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

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Citation: Dal Bello, Martina, Rindi, Luca and Benedetti#Cecchi, Lisandro. 2019. "Temporal clustering of extreme climate events drives a regime shift in rocky intertidal biofilms." Ecology, 100 (2).

As Published: http://dx.doi.org/10.1002/ecy.2578

Publisher: Wiley

Persistent URL: https://hdl.handle.net/1721.1/140589

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

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4	Article type : Articles
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7	Running head: Extreme events and alternative states
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9	Title: Temporal clustering of extreme climate events drives a regime shift in rocky intertidal
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22	Turne of the article Article ADSTRACT
22	Type of the article: Afficie 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

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi:</u> <u>10.1002/ECY.2578</u>

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 of temporal characteristics of press disturbances in regulating regime shifts. Ratajczak et al. 49 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 have a great potential to induce regime shifts (Scheffer et al. 2001). In highly stochastic 56 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 changes in the temporal clustering of extreme events, i.e. the degree of separation between
consecutive instances, can modulate ecological memory of microbial assemblages (Dal Bello et
al. 2017) and regulate the onset of regime shifts in tropical ecosystems (Holmgren et al. 2013).
Evaluating how different scenarios of extreme events can trigger a regime shift in systems with
alternative states will be a crucial step to better understand the impact of climate change on
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).

We used a field experiment and a model to test for the presence of alternative states in rocky shore photosynthetic biofilms and to explore the underlying feedback mechanisms. The 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 "Allee effect". 132

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

interspersed among areas of seemingly bare rock, where photosynthetic biofilms develop.

140 Biofilm assemblages at Calafuria include mainly cyanobacteria, with diatoms being less

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

barometric pressure alternate with heavy rainfalls, the latter resulting in the deposition of

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

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).

Another qualitative indicator for the presence of alternative states is the divergence of temporal trajectories of identically treated experimental units (Scheffer and Carpenter 2003). In particular, alternative state theory predicts that the final state of a system, vegetated or semi-bare in our case, will depend on the initial position of the state variable with respect to a threshold: units with biofilm biomass above the threshold at the first sampling date will remain in the 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 semi-bare otherwise, and 2) a testing set including the remaining 40% of the data. The training 218 219 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

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

We modelled the dynamics of biofilm using a simple growth equation describing changes of biofilm biomass (μ g chl *a* cm⁻²) as a function of temperature and a loss equation, which reflects general processes leading to biofilm mortality (e.g. consumption by grazers and dislodgment by waves):

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- 234

235

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

where *B* is the biomass of biofilm (μ g chl *a* cm⁻²), *t* is time and T is mean air temperature (°C). Function *G*(*B*) is a logistic equation that describes the growth of biofilm biomass, in which the per capita growth rate varies as a function of mean air temperature (C°). Function *F*(*B*) describes the loss of biomass due to biological or physical disturbance. Due to the narrow amplitude of tides, intertidal organisms along Mediterranean coasts may be exposed to elevated desiccation stress due to prolonged periods of calm seas and high barometric pressure. In contrast, waves and

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
- rapidly changing weather conditions are represented in the model by the term $\sigma BdW/dt$, where

245 dW/dt is a Wiener white noise process with mean 0 and variance dt and σ 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:

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

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

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

254

249

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

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

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

- 268 The loss term caused a net reduction of per capita growth rate at low biomass levels. This was
- 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 Mareografica Nazionale (ISPRA, http://www.mareografico.it). Maximum likelihood parameter 276 277 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 package deSolve was used to obtain temperature estimates at exact time points during the 283 284 integration routine. Likelihood profiles were inspected to ensure that parameters were well defined. 285

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 (μ) zero and standard deviation (σ) 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

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

302

RESULTS 303 304 Biofilm biomass exhibited two distinct states (Fig. 1). Biomass distribution in controls (no extreme events) was unimodal and centered on the value of 4.59 μ g chl a cm⁻² (95% CIs [4.23 -305 306 4.97]), which identifies the vegetated state (Fig. 1A, D, see Table 1). The distribution of biomass in the clustered scenario was also unimodal but centered on a lower value (1.23 μ g chl a cm⁻²; 307 308 95% CIs [1.08 – 1.38]), which identifies a semi-bare state (Fig. 1B, D and Table 1). Non-clustered event treatments showed instead bimodality (1.61 μ g chl a cm⁻²; 95% CIs [1.25 - 1.91] and 4.38 309 μ g chl *a* cm⁻²; 95%CIs [3.63 - 4.89]), with intermediate values of biofilm biomass (Fig. 1C, D 310 311 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

11 a response of biofilm biomass to extreme events in the model was consistent with the experimental results (Fig. 3). In the non-clustered scenario, time series of biofilm biomass showed a marked divergent pattern, with some replicates recovering and others collapsing. This resulted in a bimodal frequency distribution, with one mode of ~0 μ g chl *a* cm⁻² and the other of ~3 μ g chl *a* cm⁻² (Fig. 3a). In the clustered scenario, instead, biofilm biomass collapsed, showing a unimodal pattern with a mode corresponding to ~0 μ g chl *a* cm⁻² (Fig. 3b). In the controls, biofilm biomass showed a slight decrease over time and a unimodal pattern in the frequency distribution, with a mode of $\sim 3 \ \mu g \ chl \ a \ cm^{-2}$ (Fig. 3c). Although the model clearly produced a bimodal pattern, the frequencies distribution in the experiment did not exactly match the pattern produced by the simulation, with the experimentally observed modes slightly greater than the ones predicted by the model.

334 DISCUSSION

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

Self-replacement, the capacity of an assemblage to maintain itself over time, is a proxy for
stability of alternative states (Connell and Sousa 1983). Biofilm at our study site was mainly
composed of cyanobacteria characterised by fast-growing species with short generation time
(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 the that the two alternatives 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

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

431 ACKNOWLEDGMENTS

We thank A. Schröder and an anonymous reviewer for their constructive comments to the
manuscript. We also thank Elena Maggi, Chiara Ravaglioli and several students for assistance
with the field work. This research was supported by the University of Pisa through the PRA
programme (PRA_2017_19) and the Italian Ministry of Research and Education through the
PRIN grant 'Biocostruzioni costiere: struttura, funzione e gestione' to LBC. The first and second
authors contributed equally to this work.

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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.

Modes	Contro	ls (no extremes)	Non-clustered events	Clustered events
1		223.9490	238.5700	118.1841
2	\bigcirc	231.3705	231.1430	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 or608vegetated state) as a function of the value of biofilm biomass at the first sampling date after both609extreme events. An experimental unit is assigned to the vegetated state if its biomass value is610embraced in the 95% confidence interval of the mean control biomass at the last sampling date;611otherwise it is classified as semi-bare state. McFadden R² indicates the goodness of fit.612* p < 0.05, **p < 0.01, ***p < 0.001

	Coefficient (SE)	
Intercept	-8.46 (3.19)	**
Biomass at the first sampling date	1.88 (0.71)	**
McEaddan P^2 -52%		

McFadden R⁻

- 613
- 614

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.

Figure 2. Observed temporal trajectories of biofilm biomass under the non-clustered (left panel)
and the clustered (right panel) scenarios of extreme climatic events, indicated as days from the
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.

Figure 3. Simulated temporal trajectories of biofilm biomass (μ g chl *a* 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

air temperature from 22 to 27 °C. Warming in the simulation mirrored the observed increase in

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.

Author







