North Atlantic Oscillation drives the annual occurrence of an isolated, peripheral population of the brown seaweed *Fucus guiryi* in the Western Mediterranean Sea

Ignacio Melero [1] , **A. Enrique Salvo** [1] , **José Carlos Báez** [2] , **Elena Bañares-España** [1] , **Andreas Reul** [3] , **Antonio Flores-Moya** Corresp. [1]

[1] Faculty of Science, Department of Plant Biology, Universidad de Málaga, Málaga, Spain

[2] Centro Oceanográfico de Canarias, Instituto Español de Oceanografía, Santa Cruz de Tenerife, Spain

[3] Faculty of Science, Department of Ecology and Geology, Universidad de Málaga, Málaga, Spain

Corresponding Author: Antonio Flores-Moya
Email address: floresa@uma.es

The intertidal brown seaweed *Fucus guiryi* is distributed in the cold-temperate and warm-temperate coasts of Europe and North Africa. Curiously, an isolated population develops at Punta Calaburras (Alboran Sea, Western Mediterranean) but its presence is not permanent throughout the years, unlike the closest (ca. 80 km), perennial populations at the Strait of Gibraltar. The presence of the alga at Punta Calaburras is supposed to be due to the influence of the permanent Atlantic jet coming from the Atlantic Ocean into the Mediterranean. A twenty six years' time series (from 1990 to 2015) of occurrence of *F. guiryi* at Punta Calaburras has been analysed by correlating with oceanographic (sea surface temperature, an estimator of the Atlantic jet power) and climatic factors (air temperature, rainfall, and North Atlantic Oscillation –NAO-, and Arctic Oscillation –AO-indexes). The occurrence of the alga aggregated from 1990-1994 and 1999-2004, with sporadic events in 2006 and 2011. Binary logistic regression showed that the occurrence of the alga at Punta Calaburras is favoured under positive NAO index from April to June. It has been hypothesized that the isolated population of *F. guiryi* should show greater stress than their congeners of permanent populations, and to this end, two approaches were used to evaluate stress: one based on the integrated response to ontogeny (developmental instability, based on measurements of the fractral pattern of algal thalli) and another based on the photosynthetic response. However, the only significant differences detected were in photosynthetic quantum yield and water loss under emersion conditions. In conclusion, this study demonstrates the teleconnection between atmospheric oscillations and survival and proliferation of marine macroalgae, an aspect practically unknown before.

1 **North Atlantic Oscillation drives the annual occurrence of an isolated,**

2 **peripheral population of the brown seaweed *Fucus guiryi* in the Western**

3 **Mediterranean Sea**

4

5 Ignacio J. Melero-Jiménez[a], A. Enrique Salvo[a], José C. Báez[b], Elena Bañares-España[a], Andreas

6 Reul[c], Antonio Flores-Moya[a*]

7

8 [a] *Departamento de Biología Vegetal (Botánica), Facultad de Ciencias, Universidad de Málaga,*

9 *Campus de Teatinos s/n, 29071 Málaga, Spain*

10 [b] *Instituto Español de Oceanografía, Centro Oceanográfico de Málaga (sede Fuengirola), 29640*

11 *Fuengirola, Spain*

12 [c] *Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Campus*

13 *de Teatinos s/n, 29071 Málaga, Spain*

14

15 *Corresponding author

16 *E-mail address*: floresa@uma.es

17

18 *Keywords*: developmental instability, *Fucus guiryi*, North Atlantic Oscillation, photosynthesis,

19 stress

20

21

22   ABSTRACT

23

24   The intertidal brown seaweed *Fucus guiryi* is distributed in the cold-temperate and warm-

25   temperate coasts of Europe and North Africa. Curiously, an isolated population develops at

26   Punta Calaburras (Alboran Sea, Western Mediterranean) but its presence is not permanent

27   throughout the years, unlike the closest (ca. 80 km), perennial populations at the Strait of

28   Gibraltar. The presence of the alga at Punta Calaburras is supposed to be due to the influence of

29   the permanent Atlantic jet coming from the Atlantic Ocean into the Mediterranean. A twenty six

30   years' time series (from 1990 to 2015) of occurrence of *F. guiryi* at Punta Calaburras has been

31   analysed by correlating with oceanographic (sea surface temperature, an estimator of the Atlantic

32   jet power) and climatic factors (air temperature, rainfall, and North Atlantic Oscillation –NAO-,

33   and Arctic Oscillation –AO- indexes). The occurrence of the alga aggregated from 1990-1994

34   and 1999-2004, with sporadic events in 2006 and 2011. Binary logistic regression showed that

35   the occurrence of the alga at Punta Calaburras is favoured under positive NAO index from April

36   to June. It has been hypothesized that the isolated population of *F. guiryi* should show greater

37   stress than their congeners of permanent populations, and to this end, two approaches were used

38   to evaluate stress: one based on the integrated response to ontogeny (developmental instability,

39   based on measurements of the fractral pattern of algal thalli) and another based on the

40   photosynthetic response. However, the only significant differences detected were in

41   photosynthetic quantum yield and water loss under emersion conditions. In conclusion, this study

42   demonstrates the teleconnection between atmospheric oscillations and survival and proliferation

43   of marine macroalgae, an aspect practically unknown before.

INTRODUCTION

44

45

46   The brown seaweed *Fucus guiryi* G. I. Zardi et al. inhabits the littoral zone of cold-temperate and

47   warm-temperate seas of European and African coasts of the northern Atlantic Ocean (*Zardi et*

48   *al., 2011*). The southern limit of distribution occurs in the Canary Islands, not extending into the

49   Mediterranean Sea (*Zardi et al., 2011*). However, sometimes an isolated population develops at

50   Punta Calaburras (Alboran Sea, Western Mediterranean; Fig. 1A-B), around 80 km from the

51   nearest populations in the Strait of Gibraltar (*Conde, 1989*). It has been hypothesized that the

52   presence of *F. guiryi* (sub *F. spiralis* and *F. spiralis* var. *platycarpus*) at Punta Calaburras is

53   favoured by the current flowing from the Atlantic Ocean into the Mediterranean Sea through the

54   Strait of Gibraltar (*Bellón, 1953*; *Conde & Seoane-Camba,* 1981). Punta Calaburras is located at

55   the edge of the North Western Alboran upwelling (*Reul et al., 2005*; *Muñoz et al., 2015*; *Macías*

56   *et al.*, *2016*), where the Atlantic Jet (AJ) approaches to the coast before traveling towards the

57   Moroccan coast (Fig. 1A). This current compensates for the negative water balance in the

58   Mediterranean Sea due to the loss of water by evaporation, which is higher than the inputs by

59   precipitation and rivers (*Rodríguez, 1982; Parrilla & Kinder, 1987*). This current could transport

60   drifting thalli and few-celled embryos of *F. guiryi* from neighbour populations in the Strait of

61   Gibraltar to Punta Calaburras (*Conde & Seoane-Camba, 1981*). It must be highlighted that *F.*

62   *guiryii* (sub *F. spiralis*) was found in 1987 on the Mediterranean coast of France, Gruissan

63   (Aude), possibly due to the oyster culture in the lagoons from the Mediterranean French coasts

64   (*Sancholle*, 1987); this population was "introduced" in the Mediterranean and it is not

65   comparable to the natural population of Punta Calaburras.

66   The singularity of the isolated population of *F. guiryi* at Punta Calaburras inspired us to

67   start a uninterrupted survey in 1990 (linked to the field teaching at the university of AFM),

68   which revealed that the population was not permanent, in contrast to the nearby perennial

69   populations in the Strait of Gibraltar. Although the alga was always detected in winter, in some

70   years the population did not survive in midsummer. The occurrence of this population of *F.*

71   *guiryi* must be determined by environmental conditions, so the aim of this study was to analyse

72   the role of the oceanographic and atmospheric factors controlling the presence of the alga at

73   Punta Calaburras. For this purpose, the time series (from 1990 to 2015) of presence/absence of *F.*

74   *guiryi* at Punta Calaburras was analysed by binary logistic regression, using two kinds of

75   independent, explanatory variables. First, a proxy for the powerful incoming current from the

76   Atlantic Ocean into the Alboran Sea, which becomes evident by changes in sea surface

77   temperature (SST; lower SST with higher current flow; *Vargas–Yáñez et al., 2002; Renault et*

78   *al., 2012*). Second, the North Atlantic Oscillation (NAO) and the Arctic oscillation (AO) can

79   account for the most important climate variability in the Northern Hemisphere. In fact, it has

80   been found that both atmospheric oscillations affect the SST in the Alboran Sea (*Báez et al.,*

81   *2013*), but the link between occurrence or productivity of microalgae and seaweeds and

82   atmospheric oscillations remains almost unexplored (*Moore etal., 2008*; *Folland et al., 2009;*

83   *Gamboa et al., 2010; Smale et al., 2013; Báez et al., 2014*).

84   The population of *F. guiryi* occurring at the limit of the species' distribution,

85   geographically isolated and composed of a low number of individuals, could experience higher

86   stress than the population inhabiting the Strait of Gibraltar. This assessment is based on the

87   notion that peripheral populations of organisms are typical cases of "living at the edge"

88   (*Channell & Lomolino, 2000; Eckert et al., 2008; Peterman et al., 2013*), with organisms

89   showing signs of physiological stress (*Shumaker & Babble, 1980*). Following this idea, we made

90   a comparative study of individuals at Tarifa (Strait of Gibraltar) (Fig. 1A-C) vs. Punta

91 Calaburras, during the summer of 2011, a year when the alga occurred at both places. For this

92 purpose, we evaluated stress via a physiological approach based on photosynthesis as well as the

93 whole-organism response by assessing developmental stability. Quantum yield from

94 photosystem II photochemistry responds to the alteration of optimum conditions, which could

95 indicate that photosynthesis is being affected (*Baker & Oxborough, 2004*). On the other hand,

96 individuals integrate stress conditions throughout their ontogeny, termed developmental

97 instability (*Clarke & McKenzie, 1987; McKenzie & Clarke, 1988; Emlen et al., 1993; Palmer,*

98 *1994*). Therefore, it could be expected that a higher rate of development instability arise at the

99 limits of distribution of organisms, compared to instability in core populations, because in

100 peripheral areas combinations of environmental factors occur that adversely affect growth,

101 reproduction, and ultimately survival of organisms (*Zakharov, 1992; Clarke, 1995*).

102 Here we show that the time series from 26 years (1990-2015) of presence/absence of *F.*

103 *guiryi* at Punta Calaburras is explained by the overall mean value of the NAO from the three

104 months previous to the control month (July). When the alga occurred at Punta Calaburras it did

105 not differ from its counterparts at Tarifa in photosynthetic performance (estimated by the

106 fluorescence of chlorophyll *a* from PSII) under submerged conditions, nor in developmental

107 instability. However, a different response of photosynthetic performance was detected when the

108 alga was exposed to air, possibly related to a more rapid loss of water during air exposure in

109 individuals at Punta Calaburras.

110

111

112 **MATERIALS AND METHODS**

113

114   **Analysis of the time series of occurrence**

115

116   The presence of the population of *F. guiryi* at Punta Calaburras (36º30′28′′ N, 004° 38′ 8″ W)

117   (Fig. 1) was checked annually in February-March, and in July-August (Table 1), from 1990 to

118   2015. We detected the alga all the years of the survey in February-March, but the alga did not

119   always persist into midsummer. The time series of the occurrence of *F. guiryi* in midsummer was

120   initially analysed taking into account two aspects: the distribution and the presence of the alga

121   throughout the time series. The annual occurrence distribution was checked by the exp test

122   (*Prahl, 1999*) for a stationary Poisson process (randomly distributed throughout the time)

123   computed as:

124

$$M = \frac{1}{n}\sum_{\Delta t_i < T}\left(1 - \frac{\Delta t_i}{T}\right) \qquad \text{(eq. 1)}$$

126

127   where *n* is the waiting times, Δt_i is the time period between successive events in the sorted

128   sequence and *T* is the mean waiting time. The statistic *M* will tend to 0 for a regularly spaced

129   (overdispersed) sequence of events, and to 1 for a highly clustered sequence. If *p*<0.05, the

130   sequence is not Poisson; in this case, if the *M* statistic is smaller than the expected value it

131   indicates regularity, whereas if the *M* value is higher it indicates clustering.

132   The tendency of the annual occurrence distribution of *F. guiryi* at Punta Calaburras was

133   analysed by the Laplace test (*Cox & Lewis, 1978*) based on the *U* statistic:

134

$$U = \frac{\bar{t} - \frac{L}{2}}{L\sqrt{\frac{1}{12n}}} \qquad \text{(eq. 2)}$$

136

137   where t is the mean event time, n the number of events and L the length of the interval, estimated

138   as the time from the first to the last event, plus the mean waiting time. U is approximately

139   normally distributed with 0 mean and unit variance under the null hypothesis of constant

140   intensity. If $p<0.05$, a positive U indicates an increasing trend in intensity (decreasing waiting

141   times) whereas a negative U suggests a decreasing trend. If $p>0.05$, it indicates no trend in the

142   density of events.

143

144

145   **Analysis of the annual occurrence as a function of oceanographic and atmospheric**

146   **variables**

147

148   The relationship between the presence/absence in midsummer of *F. guiryi* at Punta Calaburras

149   from 1990 to 2015, and the temperature, rainfall, NAO, AO and SST, was addressed by binary

150   logistic regressions, widely used for establishing relationships between environmental

151   independent variables and the probability response of target variables (*Zuur et al., 2007*).

152   Temperature and rainfall mean monthly data were obtained from the Agencia Española de

153   Meteorología (Fuengirola station, 4 km to the east of Punta Calaburras). Of the atmospheric

154   oscillations, NAO is the most important mechanism responsible for the interannual climate

155   variability in SW Europe, particularly during the winter (*Hurrell, 1995; Hurrell et al., 2003*).

156   *Walker & Bliss (1932)* defined the NAO as the difference in surface atmospheric pressure

157   between Stykkisholmur station (Iceland) and Ponta Delgada (Azores Islands, Portugal). NAO

158   index is a proxy for the difference between the High of the Azores and the Low of Iceland (*Jones*

159   *et al., 1997*). The AO also affects the overall mean of weather conditions in SW Europe.

160   According to *Thompson & Wallace (1998)* the AO is characterised by a meridional dipole in sea

161   level atmospheric pressure between polar regions and mid-latitudes, and could be interpreted as

162   the surface signature of modulations in the strength of the polar vortex aloft. When the AO index

163   is positive (characterised by a strengthening of the polar vortex), surface pressure is low in the

164   polar region, and the opposite occurs when the index is negative. Monthly AO and NAO index

165   values were obtained from the free-access web address

166   http://www.cpc.ncep.noaa.gov/products/precip/CWlink/daily_ao_index/ao.shtml and

167   http://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml, respectively. Finally, the

168   power of the Atlantic current entering the Mediterranean Sea was estimated by the SST values

169   close to Punta Calaburras (the higher the flow of AJ water, the lower SST is; *Parrilla & Kinder,*

170   *1987*). Data of SST were obtained from the free-access web address

171   http://www.ma.ieo.es/gcc/playafuengirola_taireyagua_anomalias.xls (Centro Oceanográfico de

172   Málaga (sede Fuengirola), Instituto Español de Oceanografía).

173   　　　For the analysis, we tested the monthly values of environmental variables from the same

174   month as well as the overall mean figures from two to six previous months. The mathematical

175   concept underlying logistic regression is the natural logarithm of the odds ratios, called logit (*y*),

176   depending on the model:

177

178   $$\text{logit } (y) = \ln\left(\frac{p}{1-p}\right) = \alpha + \beta_1 x_1 + ... + \beta_n x_n \text{ (eq. 3)}$$

179

180   where p is the probability (risk) of the event of interest occurs, the independent variables are

181   represented with the letter x, α is the intercept of the logit function (y) and the coefficients

182   associated with each variable is identified as β:

183

184
$$p = \frac{e^{\alpha + \beta_1 x_1 + \ldots + \beta_n x_n}}{1 - e^{\alpha + \beta_1 x_1 + \ldots + \beta_n x_n}} \text{ (eq. 4)}$$

185

186   The null hypothesis posits that all of the β coefficients are equal to 0. The rejection of the null

187   hypothesis implies that at least one of the β coefficients is not 0; that is, the logistic regression

188   equation predicts the probability that the event occurs above the overall mean. We assessed the

189   significance of the variables in the model using the Wald test (*Wald, 1943*), the calibration of the

190   model using the Hosmer & Lemeshow test (*Hosmer & Lemeshow, 1980*), its discrimination

191   capacity using the area under the curve (AUC) of the receiving operator characteristics (*Lobo et*

192   *al., 2008*), and its explanatory power using the Nagelkerke R^2 (*Nagelkerke, 1991*).

193   Additionally, we used relevant probability levels to assess the environmental conditions

194   that favoured the presence of *F. guiryi*, the opening gap between the values considered as clearly

195   probable ($p>0.6$) or clearly improbable ($p<0.4$). It must be taken into account that $p = 0.5$ means

196   that the presence or the absence of the population of *F. guiryi* have a similar probability. We then

197   compared the correct classification rate of the models for years clearly probable and clearly

198   improbable for a presence of *F. guiryi*, and simultaneously we identified the levels of the

199   environmental variables associated with the relevant probability levels.

200

201

202   **Developmental instability**

203

204   Developmental instability is more sensitive than traditional measures of stress (*Graham et al.,*

205   *1993; Clarke, 1995*). It is responsive to a wide range of stressors (*Zakharov 1992; Graham et al.,*

206   *1993*) and it is ideally suited for detecting stress in the field (*Graham et al., 1993*). All the

207   species in the genus *Fucus* exhibit self-symmetry, i.e. symmetry across scale (*Corbit & Garbary,*

208   *1995*). We estimated the developmental instability in *F. guiryi* individuals by deviations of the

209   self-symmetry of thalli, by using the box-counting procedure (*Mandelbrot, 1983; Iannaccone &*

210   *Khokha, 1996*). Thalli were placed between two transparent acetate foils, avoiding overlapping

211   the fronds, and they were scanned in TIFF format (300 ppi). The scanned image were overlapped

212   on grids with exponentially increasing box sizes (0.125, 0.25, 0.5, 1, 2 and 4 cm^2). The number

213   of boxes in which at least part of the thallus occurred were counted using an image analysis

214   system Visilog 6.3 (Noesis, French). Twenty independent thalli were processed from each

215   location (Tarifa and Punta Calaburras). Because the overall positioning of the boxes can

216   influences the results of a box count (*Walsh & Watterson, 1993*; *Schulze et al., 2008*), the

217   counting of the boxes was carried out three times (named "replicates"), repositioning the thalli

218   over the acetate foils with the grids every time. We then regressed the natural log of the number

219   of occupied boxes against the natural log of the size of each box. The absolute value of the slope

220   of the regression line is the fractal dimension (a measure of the space filled by the individual).

221   Developmental instability is the degree to which the individual failed to fit the idealized

222   phenotype, and is measured as the standard error of the estimate ($S_{Y\cdot X}$, computed as the square

223   root of the residual mean square of the ANOVA regression of the linear fit). The value of $S_{Y\cdot X}$ is an

224   overall indication of the accuracy with which the fitted regression function predicts the

225   dependence of *Y* on *X*. Under non-stressful conditions, all points should lie on the regression line.

226   A two-level nested ANOVA (model: y = overall mean + locations + replicates [locations] +

227   error) was performed to compare the $S_{Y \cdot X}$ values. The factor "locations" correspond to the Tarifa

228   and Punta Calaburras populations, whereas the factor "replicates [locations]" corresponds to the

229   three independent measurements of $S_{Y \cdot X}$ of each thallus from both locations. The homogeneity of

230   variances was previously checked with Bartlett's test.

231

232

233   **Measurement of natural solar radiation and temperature in air and water**

234

235   The measurements were carried out on 16th July 2011 at Tarifa, and on the following day at

236   Punta Calaburras. Daily changes in PAR (λ = 400-700 nm) were measured using a LI-190R PAR

237   sensor connected to a LI-1400 data logger (LI-COR, Lincoln, NE). The ultraviolet A (λ = 315-

238   400 nm) and ultraviolet B (λ = 280-315 nm) bands were measured using a RM12 device (Dr.

239   Gröbel, Ettlingen, Germany) connected to the respective UVA and UVA sensors. Measurements

240   were made every 30 min, and data were fit to a single sinusoid with the free software PAST ver.

241   2.17 (*Hammer et al., 2001*); the daily doses of each channel were calculated by integrating the

242   area under the sinusoid curves. Air temperature (± 0.1 °C) in the shade was measured with a

243   sensor connected to a LI-1400 data logger. Seawater temperature (± 0.1 °C) was measured with

244   the temperature sensor of a Crison2 OXI-92 (Crison, Spain) oxymeter.

245

246

247   **In vivo measurements of chlorophyll *a* fluorescence**

248

249   A day-long record (from 06.00 to 18.00 UTC) of the photosynthetic performance of *F. guiryi*

250   was carried out on the same days as the solar radiation measurements. The sampling day was

251   selected to correspond to a spring tide, with a maximum tidal height ca. 1.2 m at Tarifa and ca.

252   0.4 m at Punta Calaburras (Fig. 2). Because the weather was sunny without clouds and with

253   similar air and seawater temperatures (see Results), we assumed that the photosynthetic

254   performance measured on the two consecutive days at the different locations could be

255   comparable. Five independent thalli were randomly collected before sunset from the higher

256   eulittoral of Tarifa and at Punta Calaburras. The thalli were cultured in a 25 L white

257   polyvinylchloride tank in natural seawater from the collection site. The tank was placed close to

258   the attachment site of the alga, in an unshaded place. To avoid nutrient depletion and changes in

259   temperature in the tank, seawater was renewed completely every 10 min. The measurements

260   were carried out every 2 h from sunrise to sunset; from 10.00 to 14.00 UTC; an extra set of

261   measurements was carried out on thalli exposed to air from 09.30 UTC.

262   Fresh weight (FW) on thalli in situ was measured with an ELB120 portable analytical

263   balance (± 0.01 g) (Shimadzu, Kyoto, Japan) after thalli were blotted dry with paper towel. The

264   same samples were transported to the laboratory and dried at 60 ºC for 48 h to determine their

265   dry weights (DW). The percentage of water content of thalli was determined as:

266

267    $$\% \text{ Water content} = \left(\frac{\text{FW} - \text{DW}}{\text{DW}}\right) \times 100 \text{ (eq. 5)}$$

268

269   Chlorophyll fluorescence was measured using a portable pulse amplitude modulated

270   PAM-2000 fluorimeter (Walz, Effeltrich, Germany) following *Schreiber et al. (1986)*. The

271   optimal or potential quantum efficiency (F_v/F_m) was measured in thalli exposed to darkness for

272   30 min, by applying the equation:

273

274
$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m} \quad \text{(eq. 6)}$$

275

276   where F_v is the variable fluorescence defined as the difference between the initial fluorescence F_0

277   (when all PSII centres are reduced) and the maximal fluorescence F_m (when all PSII centres are

278   oxidized) of a dark-adapted plant.

279   The relative electron transport rate (ETR_{rel}) was estimated as:

280

281
$$ETR_{rel} = \Phi_{PSII} \times I \quad \text{(eq. 7)}$$

282

283   where I is the incident irradiance of PAR ($\lambda = 400\text{-}700$ nm) and Φ_{PSII} is the quantum yield of

284   PSII photochemistry, defined as:

285

286
$$\Phi_{PSII} = \frac{(F_m' - F_t)}{F_m'} \quad \text{(eq. 8)}$$

287

288   where F_m' and F_t are the maximal and the actual fluorescence of a light-adapted plant.

289   The contribution of the location and the time of day, on water content of thalli (in air),

290   and F_v/F_m, Φ_{PSII} and ETR_{rel} (in air and water), was analysed by a two-way, model I ANOVA.

291   Differences, when obtained, were checked by the Student-Newman Keuls (SNK) procedure. The

292   homogeneity of variances was previously checked with the Bartlett's test. The Pearson's

293   correlation coefficient was computed for the relationships between hydration of thalli and

294   photosynthetic performance parameters.

295

296

297   **Statistical software analysis**

298

299   The exp and tendency tests in the time series were performed using the free software

300   PAST ver. 2.17 (*Hammer et al., 2001*) accessible at

301   http://nhm2.uio.no/norlex/past/download.html. The remaining statistical analyses were carried

302   out using R (2013).

303

304

305   **RESULTS**

306

307   **Analysis of the time series of occurrence**

308

309   The occurrences of the population of *F. guiryi* at Punta Calaburras through the years 1990 to

310   2015 were clustered ($M = 0.96$; M-expected $= 0.36$, $p<0.0001$), with the occurrences aggregated

311   from 1990-1994 and 1999-2004, and sporadic occurrences in 2006 and 2011 (Table 1). A trend

312   throughout of the time series was not detected (U-Laplace test $= -3.8 \times 10^{-15}$, $p = 1$).

313   We found a significant positive relationship between the NAO for the months from April

314   to June (NAO_3) of each year and the probability of the presence of *F. guiryi* ($\chi^2 = 13.530$, $df = 1$,

315    $p = 0.0002$; Wald's test = 5.994, $df = 1$, $p = 0.014$; Table 2) according to the logit y function (Fig.

316   3):

317

318                                    $$y = 3.418 \times NAO_3 + 0.239 \text{(eq. 6)}$$

319

320   The 95% confidence limits for the intercept and for the explanatory variable NAO_3 were [-0.779,

321   1.263] and [0.682, 6.138], respectively; that is to say, a logit y function in which the intercept is

322   deleted could also be used because its contribution to the model was not significant (the

323   confidence interval includes the figure 0). This model was well calibrated (Hosmer and

324   Lemeshow's test = 4.145, $df = 7$, $p = 0.7661$), meaning that the differences between observed

325   and predicted frequencies were not significant. The overall ability of discrimination and the

326   general explanatory power of the model were high (AUC = 0.876 and Nagelkerke $R^2 = 0.541$,

327   respectively).

328   Based on relevant p values, we estimated the correct classification of years in which the

329   NAO_3 index favoured the presence or the absence of *F. guiryi*. The model clearly identified three

330   of four highly probable years ($p>0.6$, corresponding to $NAO_3 > 0.048$) for the presence of *F.*

331   *guiryi* and, simultaneously, all of the clearly improbable years ($p>0.4$, corresponding to $NAO_3 < -$

332   0.189) were correctly assigned.

333

334

335   **Developmental instability**

336

337 The standard error of the regression ($S_{Y \cdot X}$) derived from the box-counting method was used as a

338 proxy for developmental instability in *F. guiryi*. The $S_{Y \cdot X}$ values ranged from 0.025 to 0.162 in

339 the thalli from Tarifa, and from 0.037 to 0.153 in the algae collected in Punta Calaburras (Fig. 4).

340 The comparison of the $S_{Y \cdot X}$ values showed that the replicates [locations] were not significantly

341 different (nested ANOVA; $F = 0.0002$, $df = 4$ and 114, $p = 1.000$) suggesting that the

342 reproducibility of the method was accurate while the contribution of the locations was at the

343 limit of significance (nested ANOVA; $F = 14.041$, $df = 1$ and 4, $p = 0.040$).

344

345

346 **In situ temperature, solar radiation and photosynthetic performance**

347

348 At Tarifa, the temperature of the air on 16th July 2011 increased from 18.2 °C in early morning to

349 an overall mean of 27.4 ± 0.3 °C between 12.30 and 14.30 UTC, and then declined throughout

350 the afternoon. The temperature of the seawater did not change significantly throughout the day,

351 with an overall mean value of 19.5 ± 0.1 °C. The air temperature records at Punta Calaburras

352 were 21.1 ºC in early morning and a maximum of 28.2 ºC at noon; the seawater temperature

353 ranged from 19.1 to 19.8ºC.

354 The daily profile of the solar irradiance recorded at Tarifa showed a symmetrical pattern

355 centered at noon, typical for a clear blue sky (Fig. 5). Daily doses of solar radiation were

356 9228.25, 1109.70 and 13.03 kJ m^{-2} for PAR, ultraviolet A and ultraviolet B, respectively (Fig. 5).

357 Solar radiation data recorded at Punta Calaburras the following day were similar (data not

358 shown), with doses differing <±3%.

359   The F_v/F_m figures ranged from 0.674 ± 0.035 to 0.732 ± 0.034 during the day in

360   permanently submerged thalli (Fig. 6A). The F_v/F_m values were similar at both locations but a

361   highly significant effect of time of day was detected (Table 3). The interaction between sampling

362   location and time of day was not significant (Table 3). Under simulated emerged conditions, the

363   values of F_v/F_m significantly decreased from 10:00 to 14:00 (Fig. 6A), with a greater decrease in

364   thalli from Punta Calaburras than those from Tarifa (Table 3). A significant interaction between

365   locations and time of day was also found (Table 3).

366   The Φ_{PSII} figures ranged from 0.307 ± 0.023 to 0.732 ± 0.028 during the day in

367   permanently submerged thalli (Fig. 6B). The Φ_{PSII} values were similar at both locations (Table 3)

368   but a highly significant effect of time of day was detected (Table 3). The interaction between

369   sampling location and time of day was not significant (Table 3). Under simulated emerged

370   conditions the values of Φ_{PSII} significantly decreased from 10:00 to 14:00 (Fig. 6B; Table 3),

371   with a greater decrease in thalli from Calaburras than those from Tarifa (Fig. 6B; Table 3). A

372   significant interaction between locations and time of day was also found (Table 3).

373   The ETR_{rel} figures ranged from 175.3 ± 11.4 to 671.7 ± 34.0 during the day in

374   permanently submerged thalli (Fig. 6C). The ETR_{rel} values were similar at both locations (Table

375   3) but a highly significant effect of time of day was detected (Table 3). The interaction between

376   sampling location and time of day was not significant (Table 3). Under simulated emerged

377   conditions the values of ETR_{rel} significantly decreased from 10:00 to 14:00 (Fig. 6C), with a

378   greater decrease in thalli from Calaburras than those from Tarifa (Fig. 6C; Table 3). A significant

379   interaction between locations and time of day was also found (Table 3).

380   The water content in algal fronds decreased when they were exposed to air (Fig. 6D),

381   with a greater decrease in thalli from Calaburras than those from Tarifa (Fig. 6D; Table 3). A

382   significant interaction between location and time of day was also found (Table 3).

383   We found that the hydration level significantly correlated ($p < 0.0001$, $n = 15$) both with

384   F_v/F_m ($r = 0.9407$) and Φ_{PSII} ($r = 0.9039$).

385

386

387   **DISCUSSION**

388

389   The study of peripheral populations of organisms "living at the edge" is an interesting research

390   topic because it allows us to explore the factors controlling the limits of growth, reproduction

391   and, finally, survival of the organisms (*Channell & Lomolino, 2000; Eckert et al., 2008;*

392   *Peterman et al., 2013*). In this sense, the isolated population of *F. guiryi* inhabiting Punta

393   Calaburras (Alboran Sea, Western Mediterranean Sea) present a model to understand the role of

394   oceanographic and atmospheric conditions on the annual occurrence of the alga. The recruitment

395   of the few-celled stages could occur from the rapid settlement of very near parent thalli (*Serrão*

396   *et al.*, *1996*) when the alga survive several continuous years. However, after the disappearance of

397   the alga a given year, the recruitment and establishment of the new population occurs from

398   embryos possibly coming from neighbouring populations in the Strait of Gibraltar inhabiting

399   around 80 km from Punta Calaburras (see Fig. 1). It could be estimated that the journey from the

400   source thalli to the settlement location could be achieved in ca. 3 days in agreement with the

401   highest AJ velocities close to 3 m s^{-1} (*Macías et al.*, 2016). It must be highlighted that, during the

402   travel from the Strait of Gibraltar to Punta Calaburras, the main stress undergoing the few-celled

403   stages of *F. guiryi* are the high levels of PAR and UV radiation; however, it has been

404 demonstrated that embryos of this alga can survive even under high doses of solar radiation

405 (*Altamirano et al.*, 2003). This hypothesis could satisfactorily explain the result of our long-term

406 survey of the population (26 years from 1990 to 2015): we observed several years when the thalli

407 did not survive to midsummer, in contrast to the nearby perennial population of the alga

408 inhabiting the Strait of Gibraltar. Moreover, we found no regularities nor tendencies in the

409 occurrence along the time series but, obviously, the "switch-on switch-off" pattern of the

410 population of *F. guiyii* from Punta Calaburras must be under environmental control. For this

411 reason, we addressed the survey of oceanographic and atmospheric factors that could be involved

412 in the annual occurrence of the alga.

413   The survival of the population of *F. guiryi* at Punta Calaburras in midsummer correlates

414 with the overall mean NAO value recorded from April to June, with presence clearly favoured

415 under positive NAO, whereas the alga did not survive under negative overall mean NAO values

416 from April to June. It must be taken into account that NAO is an atmospheric "teleconnection"

417 pattern affecting the climatic conditions in the North Atlantic region, and the derived NAO index

418 is a measure of the strength of the sea level air pressure gradient between Iceland and the Azores,

419 which integrates several climatic variables (e.g., water temperature, prevailing wind direction

420 and speed, and precipitation). In the Alboran Sea, a significant relationship has been found

421 between the negative NAO phase and an increase of SST, possibly through increase in run-off

422 (*Báez et al., 2013*). Consequently, it is possible to consider a direct effect of SST on *F. guiryi* by

423 NAO. The sequence of events for the success of the occurrence of *F. guiryi* at Punta Calaburras

424 in midsummer could be the arrival of few-celled embryos originating from the populations

425 located on the shores of the Strait of Gibraltar in winter-spring; then, the attachment and growth

426 of young thalli if the SST remains relatively low. However, it could be hypothesized that the

427 occurrence of the alga is favoured directly both by NAO, and SST resulting from NAO. Thus,

428 positive phases of the NAO during April and June produce dry springs and clear skies. On the

429 other hand, the position of the AJ is variable (*Vargas-Yáñez et al.*, 2002; *Macías et al.*, 2016)

430 with a north-south migration pattern (*Sarhan et al.*, 2000). The speed of the incoming AJ

431 increases at low pressure over the western Mediterranean (*García-Lafuente et al.*, 2002), and

432 decreasing Mediterranean Sea-level has been related to positive NAO index (*Tsimplis & Josey*,

433 2001). Increasing velocity enhance the Coriolis force and separate the AJ from the Spanish coast,

434 facilitating the upwelling of cold Mediterranean water (from 14 ºC to 17 ºC) at the Spanish coast

435 and consequently allowing the survival of *F. guiryi*. In contrast, under negative NAO index, the

436 AJ velocity might decreases and the Western Alboran gyre (see Fig. 1), characterized by warmer

437 water, migrate northward and may reach the coast at Punta Calaburras. This increases the

438 probability of short-term period of very warm water (up to 22 ºC) coast that hinder survival of *F.*

439 *guiryi* under negative NAO index. It must be highlighted that other organisms do not proliferate

440 on the substrate occupied by *F. guiryi* in Punta Calaburras.

441   The effect of long-period climate variability such as the Atlantic Multidecadal Oscillation

442 (AMO) on the growth of seaweeds has been recently recognized (*Halfar et al., 2011*), but we

443 cannot correlate our data to this phenomenon because our time series for *F. guiryi* is relatively

444 short.

445   When the population of *F. guiryi* at Punta Calaburras proliferates in midsummer, it does

446 not show evidences of physiological stress under submerged conditions when compared to the

447 neighbouring population at Tarifa. However, the photosynthetic performance of the alga at Punta

448 Calaburras is clearly less efficient in air than the counterpart population at the Strait of Gibraltar.

449 The narrower tidal range at Punta Calaburras in comparison to Tarifa (see Fig. 2) ensures that the

450 thalli of the former population remain almost permanently hydrated, whereas the Tarifa thalli

451 experience true submersion-emersion cycles, and consequently they are better adapted to air

452 exposure. In fact, the thalli at Tarifa lose water in air more slowly than those at Punta Calaburras.

453 However, the difference in water economy and photosynthetic performance when the thalli are

454 exposed to air is not reflected in the development instability. The two populations showed

455 similar values of the standard error of the estimate of the regression derived from the box-

456 counting method, suggesting that they fit to an idealized phenotype with a strong fractal pattern.

457 Possibly the fractal phenotype in *F. guiryi* is under a strong genetics control, with the influence

458 of the environment a scant contribution.

459   Summarizing, the connection between the climate variability due to the NAO seems to

460 modulate the occurrence of the annual survival of the isolated population of *F. guiryi* in Punta

461 Calaburras, with the presence of the alga favoured in midsummer if the overall mean NAO value

462 from April to June is positive. In this case, the growth of thalli does not reflect physiological or

463 integrative stress in comparison to the neighboring populations, with the exception of water and

464 carbon economy.

465

466

467 **ACKNNOWLEDGMENTS**

468

471

472

REFERENCES

Altamirano M, Flores-Moya A, Figueroa FL. 2003. Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophyceae). *Aquatic Botany* **75**: 9-20. DOI 10.1016/S0304-3770(02)00149-3.

Báez JC, Gimeno L, Gómez-Gesteira, M, Ferri-Yáñez F, Real R. 2013. Combined effects of the North Atlantic Oscillation and the Arctic Oscillation on sea surface temperature in the Alborán Sea. *PLoS One* **8:** e62201 DOI 10.1371/journal.pone.0062201.

Báez JC, Real R, López-Rodas V, Costas E, Salvo AE, García-Soto C, Flores-Moya A. 2014. The North Atlantic Oscillation and the Arctic Oscillation favor harmful algal blooms in SW Europe. *Harmful Algae* **39:** 121–126 DOI 10.1016/j.hal.2014.07.008.

Baker NR, Oxborough K. 2004. Chlorophyll fluorescence as a probe of photosynthetic productivity. In: Papageorgiou GC, ed. *Chlorophyll a Fluorescence. A Signature of Photosynthesis*. Netherlands: Springer, 65-82.

Bellón L. 1953. Atlantic seaweeds of the Malagan coast (Western Mediterranean). *Abstract Proceeding International Seaweed Symposium* **1:** 5.

Channell R, Lomolino MV. 2000. Dynamic biogeography and conservation of endangered species. *Nature* **403**: 84–86 DOI 10.1038/47487.

Clarke GM, McKenzie JA. 1987. Developmental stability of insecticide resistant phenotypes in blowfly; a result of canalizing natural selection. *Nature* **325:** 345-346 DOI 10.1038/325345a0.

Clarke GM. 1995. Relationships between developmental stability and fitness: Application for conservation biology. *Conservation Biology* **9:** 18-24 DOI 10.1046/j.1523-1739.1995.09010018.x.

Conde F. 1989. Catálogo de las algas macrobentónicas marinas de Málaga. *Acta Botanica Malacitana* **9:** 47-78.

Conde F, Seoane-Camba, JA. 1982. Corología de las especies de algas en relación a ciertos factores ecológicos en el litoral malagueño. *Collectanea Botanica* **13:** 783-802.

Corbit JD, Garbary DJ. 1995. Fractal dimension as a quantitative measure of complexity in plant development. *Proceedings: Biological Sciences* **262**: 1–6. DOI 10.1098/rspb.1995.0168.

Cox DR, Lewis PAW. 1978. *The Statistical Analysis of Series of Events*. London: Chapman and Hall.

501   **Eckert CG, Samis KE, Lougheed C. 2008.** Genetic variation across species' geographical ranges: the central–

502   marginal hypothesis and beyond. *Molecular Ecology* **17:** 1170–1188 DOI 10.1111/j.1365-

503   294X.2007.03659.x.

504   **Emlen JM, Freeman DC, Graham JH. 1993.** Nonlinear growth dynamics and the origin of fluctuating asymmetry.

505   *Genetica* **89:** 77-96 DOI 10.1007/BF02424507.

506   **Folland CK, Knight J, Linderholm HW, Fereday D, Ineson S, Hurrell JW. 2009.** The summer North Atlantic

507   Oscillation: past, present, and future. *Journal of Climate* **22:** 1082–1103 DOI 10.1175/2008JCLI2459.1.

508   **Gamboa G, Halfar J, Hetzinger S, Adey W, Zack T, Kunz B, Jacob DE. 2010.** Mg/Ca ratios in coralline algae

509   record northwest Atlantic temperature variations and North Atlantic Oscillation relationships. *Journal of*

510   *Geophysical Research* **115:** C12044 DI 10.1029/2010JC006262.

511   **García-Lafuente J, Álvarez Fanjul E, Vargas JM, Ratsimandresy AW. 2002.** Subinertial variability in the flow

512   through the Strait of Gibraltar. *Journal of Geophysical Research* **107**(C10), 3168 DOI

513   10.1029/2001JC001104.

514   **Graham JH, Freeman DC, Emlen JM. 1993.** Developmental stability: a sensitive indicator of populations under

515   stress. *ASTM Special Technical Publication* **1179:** 136-136 DOI 10.1520/STP19239S.

516   **Halfar J, Hetzinger S, Adey W, Zack T, Gamboa G, Kunz B, Williams B, Jacob DE. 2011.** Coralline algal

517   growth-increment widths archive North Atlantic climate variability. *Palaeogeography, Palaeoclimatology,*

518   *Palaeoecology* **302:** 71-80 DOI 10.1016/j.palaeo.2010.04.009.

519   **Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: Paleontological statistics software package for education and

520   data analysis. *Palaeontologia Electronica* **4:**1–9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

521   **Hosmer DW, Lemeshow S. 1980.** A goodness of fit test for the multiple logistic regression model.

522   *Communications in Statistics-Theory and Methods* **9:** 1043-1069 DOI 10.1080/03610928008827941

523   **Hurrell JW. 1995.** Decadal trends in the North Atlantic Oscillation: Regional temperatures and precipitation.

524   *Science* **269:** 676–679 DOI 10.1126/science.269.5224.676.

525   **Hurrell JW, Kushnir Y, Ottersen, G. Visbeck M. 2003.** An Overview of the North Atlantic Oscillation. In:

526   Hurrell JW, Kushnir Y, Ottersen G, Visbeck Mm, eds. *The North Atlantic Oscillation Climatic Significance*

527   *and Environmental Impact*. Washington DC: Geophysical Monograph, 134: 1–35.

528   **Iannaccone PM, Khokha M. 1996.** *Fractal Geometry in Biological Systems: An Analytical Approach*. Boca Raton:

529   CRC Press.

530   **Jones PD, Josson T, Wheeler D. 1997.** Extension to the North Atlantic Oscillation using early instrumental

531   pressure observations from Gibraltar and South-West Iceland. *International Journal of climatology* **17:** 1433-

532   1450 DOI 10.1002/(SICI)1097-0088(19971115)17:13<1433::AID-JOC203>3.0.CO;2-P.

533   **Lobo JM, Jiménez-Valverde A, Real R. 2008.** AUC: a misleading measure of the performance of predictive

534   distribution models. *Global Ecology and Biogeography* **17:** 145-151 DOI 10.1111/j.1466-

535   8238.2007.00358.x.

536   **Macías D, García-Gorriz E, Stips A. 2016.** The seasonal cycle of the Atlantic Jet dynamics in the Alboran Sea:

537   direct atmospheric forcing versus Mediterranean thermohaline circulation. *Ocean Dynamics* **66**: 137–151

538   DOI 10.1007/s10236-015-0914-y.

539   **Mandelbrot B. 1983.** *The Fractal Geometry of Nature*. New York: WH Freaman and Company.

540   **McKenzie JA, Clarke GM. 1988.** Diazinon resistance, fluctuating asymmetry and fitness in the Australian sheep

541   blowfly, *Lucilia cuprina*. *Genetics* **1:** 213-220 DOI 10/1988; 120(1):213-20.

542   **Moore SK, Trainer VL, Mantua NJ, Parker MS, Laws EA, Backer LC, Fleming LE. 2008**. Impacts of climate

543   variability and future climate change on harmful algal blooms and human health. *Environmental Health* **12:**

544   1–12 DOI10.1186/1476-069X-7-S2-S4.

545   **Muñoz M, Reul A, Plaza F, Gómez-Moreno M-L, Vargas-Yáñez M, Rodríguez V, Rodríguez J. 2015.**

546   Implication of regionalization and connectivity analysis for marine spatial planning and coastal management

547   in the Gulf of Cadiz and Alboran Sea. *Ocean and Coastal Management* **118**: 60-74 DOI

548   10.1016/j.ocecoaman.2015.04.011.

549   **Nagelkerke NJD. 1991**. A note on a general definition of the coefficient of determination. *Biometrika* **78:** 691-692

550   DOI 10.1093/biomet/78.3.691

551   **Palmer AR. 1994.** Fluctuating asymmetry analyses: a primer. In: Markow TA, ed. *Developmental Instability: its*

552   *Origins and Evolutionary Implications*. Netherlands: Kluwer Academic Publishers, 335-364.

553   **Parrilla G, Kinder TH. 1987.** Oceanografía física del Mar de Alborán. *Boletín del Instituto Español de*

554   *Oceanografía* **4:** 133–165.

555   **Peterman WE, Feist SM, Semlitsch RD, Eggert LS. 2013**. Conservation and management of peripheral

556   populations: Spatial and temporal influences on the genetic structure of wood frog (*Rana sylvatica*)

557   populations. *Biological Conservation* **158:** 351-358 DOI 10.1016/j.biocon.2012.07.028.

558   **Prahl J. 1999.** A fast unbinned test on event clustering in Poisson processes. arXiv preprint astro-ph/9909399.

559   **R Core Team (2013).** R: A language and environment for statistical computing. R Foundation for Statistical

560   Computing, Vienna, Austria. URL http://www.R-project.org/.

561   **Renault L, Oguz T, Pascual L, Visozo G, Tintoré J. 2012.** Surface circulation in the Alborán Sea (western

562   Mediterranean) inferred from remotely sensed data. *Journal of Geophysical Research* **117:** C08009 DOI

563   10.1029/2011JC007659**.**

564   **Reul A, Rodríguez V, Jiménez-Gómez F, Blanco, JM, Bautista B, Sarhan T, Guerrero F, Ruíz J, García-**

565   **Lafuente J. 2005.** Variability in the spatio-temporal distribution and size-structure of phytoplankton across

566   an upwelling area in the NW-Alboran Sea (W-Mediterranean). *Continental Shelf Research* **25**: 589-608 DOI

567   10.1016/j.csr.2004.09.016.

568   **Rodríguez J. 1982.** *Oceanografía del mar Mediterráneo*. Madrid: Pirámide SA.

569   **Sancholle M. 1988.** Présence de *Fucus spiralis* (Phaeophyceae) en Méditerranee occidentale. *Cryptogamie,*

570   *Algologie* **9**: 157-161.

571   **Sarhan T, García-Lafuente J, Vargas M, Vargas JM, Plaza F. 2000**. Upwelling mechanisms in the Northwestern

572   Alboran sea. *Journal of Marine System* **23**: 317-331.

573   **Serrão EA, Pearson G, Kautsky L, Brawley SH. 1996.** Successful external fertilization in turbulent environments.

574   Proceedings of the National Academy of Sciences of the United States of America **93**: 5286-5290.

575   **Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ. 2013.** Threats and knowledge gaps for ecosystem

576   services provided by kelp forests: a northeast Atlantic perspective. *Ecology and Evolution* **3:** 4016–4038 DOI

577   10.1002/ece3.774.

578   **Schreiber U, Schliwa U, Bilger W. 1986.** Continuous recording of photochemical and non-photochemical

579   chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis research* **10:**

580   51–62 DOI 10.1007/BF00024185.

581   **Schulze MM, Hutchings N, Simpson TL. 2008.** The use of fractal analysis and photometry to estimate the

582   accuracy of bulbar redness grading scales. *Investigative Ophthalmology & Visual Science* 49: 1398–1406

583   DOI 10.1167/iovs.07-1306.

584   **Shumaker KM, Babble GR. 1980.** Patterns of allozymic similarity in ecologically central and marginal populations

585   of *Hordeum jubatum* in Utah. *Evolution* **34:** 110-116 DOI 10.2307/2408319.

586   **Thompson DWJ, Wallace JW. 1998.** The Arctic Oscillation signature in the wintertime geopotential height and

587   temperature fields. *Geophysical Research Letters* **25:** 1297–1300 DOI 10.1029/98gl00950.

588   **Tsimplis NM, Josey SA. 2001.** Forcing of the Mediterranean Sea by atmospheric oscillations over the North

589   Atlantic. *Geophysical Research Letters* **28**: 803-806. DOI 10.1029/2000GL012098.

590   **Vargas-Yáñes M, Plaza F, García-Lafuente J, Sarhan T, Vargas JM, Vélez-Belchí P. 2002.** About the seasonal

591   variability of the Alboran Sea circulation. *Journal of Marine System*s 35: 229–248. DOI 10.1016/S0924-

592   7963(02)00128-8.

593   **Wald A. 1943.** Test of statistical hypotheses concerning several parameters with applications to problems of

594   estimation. *Transactions of the American Mathematical society* **54:** 426-482.

595   **Walsh J, Watterson J. 1993.** Fractal analysis of fracture pattern using the standard box-counting technique: valide

596   and invalid methodologies. *Journal of structural Geology* **15:** 1509-1521 DOI 10.1016/0191-8141(93)90010-

597   8.

598   **Walker GT, Bliss EW. 1932.** World weather V. Royal *Meterolological Society* **4:** 53–84.

599   **Zakharov VM. 1992**. Population phenogenetics: analysis of developmental stability in natural populations. *Acta*

600   *Zoologica Fennica* **191**: 7-30.

601   **Zardi GI, Nicastro KR, Canovas F, Ferreira Costa J, Serrão EA, Pearson GA. 2011.** Adaptive traits are

602   maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *PLoS ONE*

603   **6(6)**: e19402 DOI 10.1371/journal.pone.0019402.

604   **Zuur A, Ieno EN, Smith GM. 2007.** *Analysing Ecological Data*. New York: Springer Science and Business Media.

Table 1(on next page)

Time series (1990-2015) of presence (1)/ absence (0) of *Fucus guiryi* at Punta Calaburras.

Table 1. Time series (1990-2015) of presence (1)/ absence (0) of *Fucus guiryi* at Punta Calaburras, and overall mean values of NAO and AO indexes (dimensionless), sea surface temperature (SST; units in ºC), air temperature (AT; units in ºC) and rainfall (R; units in mm), for the previous month or the overall mean for the six previous months (suffixes 1-6).

Table 1. Time series (1990-2015) of presence (1)/ absence (0) of *Fucus guiryi* at Punta de Calaburras , and overall mean values of NAO and AO indexes (dimensionless), sea surface temperature (SST; units in ºC), air temperature (AT; units in ºC) and rainfall (R; units in mm), for the previous month or the overall mean for the six previous months (suffixes 1-6).

Year	*Fucus*	AO_1	AO_2	AO_3	AO_4	AO_5	AO_6	NAO_1	NAO_2	NAO_3	NAO_4	NAO_5	NAO_6	SST_1	SST_2	SST_3	SST_4	SST_5	SST_6	AT_1	AT_2	AT_3	AT_4	AT_5	AT_6	R_1	R_2	R_3	R_4	R_5	R_6
1990	1	0.30	0.62	1.04	1.53	1.90	1.75	-0.02	-0.78	0.15	0.48	0.66	0.73	16.2	16.7	16.2	16.0	15.7	15.7	21.4	20.3	18.8	17.9	17.3	16.6	25	153	118	324	346	469
1991	1	-0.12	0.19	0.30	0.09	-0.10	0.04	-0.82	-0.37	-0.15	-0.16	0.08	0.21	17.5	16.9	16.1	15.6	15.1	14.9	21.9	20.0	18.4	17.4	16.3	15.8	0	371	267	325	307	377
1992	1	-0.30	0.52	0.17	0.38	0.52	0.53	0.20	1.42	1.56	1.39	1.33	1.08	14.4	15.8	15.3	15.2	14.9	14.7	18.8	19.0	18.2	17.3	16.4	15.7	270	140	493	472	497	450
1993	1	-0.52	-1.06	-0.85	-0.45	-0.32	0.31	-0.59	-0.69	-0.13	0.07	0.15	0.40	16.2	15.6	15.2	15.0	14.9	14.7	20.7	19.2	17.9	16.8	16.0	15.4	0	103	209	323	384	400
1994	1	1.61	0.75	0.57	0.90	0.55	0.41	1.52	0.48	0.70	0.84	0.76	0.81	17.6	16.2	15.6	15.4	15.0	14.8	20.8	19.4	18.2	17.5	16.6	16.0	25	208	397	482	599	579
1995	0	-0.11	-0.50	-0.66	-0.39	-0.03	-0.05	0.13	-0.68	-0.74	-0.24	0.04	0.19	17.7	17.1	16.5	16.1	15.7	15.4	20.6	19.9	18.6	17.8	17.0	16.3	0	19	13	34	143	368
1996	0	0.50	0.14	-0.42	-0.68	-0.51	-0.63	0.56	-0.25	-0.22	-0.23	-0.20	-0.18	19.0	17.3	16.8	16.3	15.9	15.7	21.6	19.7	18.7	17.7	16.7	16.4	65	194	287	284	378	334
1997	0	-0.81	-0.89	-0.48	-0.09	0.31	0.18	-1.47	-0.88	-0.92	-0.33	0.08	-0.02	15.7	15.7	16.0	16.1	15.9	15.6	20.7	19.9	19.2	18.4	17.7	16.9	0	3	119	303	502	797
1998	0	-0.71	-0.14	-0.11	-0.14	-0.15	-0.47	-2.72	-2.02	-1.57	-0.96	-0.79	-0.60	17.7	16.8	15.9	15.8	15.6	15.4	21.1	19.2	18.3	17.6	16.9	16.4	45	315	252	436	531	550
1999	1	0.71	0.47	0.41	-0.07	0.04	0.05	1.12	1.02	0.36	0.33	0.32	0.40	18.5	17.0	16.3	16.0	15.6	15.4	21.8	20.3	19.2	18.1	17.0	16.3	145	450	358	346	277	234
2000	1	0.59	0.78	0.43	0.21	0.38	0.53	-0.03	0.78	0.51	0.57	0.80	0.77	20.4	18.5	16.9	16.6	16.3	15.9	22.6	20.7	19.0	18.1	17.4	16.5	147	472	522	472	729	766
2001	1	-0.02	0.22	0.45	-0.09	-0.19	-0.32	-0.20	-0.11	-0.07	-0.37	-0.21	-0.13	18.2	17.2	16.7	16.3	15.9	15.5	22.6	20.4	19.3	18.6	17.6	17.0	9	155	137	226	181	175
2002	1	0.57	0.49	0.57	0.66	0.79	0.88	0.38	0.08	0.45	0.51	0.63	0.60	16.6	16.3	16.1	15.8	15.7	15.5	21.3	20.1	18.7	17.8	17.1	16.5	916	600	909	980	785	773
2003	1	-0.10	0.46	0.25	0.42	0.36	0.22	-0.07	-0.03	-0.08	0.02	0.14	0.14	19.0	18.4	17.5	17.0	16.5	16.1	22.9	21.5	19.8	18.6	17.5	16.7	147	366	253	498	725	651
2004	1	-0.24	-0.17	-0.25	-0.11	-0.39	-0.61	-0.89	-0.35	0.15	0.37	0.27	0.17	19.1	17.5	16.8	16.4	16.0	15.8	22.5	19.9	18.6	17.6	16.7	16.3	3	246	386	488	430	462
2005	0	-0.38	-0.57	-0.40	-0.64	-0.76	-0.58	-0.05	-0.65	-0.53	-0.86	-0.70	-0.33	18.3	17.5	16.7	16.1	15.6	15.4	22.2	20.9	19.6	18.2	16.9	16.0	0	56	46	229	560	487
2006	1	1.07	0.61	0.45	-0.06	-0.08	-0.09	0.84	-0.15	0.31	-0.09	-0.17	0.07	18.7	18.3	17.3	16.4	15.9	15.7	21.6	20.8	19.6	18.6	17.7	17.3	103	233	450	736	791	816
2007	0	-0.55	0.17	0.29	0.52	0.15	0.47	-1.31	-0.32	-0.16	0.24	0.10	0.12	16.0	15.7	15.3	15.0	15.0	15.1	20.6	19.8	18.5	17.6	17.0	16.3	1283	1640	1110	1365	1293	1219
2008	0	-0.09	-0.65	-0.58	-0.29	-0.05	0.10	-1.39	-1.56	-1.39	-1.03	-0.67	-0.41	18.4	17.2	16.7	16.5	16.2	16.1	21.7	20.0	19.0	18.2	17.5	17.0	673	2209	3031	2482	2132	1777
2009	0	-1.35	-0.08	0.27	0.23	0.05	0.18	-1.21	0.24	0.09	0.21	0.18	0.15	18.2	17.2	16.4	16.0	15.6	15.3	21.8	20.9	19.3	18.2	17.2	16.5	198	474	383	451	373	311
2010	0	-0.01	-0.47	-0.40	-0.41	-1.18	-1.42	-0.82	-1.15	-1.01	-0.98	-1.18	-1.17	17.9	17.2	17.0	16.4	16.0	15.7	20.6	19.4	18.5	17.5	16.9	16.3	246	466	370	602	547	456

50	2011	1	-0.86	-0.45	0.46	0.70	0.88	0.45	-1.28	-0.67	0.38	0.44	0.49	0.26	19.2	18.6	17.8	17.2	16.9	16.7	21.5	20.6	19.5	18.4	17.5	16.8	588	761	560	809
51		700	583
52	2012	0	-0.67	-0.25	-0.18	0.12	0.09	0.04	-2.53	-1.72	-0.99	-0.43	-0.26	-0.02	18.4	17.3	16.3	15.8	15.6	15.5	22.1	20.5	19.1	17.9	16.6	16.0	32	899	1047	804
53		643	536
54	2013	0	0.55	0.52	0.45	-0.46	-0.57	-0.57	0.52	0.54	0.59	0.04	-0.06	0.01	17.1	16.3	15.9	15.4	15.0	14.9	20.3	19.1	18.0	17.2	16.4	16.0	8	294	361	271
55		217	181
56	2014	0	-0.51	-0.02	0.31	0.53	0.44	0.20	-0.97	-0.95	-0.53	-0.20	0.11	0.14	18.1	18.1	17.7	17.0	16.4	16.1	21.3	20.1	19.2	18.1	17.3	16.8	691	346	235	177
57		185	189
58	2015	0	0.43	0.59	0.80	1.06	1.06	1.06	-0.07	0.04	0.27	0.56	0.72	0.89	17.7	17.1	16.4	16.0	15.7	15.5	21.7	20.1	18.6	17.7	16.8	16.2	1175	1008	744	607
59		486	405

Table 2(on next page)

Binary logistic regression between presence/absence of *Fucus guiryi* as a function of NAO and AO indexes, sea surface temperature (SST), air temperature (AT) and rainfall (R).

Table 2.Value of χ^2-test (df = 1 in all of the cases) and associated probability, and Akaike Information Criterion (AIC), in the first step of the binary logistic regression between presence/absence of *Fucus guiryi* as a function of NAO and AO indexes, sea surface temperature (SST), air temperature (AT) and rainfall (R), for the previous month or the overall mean for the six previous months (suffixes 1-6). The asterisk shows the independent variable selected for the analyses based on the higher explanatory power.

1   **Table 2.** Value of χ^2-test ($df = 1$ in all of the cases) and associated probability, and Akaike
2   Information Criterion (AIC), in the first step of the binary logistic regression between
3   presence/absence of *Fucus guiryi* as a function of NAO and AO indexes, sea surface temperature
4   (SST), air temperature (AT) and rainfall (R), for the previous month or the overall mean for the
5   six previous months (suffixes 1-6). The asterisk shows the independent variable selected for the
6   analyses based on the higher explanatory power.

	Variables	χ^2	p	AIC
8	AO_1	3.785	0.045	36.035
9	AO_2	4.735	0.029	35.308
10	AO_3	4.656	0.031	35.387
11	AO_4	3.208	0.073	36.835
12	AO_5	3.364	0.066	36.680
13	AO_6	3.384	0.060	36.516
14				
15	NAO_1	5.532	0.018	34.511
16	NAO_2	6.824	0.008	33.220
17	NAO_3^*	13.530	0.0002	26.513
18	NAO_4	10.543	0.001	29.501
19	NAO_5	9.910	0.001	30.133
20	NAO_6	9.431	0.002	30.612
21				
22	SST_1	0.028	0.866	40.015
23	SST_2	0.386	0.543	39.658
24	SST_3	0.006	0.941	40.038
25	SST_4	0.008	0.930	40.036
26	SST_5	0.011	0.916	40.033
27	SST_6	0.019	0.890	40.024
28				
29	AT_1	0.899	0.343	39.168
30	AT_2	0.760	0.383	39.294
31	AT_3	0.670	0.769	39.991
32	AT_4	0.480	0.826	39.967
33	AT_5	0.017	0.896	40.036
34	AT_6	0.005	0.942	40.035
35				
36	R_1	1.184	0.277	38.860
37	R_2	2.378	0.123	37.665
38	R_3	1.110	0.292	38.933
39	R_4	0.433	0.511	39.611
40	R_5	0.386	0.534	39.658
41	R_6	0.263	0.608	39.781

42

Table 3 (on next page)

Two-way anovas for the comparison of the F_v/F_m, Φ_{PSII}, ETR_{rel} and water tissue content of *Fucus guiryi.*

Table 3. Two-way anovas for the comparison of the F_v/F_m, Φ_{PSII}, ETR_{rel} and water tissue content of *Fucus guiryi*, and Student-Newman-Keuls (SNK) post-hoc test for significant (95%) sources of variation.

1 **Table 3.** Two-way ANOVAS for the comparison of the F_v/F_m, Φ_{PSII}, ETR_{rel} and water tissue content of *Fucus guiryi*, and Student-
2 Newman-Keuls (SNK) post-hoc test for significant (95%) sources of variation.

Variable	Source of variation	df	SS	MS	F	p	SNK
F_v/F_m in water	Locations	1	0.0001	0.0001	2.679	0.107	
	Time of day	6	0.0492	0.0082	296.800	0.000	
6:00=8:00>18:00>10:00=16:00>14:00>12:00							
	Locations × Time of day	6	0.0002	0.0000	1.304	0.270	
	Error	56	0.0015	0.0000			
F_v/F_m in air	Locations	1	0.0269	0.0269	17.960	0.000	Tarifa>Calaburras
	Time of day	2	0.3569	0.1785	119.000	0.000	10:00>12:00>14:00
	Locations × Time of day	2	0.0172	0.0086	5.726	0.009	
	Error	24	0.0360	0.0015			
Φ_{PSII} in water	Locations	1	0.0021	0.0021	3.209	0.078	
	Time of day	6	1.5120	0.2520	384.000	0.000	
6:00>8:00>18:00>16:00>10:00>14:00=12:00							
	Locations × Time of day	6	0.0018	0.0003	0.455	0.838	
	Error	56	0.0368	0.0007			
Φ_{PSII} in air	Locations	1	0.0167	0.0167	32.480	0.000	Tarifa>Calaburras
	Time of day	2	0.2320	0.1160	225.500	0.000	10:00>12:00>14:00
	Locations × Time of day	2	0.0061	0.0030	5.889	0.008	
	Error	24	0.0124	0.0005			
ETR_{rel} in water	Locations	1	3764	3764	3.296	0.075	
	Time of day	6	2154000	358900	314.300	0.000	
8:00=12:00>10:00>14:00>16:00>6:00>18:00							
	Locations × Time of day	6	3617	603	0.528	0.785	
	Error	56	63940	1142			
ETR_{rel} in air	Locations	1	60450	60450	32.800	0.000	Tarifa>Calaburras
	Time of day	2	655700	327800	177.900	0.000	10:00>12:00>14:00
	Locations × Time of day	2	26430	13210	7.169	0.004	
	Error	24	44230	1843			
Water of tissue in air	Locations	1	326.7	326.7	49.630	0.0003	Tarifa>Calaburras
	Time of day	2	3712.0	1856.0	281.900	0.0000	10:00>12:00>14:00
	Locations × Time of day	2	175.4	87.7	13.320	0.0001	
	Error	24	158.0	6583.0			

Figure 1(on next page)

Map of the area near the Strait of Gibraltar (A) showing the two sampling points of *Fucus guiryi* on the S.W. Iberian Peninsula (Tarifa and Punta Calaburras) and herbarium MGC sheets (B, C).

Fig 1 - Map of the area near the Strait of Gibraltar (A) showing the two sampling points of *Fucus guiryi* on the S.W. Iberian Peninsula (Tarifa and Punta Calaburras). Mean Sea Surface Temperature (SST) of weekly SST images 1998-2014 (cold upwelled water in blue (17ºC) and warm water of the Westarn Alboran Anticyclon in red (20ºC) as well as a schematic circulation pattern. Herbarium MGC sheets with samples of *F. guiryi* collected at Punta Calaburras (MGC 1772; B) and Tarifa (MGC 5224; C).
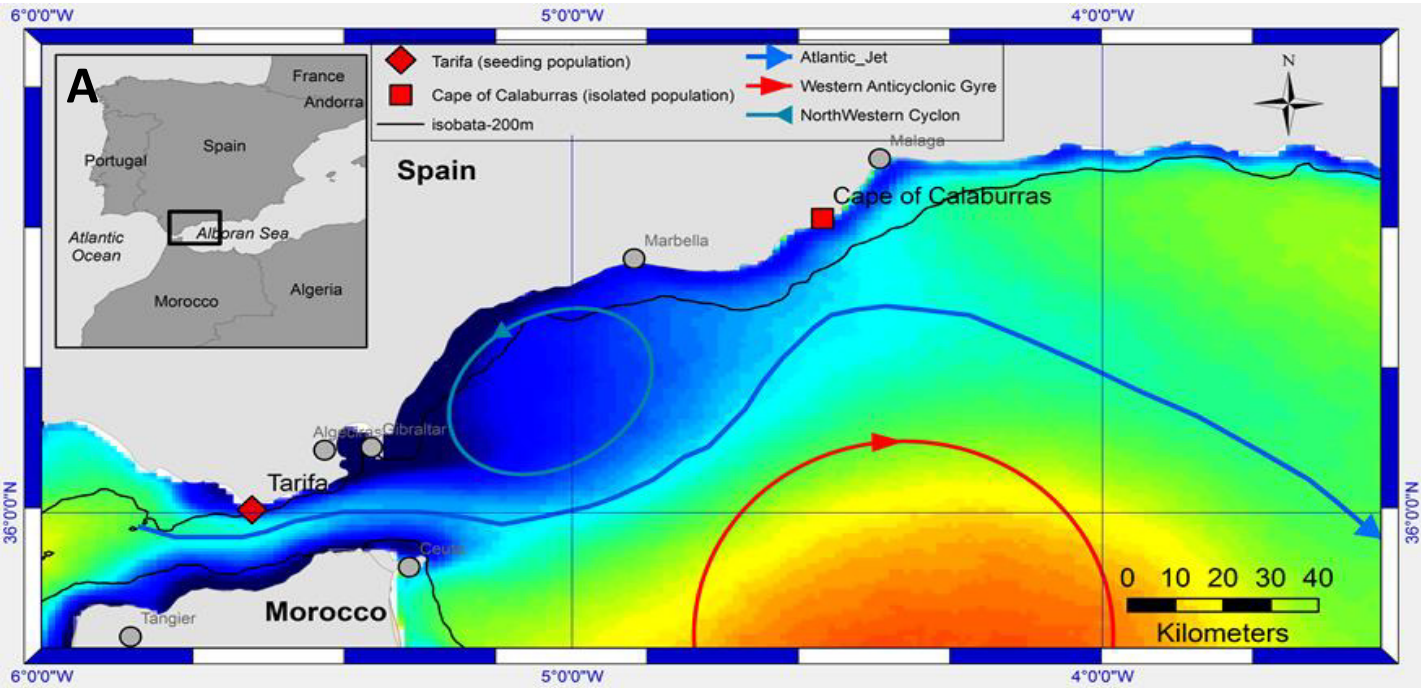
Figure 2(on next page)

Daily (UTC) tidal height.

Fig 2 - Daily (UTC) tidal height on 16th July 2011 at Tarifa, and vertical ranges of *Fucus guiryi* at Tarifa and Punta Calaburras.
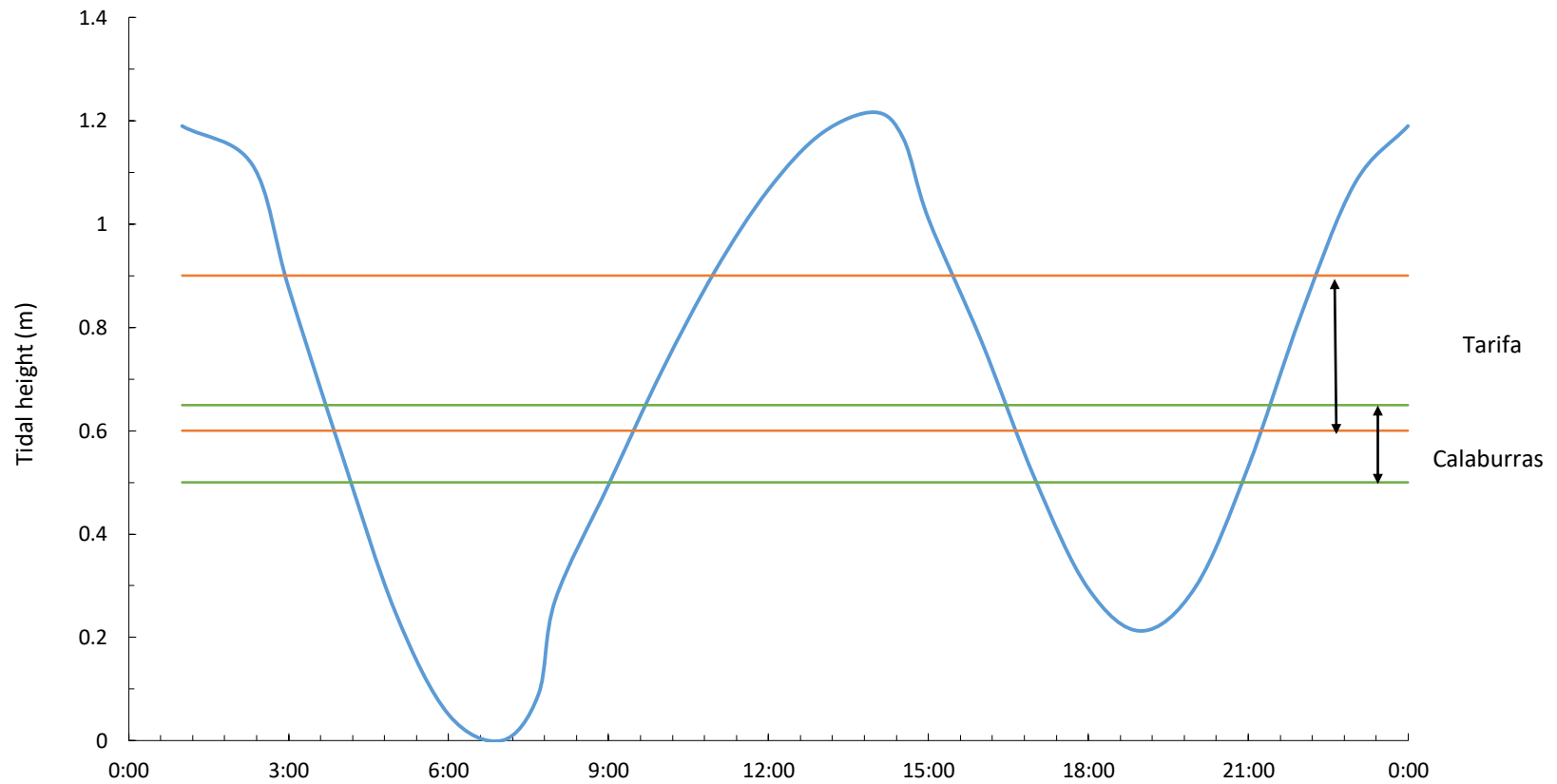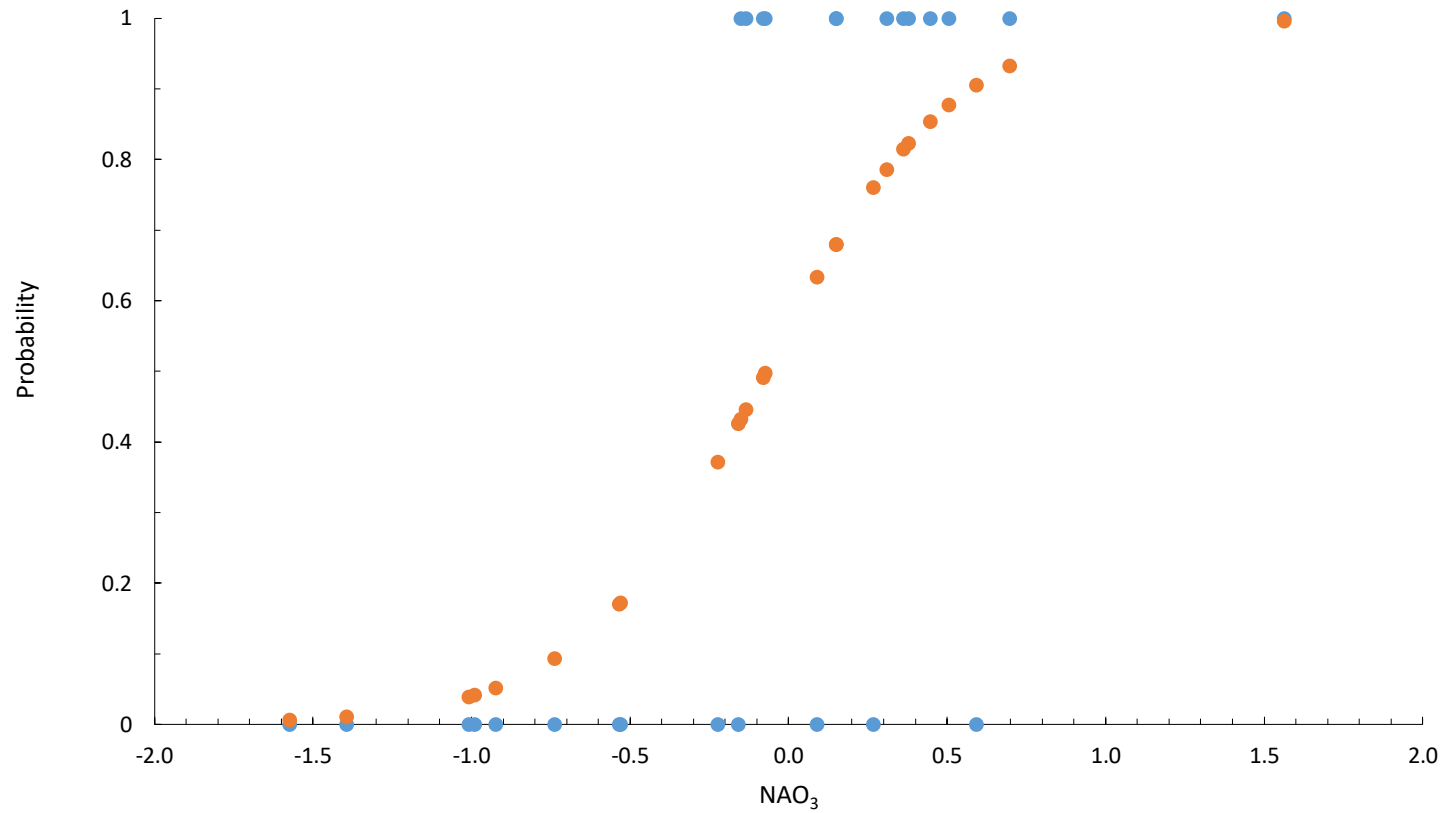
Figure 3 (on next page)

Probability (ornage circles) and presence (1)/absence (0) (blue circles) of *Fucus guiryi* at Punta Calaburras from 1990 to 2015, as a function of the North Atlantic Oscillation.

Fig 3 - Probability (orange circles) and presence (1)/absence (0) (blue circles) of *Fucus guiryi* at Punta de Calaburras from 1990 to 2015, as a function of the North Atlantic Oscillation averaged for the months between April and June (NAO_3), as explanatory independent, environmental variable.
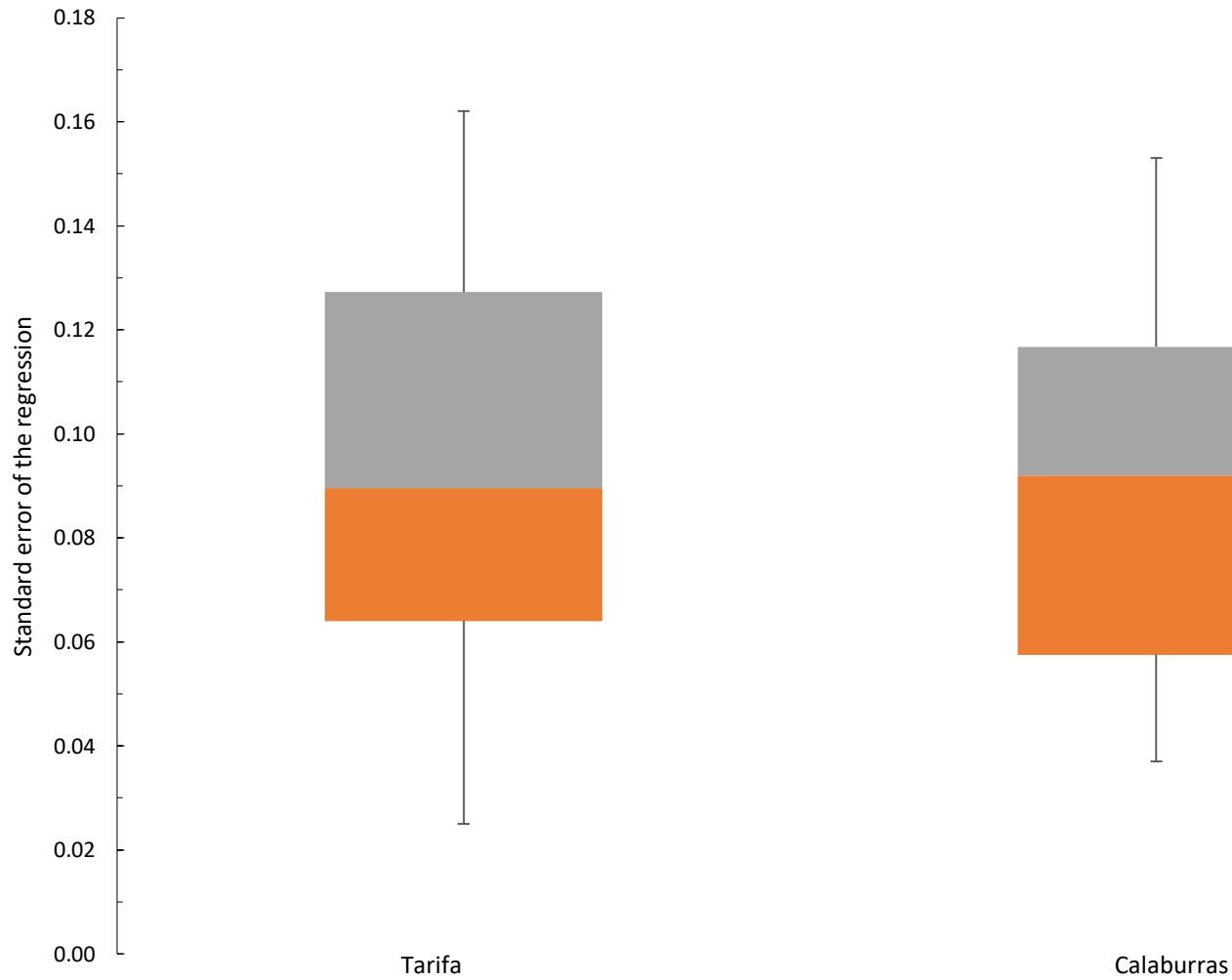
Figure 4(on next page)

Box-whiskers plot (*n* = 60) for the standard error of the regression derived from the box-counting method.

Fig 4 - Box-whiskers plot (the smallest observation, lower quartile, median, upper quartile and largest observation; *n* = 60) for the standard error of the regression derived from the box-counting method in *Fucus guiryi* from Tarifa and Punta de Calaburras.

Figure 5(on next page)

Daily course of PAR, and UV-A and UV-B radiation at Tarifa, on 16th July 2011.

Fig 5 - Daily course of PAR, and UV-A and UV-B radiation at Tarifa, on 16th July 2011.
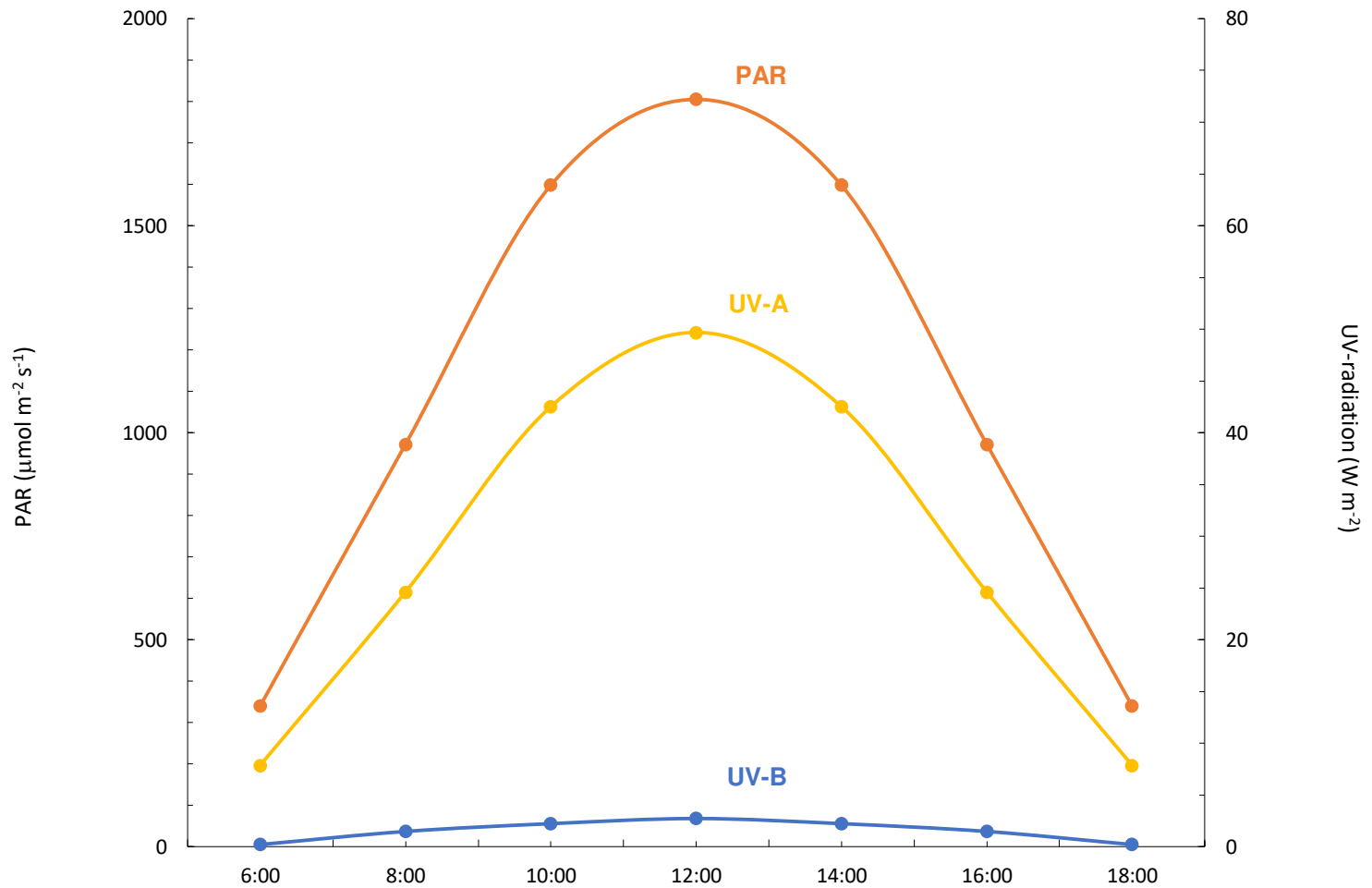
Figure 6(on next page)

Daily course (UTC) of the F_v/F_m (A) , Φ_{PSII} (B), ETR_{rel} (C) and water tissue content (D) of *Fucus guiryi.*

Fig 6 - Daily course (UTC) of the F_v/F_m (A) , Φ_{PSII} (B), ETR_{rel} (C) and water tissue content (D) of *Fucus guiryi* from Tarifa (blue symbols) and from Punta Calaburras (orange symbols) in air (squares) and water (circles). The measurements were carried out on 16th July 2011 in Tarifa, and the next day in Punta de Calaburras.