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How experimental physiology and ecological niche modelling can inform the management of marine bioinvasions?

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1 HOW EXPERIMENTAL PHYSIOLOGY AND ECOLOGICAL NICHE MODELLING
2 CAN INFORM THE MANAGEMENT OF MARINE BIOINVASIONS?

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36   ABSTRACT

37   Marine bioinvasions are increasing worldwide by a number of factors related to the

38   anthroposphere, such as higher ship traffic, climate change and biotic communities' alterations.

39   Generating information about species with high invasive potential is necessary to inform

40   management decisions aiming to prevent their arrival and spread. *Grateloupia turuturu,* one of

41   the most harmful invasive macroalgae, is capable of damaging ecosystem functions and services,

42   and causing biodiversity loss. Here we developed an ecological niche model using occurrence

43   and environmental data to infer the potential global distribution of *G. turuturu*. In addition,

44   ecophysiological experiments were performed with *G. turuturu* populations from different

45   climatic regions to test predictions regarding invasion risk. Our model results show high

46   suitability in temperate and warm temperate regions around the world, with special highlight to

47   some areas where this species still doesn't occur. Thalli representing a potential temperate region

48   origin, were held at 10, 13, 16, 20 and 24ºC, and measurements of optimal quantum field

49   (Fv/Fm) demonstrated a decrease of photosynthetic yield in the higher temperature. Thalli from

50   the population already established in warm temperate South Atlantic were held at 18, 24 and

51   30°C with high and low nutrient conditions. This material exposed to the higher temperature

52   demonstrated a drop in photosynthetic yield and significant reduction of growth rate. The

53   congregation of modelling and physiological approach corroborate the invasive potential of *G.*

54   *turuturu* and indicate higher invasion risk in temperate zones. Further discussions regarding

55   management initiatives must be fostered to mitigate anthropogenic transport and eventually

56   promote eradication initiatives in source areas, with special focus in the South America. We

57   propose that this combined approach can be used to assess the potential distribution and

58   establishment of other marine invasive species.

59

60   Keywords: *Grateloupia turuturu*, invasive marine algae, populational ecology,

61   ecophysiology, anthropogenic impacts.

62

63   1. INTRODUCTION

64   Marine bioinvasions are receiving great attention as many of them are producing negative

65   socio-environmental-economic impacts worldwide (Katsanevakis et al., 2014). Offshore oil

66   exploitation, aquaculture activities and ballast water are major vectors responsible for growing

67   events of non-indigenous species arrival and spread (Creed et al., 2017; Hulme, 2009). Climate

68   change further facilitates the dispersal of marine organism by increasing the frequency and

69   intensity of extreme climatic/oceanographic events (Sissini et al., 2014, 2017; Ummenhofer and

70   Meehl, 2017). A diverse and abundant array of vectors for the transport of organisms across

71 biogeographic boundaries coincides with disrupted native communities of marine organisms,

72 caused by both global (ocean acidification and warming) and local stressors (coastal pollution,

73 overfishing) (Shea, 2002), which is facilitating the establishment of non-indigenous species on

74 coastlines all around the world (Occhipinti-Ambrogi 2007). The geographical distribution of

75 marine macroalgae is determined by their physiological tolerance to environmental conditions

76 (Breeman, 1988; Hoek, 1982), enabling the prediction of suitable habitats for invasive species

77 (Elith et al., 2006; Marcelino and Verbruggen, 2015). Ecological Niche Models (ENM) relate

78 occurrence data with environmental gradients to produce habitat suitability maps (Peterson,

79 2003). An ability to assess the risk of invasion and the threats posed by foreign organisms helps

80 managers and stakeholders put preventive measures in place and optimize biosecurity monitoring

81 (Marcelino and Verbruggen, 2015). The known occurrence of species informs predictive

82 distribution models, which can be misleading since this depends on sampling effort and

83 identification expertise which varies widely depending on the species and regions investigated

84 (Verbruggen et al., 2009). ENMs do not consider the physiological traits of species when

85 modelling distributions, likely missing valuable information about the mechanisms and processes

86 allowing the presence and establishment of populations (Marcelino and Verbruggen, 2015).

87 Therefore, physiological information can be used to validate and further improve ENMs to create

88 a more accurate prediction of habitat suitability (Martínez et al., 2015).

89 Considering species traits related with dispersal, establishment and ecological impact, the

90 fifth most dangerous invasive alga in a global perspective is *Grateloupia turuturu* Yamada

91 (1941) (Nyberg and Wallentinus, 2005), commonly known as the "Devil's tongue weed" (Mulas

92 and Bertocci, 2016). *G. turuturu* can displace native species in rocky intertidal habitats (Freitas

93 et al., 2016; Mulas and Bertocci, 2016), has advantage establishing in recently perturbed

94 environments, where native assemblages are disrupted (Mulas and Bertocci, 2016), and has been

95 reported for reducing the abundance and richness of invertebrates (Janiak and Whitlatch, 2012;

96 Jones and Thornber, 2010). Native to Japan, *G. turuturu* has greatly expanded its distribution

97 range to other countries (e.g. Aguilar-Rosas et al., 2012; D'Archino et al., 2007; Mathieson et al.,

98 2008; Simon et al., 2001; Tsiamis et al., 2015), and was recently registered for the coast of South

99 Brazil, being the first record of this species in the South Atlantic (Azevedo et al., 2015). *G.*

100 *turuturu* has been described to tolerate a wide range of temperature (from 4 to 28ºC), salinity

101 (from 15 to 37), nutrients (Simon et al., 2001) and can be found down to a depth of 7m (Sweet

102 2011). However, even with the description of *G. turuturu* being tolerant to a great spectrum of

103 environmental conditions in the North Atlantic and Pacific, there is no descriptions of the South

104 Atlantic population tolerance to the local oceanographic conditions, nor experiments that

105 describe the biochemical processes of its ecophysiological performance. For this reason,

106 ecophysiological experiments are necessary to better understand the influence of temperature and

107 nutrients in *G. turuturu* physiology and assess its probability of success in regions with

108 conditions similar to the native range.

109   In this study we developed an ENM to estimate the global potential distribution of *G.*

110 *turuturu*. In addition, we determined the ecophysiological thresholds of *G. turuturu* as a tool to

111 infer about the species resilience and temperature tolerance. Our hypothesis is that *G. turuturu*

112 will present higher habitat suitability and physiological performance in temperate regions. By

113 combining ENMs and the physiological thresholds evaluation, we desire to propose this

114 associated approach to foster coastal management strategies related not only to *G. turuturu*

115 bioinvasion, but also to invasions by other marine species.

116

117   2. MATERIALS AND METHODS

118   2.1 Ecological Niche Modelling

119   To develop the ENM for *G. turuturu*, six environmental benthic data layers (i.e., along

120   the bottom of the ocean) were extracted from Bio-ORACLE (Assis et al., 2017b; Tyberghein et

121   al., 2012) based on the biological relevance of macroalga (Assis et al., 2017a, 2014; Neiva et al.,

122   2015) (environmental predictors used in the model are described in Table 1). Depth was not

123   included as a predictor as it has no direct biological meaningfulness in the ecophysiological

124   response of macroalgae, and many previous works modelling marine macroalgae also do not

125   include it (Jueterbock et al., 2016, 2013; Tyberghein et al., 2012). Bio-ORACLE data has been

126   widely used for marine ecological modelling (Marcelino and Verbruggen, 2015) since it contains

127   climatic and geophysical data with a global spatial resolution of 0.08º (9.2 km). Detailed

128   georeferenced occurrence data from both invaded and native ranges was obtained from the

129   public Global Biodiversity Information Facility (GBIF, 2018), and literature data (available in

130   the supplementary material ESM1) (e.g. Tyberghein et al. 2012; Escobar et al. 2016). The

131   inclusion of the invaded range in the model increases model accuracy, as models trained using

132   data only from the native range usually fail to predict the full invasion extent of a species

133   (Beaumont et al., 2009; Broennimann and Guisan, 2008). Duplicated occurrences, outside

134   marine range or too close to each other (5 km²) were excluded to prevent biases and reduce

135   spatial autocorrelation in the analyses, remaining 106 records (Figure 1). The radius of 5 km²

136   was chosen as an conservative distance of about half of the resolution of the climatic data used in

137   the model, seeking spatial independence between the environmental data of occurrences (Phillips

138   et al. 2009; Kramer-Schadt et al. 2013). The modelling approach combined multiple cross-

139   validation interactions using independent datasets with Boosted Regression Trees (BRT), a

140 statistical algorithm able to fit complex non-linear relationships between a response (occurrence

141 records) and predictor variables (environmental data), while avoiding overfitting through

142 optimized parameterization procedures and specific forcing of monotonic responses (Elith,

143 Leathwick, & Hastie, 2008). BRT algorithm is known for high predictive performance (De'ath,

144 2007; Neiva et al., 2014), improving its performance by combining many models for prediction,

145 which makes it a more accurate algorithm when compared to other regression methods (Elith,

146 Leathwick, & Hastie, 2008). In the lack of absence data, presence-only modelling requires the

147 generation of pseudo-absences or background information (e.g., Maxent; Phillips et al. 2006).

148 This information was produced using the method developed by Senay et al. (2013) for improved

149 ecological modelling, which comprise the calculation of environmentally dissimilar regions to

150 the presence points and the random selection of pseudo-absences in clustered climatic predictors

151 to reduce the use of redundant information. To account for likely sources of sampling bias,

152 pseudo-absences were chosen from a kernel probability surface developed with the records of

153 occurrence over a grid with the same resolution of environmental data (e.g., Phillips et al. 2009;

154 Assis et al. 2018a)

155 Cross-validation interactions were developed by partitioning species records (i.e.,

156 presences and also pseudo-absences) into 10 independent longitudinal bands to evaluate the

157 performance of models (Wenger and Olden, 2012). Models fitted with one withheld band at a

158 time, and True skill statistics (TSS) compared this data to the outcomes of a predicted surface of

159 distribution reclassified to reflect presence and absences (binomial) with a threshold maximizing

160 TSS. Over-fitting in the models was reduced by tuning the algorithm parameters (i.e., learning

161 rate, number of trees and tree complexity; see Elith et al. 2008; Assis et al. 2016, 2018a for

162 details) and also by forcing monotonic responses (positive for minimum nutrients, salinity, light

163 and temperature; negative for maximum temperatures). Final predictions using optimal

164 parameters were reclassified to binomial responses and its final accuracy was reported with AUC

165 (Area Under the receiver operating characteristic Curve, Fielding and Bell 1997), sensitivity

166 (true positive rate) and TSS (True Skill Statistics, Allouche et al. 2006). The significance of

167 models was determined by inferring the relative contribution of each environmental predictor to

168 the performance of the models (Elith et al., 2008). Physiological limits points (maximum and

169 minimum, depending on the predictor) were determined by extracting the individual fitting

170 function of each predictor alone, while fixing the alternative predictors in their average (Elith et

171 al., 2008; Marcelino and Verbruggen 2015).

172   ENM analyses were performed with R (R Development Core Team, 2017) using the

173 packages dismo, gbm, parallel, raster, SDMTools, and sm.

174

175   2.2   Ecophysiological experiments

176   Two populations of *G. turuturu* were ecophysiologically evaluated considering different

177 acclimatization histories. A warm temperate adapted population (WAP) was sampled

178 (approximately 100 g of fresh weight (FW)) on August 22nd, 2016 from the intertidal zone of

179 Itapirubá beach (28°20'14.32"S, 48°42'4.87"W), in the city of Imbituba, Santa Catarina, Southern

180 Brazil. A temperate adapted population (TAP), approximately 60 g FW, was also sampled in

181 Saint Malo beach (48°39'32.8"N 2°00'24.6"W), France, on February 20th, 2017, region

182 representing a possible source of *G. turuturu* germlings through oyster seeds or ballast water.

183 Since a determined biomass of *G. turuturu* was collected, which included individual blades and

184 blades attached together by a holdfast, all samples were separated in individual blades. The

185 sampled blades of both populations presented around 6 cm height and 2 cm in width, were not

186 fertile, collected in winter, immediately transported in plastic containers with seawater to the lab,

187 and manually cleaned from epiphytes or associated fauna after sampling.

188 To determine the control conditions for each of the experimental designs, we considered

189 the different adaptation conditions of each population. Therefore, the temperate and warm

190 temperate populations were exposed to conditions observed in their respective occurrence

191 regions (~10ºC in France in February (Dubois et al., 2007), and ~18ºC in Imbituba in August

192 (Gouvêa et al., 2017)). Both populations were cultivated in 250 mL Erlenmeyer flasks, in UV

193 sterilized seawater enriched with Von Stosch medium in a 50% concentration, without EDTA

194 chelating salt (adapted from Edwards 1972) until the beginning of the experiment. To prevent the

195 lack of essential nutrients water was replaced daily. Aeration was provided individually in the

196 flasks, also stirring the water in each flask to disrupt the diffusive boundary layer. The

197 temperature was adjusted by heaters (Radical 1 HP, Brazil) positioned in trays with water, where

198 the treatment flasks were kept in a water bath. Each tray contained one data logger (HOBO

199 Pendant Temperature/Light Data Loggers, Onset, Bourne, USA) that recorded the water

200 temperature every 10 minutes. A 12-hour light/dark photoperiod was used, and irradiance was

201 provided by fluorescent lamps with an average of 65 ± 5 μmol photons $m^{-2}\,s^{-1}$.

202 The experimental design performed in Brazil had as main purpose to observe short-term

203 ecophysiological effects of temperature and nutrients, key environmental drivers selected

204 according to conditions found in adjacent tropical environments where the species has not been

205 reported yet (Gouvêa et al., 2017). The WAP sampled blades were brought to the laboratory, and

206 acclimated for 21 days to laboratory irradiance, maintaining field temperature (18ºC). Initial and

207 final 1g FW samples were frozen with liquid nitrogen for the analysis of biochemical descriptors.

208 The experiment lasted for seven days. The experimental setup in Brazil utilized three

209 temperatures (18, 24 and 30ºC) and two nutrient conditions: one without medium culture (Low

210 Nutrient Concentration – LNC) and another with 50% of Von Stosch (VS) medium (High

211 Nutrient Concentration – HNC), resulting in 6 factorial treatments. Each treatment contained 10

212 replicas (n = 10), consisting of individual blades of *G. turuturu* weighted to sum 1g FW per

213 Erlenmeyer flask.

214   The experiment performed with the temperate adapted population, a potential source of

215 *G. turuturu*, has as main purpose the investigation of the short-term ecophysiological effects of

216 temperature, an environmental driver traditionally related with the distribution constraints of

217 marine algae (Hoek, 1982). The chosen temperature values used in the experiment are present in

218 the region of the North Atlantic where the algae were sampled (Dubois et al., 2007), expect for

219 the higher temperature used. The higher temperature used was chosen to overlap with conditions

220 observed in the warm temperature region, providing the necessary scenario for the evaluation of

221 the potential ecophysiological continuum, contribution to the discussion about invasive potential

222 of *G. turuturu.* For the TAP experiment realized in France, *G. turuturu* blades were acclimated

223 for three days at 10ºC, temperature observed in the water of the sampling location. *G. turuturu*

224 was exposed for 60 hours to 5 different temperatures: 10 (control), 13, 16, 20 and 24ºC. Each

225 treatment contained 6 replicas (n = 6), also consisting of individual blades of *G. turuturu*

226 weighted to sum 1g FW. The experiment duration was limited by laboratory structure and the

227 availability at Saint Malo. Figure S1 on the supplementary material ESM2 illustrates the

228 different experimental designs of both populations.

229

230   2.3 Physiological analyses

231   Physiological analysis performed in both populations were measurements of optimal

232   quantum field (Fv/Fm). Growth rate and photosynthetic pigments were only evaluated in the

233   WAP experiment, as the biomass sent to Brazil lost minimum preservation conditions due to

234   transportation delay. Considering the relevance of Fv/Fm as a descriptor, we chose to keep the

235   results obtained in the TAP experiment.

236

237   2.3.1 Photosynthetic yield

238   In the WAP experiment, effective quantum yield (Fv/Fm) was estimated by the

239   fluorescence of *in vivo* chlorophyll *a*. Three measurements of Fv/Fm were made in each replica

240   in different parts of the thallus, resulting in 3 subreplicas of each one of the 10 replicas.

241   Measurements of Fv/Fm were taken with a pulse-amplitude modulated chlorophyll *a* fluorometer

242   (Diving PAM – Walz Germany) after acclimation in the dark for 20 minutes in the first day, then

243   every two days, and in the last day of the experiment (for further explanation of the methodology

244   followed, see de Ramos et al., 2018; Figueroa et al., 2006). For the TAP experiment,

245   measurements of Fv/Fm were made in the dark only in the end of the 60 hours.

246

247   2.3.2 Pigment extraction

248   At the end of the experiment, all the replicates from the WAP experiment were frozen

249   and used for pigments quantification. Each replica was divided in 3 portions of 100 mg, allowing

250   the reading of 3 subreplicas, which resulted in 30 extractions for each treatment. The extraction

251   followed Schubert et al. (2011), where phycobiliproteins, chlorophyll *a* and total carotenoids

252   were extracted from the same 100 mg biomass sample grinded in liquid nitrogen.

253   Phycobiliproteins were extracted first with phosphate buffer (pH 6.8) and kept in the dark at 4ºC

254 for 2 hours until centrifugation for 4 minutes at 13000 rpm in the MiniSpin centrifuge

255 (Eppendorf) at room temperature. Subsequently, the supernatant was used to determine the

256 concentration of phycobiliproteins according to Beer and Eshel (1985). Afterwards, methanol

257 100% was placed on the same biomass and kept in the dark at 4ºC overnight, and then

258 centrifuged as stated before. Then, chlorophyll *a* and total carotenoids were quantified according

259 to Torres et al. (2014). Absorbance was determined in the SpectraMax Paradigm Multi-Mode

260 Microplate Reader (Molecular Devices), of the Laboratório Multiusuário de Estudos em Biologia

261 (LAMEB).

262

263   2.3.3 Growth rate (GR)

264   To determine the daily growth rate (GR), replicates were spun for 15 seconds in a salad

265 spinner to reduce the excess of water, and then weighted in an analytical balance. The replicates

266 were weighted in the first and last days to determine the GR by the following equation (Lignell

267 and Pedersén, 1989):

268 $$\text{GR} \left(\%.\text{day}^{-1} \right) = \left[\left(\frac{Wt}{Wi} \right)^{1/t} \right] \times 100\% \qquad (1)$$

269   Where Wi and Wt are respectively the initial and final fresh weight after *t* days (t = 7

270 days).

271

272   2.4 Statistical analysis

273   For the WAP experiment, the independent variables considered were time (beginning and

274 end), nutrient concentration (HNC and LNC) and temperature (18, 24 and 30ºC). For Fv/Fm,

275 time was considered for the 1st, 3rd, 5th and 7th day. For significant differences, post-hoc test of

276 Tukey was used to identify significantly different groups ($p<0.05$). The TAP experiment had as

277 variables only the different temperatures, 10, 13, 16, 20 and 24ºC, with 6 replicates ($n=6$).

278   Each treatment of the WAP experiment had 10 replicates ($n=10$), which were used for the

279 analysis of GR. However, in the reading of pigments and Fv/Fm, some of the replicas presented

280 negative values or values too close to zero, representing errors in the readings. This required the

281 removal of 3 replicas in each treatment (resulting in a $n=7$), to maintain the same number of

282 replicates for statistical analysis. Before analysis, data were tested for normality and residuals for

283 half-normality. Statsoft Statistica 13.0 was used to perform multifactorial Analysis of Variance

284 (ANOVA, $p<0.05$). Due to the removal of outliers, the data did not require transformation, as it

285 attained the normality requirements for the ANOVA parametric tests calculated.

286

287   3. RESULTS

288   3.1 Ecological Niche Modelling

289   The final predictive model had high performance (AUC: 0.96; Sensitivity: 0.95; TSS:

290 0.93) and showed that *G. turuturu* has great habitat suitability in temperate regions of Northern

291 Pacific, Northern Atlantic, South America, Australasia (Oceania) and a small portion of Southern

292 Africa (Fig. 2). Light at bottom and maximum ocean temperature were the most important

293 predictors to the ecological niche of *G. turuturu*, while nutrients (as nitrates and phosphates)

294 were the least important (Table 1). Response curves showed habitat suitability with light above

295 4.4 E . m^{-2} . yr^{-1} and ocean temperature bellow 26.55ºC, however, with a starting drop at 21.23ºC

296 (Fig. 3). Response curves of the other variables used in the model are available in the

297 supplementary material ESM2 (Figures S2, S3, S4, S5 and S6).

298

299   3.2 Ecophysiological experiments and physiological analyses

300   3.2.1   Photosynthetic yield

301   In the WAP experiment, there was significant interaction ($p<0.05$) between temperature,

302   time and nutrient concentration for Fv/Fm (Table 2). A decline in Fv/Fm was noticeable as the

303   temperature increases and through the experiment time in WAP (Fig. 4). The treatment of 30ºC

304   with HCN showed the lowest values in the fifth and seventh day of treatment in comparison with

305   the treatments with lower temperatures. For 30ºC with LNC, there was a smaller decline in

306   Fv/Fm, but still presented Fv/Fm values lower than the treatments of 24 and 18ºC. Similarly,

307   there was a contrast between the LNC and HNC treatments at 24ºC, with lower values of Fv/Fm

308   observed at HNC. In France, statistical analysis of TAP experiment results of Fv/Fm showed

309   significant difference between temperatures (Table 2). The response of *G. turuturu* to the short-

310   term exposure in the TAP experiment to different temperatures was of lower Fv/Fm in the

311   treatment of 24ºC (Fig. 5).

312

313   3.2.2   Photosynthetic pigments

314   No significant interaction ($p>0.05$) was found for both GR and pigments with time,

315   temperature, and nutrient concentration (i.e., no three-factorial interaction for GR and pigments).

316   However, regarding chlorophyll *a*, total carotenoids and GR, when the effects of nutrient

317   concentration are taken out, the interaction between time and temperature was significant

318   ($p<0.05$). For phycoerythrin and chlorophyll *a* the interaction between the effects of time and

319   nutrient concentrations was significant (Table 3). For chlorophyll *a*, the treatments of 24ºC and

320   30ºC in the end of the experiment were statistically distinct from the samples of the beginning

321   and 18ºC (Fig. 6a). The higher concentration of chlorophyll *a* was found in 30ºC, followed by

322 24ºC. Similarly, the higher concentration of total carotenoids was found in the last day at 30ºC,

323 and all the other treatments had the same lower concentration (Fig. 6b). Additionally, for

324 chlorophyll *a* there was statistical difference between different nutrient concentrations. On the

325 end of the experiment, the HNC treatment presented higher values of chlorophyll *a*, as in LNC

326 the concentration remained close to the one in the beginning of the experiment (Fig. S7 on the

327 supplementary material ESM2). On the other hand, there was a decrease in phycoerythrin

328 concentration in the last day in LNC, while at the treatment of HNC remained constant

329 throughout the experiment (Fig. 7). Concentration of phycocyanin was not detectable.

330

331   3.2.3   Growth rate

332   For daily growth rate, only temperature and time were significant (Table 3). The algae

333 exposed to 30ºC showed lower GR when compared to the algae in lower temperatures (Figure

334 6c).

335

336   2.4   DISCUSSION

337   The investigation of the potential geographic distribution through ENM and physiological

338 thresholds tested through experiments of *G. turuturu* indicated higher habitat suitability of this

339 invasive species in temperate and warm-temperate regions around the world. Experimental

340 results demonstrated a decrease in photosynthetic yield in algae exposed to higher temperatures,

341 along with a stress response observed in pigments and growth rate. At the same time, ENM

342 showed a possible expansion of the distribution of *G. turuturu* to new countries around the globe,

343 especially in regions with temperatures close to the ones that the algae better adapted in the

344 experiments.

345   The variables that most contributed to explain the distribution of *G. turuturu* were the

346   availability of light at bottom and maximum ocean temperatures. Light at bottom is determined

347   with an exponential decay function of photosynthetic active radiation, diffuse attenuation

348   coefficient and depth (Assis et al., 2017b) and allows restricting the species vertical distribution.

349   Indeed, the 4.04 E. m^{-2}. yr^{-1} threshold is comparable with the minimum annual irradiance

350   inferred for other species of algae (Gattuso et al., 2006). The limitation of 26.55ºC maximum

351   temperature inferred in ENM was also in line with previous descriptions of Simon et al. (2001)

352   for the species occurrence in Brittany (France), where waters can reach up to 28ºC in summer.

353   The relative discrepancy in these values can arise from the short term variations of temperature

354   in the water pools where Simon et al. (2001) described the occurrences, which are not captured

355   in the averaged climatic layers used for modelling. In fact, as our experiment results show, the

356   warm temperate population of *G. turuturu* can maintain its photosynthetic yield for at least three

357   days in this temperature, demonstrating that this alga can survive to short-time exposure to high

358   temperatures.

359   Our results allow the assertion that non-fertile adult blades of *G. turuturu* will likely not

360   have success in expanding its niche to regions with an average temperature around 30ºC or

361   survive to heatwaves (short periods with an average of 5 days of anomalous warming of the

362   ocean) (Hobday et al., 2016) in regions with milder temperatures. As for temperate and warm-

363   temperate regions, our model predicts habitat suitability for *G. turuturu* for temperatures

364   reaching up to approx. 26ºC, and the WAP experiment results demonstrated that this alga can

365   maintain its photosynthetic yield and growth at 24 ºC. However, if in 24ºC an event of

366   introduction of a higher amount of nutrients in the water column occurs, *G. turuturu* may suffer a

367   drop in photosynthetic yield depending on the exposure period. Consequently, the invasive

368   potential of this alien species in regions with an average temperature around 24ºC may be

369   smaller than its potential in colder water regions, as noted in the 18ºC treatment, where *G.*

370   *turuturu* had no response to the higher nutrient concentration.

371   In addition, the temperate population experiment demonstrated *G. turuturu* similar Fv/Fm

372   responses to temperature as the response curve from the model, with higher photosynthetic yield

373   in 10, 13, 16 and 20ºC. The experiment with the blades collected in Brazil also showed higher

374   Fv/Fm in the lower temperature used. These results indicate that both populations of *G. turuturu*

375   present better physiological performance in regions with mild temperatures, i.e. temperate

376   climates. Interestingly, in higher temperatures the two populations responded differently. As

377   stated above, the WAP population presented tolerance to a short-time exposure to 30ºC, and to

378   24ºC for the duration of the experiment (LNC only), while the TAP population had a significant

379   drop in performance in 24ºC. Although the different responses may be relative to the fact that

380   these are two distinct invasive populations (Allendorf and Lundquist, 2003), both demonstrated

381   the tendency to experience a drop in Fv/Fm in higher temperatures. The higher temperature used

382   in the warm temperate experiment demonstrated that *G. turuturu* will also present reduced

383   growth rate in regions with average temperatures around 30ºC. Even with the higher chlorophyll

384   *a* content, *G. turuturu* was not able to maintain its photosynthetic yield in 30ºC. Furthermore, the

385   higher concentration of total carotenoids showed a stress response of the algae to the higher

386   temperature but did not prevent the reduction of growth. However, previous studies of the

387   species demonstrated that younger stages of *G. turuturu*'s life cycle have a wider temperature

388   tolerance range than older stages, with germiling growth being the same at 15ºC and 30ºC

389   (Kraemer et al., 2017). For this reason, juvenile stages and/or spores may be more aggressive in a

390   bioinvasion process if transported by an oceanographic vector. And once in a new habitat, the

391   acclimation conditions during the invasive process may be determinant in the success of

392   establishment, when temperature limits are between the ones here evaluated for adult blades.

393   The ecophysiological experiment in Brazil showed that *G. turuturu* can tolerate a wider

394   range of nutrient concentration without reducing its photosynthetic yield in 18ºC, which was

395   expected based on previous descriptions of this algae (Simon et al., 2001). The region where the

396   species is found in southern Brazil is characterized by the presence of upwelling of colder,

397   nutrient-rich waters from the South Atlantic Central Water, and during winter receives influence

398   from the nutrient-rich La Plata river plume and the cold waters of the Sub-Antarctic currents

399   (Pereira et al., 2009). Simultaneously, Martins et al. (2012) reported the algae diversity decrease

400   in southern Brazil caused by urbanization and coastal pollution, damaging the resilience of the

401   benthic community. Consequently, the local conditions of this region meet the suitable

402   conditions demonstrated by the experiment and the model for the establishment and expansion of

403   *G. turuturu* occurrence. Hence, countries with similar coastal characteristics with no register of

404   the species must be aware and act to prevent the arrival and establishment of *G. turuturu.*

405   Nevertheless, in addition to environmental conditions, the success of invasions is highly

406   dependable on biological interactions and the local benthic assemblage. The Brazilian Province,

407   ranging from 0^0 to 28^0S latitude, has communities dominated mostly by macroalgae and turf

408   algae, and presents high diversity of taxa (Aued et al., 2018). In southern Brazil, *G. turuturu*

409   occupies the same niche as *Pyropia acanthophora* (E.C.Oliveira & Coll), both occupying the

410   same position in the rocky shores of Santa Catarina (de Ramos et al., 2018). Mulas and Bertocci

411   (2016) report that, although *G. turuturu* is not a driver of change in assemblages, it takes

412   advantage of the loss of algal canopies to spread and establish in new communities, reducing the

413   abundance of turf algae in some localities. This indicates that *G. turuturu* might have a facilitated

414   establishment in assemblages dominated by algae and prone to loss of algae cover, in regions

415   within the environmental ranges found in this study. However, we have not investigated the

416   characteristics and impacts of *G. turuturu*'s invasion in the local benthic assemblage, and further

417   studies are necessary to fully comprehend the effects of biological interactions in the invasion

418   process of this alga.

419   Temperature was chosen as one of the variables in the experiments and model due to its

420   importance in determining the geographic distribution of macroalgae (Hoek, 1982). The changes

421   in the average sea surface temperature caused by climate change are characterized as a global

422   stressor to autochthonous communities. *G. turuturu*, as demonstrated in this study, can have

423   advantage in such scenario, expanding its distribution to locations with predicted regional

424   cooling (e.g. northern Chile and South Africa, Falvey and Garreaud 2009; Bolton and Anderson

425   2012) or warming of cold-water regions (Poloczanska et al., 2013; Vergés et al., 2014). Models

426   as the one here used are the best approach to translating future climatic changes into ecological

427   responses, guiding strategies of conservation and management (Wiens et al., 2009). Moreover,

428   the effects of a local stressor, higher nutrient concentration, were assessed in *G. turuturu* through

429   ecophysiology evaluation. By combining both approaches, we could determine the possible

430   geographic expansion of *G. turuturu* under both local and global stressors.

431   Habitat models for invasive seaweeds need a high transferability, expanding predictions

432   to regions where the species could potentially invade. Verbruggen et al. (2013) demonstrated that

433   for global predictions the most important procedure to increase model transferability is choosing

434   fewer predictors with high ecological significance for the species. Furthermore, the use of

435   ecophysiological knowledge about the species is highly advisable to assess the significance of

436 predictions (Araújo and Guisan, 2006). Accordingly, we used ENM and fewer variables to model

437 the global distribution of *G. turuturu* together with the evaluation of its ecophysiology.

438 　　With increasing environmental changes and availability of transport vectors, it is essential

439 the use and improvement of approaches capable of predicting possible new events of

440 bioinvasion, as done in this work. Invasive species represent great harm to ecosystem services,

441 with provision services being the most affected by them, such as food provision and genetic

442 variability (Vilà et al., 2010). It becomes clear that these predicting tools and the knowledge of

443 invasive species and their habitats must be used as the foundation of international regulatory

444 agreements, pursuing the protection of natural resources and biodiversity (Hulme et al., 2008).

445 The results found in this study enable stakeholders and policymakers to discuss and foster

446 management actions regarding prevention or eradication in case of *G. turuturu* expanding its

447 distribution to new regions, especially for South America where there are few registers of its

448 occurrence (Azevedo et al., 2015). As management of a consolidated bioinvasion event is

449 expensive and difficult, prevention should be of the highest priority. Therefore, the present work

450 takes place as an important tool of bioinvasions' prevention in a scenario of quick changes in

451 environmental conditions and dispersion patterns, in which the delay of response by international

452 and local governments can cost the planet ecosystem functions and services that it relies upon.

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723

724 Figure captions

725 *Coloured figures only in the online version.*

726 **Fig. 1** Map of *G. turuturu* occurrences used in the model. Sampling locations are represented in

727 white diamonds, whilst black dots represent occurrences in the known introduced range and grey

728 dots represent occurrences in the native range. A close-up was made in South America to show

729 more clearly the introduced and sampled locations in this region.

730 **Fig. 2** Map of *G. turuturu* habitat suitability in the planet (a), with zoom in South America (b),

731 and Europe/North Africa (c). Regions in dark red represent regions suitable for the occurrence of

732 *G. turuturu,* whilst regions in light grey represent regions with unsuitable environmental

733 conditions for *G. tururu.* Land is represented in grey, and zones without environmental data are

734 represented in white (no data).

735 **Fig. 3** Response curve of *G. tururu* to maximum ocean temperature. Decline point is set at

736 21.23ºC and the limiting point at 26.55ºC, demonstrating low habitat suitability of *G. tururu* to

737 higher temperatures.

738 **Fig. 4** Average ± standard deviation (*n*=7) of Optimal Quantum Yield (Fv/Fm) for all

739 temperatures (18, 24 and 30°C) and nutrient conditions (low nutrient condition – LNC, and high

740 nutrient concentration – HNC) in the first, third, fifth and seventh day of the warm temperate

741 adapted population (WAP) experiment. Letters indicate Tukey's test significantly different

742 groups (*p*<0.05).

743 **Fig. 5** Average ± standard deviation (*n*=6) of Optimal Quantum Yield (Fv/Fm) in the temperate

744 adapted population (TAP) experiment in the beginning of the experiment (initial control), then

745 the values of each treatment after three days at 10 (control), 13, 16, 20 and 24°C. Letters indicate

746 Tukey's test significantly different groups (*p*<0.05).

747 **Fig. 6** Response of *G. tururu* (warm temperate adapted population - WAP) to different

748 temperatures in treatments. Different letters represent Tukey's test significantly (*p*<0.05)

749 different groups. Bars represent average ± standard deviation. (a) Chlorophyll *a* concentration

750 (μg chlorophyll-*a* per g of fresh weight) in the experiment's beginning and end (*n*=7). (b) Total

751 Carotenoids concentration (μg total carotenoids per g of fresh weight) in experiment's beginning

752 and end (*n*=7). (c) Percentage of *G. tururu* daily growth rate during the experiment (*n*=10).

Fig. 7 Average ± standard deviation (n=7) of phycoerythrin concentration (µg phycoerythrin per g of fresh weight) at different nutrient conditions (warm temperate adapted population - WAP). Letters indicate Tukey's test significantly different groups (p<0.05).

Supplementary material

ESM1: Excel file with all the occurrences and their sources used in the Ecological Niche Model.

ESM2: Word document with additional figures, such as the experimental design setup, response curves of the model and chlorophyll a concentration in each nutrient condition (WAP experiment).

HOW EXPERIMENTAL PHYSIOLOGY AND ECOLOGICAL NICHE MODELLING CAN INFORM THE MANAGEMENT OF MARINE BIOINVASIONS?

Gabrielle Koerich[a,c*], Jorge Assis[b], Giulia Burle Costa[a,c], Marina Nasri Sissini[a,c], Ester A. Serrão[b], Leonardo Rubi Rörig[a], Jason M. Hall-Spencer[d], José Bonomi Barufi[a], Paulo Antunes Horta[a*]

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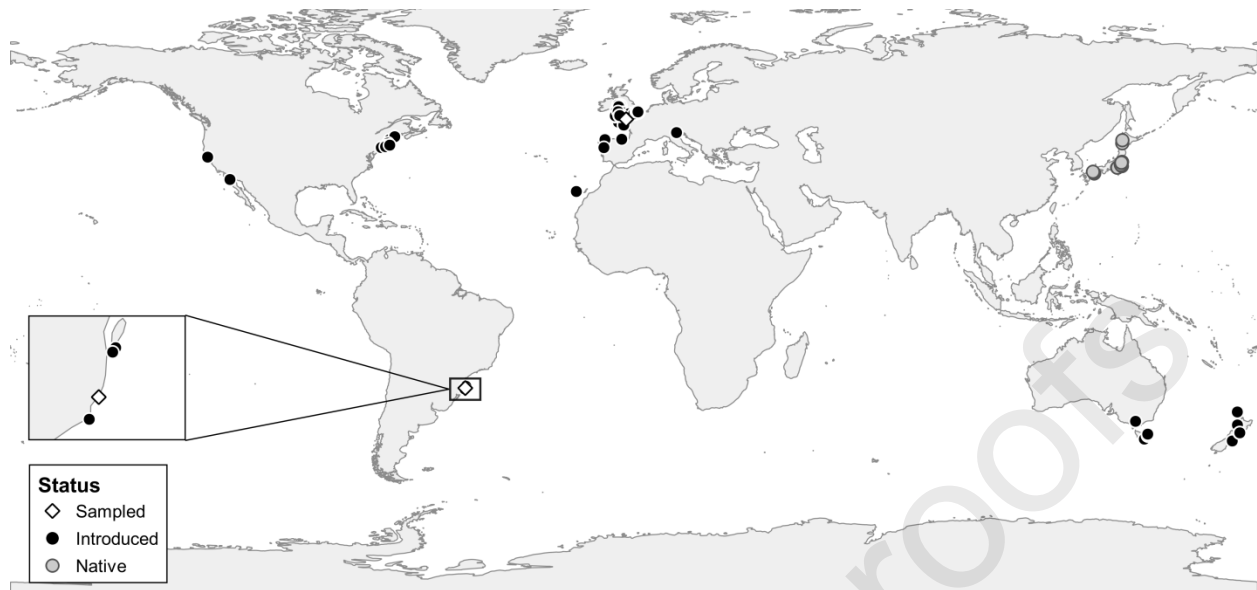780 PL4 8AA United Kingdom

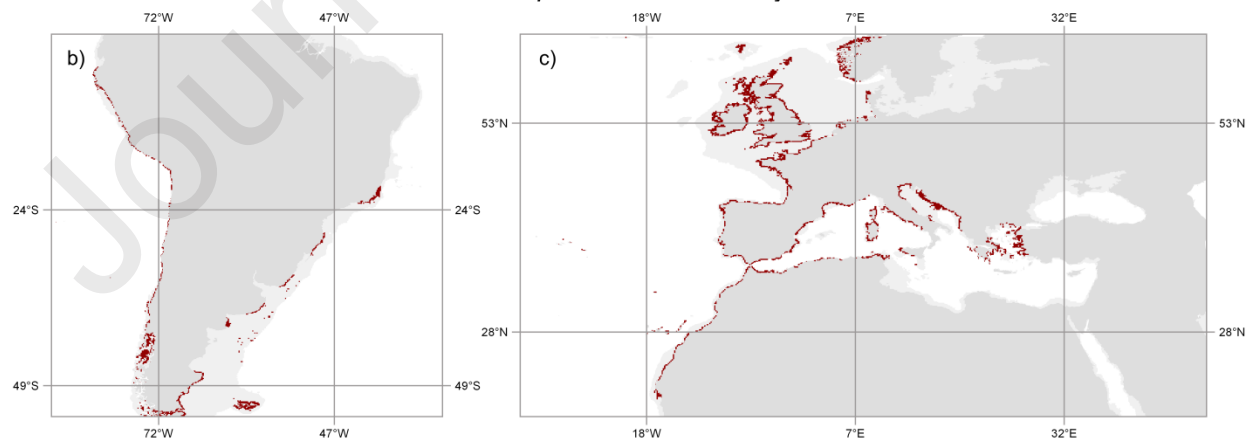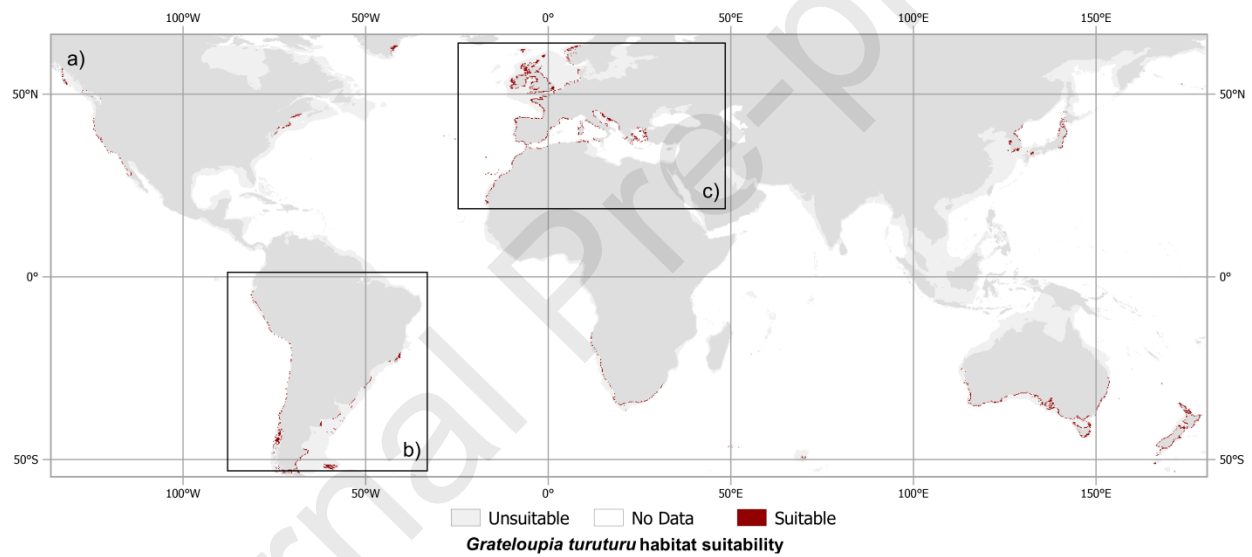781 *Corresponding author:

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783 Phone address: +55(48) 3721-4767
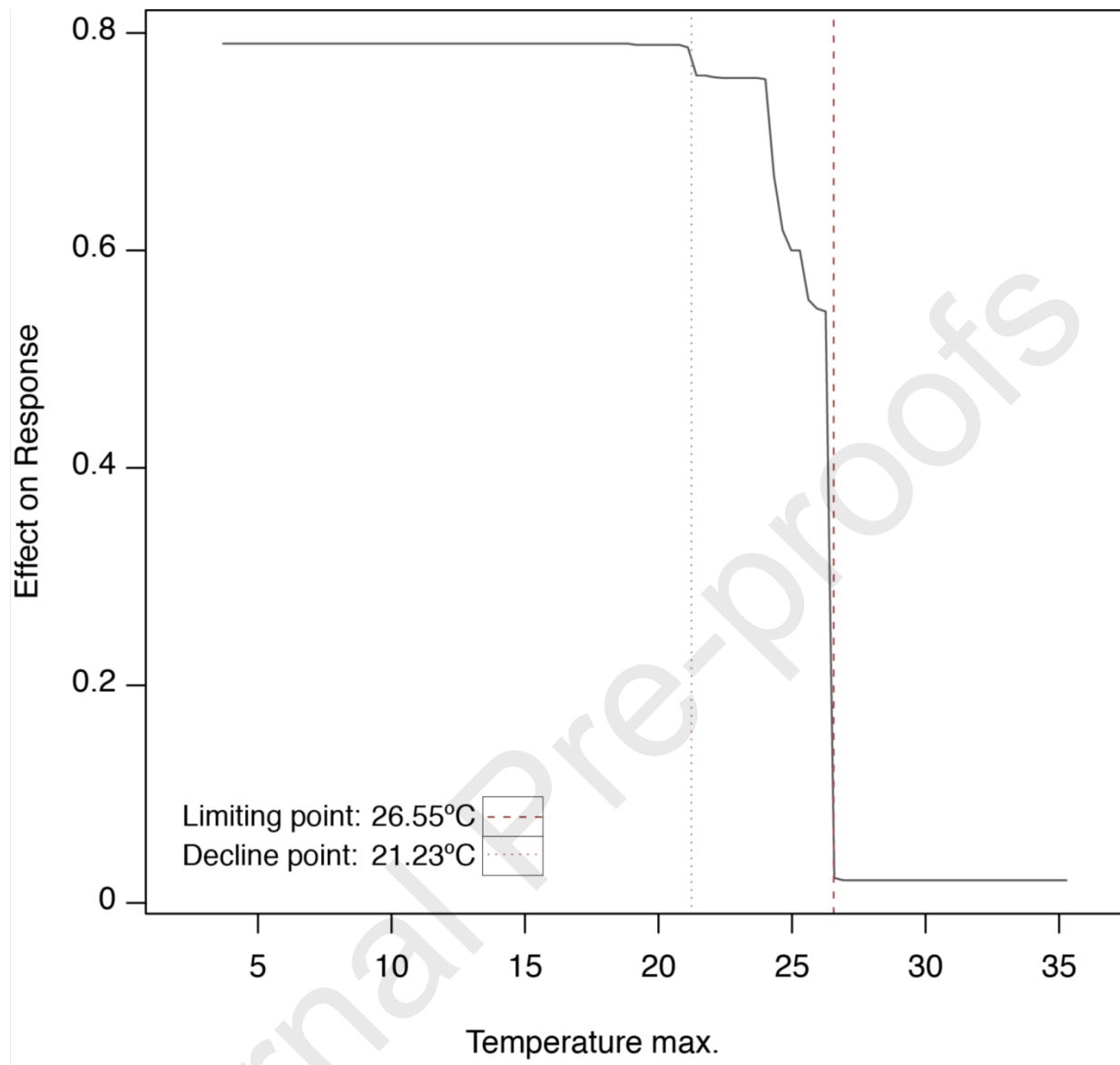
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785 CRediT author statement

786   **Gabrielle Koerich:** Investigation, Formal Analysis, Data Curation, Writing - Original
787 draft preparation, Writing - review & editing. **Jorge Assis**: Methodology, Formal Analysis,
788 Writing - review & editing. **Giulia Burle Costa:** Investigation, Data Curation, Writing - review
789 & editing. **Marina Nasri Sissini:** Data Curation, Writing – Review & Editing. **Ester A. Serrão:**
790 Writing- Reviewing and Editing, Resources. **Leonardo Rubi Rörig:** Writing - review & editing,
791 Resources. **Jason M. Hall-Spencer:** Writing - review & editing. **José Bonomi Barufi:** Writing -
792 review & editing, Resources. **Paulo Antunes Horta:** Conceptualization, Writing - review &
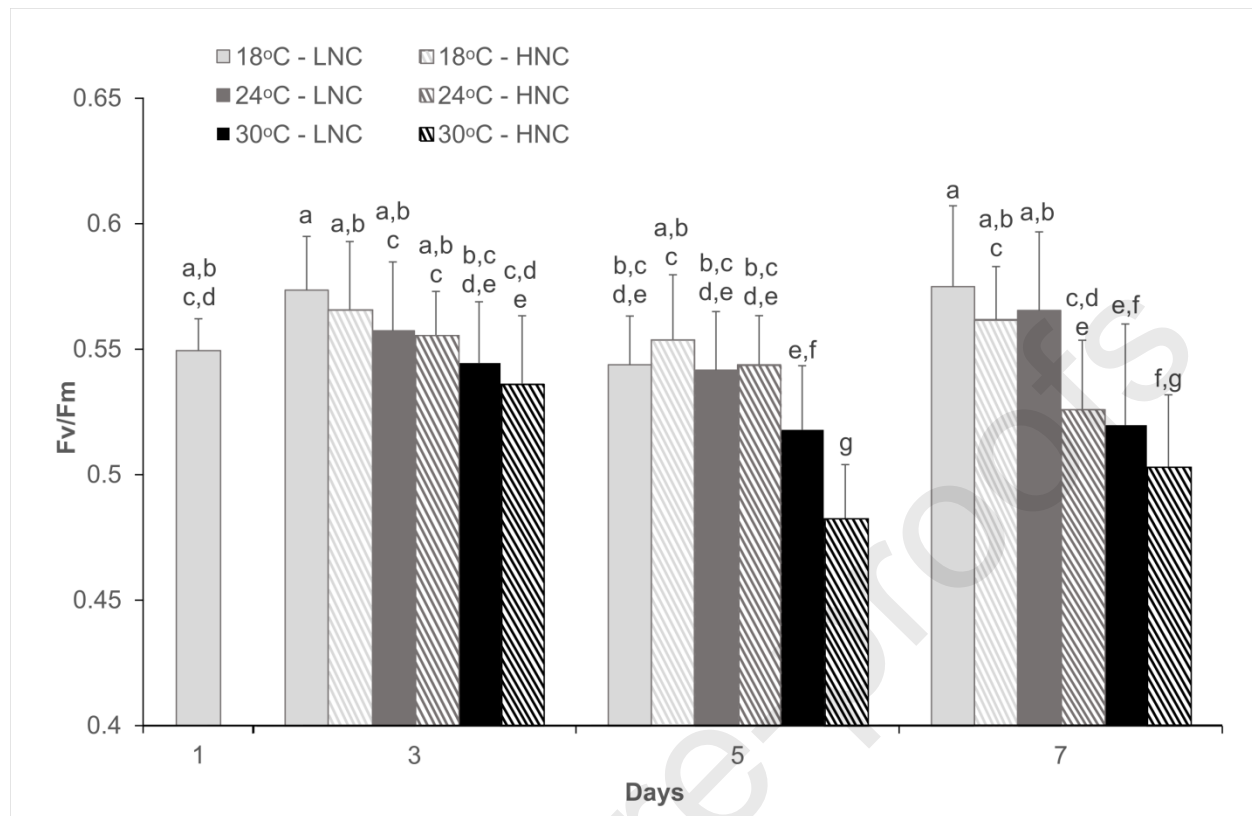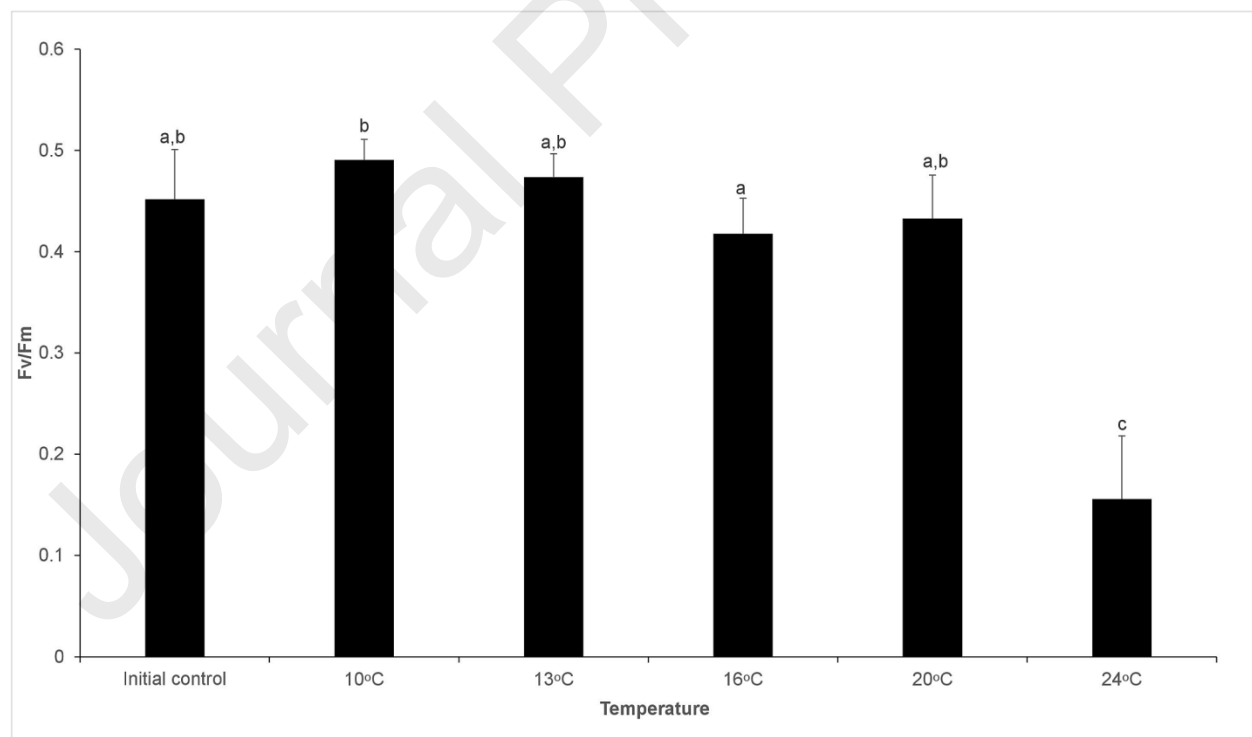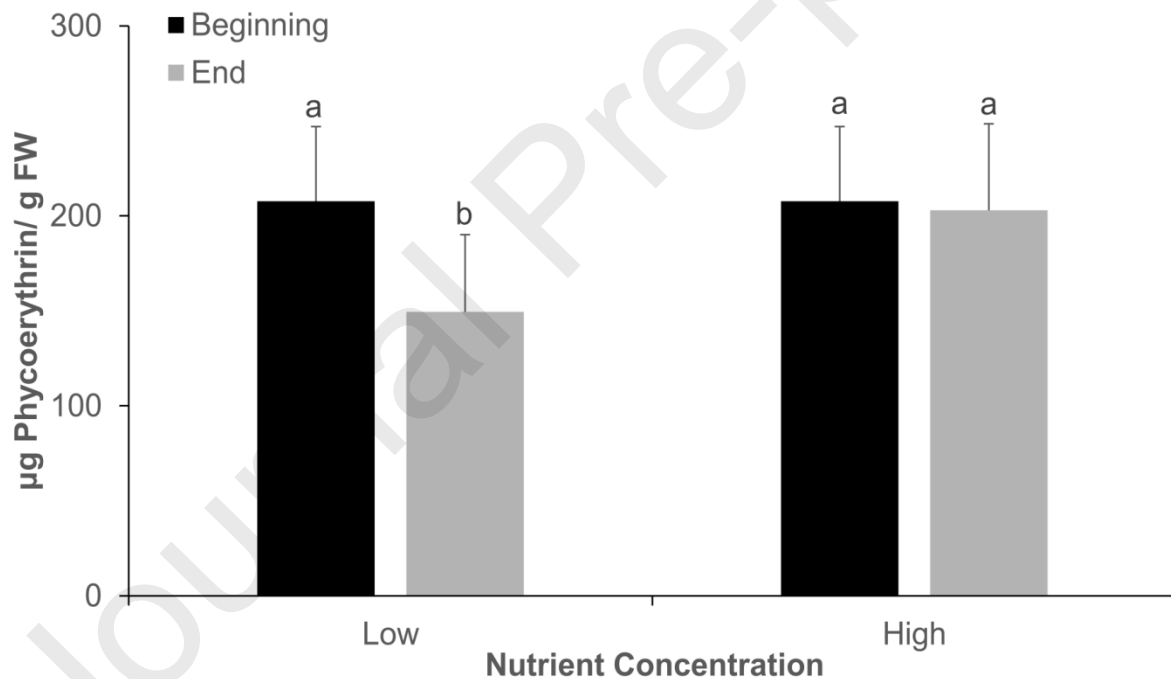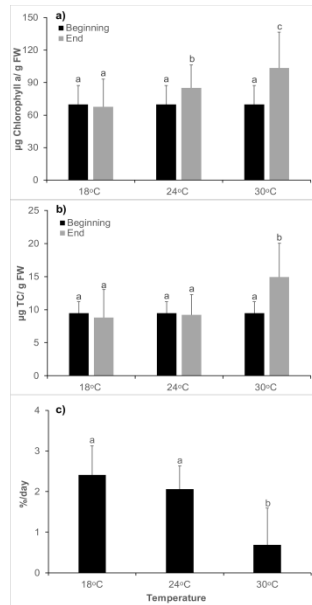793 editing, Funding acquisition, Resources.

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Grateloupia turuturu habitat suitability

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803   HOW EXPERIMENTAL PHYSIOLOGY AND ECOLOGICAL NICHE MODELLING

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806   Serrão[b], Leonardo Rubi Rörig[a], Jason M. Hall-Spencer[d], José Bonomi Barufi[a], Paulo Antunes

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819 Phone address: +55(48) 3721-4767

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824 **Table 1** Environmental predictors used in ENM, units, tipping points and relative

825 importance to the models

Environmental predictor	Unit	Tipping points	Relative importance (%)
Long-term min. Light at bottom	E . m⁻² . yr⁻¹	4.04	
Long-term max. Ocean Temperature	°C	26.55	
Long-term min. Ocean Temperature	°C	2.20	
Long-term min. Ocean Salinity	PSS	31.05	
Long-term min. Nitrate concentration	mmol . m⁻³	0.42	
Long-term min. Phosphate concentration	mmol . m⁻³	0.06	

826

827

828 **Table 2** Results of ANOVA examining the effects of temperature (T), nutrient concentration (N)
829 and time (D) in Optimal Quantum Yield (Fv/Fm) values of both populations studied, the warm
830 temperate adapted population (WAP) and temperate adapted population (TAP). Significance is
831 demonstrated as: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

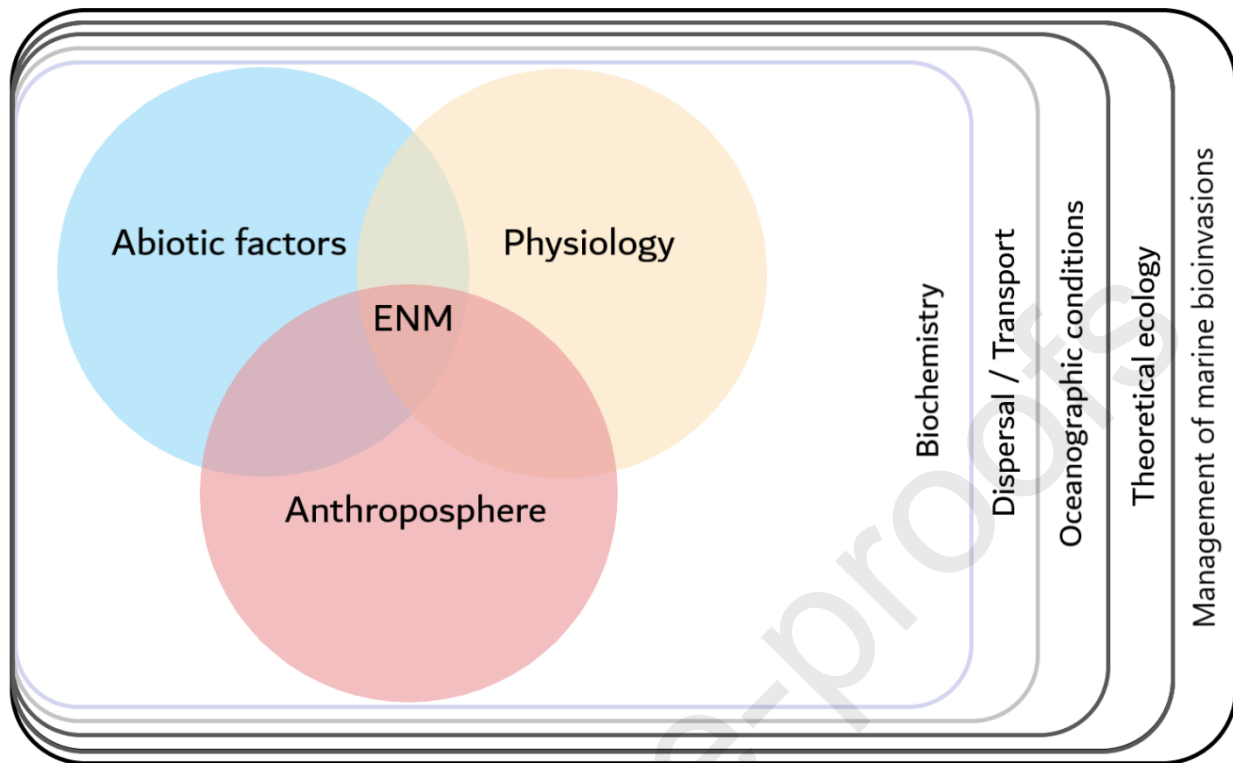| Effect / Variable	Fv/Fm - WAP			Fv/Fm - TAP		
	df	MS	F	df	MS	F
T	2	0.023	149785107.000***	5	0.093	53.819***
N	1	0.001	0.538			
D	3	0.023	145662023.000***			
TxN	2	0.001	0.883			
TxD	6	0.006	363232755.000**			
NxD	3	0.002	110942947.000			
TxNxD	6	0.007	429910213.000***			

832

833 **Table 3** Results of ANOVA examining the effects of temperature (T), nutrient concentration (N)
834 and time (D) in concentration of chlorophyll-*a*, total carotenoids, phycoerythrin and growth rate
835 values of the warm temperate adapted population (WAP). Significance is demonstrated as: *

Effect / Variable	df	Chlorophyll-a		Total Carotenoids		Phycoerythrin		Growth Rate	
		MS	F	MS	F	MS	F	MS	F
T	2	0.007	13.523***	0.000	21.901***	0.002	1.050	0.009	27.824***
N	1	0.003	5.848*	0.000	0.033	0.030	16.896***	0.001	2.203
D	1	0.012	23.991***	0.000	39.442***	0.042	23.451***		
TxN	2	0.001	2.623	0.000	1.634	0.000	0.258	0.000	0.499
TxD	2	0.006	12.478***	0.000	22.318***	0.002	1.050		
NxD	1	0.002	4.437*	0.000	0.010	0.030	16.896***		
TxNxD	2	0.001	2.289	0.000	1.839	0.000	0.258		

836 $p<0.05$, ** $p<0.01$, *** $p<0.001$

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838

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856

857   Highlights

858   •   The process of bioinvasion is a combination of abiotic conditions and biotic factors under
859   anthropogenic influence
860   •   We developed an Ecological Niche Model to investigate the possible distribution of a
861   harmful red algae, and crosschecked these results with its ecophysiologicals evaluations,
862   considering two populations with different biogeographical acclimation histories.
863   •   We observed higher habitat suitability for *G. turuturu* in warm temperate and temperate
864   regions, result that was corroborated by ecophysiological experiments
865   •   In a changing world, where marine bioinvasions are increasing in number, assessing the
866   risk of invasion by integrating ecological theory and physiological observations is of
867   great use to inform management and conservation decisions.

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