Sandra RAMIREZ-CALERO, BENSOUSSAN N, LÓPEZ-SENDINO P, GÓMEZ-GRAS D, MONTERO-SERRA I, PAGÈS-ESCOLÀ M, MEDRANO A, LÓPEZ-SANZ A, FIGUEROLA, L, LINARES C, LEDOUX JB, GARRABOU J.
Institute of Marine Sciences, ICM-CSIC, Pg. Marítim de la Barceloneta 37-49, 08003, Barcelona, Spain
E-mail: sandraramirezcalero@gmail.com

TEMPORAL VARIABILITY IN THE RESPONSE TO THERMAL STRESS IN THE RED GORGONIAN, *P. CLAVATA*: INSIGHTS FROM COMMON GARDEN EXPERIMENTS

Abstract
Recurrent mass mortality events (MMEs) linked to marine heatwaves (MHWs) have been observed in the Mediterranean Sea affecting thousands of kilometers of coastline. Coralligenous habitats were among the most impacted during these events. Information on how the exposure to recurrent MHWs is affecting the coralligenous is critical to anticipate the consequences of climate change and implement actions to enhance their resilience. Combining field surveys with experiments in controlled conditions allowed to dilucidate the differential responses to thermal stress among species, populations and individuals and to explore the spatial and taxonomic variability response to thermal stress linked to MHWs. Yet, the temporal variability in the response to thermal stress remains to be characterized. Thus, we aim to fill this gap focusing on the temporal variability in the response to thermal stress of the coralligenous key habitat-forming species Paramuricea clavata (Plexauridae). We replicated thermal stress experiments during 3 consecutive years following a common garden setup (control vs. thermal stress) involving the same individuals from the same three populations. Considering different phenotypic responses including the level of tissue necrosis during the time of the experiment and the survival of the individuals, we found that the average percentage of tissue necrosis per population variated greatly across years while the probability of survival was considerably reduced in 2017. During the experiments, several individuals from the 3 populations systematically showed reduced level of tissue necrosis suggesting resistance to thermal stress. Overall our data will contribute to help better inform further conservation strategies of habitat-forming coral species in the Mediterranean Sea.

Key-words: Marine heat-waves, octocorals, temporal series, population genetics, conservation.

Introduction
The Mediterranean Sea, considered as a hotspot of marine biodiversity and climate change (Cramer *et al*., 2018), has been affected by recurrent mass mortality events (MMEs) linked to marine heat waves (MHWs) with differential impacts among species, populations and individuals (Garrabou *et al.,* 2022). Habitat-forming octocorals such as *Paramuricea clavata* (Risso, 1827) were strongly impacted with up to 80% of individuals affected in some localities (Cerrano *et al*., 2000; Garrabou *et al*., 2009; 2021; 2022). This Mediterranean octocoral is characterized by slow population dynamics and restricted dispersal abilities (Linares, *et al*., 2008; Arizmendi-Mejía *et al*., 2015), thus, the high mortality rates induced by MMEs question its evolutionary trajectory. Moreover, *P. clavata* has a key ecological role increasing habitat complexity in biogenic coralligenous communities. Thus, its decline owing to MMEs may have cascading effects on associated communities and related ecosystem functioning (Gómez-Gras *et al.,* 2021).

Differential responses to MMEs among individuals and populations have been reported from the field and from experimental approaches in this species (Garrabou & Harmelin, 2002; Crisci *et al*., 2011; Linares *et al*., 2015), suggesting spatial variability in the response to thermal stress with some individuals/populations able to better resist to MMEs. Yet, these studies have not included observations across consecutive years. Accordingly, the level of temporal variability in the response to thermal stress is totally unknown. This study aims to analyze the differential response to thermal stress between individuals and populations of *Paramuricea clavata* across three consecutive years, to: i) characterize the yearly temporal response variability; ii) refine estimation regarding the response variability at local scale (1 km^2) and iii) explore the potential underlying genetic and environmental factors.

Materials and methods

Samples of *P. clavata* were collected yearly from 2015 to 2017 in early September from three different localities at the Medes Island (Spain, 42°02'60.00" N 3°12'60.00" E). Thirty healthy adult colonies (>50 cm) were initially tagged in 2014 at each location within a depth range of 15-20 m. From each marked colony, apical fragments were collected every year and allocated in the Aquarium Experimental Zone (ZAE) of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona. After one week of acclimation in an open aquarium system at 17-18 °C, each colony branch was divided into two and the common garden experiments were conducted with one control (18 °C) and one stress treatment (25 °C).

For control treatment, seawater temperature was maintained between 16-18 °C during the whole experiment. For the stress treatment, the temperature was increased stepwise from 18 to 25 °C over a period of 3 days. Once the temperature reached 25 °C, thermal conditions were maintained for 25 days following Crisci *et al*., (2017). Feeding was carried out three times per week in each tank.

Phenotypic response and Statistical analysis

Firstly, the percentage of tissue necrosis (extent of injury) was used as a proxy of colony response to thermal stress. Each individual was monitored visually every day until the end of the experiment. Then, we estimated the mean (and SD) extent of injury for each population and each year by using the average of injured tissue across all individuals. Secondly, the survival probabilities for each colony and population during the time of the experiment (*i.e.* having less than 100% of injured surface) was evaluated using a series of COX models under the *survival* R-package (Therneau *et al.*, 2022). Finally, since we sampled the same individuals every year, we also reported the daily trajectory of tissue necrosis for each colony each year separately in order to look for temporal variability at the population and individual levels. Following Garrabou *et al*., 2022, we identified individuals as potentially "resistant" if the levels of tissue necrosis were between 0 and 30% at the end of the experiment of any of the tested years. Remaining individuals were considered as "sensitive".

Results

Tissue necrosis was observed for all populations in the three experiments. In 2015 and 2016, the extent of injury was below 40% at the end of the experiment (day 25^{th}). In contrast for 2017, colonies were totally affected (100% of tissue necrosis) by the 18^{th} day, except for Pota del Llop where a 100% of tissue necrosis was reached at day 24th.

(Fig. 1a). In addition, there were no significant differences in the survival probability comparing between localities for any given year. Significant differences in the survival probability of colonies from the three localities were found across years (*p<0.001*). In 2015 and 2016, the probability of surviving slightly decreased only after day 15. In contrast, in 2017, the probability of survival sharply decreased after day 10. The probability of surviving in 2015 and 2016 was higher in comparison with 2017 (Fig. 1b).

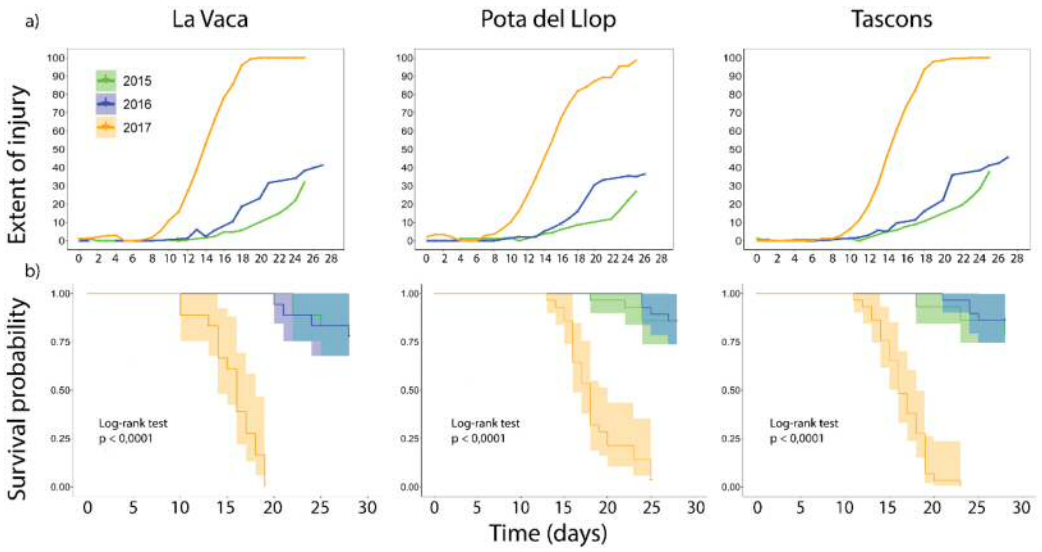

Fig. 1:. a) Average tissue necrosis in common garden experiments in La Vaca, Pota del Llop and Tascons from 2015 to 2017. b) Differences in survival probability (*i.e.* mortality) across years in *P. clavata* when exposed to thermal stress Kaplan-Meier survival curve (*p<0,0001*).

Figure 2 shows the temporal variability of individual tissue necrosis among the three years. In each population, the level of tissue necrosis varied greatly among individuals. Considering the three years, around 40% of the total individuals (black arrows) in each population show between 0-30% of tissue necrosis in at least one of the tested years at the beginning of the second week of the experiment (~13 day). Thus, they were considered as potentially more resistant.

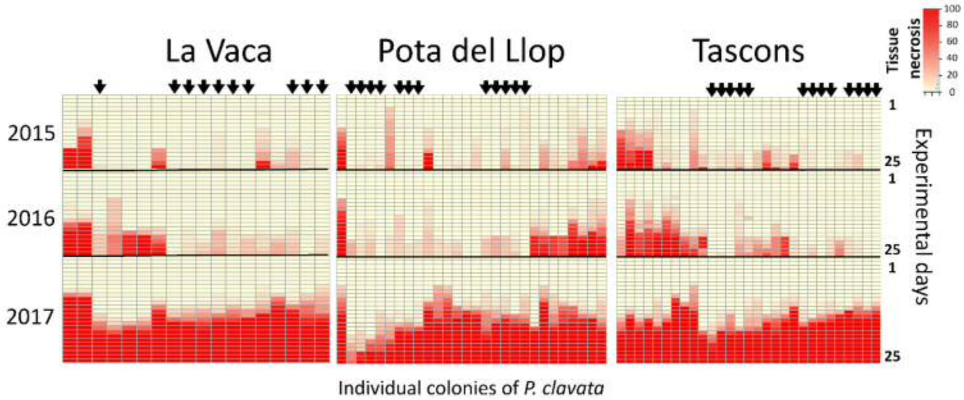

Fig. 2: Trajectory of tissue necrosis in every experimental year for individual colonies of *P. clavata* at each locality. Each column represents the tissue necrosis in one individual during the 25 days of the experiment for each year. Black arrows indicate potential "resistant" individuals.

Discussion

In this study we conducted the characterization of the spatial and temporal variability in the responses to thermal stress between individuals and populations of the red gorgonian *P. clavata*. We considered a local scale within 1 km^2 with three populations, while the temporal scale accounts for three consecutive years. We found no significant response to thermal stress when comparing the survival probability among populations for any given year. These results are in line with previous thermal stress experiments with the same species. In fact, as in our study, the response of populations from the same geographic area were not significantly different (Crisci *et al.,* 2017). The factors and processes underlying this level of spatial variability remain poorly understood in *P. clavata*. Nevertheless, the influences of local thermal environment and recent thermal history on the spatial variability to thermal stress seem to be low (Gomez-Gras *et al*., 2022). This result contrasts with different studies conducted in tropical corals species and point towards the implication of other processes, in particular genetic drift, driving the differential response among populations (Crisci *et al.,* 2011; Linares *et al*., 2013;Howells *et al*., 2012; Palumbi *et al.,* 2014).

We demonstrate a high variability in the percentage of tissue necrosis among individuals from the same population for a given year. This result is also in line with previous studies with *P. clavata* (Crisci *et al.,* 2017). For instance, physiological aspects, including reproductive status (male vs. female) of the colonies were also suggested as important drivers controlling the differential response among individuals (Coma *et al.,* 2009; Arizmendi-Mejía *et al.,* 2015). In the present case, all the sampled colonies were adults (> 50cm), and they were feed during all the experiments, most likely buffering the impact of physiological and reproductive status. Therefore, on-going population genetics and transcriptomics studies should provide further insights into this level of variability. Noteworthy, this individual sensitivity has been proposed as a fuel for adaptation and directional selection of resistant individuals to new thermal regimes in other coral populations over time (Morikawa & Palumbi, 2019).

Finally, we demonstrate a strong temporal variability in the response to thermal stress for all three populations. We found that the survival probability of colonies was strongly reduced in 2017 experiment. Even though, we found several potentially resistant individuals of *P. clavata* at every locality, we observed that mortality is reached faster at year 2017 in at least two of the three populations (100% tissue necrosis by day 18^{th}). Two non-exclusive hypotheses may potentially explain this result: i) impact of past thermal conditions (*i.e.* years before the experiment); ii) impact of recent thermal conditions (*i.e.* summer before the experiment). In 2017, the study area suffered a severe marine heatwave in the sea surface (Bensoussan *et al.,* 2019). Besides preliminary results comparing 2017 with 2015 and 2016 thermal conditions at the depth where the populations dwell (15-20m) point toward a stronger and longer thermal disturbance (>23°C) observed during June to September of 2017 in comparison with 2015 and 2016. In any case, the populations in the field were slightly affected by mortality (Hereu *et al*., 2020). This study indicates that determining the thermal response of populations and individuals may not be straightforward, as we showed that it may depend on the yearly environmental conditions. Despite of this, we also found some potential sensitivity/resistance patterns that may support climate adaptation conservation measures.

Bibliography

ARIZMENDI-MEJÍA R., LEDOUX JB., CIVIT S., ANTUNES A., THANOPOULOU Z., GARRABOU J., LINARES C. (2015) - Demographic responses to warming: reproductive maturity and sex influence vulnerability in an octocoral, *Coral Reefs*, 34(4), pp. 1207–1216.

BENSOUSSAN N., CEBRIAN E., DOMINICI JM., KERSTING DK., KIPSON S., KIZILKAYA Z., OCANA O., PEIRACHE M., ZUBERER F., LEDOUX JB., LINARES C., ZABALA M., NARDELLI BB., PISANO A., GARRABOU J (2019) - Using CMEMS and the Mediterranean Marine Protected Areas sentinel network to track ocean warming effects in coastal areas, *Journal of Operational Oceanography*, 12, pp. S65.

CERRANO C., BAVESTRELLO G., BIANCHI C.N., CATTANEO-VIETTI R., BAVA S., MORGANTI C., MORRI C., PICCO P., SARA G., SCHIAPARELLI S., SICCARDI A., SPONGA F. (2000) - A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999, *Ecology Letters*. 3(4), pp. 284–293.

COMA R., RIBES M., SERRANO E., JIMÉNEZ E., SALAT J., PASCUAL J. (2009) - Global warming-enhanced stratification and mass mortality events in the Mediterranean, *Proceedings of the National Academy of Sciences of the United States of America*, 106(15), pp. 6176–6181.

CRAMER W., GUIOT J., FADER M., GARRABOU J., GATTUSO J., IGLESIAS A., LANGE M., LIONELLO P., LLASAT M., PAZ S., PEÑUELAS J., SNOUSSI M., TORETI A., TSIMPLIS M., XOPLAKI E. (2018) - Climate change and interconnected risks to sustainable development in the Mediterranean, *Nature Climate Change 2018 8:11*. 8(11), pp. 972–980.

CRISCI C., BENSOUSSAN N., ROMANO JC., GARRABOU J. (2011) - Temperature anomalies and mortality events in marine communities: Insights on factors behind differential mortality impacts in the NW Mediterranean, *PLoS ONE*. 6(9), p. e23814.

CRISCI C., LEDOUX J.B., MOKHTAR-JAMAÏ K., BALLY M., BENSOUSSAN N., AURELLE D., CEBRIAN E., COMA R., FÉRAL J.P., LA RIVIÈRE M., LINARES C., LÓPEZ-SENDINO P., MARSCHAL C., RIBES M., TEIXIDÓ N., ZUBERER F., GARRABOU J., (2017) - Regional and local environmental conditions do not shape the response to warming of a marine habitat-forming species, *Scientific Reports*, 7(1), pp. 1–13.

GARRABOU J., COMA R., BENSOUSSAN N., BALLY M., CHEVALDONNÉ P., CIGLIANO M., DIAZ D., HARMELIN J.G., GAMBI M.C., KERSTING D.K., LEDOUX J.B., LEJEUSNE C., LINARES C., MARSCHAL C., PÉREZ T., RIBES M., ROMANO J.C., SERRANO E., TEIXIDO N., TORRENTS O., ZABALA M., ZUBERER F., CERRANO C. (2009) - Mass mortality in Northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave, *Global Change Biology*. 15(5), pp. 1090–1103.

GARRABOU J., LEDOUX J., BENSOUSSAN N., GÓMEZ-GRAS D., LINARES C. (2021) - Sliding Toward the of Mediterranean Coastal Marine Rocky Ecosystems, in *Ecosystem Collapse and Climate Change*. pp. 291–324.

GARRABOU J., GÓMEZ-GRAS D., MEDRANO A., CERRANO C., PONTI M., SCHLEGEL R., BENSOUSSAN N., TURICCHIA E., SINI M., GEROVASILEIOU V., TEIXIDO N., MIRASOLE A., TAMBURELLO L.,CEBRIAN E., RILOV G., LEDOUX J., SOUISSI J., KHAMASSI F.,GHANEM R., BENABDI M.,GRIMES S., OCAÑA O., BAZAIRI H., HEREU B., LINARES C., KERSTING D., ROVIRA G., ORTEGA J., CASALS D., PAGÈS-ESCOLÀ M., MARGARIT N., CAPDEVILA P., VERDURA J., RAMOS A., IZQUIERDO A., BARBERA C., RUBIO-PORTILLO E., ANTON I., LÓPEZ-SENDINO P., DÍAZ D., VÁZQUEZ-LUIS M., DUARTE C., MARBÀ N., ASPILLAGA E., ESPINOSA F., GRECH D., GUALA I., AZZURRO E., FARINA S., GAMBI M., CHIMIENTI G., MONTEFALCONE M., AZZOLA A., MANTAS T., FRASCHETTI S., CECCHERELLI G., KIPSON S., BAKRAN-PETRICIOLI T., PETRICIOLI D., JIMENEZ C., KATSANEVAKIS, S., KIZILKAYA Z., INCI T., KIZILKAYA Z., SARTORETTO S., ELODIE R., RUITTON S., COMEAU S., GATTUSO J. HARMELIN J. (2022) - Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea, *Global Change Biology*. pp. 1–18.

GARRABOU J., HARMELIN J. G. (2002) - A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean: Insights into conservation and management needs, *Journal of Animal Ecology*, 71(6), pp. 966–978.

GÓMEZ-GRAS D., BENSOUSSAN N., LEDOUX JB., LÓPEZ-SENDINO P., CERRANO C., FERRETTI E., KIPSON S., BAKRAN-PETRICIOLI T., CRUZ F., GÓMEZ-GARRIDO J., ALIOTO TS., GUT M., SERRAO EA., , PAULO D., BOAVIDA J., COELHO M., PEARSON GA., MONTERO-SERRA I., PAGÈS-ESCOLÀ M., MEDRANO A., LÓPEZ-SANZ A., MILANESE M., LINARES C., GARRABOU J. (2022) Exploring the response of a key Mediterranean habitat-forming gorgonian to heat stress across biological and spatial scales. *In prep.*

GÓMEZ-GRAS D., LINARES C., DORNELAS M., MADIN J., BRAMBILLA V., LEDOUX J., LÓPEZ-SENDINO P., BENSOUSSAN N. GARRABOU J. (2021) - Climate change transforms the functional identity of Mediterranean coralligenous assemblages, *Ecology Letters*. 24(5), pp. 1038–1051.

HEREU B., ROVIRA G. CASALS D., ORTEGA J., MARGARIT N., MEDRANO A., PAGÈS-ESCOLÀ M., ZENTNER Y., LINARES C. (2020) - Seguiment anual de briozous, gorgònia vermella i coves a la Reserva Natural Parcial Marina de les Medes del Parc Natural del Montgrí, les Illes Medes i el Baix Ter. Memòria 2020. Generalitat de Catalunya. Departament de Territori i Sostenibilitat. Direcció General de Polítiques Ambientals i Medi Natural. 96 pp.

HOWELLS E.J., BELTRAN V.H., LARSEN N.W., BAY L.K., WILLIS B.L., VAN OPPEN M.J.H. (2012) - Coral thermal tolerance shaped by local adaptation of photosymbionts, *Nature Climate Change*, 2(2), pp. 116–120.

LINARES C. CEBRIAN E., KIPSON S., GARRABOU J. (2013) - Does thermal history influence the tolerance of temperate gorgonians to future warming?, *Marine Environmental Research*. 89, pp. 45–52.

LINARES C. DOAK D., COMA R., DIAZ D., ZABALA M., DOAK F., MOLL C. (2015) - Life History and Viability of a Long-Lived Marine Invertebrate : The Octocoral *Paramuricea clavata*, *Ecology*, 88(4), pp. 918–928.

LINARES C., COMA R., ZABALA M. (2008) -Effects of a mass mortality event on gorgonian reproduction, *Coral Reefs*, 27(1), pp. 27–34.

MORIKAWA M. K., PALUMBI S. R. (2019) -Using naturally occurring climate resilient corals to construct bleaching-resistant nurseries, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 116(21), pp. 10586–10591.

PALUMBI S., BARSHIS D., TRAYLOR-KNOWLES N., BAY R. (2014) - Mechanisms of reef coral resistance to future climate change, *Science*, 344(6186), pp. 895–897.

THERNEAU T., LUMLEY T., ATKINSON E., CROWSON C. (2022) - Package ‘survival’ Title Survival Analysis Priority recommended.