 2015). Consequently, there is little consensus on the ideal method for estimating thermal limits, which limits comparisons across studies and engenders confusion and misuse of terminology (Clusella-Trullas et al., 2021; Cooper et al., 2008; Hoffmann et al., 2003).

To explicitly account for the variability in intensity and duration of thermal stress experienced by organisms in nature, Rezende et al. (2014) have recently formalised the concept of the thermal death time (TDT). Building from a rich history of earlier studies that recognized the importance of exposure duration on the estimation of ectotherms' thermal limits (Ansell et al., 1981; Bigelow, 1921; Coles et al., s. d.; Foster, 1969; Fry et al., 1946; Kilgour & McCauley, 1986; Nedved et al., 1998; Smith, 1957; Urban, 1994), the TDT framework provides a standardized method to reconcile static and dynamic experimental assays (Jørgensen et al., 2021a, 2021a), allowing generalized comparisons across species (Molina et al., 2023; Vives⬚Ingla et al., 2023; Willot et al., 2022), populations (Castañeda et al., 2015; Li et al., 2023), life stage (Truebano et al., 2018), life-history traits, including prior acclimation (Tarapacki et al., 2021) or nutrient quality treatments (Koussoroplis et al., 2023).

87    Moreover, the integration of TDT and TPC models (Ørsted et al., 2022a) provides a consistant and

88    global picture of  organism's thermal niche. But most importantly, this framework enables the

89    identification of the critical temperature (Tc), i.e. at the very extrem edge of the *permissive*

90    temperature range (i.e., possible life completion). Once environmental temperatures (Tenv) exceeds

91    this sublethal temperature (Tc); and the *stressful* temperature range crossed (i.e., accumulation heat

92    failure); it becomes possible to predict mortality rates under variable thermal regimes (Jørgensen et al.,

93    2021a), and offers possibilities to more detailed, mechanistic explorations at the molecular level.

94        Tropical marine ectotherms are expected to be most severely affected by global warming

95    (Deutsch et al., 2008; Dillon et al., 2010; Jørgensen et al., 2022; Nguyen et al., 2011; Pinsky et al.,

96    2019; Tewksbury et al., 2008). Still, current assessments of thermal performance of tropical marine

97    species are incomplete, focusing mostly on the adult life stage, and thus ignoring the thermal

98    sensitivity of more vulnerable early-life stages  (Dahlke et al., 2020). This finding is particularly

99    worrying for tropical marine ectotherms with early dispersive stages, governing population

100    abundances and dynamics. In French Polynesia, pearl production is essentially based on settlement of

101    pelagic larva of *Pinctada margaritifera*, on immersed supports (i.e. spat collectors; Southgate &

102    Lucas, 2008). While extensive work has been done for this socio-economically important species;

103    including the calibration of a Dynamic Energy Model (DEB) for growth, survival, reproduction and

104    larval development (Sangare et al., 2019, 2020; Thomas et al., 2011); the upper thermal limits of the

105    early sensitive life stages remain largely uncharacterised .

106        To fill this knowledge gap, we used an integrative framework combining insights from the TDT

107    curves and physiological reaction norms derived from organisms' energy budget data, to precisely

108    capture *P. margaritifera* stage -specific critical temperature (Tc). By providing these quantitative

109    means, this study aimed to make *in situ* predictions of accumulation of thermal injury across time and

110    space, and establish a necessary foundation to further our understanding of species resilience

111    (acclimatization and genetic-adaptation potential) to climate warming trends and punctual extreme

112    events.

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114    **2.  Materials and methods**

115    Three different experiments (E1, E2 and E3, details below) were performed using hatchery-produced

116    individuals issued from two reproduction events (see cohorts A and B in **Supplementary**

117 **information**). The E1 experiment aimed to assess larval development completion (i.e., performance

118 and upper limits) under a wide range of temperatures and intensities (i.e. time of exposure). In turn,

119 the E2 and E3 allowed investigating the thermal sensitivity of spats experiencing stressful and

120 permissive temperatures, respectively.

121

122 **2.1. Thermal stress assays**

2.1.1. 123 *Upper limits and thermal performance of larvae (E1).* This experiment had a two-fold objective:

124 (i) describe thermal performance breath during the embryogenesis (i.e., 1-6 hours post fertilization

125 [hpf]) and pelagic larval development phases (i.e., 1-24 hpf), and (ii) build a TDT curve for the pelagic

126 larval development. To achieve these objectives, a full-factorial experiment was performed, including

127 seven temperatures (22, 26, 30, 32, 34, 36, and 38 °C) x two exposure durations (6 and 24 h), as well

128 as a control (28 °C - 24 h). In order to buid a TDT curve, two exposure durations (3 and 12 h) were

129 additionnaly investigated for *warm* temperature conditions (i.e. > 28 °C). We chose 24 h as the longest

130 exposure duration based on the time taken by embryos to develop into straight-hinge D-shaped larvae,

131 with completely developed shells, as established in the laboratory (Doroudi & Southgate, 2003). Each

132 combinaition was run in triplicate. We used one-hpf zygotes that were incubated in 15-mL Falcon[®]

133 tubes containing 10 mL of 10-µm filtered, UV-treated seawater at ~28 °C (density per tube ~ 30

134 zygotes mL$^{-1}$) immersed in temperature-controlled experimental tanks. The zygotes were exposed to a

135 fast warming/cooling (1.5 °C.min$^{1}$), reaching their respective treatment temperatures within 10

136 minutes. After the treatment period, each sample was preserved by adding 100 µL of formol. The D-

137 shaped larvae were counted and photographed using a LEICA H80 stereo microscope.

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2.1.2. 139 *Upper thermal limits of juveniles (E2).* We examined the effect of 11 *warm* temperatures, ranging

140 from 32 to 42 °C, on the survival of 6-month-old spats. Animals were randomly collected from the

141 hatchery, assigned to independent experimental tanks (n = 30 tank$^{-1}$, mean individual Fresh Weight

142 [$_{ind.FW}$]= 0.13 ± 0.06 g) , and allowed to acclimate for four days. Then, animals were exposed to a

143 gradual warming [range 0.7-4.3 °C.h$^{-1}$], reaching their respective randomly-assigned temperature

144 treatments within 6 hours (to mimic the low-tide duration period). Oysters were then maintained at the

145 target temperatures until mortality in the tanks reached 50 % (i.e., LT$_{50}$). Oyster status (alive/dead)

146 was checked every 30 minutes during the initial 24 h, and every 2-3 h thereafter. Assessment was done

147   visually and by mechanical stimulation (gently poking the mantle). When animals did not respond to

148   stimuli (valves were opened and did not respond to contact), time was recorded and the individual was

149   removed from the tank. The experiment was stopped after 30 days, and duplicated over time.

150

2.1.3.  151   *Thermal performance curve of juveniles (E3).* We examined the effect of 10 temperatures, ranging

152   from 27 to 36 °C (1 °C increments; duplicated), on the survival sub-lethal physiological performance

153   of

154   6-month-old animals. Spats were assigned randomly to independent experimental tanks (n = 250 tank$^{-1}$

155   , mean $_{ind.FW}$ = 0.70 ± 0.50 g), and allowed to acclimate for 30 days. Once the acclimatory phase

156   completed, animals were exposed to a gradual cooling [0.2 °C.h$^{-1}$] /warming [range 0.2-1.3 °C.h$^{-1}$] of

157   the seawater within 6 hours. After 7 days of exposure, we estimated the scope-for-growth (*SFG*) of 6

158   individuals per treatment, by measuring respiration and ingestion rates using a closed-system

159   respirometry approach (e.g., see Fly & Hilbish, 2013; detailed equations and energetic values

160   conversion in **Supplementary information**).

161

162   **2.2. Statistical analyses**

163   All data analyses and modelling were done using R version 4.3.1 (R Core Team, 2023). Thresholds of

164   statistical significance (α) were set at 0.05, unless specifically stated.

165

2.2.1.  166   *Survival curves.* In the experiment E1, exposure durations were not sufficient to identify directly LT$_{50}$

167   (i.e., the exposure duration at which probability of mortality is equal to 50%), and implement the TDT

168   curve. Therefore, as a first step, relative larval survival (E1; *warm* conditions) was plotted against time

169   of exposure and fitted to a temperature-specific logistic regression model (generalized linear model,

170   binomial distribution). The LT$_{50}$ was then estimated using the car R package.

171

2.2.2.  172   *Thermal performance curves (TPCs).* Mean survival data (E1) were modelled in relation to

173   temperature (glm, binomial distribution) for the (i) embryogenesis and (ii) pelagic larval-development

174   phases, seperately. In turn, spat metabolic rates (E3) were modelled as a thermal performance curve

175   using univariate regressions (lm, normal distribution). We computed various models including linear,

176   quadratic and cubic effects of temperature, and then compared their skill based on likelihood-ratio

177    tests (LRT) and model outputs comparison (Akaike and Bayesian information criteria). Finally,

178    residuals of the selected models were checked, using the DHARMa R package and diagnostic plots

179    (Residuals *vs*. Fitted, QQ-plot, Scale-Location, Residuals *vs*. Leverage), for binomial and normal

180    distributed models, respectively.

181

2.2.3.  182    *Thermal death time curves (TDT).* For each life stage, measured elapsed times to reach 50 % mortality

183    (i.e., $LT_{50}$; in minutes, $\log_{10}$-transformed) were plotted against the exposure temperature (*temp*; in °C).

184    TDT curves were then generated by fitting ordinary linear regressions (Rezende et al., 2014):

185

186    $temp = \beta - \alpha \log_{10}(LT_{50})$                    *eq. (1)*

187

188    with β the intercept and α the slope. The TDT metrics (Rezende et al., 2014), namely the upper

189    thermal limit for a specific duration of exposure (*t*; in min), *CTmax(t)* (°C), and the sensitivity (z) are

190    calculated from the linear model output parameters:

191

192    $CTmax(t) = (\log_{10} t - \beta / \alpha)$ and $z = -1 / \alpha$          *eq. (2)*

193

194    Because exposure durations differed between experiments E1 and E2, and not sufficient to identify the

195    TDT breakpoint temperature (Jørgensen et al., 2021), we estimated Tc as the maximum temperature

196    which did not induce acute heat failure.

197

2.2.4.  198    *Estimation of cumulative thermal injury.* The TDT parameters were then used to estimate the life

199    stage-specific cumulative thermal injury expected under natural, fluctuating temperature conditions.

200    Accumulation of thermal stress (condition Tenv > Tc) were calculated as a function of time, using free

201    access R-scripts (https://github.com/MOersted/Termal-tolerances), based on the equation described in

202    Jørgensen et al., (2021):

203

204    $\text{Accumulated thermal injury} = \sum_{i=1}^{T_{env} > T_c} \dfrac{100 \cdot (t_{i+1} - t_i)}{10^{\left(-\frac{1}{z} \cdot \max(T_i; T_{i+1}) + \beta\right)}}$     *eq. (3)*

205

206    We investigated cumulative thermal injury at three contrasting sites across French Polynesia based on

207    *in situ* temperature records. Nuku Hiva (140° 05' W, 8° 51' S) represents the most extreme site, where

208    *P. margaritifera* inhabits tide pools with temperatures varying between 26 to >34 °C within a day

209    (Reisser et al., 2019). Then, the atoll of Reao (136°37' W, 18°51' S) is a relatively warm lagoon due to

210    its shallow depth and the limited exchange of water with the open ocean. Finally, the closed atoll of

211    Takapoto (145°21' W, 14°63' S) represents a control site, notably due to its low seasonal variations (~

212    5 °C, recorded in 2021) and active pearl farming acitivty. To estimate the cumulative thermal injury at

213    each of these sites, we used seawater temperature recorded during the warmest months. Measurements

214    were done over eight days using iButton thermal loggers at Nuku Hiva (02 - 10/03/2023) and Reao (02

215    - 10/03/2023), and extracted from Liao *et al.* (2023) for Takapoto.

216

217    **3.   Results**

218    **3.1.  Modelling and estimating thermal thresholds.**

3.1.1.  219    *Survival curves.* For the *warm* conditions of 36 and 38 °C, all larvae died within 180 minutes. These

220    treatments were  thus not considered in the subsequent analyses. All (binomial fixed effect) models for

221    *warm* conditions showed a negative effect of exposure duration on larval survival. The 50% mortality

222    (i.e., $LT_{50}$) at 30, 32, and 34 °C was estimated at 1614, 1037 and 155 minutes, respectively.

223

3.1.2.  224    *TDT curves.* Linear regressions of $\log_{10}(LT_{50})$ against temperature were generated for each life stage.

225    Curves had a high coefficient of determination ($R^2$) ranging from 0.89 to 0.94 (**Table 1**). TDT curves

226    revealed that juveniles exhibited a higher CTmax(1h) (42.3 °C) than larva (36.1 °C). Thermal

227    sensitivity (*z*) was also higher for the early developmental stage 3.36 °C than for juvenile spats (2.75

228    °C). The effects of temperature and life stage on $LT_{50}$ were examined in a two-way ANOVA. Analysis

229    results confirmed the significant effect of temperature and life stage on $LT_{50}$ ($p > 0.001$), but no

230    evidence of an interaction was detected ($p = 0.23$).

231

3.1.3.  232    *Integrative thermal tolerance landscape.* The integrative framework, combining life stage-specific

233    physiological and survival data, is presented in **Figure 1**. All thermal performance curves were best

234    characterised with a quadratic function, except for respiration rate in spats (best fit :linear effect). The

235    temperature of optimal performance (Topt) was 26.3 and 28.1 °C for embryos and D-shaped larva,

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236    respectively. In turn, the spat Topt for ingestion rate, and scope for growth were 29.8 and 29.6 °C,

237    respectively. By overlapping the physiological performance curves (permissive range) with TDT

238    curves (stressful range), we estimated Tc at 29 and 34°C, for the early- (larva) and later- (spat) life

239    stages, respectively.

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241

242    **3.2.  Injury accumulation in natural fluctuating environments.**

243    We then used the thermal tolerance metrics estimated here to calculate the thermal injury accumulated

244    by larvae and spats under ecologically realistic, *in situ* conditions. Sea-water temperature

245    measurements (8 days), confirmed contrasting sites over the French Polynesian territory (**Figure 2**).

246    Nuku Hiva showed the most extreme thermal profiles (mean 29.13°C $\pm$ 2.50 SD, min.: 24.10°C,

247    max.: 42.40°C), while Reao mean 30.50°C $\pm$ 0.31 SD, min.: 29.74°C, max.: 31.52°C) and

248    Takapoto (mean 28.46°C $\pm$ 1.05 SD, min.: 25.65°C, max.: 31.39°C) revealed to be the lagoon with

249    the highest and the lowest mean temperature, respectively. Based on these divergent thermal regimes,

250    results of cumulative injury varied with ontogeny and site (**Table 1**). Larvae accumulated more

251    thermal injury than spats at every site. Based on natural thermal regimes recorded at Nuku Hiva (tide

252    pools) and Reao (shallow lagoon), larvae reached 100% of accumulated injury (i.e., heat failure/death

253    of the organism) within 840 and 1350 minutes, respectively. At the pearl-farming lagoon of Takapoto,

254    larvae accumulated 5.1% injury within 8 days. Later-life spats only exhibited accumulated injury

255    when exposed for 8 days to the thermal profiles of Nuku Hiva (30.2%) and Reao (1.2%); while no

256    thermal injury was detected based on Takapoto's *in situ* sea-water temperature.

257

258    **4.  Discussion**

259    *Critical thermal limits.* Using *Pinctada margaritifera* as a model species, we have quantified heat

260    failure and performance under a broad range of temperatures, which allowed a fine-scale

261    characterization of the species tolerance landscape, and a clear-cut estimation of the critical

262    temperature (Tc) delimiting the permissive from the stressful temperature range.

263    By working with two life stages (early planktonic *vs.* spat), our study revealed an ontogenetic shift in

264    lethal (CTmax) and sub-lethal (Tc) thermal limits, with higher vulnerability for early (planktonic) life

265    stages, than for 6-month juveniles. Our results are in line with numerous studies on marine ectotherms;

266 e.g., in fish (Dahlke et al., 2020), shallow-water echinoids (Collin et al., 2021), or tubeworms

267 (Rebolledo et al., 2020). Different hypotheses have been proposed to explain the observed life stage-

268 dependent thermal tolerance; e.g., oxygen-limitation due to the ventilatory and cardiorespiratory

269 system's development (Pörtner, 2002), or allometric constraints (Pörtner & Farrell, 2008). By

270 providing such quantitative means, it now seems possible to further explore thermal tolerance at

271 numerous life stages (from fertilization to spawners), and confirm such hypotheses. Ultimately, our

272 results suggest that accurate predictions of population dynamics and evolutionary bottlenecks under

273 ongoing climate change must consider nuances of species fundamental thermal niches, including the

274 thermal limits of these early develpmental stages.

275

276 *In situ thermal injury accumulation.* Translating laboratory observations of organisms' heat stress

277 responses to the natural environment remains challenging, in part, because the duration and intensity

278 of thermal stress events are often unpredictable in the wild. The recent experimental validation that

279 thermal injury is an additive process (above Tc) has provided an unprecedented quantitative tool for

280 explicitly accounting for thermal heterogeneity in nature, thus improving our ability to assess species

281 vulnerability to climate change (Jørgensen et al., 2021b).

282 We used this approach to evaluate the severity of thermal stress exhibited by larva and juvenile

283 *P. margaritifera* experiencing three different natural fluctuating thermal regimes. As expected,

284 bivalves accumulated high levels of thermal injury (100% for larvae, and 30% for spats) when

285 exposed to the most 'extreme' site (Nuku Hiva; T°C > 38°C). Admittedly, this framework does not

286 account for recovery ability (when $T_{env}$ fluctuate between permissive and stressful range temperature)

287 which might overestimate injury accumulation (Ørsted et al., 2022b). The conceptual underpinnings of

288 this temperature and duration-dependent repair function remain elusive, in part due to inherent

289 difficulties associated with rapid hardening (i.e., transient response conferring enhanced heat tolerance

290 following a sub-lethal exposure) physiological processes (Ørsted et al., 2022b). Still, considering the

291 short time of exposure calculated to induce heat failure in larvae (12 and 22 h for Nuku Hiva and

292 Reao, respectively), we expect that recovery period might be limited and insufficient to prevent high

293 selective pressure on early stage. In addition to the high degree of mortality resulting from the

294 transition from a pelagic to benthic stage (Jenkins et al., 2009), we expect a important reduction of

295 settlement during the warmest months at these sites. The significant match between the estimated

296 critical temperature (Tc ~29°C) and the mean annual sea-surface temperature in Takapoto Atoll (28.3

297 ± 0.8 °C) suggest that species are living closer to their upper thermal limits than previously estimated

298 (Le Moullac et al., 2016). Overall, this approach provides (i) the foundations for comparing the

299 species fundamental *vs.* realized thermal niches, which can help investigating evolutionary and

300 biogeographic ecological processes, and (ii) a promising tool to quantify the impacts of extreme events

301 (e.g., marine heat waves).

302

303 **5. Conclusion**

304 The present study reinforces the importance of defining ontogeny-specific thermal tolerance limits, to

305 avoid underestimating the vulnerability of individuals and populations threatened by global warming.

306 Indeed, thermal stress assays done using *P. margaritifera* revealed an ontogenetic shift in lethal

307 (CTmax) and sub-lethal (Tc) thermal limits, with higher vulnerability for early-life (planktonic) stages,

308 than for 6-month spats.

309     By integrating these estimations of thermal limits and the thermal injury accrued additivly over

310 time (Jørgensen et al., 2021a), we provide predictions of heat-failure risk for three contrasting sites in

311 French Polynesia. Results obtained for relatively stable thermal regime's atolls (Reao and Takapoto),

312 were consistent with specie's natural observations (absence and presence, respectively). However,

313 injury calculated for the specific tidal habitat of Nuku Hiva (i.e., Tenv crossing permissive and

314 stressful temperature ranges) inducates thermal stress in spats, and high selective pressure on early

315 stage during warmest months.

316     Still, the exploration of the full tolerance landscape of organisms offers possibilities for more

317 advanced mechanistic explorations. Indeed, sublethal effects at high temperature (e.g., oxidative stress,

318 depletion of energy reserves, infertility, etc) are expected to increase thermal sensitivity with longer

319 exposure (Kingsolver & Woods, 2016), resulting lower growth and reproductive output. Indeed,

320 predicted elevation of temperature will not necessarily induce mortality (presence or absence), but

321 may rather induce nuanced changes in population abundance, geographic range limits and/or work as a

322 driver of local adaptation. Such impacts may be particularly important for climate-sensitive economic

323 sector – such as molluscan shellfish farming (Fly & Hilbish, 2013), and  specially those dependent on

324 wild-spat collection (Doubleday et al., 2013). Combining tools capable of quantifying such sublethal

325 effects (e.g., Thomas & Bacher, 2018), recovery effectiveness (Jørgensen et al., 2021b), as well as

326 species thermal limits - would be a major step forward for prediction accuracy, and the guidance of

327 resource management and conservation programs.

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**Additional files**

347 Additional file 01: Supplementary information

348

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554    **Tables and figures**

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556    **Table 1. Parameters of the TDT curves** for the early-life (1 - 24 hpf) and later-life stage (6 months-

557    old spat) of *Pinctada margaritifera*. Total thermal injury (%) accumulated over a 8 days period, based

558    on natural small-scale thermal varitions at three sites in French Polynesia .

| Life-stage | TDT parameters | | | | Cumulative thermal injury (%) | | |
|---|---|---|---|---|---|---|---|
| | CTmax(1h) | Thermal sensitivity (z) | $R^2$ | *est.* Tc | Nuku Hiva | Reao | Takapoto |
| Early | 36.1 | 3.36 | 0.89 | 29 | 100 | 100 | 5.1 |
| Later | 42.1 | 2.75 | 0.94 | 34 | 30.2 | 1.2 | 0 |

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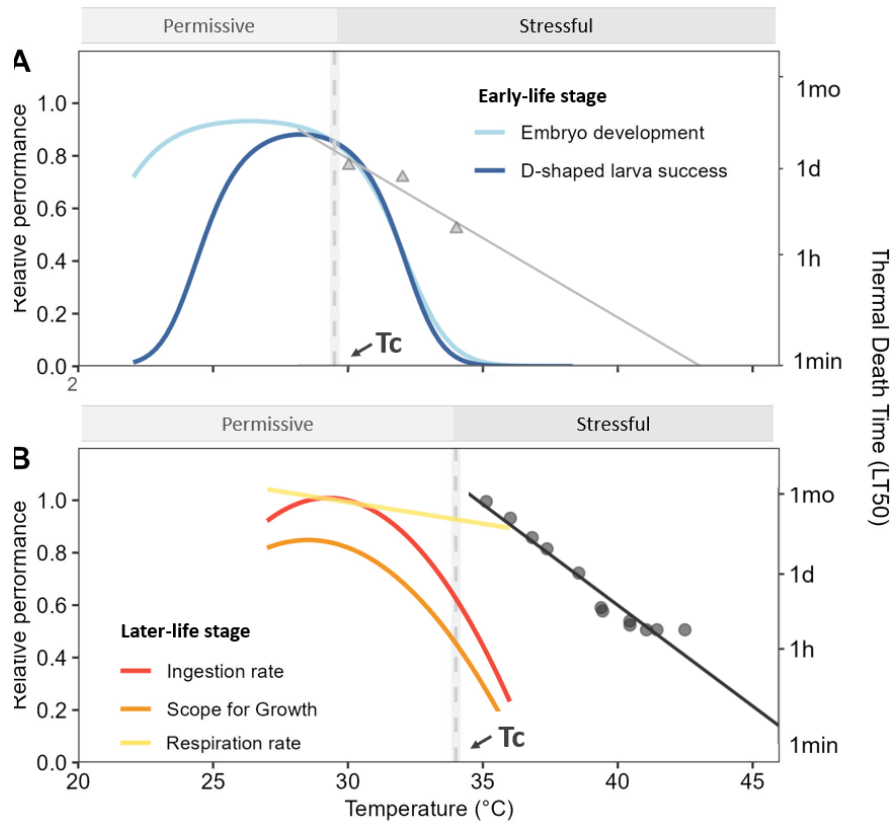567

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**Figure 1.** *Pinctada margaritifera* **Thermal Tolerance Landscape for (A) Early** (1-24 hpf) and **(B)**

**Later** (6 months-old spat) life stages. Thermal performance curves are fitted to relative mean

performance (left y-axis) through binomial and linear regression models, respectively. TDT curves

(right y-axis) are fitted to $\log_{10}$-transformed time causing a 50 % mortality ($LT_{50}$) for pelagic larval

development phase (grey triangles), and spats (black dots). TDT parameters can be found in Table 1.

The critical temperature (Tc; grey dashed line), is shown to indicate the *stage-specific* transitional zone

between the permissive and stressful temperature range.

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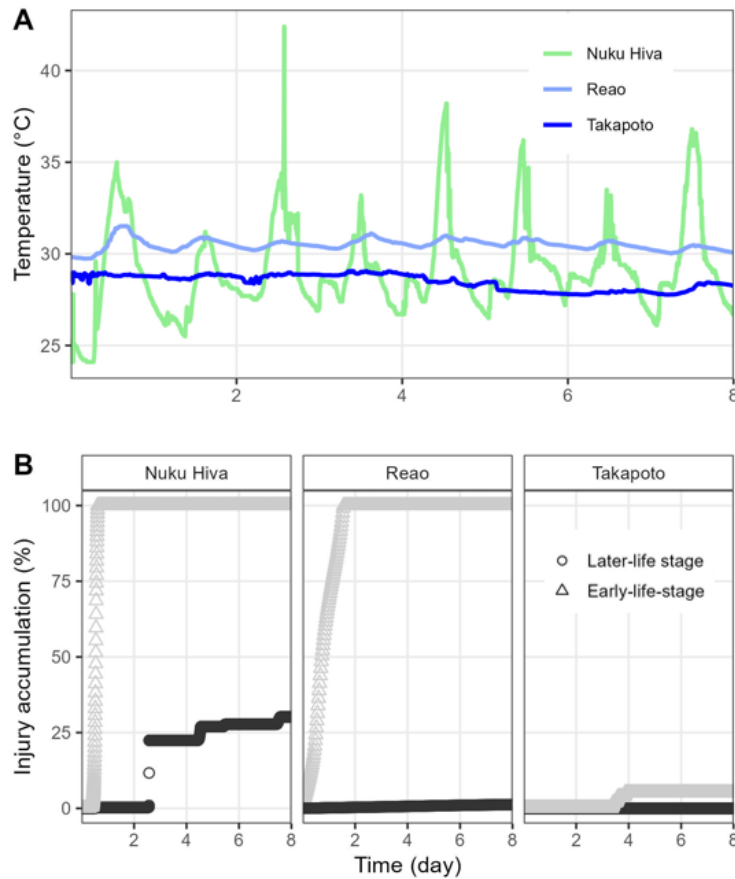
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Figure 2. (A) **Natural temperature fluctuations** recorded *in situ* during the warmest months (March) at three sites, showing different thermal regimes. **(B) Stage-specific predicted accumulated injury** (see the formula in section 2.2.4.) for the two life stages: spats (black circles) and larva (grey triangles), throughout a week (8 days). 100% injury accumulation equals to heat failure (i.e., death of the organism).

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