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*Temporal clustering of extreme climate events drives a regime shift in rocky intertidal biofilms*

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9 **Title:** Temporal clustering of extreme climate events drives a regime shift in rocky intertidal  
10 biofilms

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22 Type of the article: Article ABSTRACT

23 Research on regime shifts has focused primarily on how changes in the intensity and duration of  
24 press disturbances precipitate natural systems into undesirable, alternative states. By contrast, the  
25 role of recurrent pulse perturbations, such as extreme climatic events, has been largely neglected,  
26 hindering our understanding of how historical processes regulate the onset of a regime shift. We

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27 performed field manipulations to evaluate whether combinations of extreme events of  
28 temperature and sediment deposition that differed in their degree of temporal clustering  
29 generated alternative states in rocky intertidal epilithic microphytobenthos (biofilms) on rocky  
30 shores. The likelihood of biofilms to shift from a vegetated to a bare state depended on the  
31 degree of temporal clustering of events, with biofilm biomass showing both states under a  
32 regime of non-clustered (60 days apart) perturbations while collapsing in the clustered (15 days  
33 apart) scenario. Our results indicate that time since the last perturbation can be an important  
34 predictor of collapse in systems exhibiting alternative states and that consideration of historical  
35 effects in studies of regime shifts may largely improve our understanding of ecosystem dynamics  
36 under climate change.

37

38 Keywords: alternative states, extreme events, regime shift, epilithic microphytobenthos, biofilm,  
39 climate change, temporal clustering, abrupt changes

#### 40 INTRODUCTION

41 Ecosystems often display non-linear responses to both gradual and abrupt changes in driving  
42 variables (e.g. temperature, nutrient loading), undergoing catastrophic transitions known as  
43 regime shifts (Scheffer et al. 2001, Scheffer and Carpenter 2003). Most theoretical and  
44 experimental work on regime shifts has focused on gradual changes in the intensity of a press  
45 disturbance (the driver variable), showing that many ecosystems can absorb such changes and  
46 maintain their current state up to a threshold beyond which they transition to an alternative, less  
47 desirable state (Petraitis and Dudgeon 2004, Dakos et al. 2008, Scheffer et al. 2012, Benedetti-  
48 Cecchi et al. 2015, Rindi et al. 2017). Only recently, ecologists have recognized the importance  
49 of temporal characteristics of press disturbances in regulating regime shifts. Ratajczak et al.  
50 (2017) showed that the duration of the perturbation is crucial for the onset of regime shifts in  
51 systems that respond slowly to external change and that exhibit strong coupling between past and  
52 present dynamics. In contrast, our understanding of the role of recurrent pulse events and how  
53 the history of previous perturbations affects the susceptibility of ecosystems to undergo a regime  
54 shift is still limited.

55 Pulse events such as fires, the outbreak of natural enemies and extreme climatic events  
56 have a great potential to induce regime shifts (Scheffer et al. 2001). In highly stochastic  
57 environments, species coexistence is promoted by the capacity of species to respond  
58 differentially to environmental fluctuations. Each population, then, is able to store the gains  
59 coming from good periods and use them to survive losses in bad periods, a phenomenon known  
60 as storage effect, which ultimately allows a community to maintain biodiversity (Chesson 2000).  
61 Pulse perturbations, however, may exceed tolerance limits of organisms, causing impairment of  
62 function or outright mortality of individuals (Schröder et al. 2005). If also resting stages are  
63 affected, pulse events may prevent species coexistence by disrupting storage effects. Pulse  
64 disturbances can also influence community dynamics and biodiversity by selectively removing  
65 community dominants, thereby freeing up resources for other species and reducing community's  
66 biotic resistance to invasive species (Walker et al. 2005, Mumby et al. 2011). Any of these  
67 changes may translate into a system being suddenly pushed beyond the unstable region  
68 separating the basins of attraction of the contrasting states, resulting in a regime shift (Scheffer et  
69 al. 2001).

70 The likelihood of a pulse perturbation to push a system into an alternative state depends  
71 upon its location with respect to the critical threshold; the more the system is close to the  
72 threshold, the higher is the likelihood of a transition (Folke et al. 2004, van der Bolt et al. 2018).  
73 Moreover, ecosystems are exposed to multiple, recurrent perturbations, so that the likelihood of a  
74 regime shift may also depend on the particular regime of disturbance the system has experienced  
75 (Paine et al. 1998). Specifically, the characteristics of a regime of pulse disturbances that may  
76 leave strong historical signatures on ecosystem dynamics include the nature, the order and the  
77 timing of occurrence of perturbations (Benedetti-Cecchi et al. 2015, Dantas et al. 2016, Dal Bello  
78 et al. 2017). Although alterations of disturbance scenarios are already receiving a great amount  
79 of attention in the ecological literature, how variation in the regime of pulse perturbations affect  
80 regime shifts has been largely neglected.

81 Extreme climatic events are becoming more common and severe as a consequence of  
82 climate change (Fischer and Knutti 2015) and they can induce abrupt transitions in terrestrial and  
83 aquatic ecosystems (Holmgren et al. 2006, Wernberg et al. 2016). There is general consensus  
84 that the effects of extreme events vary with their nature and temporal regimes (Benedetti-Cecchi  
85 et al. 2006, Mumby et al. 2011, Williams et al. 2011). Moreover, recent studies showed that

86 changes in the temporal clustering of extreme events, i.e. the degree of separation between  
87 consecutive instances, can modulate ecological memory of microbial assemblages (Dal Bello et  
88 al. 2017) and regulate the onset of regime shifts in tropical ecosystems (Holmgren et al. 2013).  
89 Evaluating how different scenarios of extreme events can trigger a regime shift in systems with  
90 alternative states will be a crucial step to better understand the impact of climate change on  
91 ecosystems.

92 Here, we address this challenge using rocky intertidal epilithic microphytobenthos  
93 (biofilms) as model system. We focused on extreme events of temperature and sediment  
94 deposition after heavy rains, since these are major drivers of biofilm abundance and distribution  
95 (Thompson et al. 2004, Dal Bello et al. 2017). We used photosynthetic biofilms primarily  
96 because it is a tractable system for field experiments, being the result of the activity of fast  
97 growing organisms, which display rapid responses to perturbations (Christofoletti et al. 2011).  
98 Moreover, we expected alternative states in biofilms due to stabilizing mechanisms that operate  
99 both at high and low values of biomass. High biomass values sustain high photosynthesis rates,  
100 which, in turn, support enhanced production of extracellular polymeric substances (EPS) (Wulff  
101 et al. 2000, Wolfstein and Stal 2002). EPS, being the major components of the dense matrix in  
102 which microalgal cells are embedded, provide protection against stressful conditions, e.g. heat  
103 stress during low tides and further boost photosynthesis and biomass accumulation (Flemming  
104 and Wingender 2010). This positive feedback can be eroded by processes that either remove  
105 biomass or degrade EPS, e.g. high temperatures, abrasion due to sediment scouring, wave action  
106 (Decho 2000, Thompson et al. 2004). We propose that such losses trigger runaway changes  
107 propelling the switch from a “vegetated” to a “bare” (or “semi-bare”) state. The semi-bare state  
108 will be then maintained due to the uncoupling of photosynthesis and EPS production at low  
109 biofilm biomass values (“*Allee effect*”). Such feedback can work both ways: the more the  
110 biomass, the higher the growth and the less the biomass, the lower the growth. Positive feedback  
111 loops like this one may be responsible for the catastrophic effect of extreme events, similarly to  
112 what observed in microcosm experiments with yeasts populations, which show cooperative  
113 growth and a negative growth rate at low cells density (Dai et al. 2012).

114 We used a field experiment and a model to test for the presence of alternative states in  
115 rocky shore photosynthetic biofilms and to explore the underlying feedback mechanisms. The  
116 field experiment tested the hypothesis that series of extreme events of temperature and sediment

117 deposition that differed in their degree of temporal clustering induced alternative states in  
118 biofilm assemblages. Multimodality in the frequency distribution of biofilm biomass (Scheffer et  
119 al. 2012, Sirota et al. 2013) and divergence in the temporal trajectories of experimental units  
120 belonging to the same treatment (Scheffer and Carpenter 2003, Schröder et al. 2005) are both  
121 indirect indications for the presence of alternative states, here a vegetated and a semi-bare state  
122 (Schröder 2009). Based on the results of a previous study (Dal Bello et al. 2017), we anticipate  
123 that the vegetated state would correspond to the biomass in the controls, while the semi-bare  
124 state would reflect reduced biofilm biomass in the clustered perturbation scenario. This is  
125 expected because extreme events clustered in time may push the system below a threshold  
126 biomass value, impairing the ability of biofilm to recover to the vegetated state. Moreover, we  
127 expect two modes in the non-clustered scenario, where two perturbations separated in time may  
128 be able to push some experimental units in the semi-bare state, while others, due to small initial  
129 differences in biomass, may remain in the vegetated state. To further explore the effects of  
130 temporal clustering of extreme events on biofilm biomass, we parametrized a simple model that  
131 incorporated the positive feedback between photosynthesis and EPS production through an  
132 “Allee effect”.

133

## 134 MATERIALS AND METHODS

### 135 *Study area*

136 The experiment was done along the coast of Calafuria (Livorno, 43°30' N, 10°19' E) between  
137 April and August 2013. The coast consists of gently sloping sandstone platforms with high-shore  
138 levels (0.3 - 0.5 m above mean low-level water) colonized by assemblages of barnacles  
139 interspersed among areas of seemingly bare rock, where photosynthetic biofilms develop.  
140 Biofilm assemblages at Calafuria include mainly cyanobacteria, with diatoms being less  
141 abundant (Maggi et al. 2017). The most important grazer at this height on the shore is the  
142 littorinid snail *Melaraphe neritoides* (L). During the experiment, however, grazing pressure over  
143 biofilm assemblages was nearly absent (Dal Bello et al. 2017).

### 144 *Experimental design*

145 Along Mediterranean rocky shores, highly thermally stressing periods of calm sea and high  
146 barometric pressure alternate with heavy rainfalls, the latter resulting in the deposition of  
147 sediments at tidal heights where photosynthetic biofilms develop (Airoldi 2003, Benedetti-

148 Cecchi et al. 2006, Dal Bello et al. 2017). In order to mimic this pattern, we imposed different  
149 series of extreme events of warming and sediment deposition. A scenario characterized by non-  
150 clustered events was created by imposing two extreme disturbances 60 days apart, while two  
151 disturbances 15 days apart characterized the clustered condition. The non-clustered scenario was  
152 conceived to allow biofilm biomass to recover between the two events, whilst recovery was  
153 considered unlikely in the time window separating clustered events. Since biofilm is composed  
154 of fast-growing species with short generation time, an interval of 60 days was sufficiently long to  
155 allow recovery and therefore the two perturbations could be considered as separate events. For  
156 each level of clustering, we imposed all the possible combinations of warming and sediment  
157 deposition: two consecutive sediment deposition events, two consecutive extreme warming  
158 events, one extreme sediment deposition event followed by an extreme warming episode, an  
159 extreme warming event followed by an extreme sediment deposition episode. Extreme warming  
160 was obtained by artificially increasing air temperature over experimental plots using aluminum  
161 chambers equipped with stoves. The treatment consisted in maintaining the air temperature  
162 inside the chambers as close as possible to 32 °C during the two hours corresponding to the peak  
163 in daily temperatures, i.e. around midday in all instances. The temperature chosen represents the  
164 100-years return time temperature for the months in which the experiment was performed (Katz  
165 et al. 2005). Procedural controls for artifacts (CA) were set-up to control for the effects of  
166 shading on biofilm biomass due to the use of non-transparent heating chambers. CA plots were  
167 therefore kept in shaded conditions but without heating for the duration of the warming treatment  
168 by means of cardboard chambers. Sediment addition on experimental plots was used to simulate  
169 the effects of runoff after a heavy rainfall event. The treatment consisted in adding a 5mm-thick  
170 layer of sediment collected *in situ* and diluted in fresh water to produce the colloidal material that  
171 is naturally deposited on rocky shores after severe precipitation events. Three experimental plots  
172 were assigned to each combination of extreme events of disturbance. Three unmanipulated plots  
173 were used as controls (C) and six plots were used as procedure controls of artefacts (CA).  
174 Experimental plots were located 2-10 meters apart and consisted of areas of substratum of 30 x  
175 50 cm marked at their corners with raw-plugs inserted into the rock for subsequent relocation.

176 *Data collection and analyses*

177 Biofilm biomass was quantified indirectly by means of an image-based remote sensing technique  
178 that uses chlorophyll *a* concentration as a proxy. Chlorophyll *a* was estimated from the ratio of

179 reflectance at near-infrared (NIR) and red bands (Ratio Vegetational Index - RVI) obtained by  
180 means of an IR-sensitive camera, following the method proposed by Murphy et al. (2006).  
181 NIR/red ratios are linked to the chlorophyll content in the rock by a linear relationship,  
182 calculated on the basis of laboratory chlorophyll *a* extractions from Calafuria sandstone cores  
183 (Dal Bello et al. 2015).

184 Experimental plots were monitored in time after the imposition of both experimental  
185 perturbations, with the non-clustered scenario sampled at days 70, 84, 108,133 and the clustered  
186 scenario sampled at days 81, 91, 109, 138, counting from day 0 (i.e. when the experiment started  
187 and we imposed the first extreme of the non-clustered scenario) (see Appendix 1: Fig. S1).  
188 Controls were sampled also at days 5, 20 and 55, in addition to days 70, 84, 91, 108 and 133  
189 (Appendix 1: Fig. S1). Once in the lab, each image was handled with a routine in ImageJ  
190 software to haphazardly select 5 subplots of 256 x 256 pixels and to provide a mean estimate of  
191 biofilm biomass for each of them.

192 The presence of alternative states was tested indirectly through the evaluation of  
193 multimodality in the frequency distribution of biofilm biomass (Scheffer et al. 2012, Sirota et al.  
194 2013). The number of modes in the frequency distribution of biofilm biomass values was  
195 estimated at the first sampling date after the second perturbation event for both non-clustered and  
196 clustered scenario (days 70 and 81 from the start of the experiment, respectively), while we used  
197 data from the four dates after the second perturbation event to assess divergence among temporal  
198 trajectories of biofilm biomass. The number of modes has been identified with normal mixture  
199 modelling and model-based clustering using Mclust package in R. We used bootstrapping to  
200 calculate 95% confidence intervals. For each level of temporal clustering (control, clustered and  
201 non-clustered), observations were resampled 999 times and modes were estimated. 95%  
202 confidence intervals were calculated as 2.5th and 97.5th percentile of the vector of bootstrapped  
203 modes (Davison et al. 1997).

204 Another qualitative indicator for the presence of alternative states is the divergence of  
205 temporal trajectories of identically treated experimental units (Scheffer and Carpenter 2003). In  
206 particular, alternative state theory predicts that the final state of a system, vegetated or semi-bare  
207 in our case, will depend on the initial position of the state variable with respect to a threshold:  
208 units with biofilm biomass above the threshold at the first sampling date will remain in the  
209 vegetated state, while units below that threshold will shift to the semi-bare state (Schröder et al.



210 2005). To test this, we adopted a binary classification technique commonly used in machine  
211 learning: given the value of biofilm biomass at the first sampling date after both extreme events,  
212 the algorithm decides whether that particular unit will end up in the semi-bare (0) or in the  
213 vegetated state (1). In this case the algorithm was a binomial generalized linear model that we fit  
214 to our data using the *glm* function in the R package stats (version 3. .5.1). We divided the data  
215 belonging to the non-clustered scenario into two groups: 1) a training set, consisting of 60% of  
216 data points, in which an experimental unit was classified as vegetated if its biomass was  
217 embraced in the confidence interval of the mean control biomass at the last sampling date or  
218 semi-bare otherwise, and 2) a testing set including the remaining 40% of the data. The training  
219 set was used to fit the binomial generalized linear model, whose accuracy was then tested over  
220 the testing set.

#### 221 *Model formulation and parameterization*

222 We developed a simple mathematical model to explore whether different temporal regimes of  
223 temperature extremes could induce alternative states in biofilm biomass. We considered only one  
224 stressor variable since extremes warming and sediment deposition events have comparable  
225 effects on biofilm biomass (Dal Bello et al. 2017). The goal here was to assess biofilm dynamics  
226 under different temporal scenarios of temperature extremes and to test whether the degree of  
227 temporal clustering could generate alternative states. This model provided a qualitative  
228 benchmark with which to compare the experimental results.

229 We modelled the dynamics of biofilm using a simple growth equation describing changes  
230 of biofilm biomass ( $\mu\text{g chl } a \text{ cm}^{-2}$ ) as a function of temperature and a loss equation, which  
231 reflects general processes leading to biofilm mortality (e.g. consumption by grazers and  
232 dislodgment by waves):

$$234 \quad \frac{dB}{dt} = G(B) - F(B) + \sigma B \frac{dW}{dt} \quad (\text{Eq. 1})$$

235  
236 where  $B$  is the biomass of biofilm ( $\mu\text{g chl } a \text{ cm}^{-2}$ ),  $t$  is time and  $T$  is mean air temperature ( $^{\circ}\text{C}$ ).  
237 Function  $G(B)$  is a logistic equation that describes the growth of biofilm biomass, in which the  
238 per capita growth rate varies as a function of mean air temperature ( $^{\circ}\text{C}$ ). Function  $F(B)$  describes  
239 the loss of biomass due to biological or physical disturbance. Due to the narrow amplitude of  
240 tides, intertidal organisms along Mediterranean coasts may be exposed to elevated desiccation

241 stress due to prolonged periods of calm seas and high barometric pressure. In contrast, waves and  
 242 rough sea conditions can keep intertidal organisms constantly wet, even during low tides  
 243 (Benedetti-Cecchi et al. 2006). Frequent shocks to biofilm biomass due to such contrasting and  
 244 rapidly changing weather conditions are represented in the model by the term  $\sigma B dW/dt$ , where  
 245  $dW/dt$  is a Wiener white noise process with mean 0 and variance  $dt$  and  $\sigma$  is the scale parameter  
 246 of the noise process, which was arbitrarily set to 0.04.

247 As anticipated before, the  $G(B)$  function is a logistic equation describing the growth of  
 248 biofilm biomass:

$$249 \quad G(B) = r(T)B \left(1 - \frac{B}{K}\right) \quad (\text{Eq. 2})$$

250 where  $r(T)$  is a two-phase thermal performance curve modelling the variation of growth rate as a  
 251 function of temperature and  $K$  is maximum biofilm biomass (Deutsch et al. 2008, Vasseur et al.  
 252 2014) (Appendix S1: Fig. S2).

$$253 \quad r(T) = \begin{cases} r_{max} \left[1 - \frac{(T-T_{opt})}{T_{opt}-T_{max}}\right]^2 & T \geq T_{opt} \\ r_{max} \left[e^{\left[\frac{(T-T_{opt})}{2\sigma_p}\right]^2}\right] & T < T_{opt} \end{cases} \quad (\text{Eq. 3})$$

254 where  $r_m$  is the maximum growth rate of biofilm biomass,  $T$  is air temperature,  $T_{opt}$  is the mean  
 255 air temperature at which the growth rate is maximum ( $r(T_{opt})=r_{max}$ ),  $T_{max}$  is the temperature limit  
 256 beyond which the growth rate becomes negative, and  $\sigma_p$  is a parameter controlling the rate of  
 257 increase of growth rate in the ascending part of the curve. This relationship is in line with  
 258 experimental evidence and observations that higher values of air temperature ( $T$  °C) strongly  
 259 decreased the growth rate of rocky intertidal biofilms (Sanz-Lázaro et al. 2015, Dal Bello et al.  
 260 2017).

261 The model included an “*Allee effect*” implying a lower growth rate at low levels of  
 262 biomass. We assumed that the mortality rate of biofilm increased below a certain value of  
 263 biomass, due to the decrease in EPS production and the consequent increase in desiccation stress  
 264 and reduction of protection against UV radiation (Potts 1999, Wulff et al. 2000, Wolfstein and  
 265 Stal 2002):

$$267 \quad F(B) = m_a B \left(\frac{h_A}{B+h_A}\right) \quad (\text{Eq. 4})$$

268 The loss term caused a net reduction of per capita growth rate at low biomass levels. This was  
269 achieved through a Monod equation with a half-saturation constant  $h_a$ , which defines the  
270 biomass level below which this loss term is halved.

### 271 *Model parametrization and simulations*

272 Parameters were estimated empirically by fitting the model to time series of biofilm biomass at  
273 the study site (Appendix S1: Table 1). On nine occasions between April and August 2013 we  
274 sampled six plots the same size as the experimental units (30 x 50 cm) and biofilm biomass was  
275 evaluated as described in the previous section. Daily temperature data were obtained from Rete  
276 Mareografica Nazionale (ISPRA, <http://www.mareografico.it>). Maximum likelihood parameter  
277 estimates were obtained with the *mle2* function of the *bmle* library in R, assuming lognormal  
278 errors (Bolker 2008). Predicted time series were obtained by integrating over time initial biofilm  
279 biomass. We used the *ode* function of R package *deSolve*, with backward differentiation formula  
280 (Soetaert et al. 2012). We used plot averages of biofilm biomass for this analysis because  
281 subplots within plots differed among dates, so only data aggregated at the plot level could be  
282 tracked through time (Appendix S1: Fig. S2). The interpolating function *aproxfun* in the R  
283 package *deSolve* was used to obtain temperature estimates at exact time points during the  
284 integration routine. Likelihood profiles were inspected to ensure that parameters were well  
285 defined.

286 To evaluate the effect of extreme climatic events in the model, we first generated a  
287 baseline condition where air temperature increased from 23 to 27.5 °C, which resembled the  
288 increase in temperature observed during the experiment (data obtained by from Rete  
289 Mareografica Nazionale, ISPRA, <http://www.mareografico.it>). Moreover, to reproduce the  
290 variability in mean temperature similar to that observed over the study period, we superimposed  
291 to temperature time series a white noise process with mean ( $\mu$ ) zero and standard deviation ( $\sigma$ )  
292 equal to 1.5 °C. Time series of air temperature were finally modified to integrate the maximum  
293 air temperature measured in the experimental warming session (aerial temperature of 32 °C). As  
294 in the experiment, we produced two temporal patterns of extreme events, a clustered pattern in  
295 which we imparted two warming events separated by 15 days (day 76 and day 91) and a non-  
296 clustered scenario consisting of the same temperature extreme separated by 60 days (day 10 and  
297 day 70; Appendix S1: Fig. S1). We constructed a set of simulated time series for each scenario  
298 running Eq. 1 from 50 different initial conditions randomly selected from a normal distribution

299 ( $\mu = 3.5, \sigma = 0.5$ ), for 150 time-steps. Also, a third set of simulations without the imposition of  
300 extreme events was produced. Simulations were performed by using an Euler-Murayama method  
301 with Ito calculus (Iacus 2009).

302

## 303 RESULTS

304 Biofilm biomass exhibited two distinct states (Fig. 1). Biomass distribution in controls (no  
305 extreme events) was unimodal and centered on the value of  $4.59 \mu\text{g chl } a \text{ cm}^{-2}$  (95% CIs [4.23 -  
306 4.97]), which identifies the vegetated state (Fig. 1A, D, see Table 1). The distribution of biomass  
307 in the clustered scenario was also unimodal but centered on a lower value ( $1.23 \mu\text{g chl } a \text{ cm}^{-2}$ ;  
308 95% CIs [1.08 - 1.38]), which identifies a semi-bare state (Fig. 1B, D and Table 1). Non-clustered  
309 event treatments showed instead bimodality ( $1.61 \mu\text{g chl } a \text{ cm}^{-2}$ ; 95% CIs [1.25 - 1.91] and  $4.38$   
310  $\mu\text{g chl } a \text{ cm}^{-2}$ ; 95% CIs [3.63 - 4.89]), with intermediate values of biofilm biomass (Fig. 1C, D  
311 and Table 1). Graphical scrutiny of the results suggests that warming and sediment deposition  
312 have similar effects on the distribution of biofilm biomass (Appendix S1: Fig. S3).

313 Inspection of the temporal trajectories of biofilm biomass revealed that, despite a slight  
314 decline, controls remained in the vegetated state during the course of the study, while clustered  
315 treatments were consistently in the semi-bare state. The non-clustered scenario showed a  
316 divergent pattern, with some experimental units recovering to biomass values observed in  
317 controls and other units declining towards values measured in the clustered treatments (Fig. 2).  
318 In the non-clustered scenario, whether a unit recovered to the vegetated state or declined to the  
319 semi-bare state depended on its value of biomass at the first sampling date (Appendix S1: Fig  
320 S4). In particular, a unit increase in biofilm biomass increased the probability (log odds) to end  
321 up in the bare state by 1.88 (Table 2). Finally, the model predicted the final state of experimental  
322 units in the testing set with reasonable accuracy (AUC=0.9, Appendix S1: Fig. S5).

323 The response of biofilm biomass to extreme events in the model was consistent with the  
324 experimental results (Fig. 3). In the non-clustered scenario, time series of biofilm biomass  
325 showed a marked divergent pattern, with some replicates recovering and others collapsing. This  
326 resulted in a bimodal frequency distribution, with one mode of  $\sim 0 \mu\text{g chl } a \text{ cm}^{-2}$  and the other of  
327  $\sim 3 \mu\text{g chl } a \text{ cm}^{-2}$  (Fig. 3a). In the clustered scenario, instead, biofilm biomass collapsed, showing  
328 a unimodal pattern with a mode corresponding to  $\sim 0 \mu\text{g chl } a \text{ cm}^{-2}$  (Fig. 3b). In the controls,  
329 biofilm biomass showed a slight decrease over time and a unimodal pattern in the frequency

330 distribution, with a mode of  $\sim 3 \mu\text{g chl } a \text{ cm}^{-2}$  (Fig. 3c). Although the model clearly produced a  
331 bimodal pattern, the frequencies distribution in the experiment did not exactly match the pattern  
332 produced by the simulation, with the experimentally observed modes slightly greater than the  
333 ones predicted by the model.

## 334 DISCUSSION

335 Our findings suggest that the history of extreme events and the time since the last perturbation  
336 may affect the susceptibility of rocky intertidal photosynthetic biofilms to undergo a regime  
337 shift. The analysis of the frequency distribution of biofilm biomass indicated the occurrence of  
338 two alternative states under a regime of non-clustered extremes: a semi-bare state characterized  
339 by low biomass and a vegetated state where biomass was high, separated by an unstable range of  
340 biomass values. In contrast, clustered extremes induced the collapse of biofilm biomass  
341 precipitating the system in the semi-bare state.

342 Assessing multimodality in the frequency distribution of state variables has been often  
343 used as a qualitative flag to assess the consistency between empirical data and theoretical  
344 expectations of catastrophic transitions (Scheffer et al. 2012). Assessing whether a system shows  
345 alternative states also involves testing for the temporal random divergence of identically treated  
346 experimental units (Schröder et al. 2005). This implies that, in a bistable system strongly  
347 influenced by stochastic perturbations, some experimental units will tend to one state and others  
348 will converge towards the other state and the outcome depends on initial conditions. Yet,  
349 observing a state transition and lack of recovery following the application of pulse perturbations  
350 provides a stringent test for alternative states in natural systems (test for non-recovery, Suding et  
351 al. 2004, Schröder et al. 2005). Biofilm biomass in the clustered scenario exhibited a state  
352 transition toward the semi-bare state and a complete lack of recovery which persisted for two  
353 months following the imposition of extreme events. Our experimental results together with  
354 model simulations were consistent with these expectations, showing how experimental units with  
355 intermediated values of biomass followed divergent trajectories, culminating to either the semi-  
356 bare or the vegetated state in the non-clustered scenario.

357 Self-replacement, the capacity of an assemblage to maintain itself over time, is a proxy for  
358 stability of alternative states (Connell and Sousa 1983). Biofilm at our study site was mainly  
359 composed of cyanobacteria characterised by fast-growing species with short generation time  
360 (from days to weeks) (Whitton 2012, Maggi et al. 2017). The persistence of the two alternative

361 states for a time encompassing several generations of the species composing biofilm (two  
362 months in our study) suggests that the two alternative states may be considered stable *sensu*  
363 Connell and Sousa (1983). On the contrary, in our study we did not investigate whether  
364 alternative states were locally stable, for instance, whether the semi-bare state recovered to a  
365 vegetated state upon the arrival of new individuals from the water column (Beisner et al. 2003).  
366 One approach would involve the application of a small perturbation (e.g. a small clearing) at  
367 each of the two contrasting states to test whether or not they returned to the original condition.  
368 Previous studies have shown that biofilm may experience drastic changes in biomass and recover  
369 from apparently catastrophic transitions within a relatively short time scale (Alsterberg et al.  
370 2007, Larson and Sundbäck 2012). Although we cannot entirely rule out that vegetated and the  
371 semi-bare state represent alternative transient states (*sensu* Fukami and Nakajima 2011), our  
372 results support the hypothesis that biofilm may shift from a vegetated to a semi-bare state in  
373 response to multiple pulses of temperature and sediment deposition.

374 Out results are important in light of the predicted increase in the frequency of extreme  
375 climatic events under climate change (IPCC 2013). The degree of temporal clustering of  
376 extremes is expected to increase, as signalled by increased variance in the interval of time  
377 between events in tropical ecosystems (Mumby et al. 2011, Holmgren et al. 2013), grasslands  
378 (Fuchslueger et al. 2016) and Mediterranean coastal areas (Volosciuk et al. 2016). Changes in  
379 temporal clustering can moderate the severity of ecological impacts caused by extreme events  
380 (Benedetti-Cecchi et al. 2006, Holmgren et al. 2006, Kreyling et al. 2011, Mumby et al. 2011)  
381 and modulate the ecological memory of natural systems (Dal Bello et al. 2017). Here we  
382 highlight that the degree of temporal clustering of extremes may regulate the occurrence of  
383 regime shifts.

384 Exogenous periodic forces and seasonality may affect the ability of a natural system to  
385 respond to extreme events and, in general, to stochastic pulse perturbations. Our study shows that  
386 biofilm biomass decreased along the course of the experiment, from spring to summer. A similar  
387 decline in biofilm biomass has been described in other studies and likely reflects the effect of  
388 increasing temperature and light intensity (Nagarkar and Williams 1999, Jackson et al. 2010).  
389 Biofilm assemblages likely experienced progressively stressful conditions during the course of  
390 the experiment, which made them more susceptible to collapse as summer proceeded. As  
391 temperature increased during the experiment, the capacity of biofilm to recover from a

392 temperature extreme drastically decreased, making it more susceptible to a subsequent  
393 perturbation. In agreement with these experimental results, the biofilm model indicated that  
394 seasonal warming amplified the impact of temporally clustered perturbations. When sudden  
395 perturbations occur in combination with unfavourable environmental conditions (e.g. higher  
396 summer temperatures), their compounded effects may have dramatic consequences. Such  
397 contingencies may, thus, play a pivotal role in determining the occurrence of tipping points and  
398 alternative states in natural systems.

399 Thermal buffering provided by conspecifics is a widespread facilitative mechanism in  
400 rocky intertidal communities (Stachowicz 2001). Biofilms should benefit from living at high  
401 density due to higher EPS production, which in turn enhances survival and boosts growth (Potts  
402 1994, Steele et al. 2014). Our experimental results showed how extreme temperatures may push  
403 biofilm biomass toward a threshold level, below which growth rates can no longer compensate  
404 for increased mortality. As shown in another study, EPS production decreases with declining  
405 growth rates of biofilm, hence increasing the risk of lethal damages due to enhanced thermal  
406 stress when a critical level of low biofilm biomass is reached (Wulff et al. 2000). At this point  
407 the production of EPS becomes too low and it is no longer effective in protecting biofilm from  
408 stressful conditions. This mechanism generates feedbacks, so that the resulting loss of biomass  
409 further weakens the facilitative effect of EPS. Our experimental and model results support the  
410 view that the combined effect of greater mortality at low biomass ("*Allee effect*"), a mechanism  
411 that may reflect the reduction of EPS production, along with seasonal changes in aerial  
412 temperature markedly affect biofilm biomass temporal dynamics.

413 Biofilm assemblages consist of microscopic photosynthetic organisms and, despite their  
414 small size they strongly contribute to the primary productivity of intertidal rocky shores  
415 (Thompson et al. 2004). A wealth of studies showed that changes in primary productivity affect  
416 higher trophic levels (Wernberg et al. 2016, Guo et al. 2017, but see Liess et al. 2015 for a  
417 counter example). Since fast growing microbial populations are an important component of  
418 primary producers in virtually all ecosystems, increasing temporal clustering of extreme events  
419 will likely have pervasive impacts on food webs, altering biological interactions and affecting the  
420 stability of whole ecosystems. Our results should therefore prompt new studies investigating the  
421 cascading effects of regime shifts in primary producer communities.

422 Current research on regime shifts has mainly focused on investigating how gradual  
423 changes in ecological drivers precipitate natural systems into undesirable, alternative states. Only  
424 recently, ecological research turned its attention to the examination of the effects of other types  
425 of disturbances, such as recurrent pulse events. Here, we show that ecosystem dynamics can be  
426 largely affected by extreme events, with the likelihood of a regime shift primarily depending on  
427 the time separating consecutive events. However, further work is needed to determine the  
428 generality of these results to better understand and predict ecosystem dynamics in a rapidly  
429 changing world.

430

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 600 1152.

601  
 602 **Table 1.** BIC criterion of models with a different number of fitted density distributions (here we  
 603 show the first 4) for control, non-clustered and clustered scenarios. The model with the smallest  
 604 BIC (in bold) has the best fit.

<i>Modes</i>	Controls (no extremes)	Non-clustered events	Clustered events
1	<b>223.9490</b>	238.5700	<b>118.1841</b>
2	231.3705	<b>231.1430</b>	120.7077
3	241.5501	243.4187	‡
4	251.3868	249.2830	‡

605 ‡ No convergence

606  
 607 **Table 2.** Binomial generalized linear model on the final state of experimental units (semi-bare or  
 608 vegetated state) as a function of the value of biofilm biomass at the first sampling date after both  
 609 extreme events. An experimental unit is assigned to the vegetated state if its biomass value is  
 610 embraced in the 95% confidence interval of the mean control biomass at the last sampling date;  
 611 otherwise it is classified as semi-bare state. McFadden  $R^2$  indicates the goodness of fit.

612 \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

	Coefficient (SE)	
<b>Intercept</b>	-8.46 (3.19)	**
<b>Biomass at the first sampling date</b>	1.88 (0.71)	**

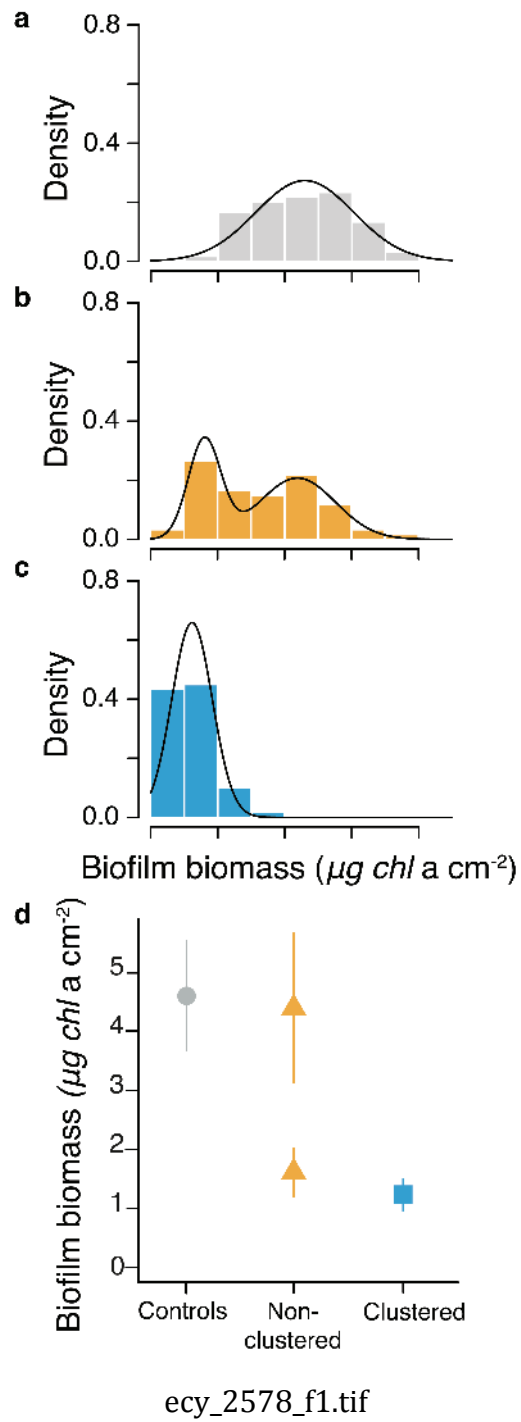
McFadden  $R^2 = 52\%$

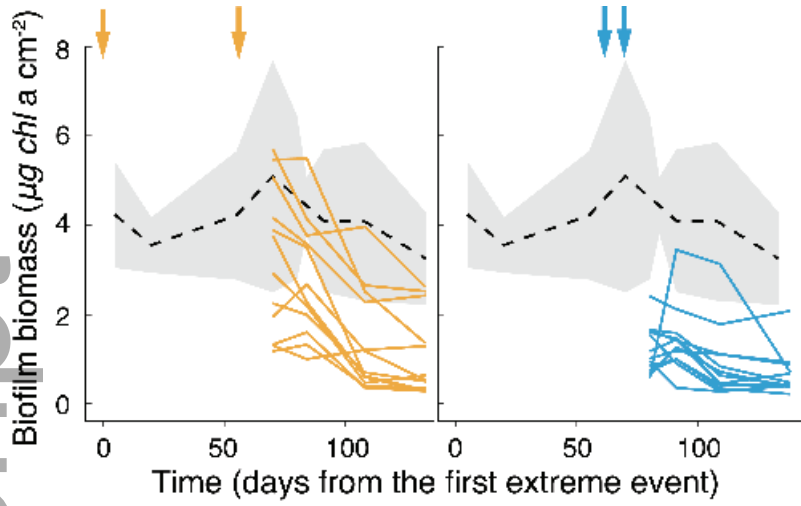
613  
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 615 **LEGEND TO FIGURES**  
 616 **Figure 1.** Frequency distribution of biofilm biomass and probability density functions (solid  
 617 lines) separately for controls (panel a), non-clustered (panel b) and clustered events treatments  
 618 (panel c). In panel d, the modes for each experimental condition are shown together with  
 619 bootstrapped 95% confidence intervals.



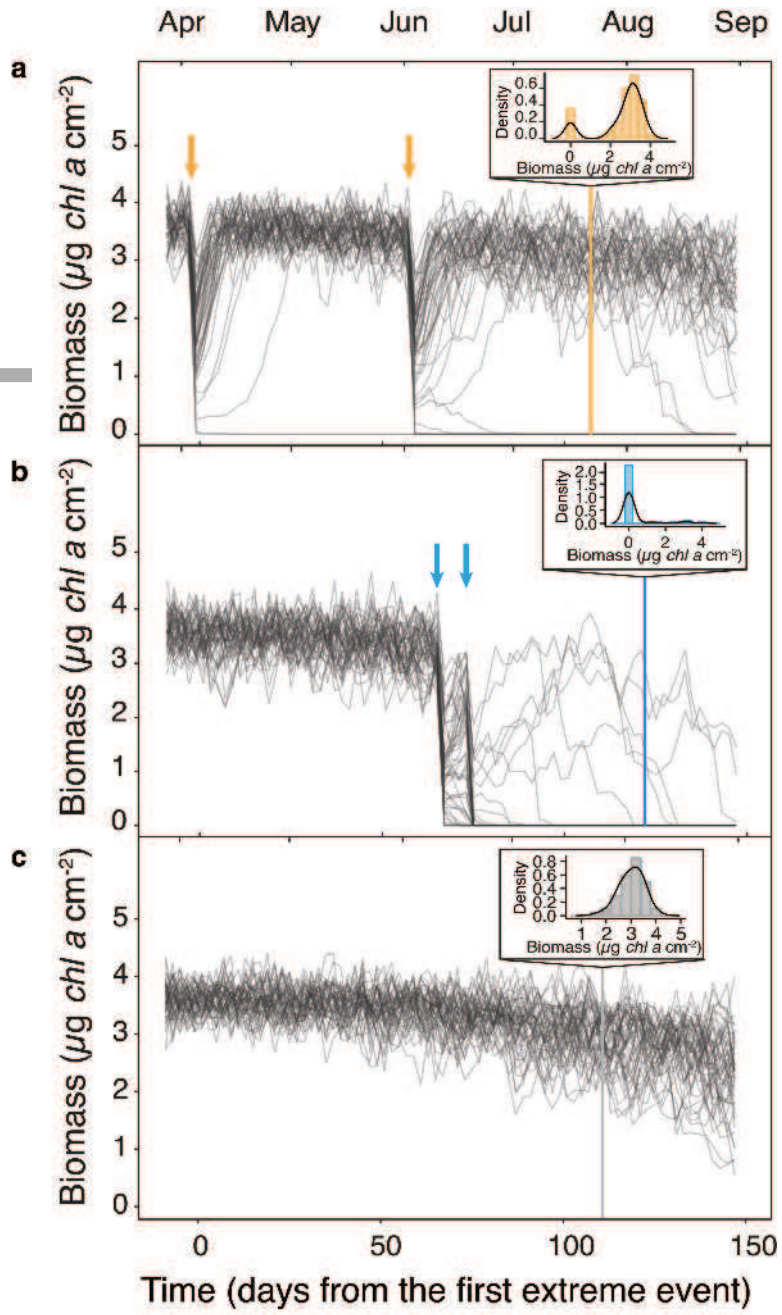
620 **Figure 2.** Observed temporal trajectories of biofilm biomass under the non-clustered (left panel)  
621 and the clustered (right panel) scenarios of extreme climatic events, indicated as days from the  
622 first experimental perturbation. The control treatment is used for reference and is shown as 95%  
623 confidence interval region (light grey) and averaged temporal trajectory (black). Arrows indicate  
624 the timing of the perturbations for non-clustered (orange) and clustered (blue) events.

625 **Figure 3.** Simulated temporal trajectories of biofilm biomass ( $\mu\text{g chl } a \text{ cm}^{-2}$ ) for (a) non-  
626 clustered and (b) clustered warming regimes. In panel (c) there are controls. Time series were  
627 computed from simulations with 50 replicates over a time span of 160 days for increasing mean  
628 air temperature from 22 to 27 °C. Warming in the simulation mirrored the observed increase in  
629 temperature during the study period (data obtained from Rete Mareografica Nazionale ISPRA,  
630 <http://www.mareografico.it>). Down-facing arrows indicate the timing of perturbations. We  
631 simulated two temporal patterns of ECEs: a clustered pattern in which we imparted two warming  
632 events (aerial temperature of 32 °C) separated by 15 days, and a non-clustered scenario  
633 consisting of the same temperature extremes separated by 60 days. The initial periods of 10 days  
634 were excluded from the visualization to remove transient dynamics. The insets show the  
635 frequency distributions and probability density functions (solid lines) of biofilm biomass under  
636 non-clustered and clustered warming regimes calculated for the day indicated by the colored bar.





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