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Short-term exposure to concurrent biotic and abiotic stressors may impair farmed molluscs performance

Mar Bosch-Belmar [a,*], Antonio Giacoletti [a], Chiara Giommi [b], Albert Girons [c], Giacomo Milisenda [d,*], Gianluca Sarà [a]

[a] Department of Earth and Marine Sciences (DISTEM), University of Palermo, Palermo, Italy
[b] Department of Integrative Marine Ecology (EMI), Stazione Zoologica Anton Dohrn, CRIMAC, Calabria Marine Center, Amendolara, Italy
[c] ICTIOVET S.L., Barcelona, Spain
[d] Department of Integrative Marine Ecology (EMI), Stazione Zoologica Anton Dohrn, Sicily Marine Center, Palermo, Italy

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ABSTRACT

Global warming, through increasing temperatures, may facilitate the spread and proliferation of outbreak-forming species which may find favourable substrate conditions on artificial aquaculture structures. The presence of stinging organisms (cnidarian hydroids) in the facilities fouling community are a source of pollution that can cause critical problems when in-situ underwater cleaning processes are performed. Multiple stressor experiments were carried out to investigate the cumulative effect on farmed mussels' functional traits when exposed to realistic stressful conditions, including presence of harmful cnidarian cells and environmental conditions of increasing temperature and short-term hypoxia. Exposure to combined stressors significantly altered mussels' performance, causing metabolic depression and low filtering activity, potentially delaying, or inhibiting their recovery ability and ultimately jeopardizing organisms' fitness. Further research on the stressors properties and occurrence is needed to obtain more realistic responses from organisms to minimize climate change impacts and increase ecosystem and marine economic activities resilience to multiple stressors.

1. Introduction

Climate change (CC) and anthropogenic activities are provoking profound and irreversible changes in coastal marine ecosystems. The emergence of multiple stressful factors such as increasing temperature and organic enrichment, decreasing oxygen concentration and seawater pH are shaping ecosystem functioning as well as their ability to provide goods and services (Barange et al., 2018; FAO, 2020). Marine aquaculture is one of the most vulnerable ecosystem-dependent sectors to CC effects (Barange et al., 2018; Bosch-Belmar et al., 2020; Froehlich et al., 2018; Maulu et al., 2021). The impacts of CC on the aquaculture industry have been extensively studied and reviewed both at local and global scales and may present themselves at different magnitudes depending on farmed species traits, local environmental conditions, and the action of coinciding stressors (Gunderson et al., 2016; Sarà et al., 2018a, 2018b). Increasing temperature and local nutrient enrichment (due to leftover food and fish feces) are among the most common sources of disturbance on marine aquaculture (Pernet and Browman, 2021; Sarà et al., 2006), impacting the surrounding environment (through water column stratification changes and hypoxic conditions) and farmed species (provoking organism performance impairment and increasing susceptibility to diseases (Bosch-Belmar et al., 2016; Hassan et al., 2021). In the context of emerging and pervasive environmental change, integrated multitrophic aquaculture (IMTA) has been recognized as a sustainable form of aquaculture given the reduction in impacts and potential ecological and economical increased system resilience that this culture alternative may provide (Alexander et al., 2016; IFAD, 2014).

The action of multiple stressors (MS) on marine aquaculture systems may alter biofouling community composition, leading to the spread and introduction of non-native species or the increase in proliferation of outbreak-forming species (Bannister et al., 2019; Barange et al., 2018; Mangano et al., 2019; Sarà et al., 2007).

Benthic colonial cnidarians represent a significant component within marine biofouling communities, and are a serious concern for aquaculture managers due to their significant impact on finfish and shellfish stocks by impairing organisms' fitness components (i.e., survival and growth) (Baba et al., 2007; Bannister et al., 2019; Baxter et al., 2012; Sievers et al., 2017). Studies focused on impacts from cnidarian fouling

* Corresponding authors.
E-mail addresses: mariadelmar.boschbelmar@unipa.it (M. Bosch-Belmar), giacomo.milisenda@szn.it (G. Milisenda).

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on farmed mussels, reported predation on molluscs larvae, as well as space and food competition causing reduced shell growth and lower population densities (Fitridge and Keough, 2013; Sievers et al., 2013). In the Mediterranean Sea, recent research highlighted the potential detrimental effect of cnidarian fouling on farmed species by the warm-water affinity hydroid, *Pennaria disticha,* one of the most problematic species due to its high growth rate, huge colonies, and painful stings (Bosch-Belmar et al., 2019, 2021). Methods and strategies to prevent or eliminate biofouling aggregations from aquaculture facilities structures are various and constantly evolving (Bannister et al., 2019; Fitridge et al., 2012). Nevertheless, some of the present-day mitigation methods (cage nets change or in-situ cleaning process) entail high economic costs to the companies or can generate additional problems to farm production (Bloecher et al., 2018; Bosch-Belmar et al., 2017; Sievers et al., 2017). The use of on-site underwater cleaning techniques using high water pressure, release huge quantities of fouling fragments into the water column, including hydroid pieces containing harmful cells (i.e., cnidocytes) (Baxter et al., 2012; Carl et al., 2011). This generates stressful environmental conditions for most pelagic and benthic farmed and wild species, inflicting tissue injuries that can cause respiratory problems and osmoregulation disorders (Baxter et al., 2012). The described situation may impact the economic performance of aquaculture facilities and impair the health of the local wild biodiversity. How the interaction between this kind of biotic stressor and other abiotic sources of disturbance may affect farmed organisms' performance remains understudied. Determining the effects of such complex interactions is essential to better understand disturbance processes and may help to identify ecological priorities for conservation actions at the local level as well as to support effective management of economic human activities linked to aquaculture, under both current and future CC scenarios.

Here, we designed a series of single and multiple stressor experiments to investigate the effects on farmed mussels' functional traits (Díaz and Cabido, 2001; Schoener, 1986), when exposed to harmful cnidarian cells within environmental conditions of increasing temperature and short-term hypoxia. We simulated an on-site cleaning process of an IMTA system including stocks of filter-feeders' (*Mytilus galloprovincialis*) that can be potentially impacted by the in-situ underwater cleaning process waste.

2. Materials and methods

The study involved three different experimental trials, a single stressor and two multiple stressor experiments. The single stressor experiment was designed to investigate the effects on *Mytilus galloprovincialis* functional traits when exposed to *Pennaria disticha* cnidocytes. This simulated the periodic biofouling cleaning activity on IMTA aquaculture cages, as hydroids represent one of the most important components. Multiple stressor experiments focused on the interaction between the effect of *P. disticha* and increasing temperatures, and short-term hypoxia events (to simulate the late summer period). In all cases, the study on functional traits was carried out by measuring individual filtration ability (clearance rate, CR) and oxygen consumption (respiration rate, RR). In the single stressor trial gill damage was also assessed (inspection of the gill epithelium morphology).

2.1. Experimental set-up

Specimens of *M. galloprovincialis* of commercial size (55.2 ± 4.8 mm) were collected in September 2020 from an aquaculture marine facility located in Faro Lake (Messina, Italy - 38°15′59.95″N, 15°38′19.56″E), packed in an insulated container and cooled with dry ice, to keep them moist during transportation to the Laboratory of Ecology - University of Palermo. Mussels were carefully cleaned and placed in a 300 l tank equipped with a biological filter, filled with natural seawater at sampling site temperature (22–23 °C) and salinity (37–38 psu), to allow acclimation for approximately two weeks (Sarà et al., 2013). Specimens were fed daily ad libitum with cultured *Isochrysis galbana* (Sarà et al., 2013). Temperature was continuously monitored using HOBO Pendant® loggers (mod. MX2201, ± 0.5 °C accuracy), while oxygen concentration was monitored using PyroScience FirestingO_2 oxygen loggers. Water pumps were responsible for water movement and recirculation, and 10% of the water was changed daily, removing all faecal material from the bottom of the tank.

Pennaria disticha colonies were collected along the coast of Palermo, Italy (38°11′12.786″N; 13°21′41.1336″E). Hydroids were gently sampled cutting off the hydrorhiza to preserve the hydrocaulus and were brought back to the laboratory and placed in 60 l tanks filled with filtered seawater and aerated by water pumps.

2.1.1. Single stressor

Once acclimated, 124 *M. galloprovincialis* specimens were randomly selected and divided into 4 equal-sized groups ($n = 31$), transferred to 4 independent rectangular glass tanks of 60 l capacity, each equipped as previously described, and kept in an air-conditioned room at 23 °C. Mussels from tanks 1–2 were exposed to fragments of *P. disticha* (~ 8 g of cut hydrocaulus and calyx containing stinging cells, per treatment) (treatment hereafter called PEN) for a period of 4 h simulating the average duration of an in-situ underwater cleaning operation (Bloecher et al., 2018). Tanks 3–4 acted as a control (not exposed to hydroid fragments, NoPEN). *Pennaria disticha* concentration was chosen according to Bloecher et al. (2018) calculations accounting for the farming cages dimension and the mean density of hydroid colonies/polyps attached to the net.

Specimens were fed before and during the exposure to PEN treatment simulating natural field conditions. Mussels' RRs and CRs were measured immediately after the end of the stressor exposure and after 4 h, 12 h, 24 h and 48 h, while at 0 h, 12 h, 24 h and 1 week, three specimens from each treatment were sampled and gill tissue was dissected and fixed in 10% neutral-buffered formalin for histological analysis.

2.1.2. Multiple stressor

Once acclimated, 160 specimens were randomly divided into 4 equal-sized groups ($n = 40$) and transferred to 4 independent 60 l rectangular glass tanks. Tanks were kept in an air-conditioned room and temperature was gradually increased from 23 °C to 28 °C, over 48 h to simulate late summer conditions in which naturally periodic hypoxic events can occur (Díaz and Rosenberg, 2011; Reid et al., 2019). Two tanks were used to test the effect of contact with *Pennaria* in high temperature conditions (tanks exposed to *Pennaria* at high temperature conditions, PEN + HighTemp treatment). The remaining two tanks were used to investigate the additional effect of hypoxia (tanks exposed to *Pennaria* with high temperature and hypoxic conditions, PEN + HighTemp + Hyp). Short-term exposure (4 h) to hypoxic conditions (2 mg l^{-1} O_2) was achieved by bubbling gaseous nitrogen (N_2) directly into the tanks and a transparent film was used to prevent gas exchanges with the environment. Mussels' RRs and CRs were measured directly at the end of the MS exposure and after 4 h, 24 h and 48 h.

2.2. Measures of stressors' effect

2.2.1. Respiration rate

The rate of oxygen consumption (mg O_2 h^{-1} g^{-1}DW) was determined using respirometric chambers (0.3 l) containing filtered (Whatman GF/C 0.45 μm) saturated-air, 38 psu seawater. To ensure the constant mixing of water, each chamber was stirred with a bar magnet and an individual stirring device (Sarà et al., 2013). Temperatures were kept stable by means of a circulated thermal bath (Grant Optima TX150) and additionally monitored throughout the recording period using HOBO Pendant® loggers. A total of $n = 50$ and $n = 40$ mussels were used respectively for the single and the multiple stressor experiments, and a total of 5 individuals were used at each experimental time for each

treatment (randomly chosen between the two corresponding tanks). The decline in oxygen concentration was continuously recorded for at least 1 h, excluding an initial period (~ 10 min) when usually there is a more rapid decline in oxygen caused by a disturbance of the sensor's temperature equilibration. RR was calculated according to Sarà et al. (2013):

$$RR = (C_{t0} - C_{t1}) \text{ x } Vol_r \text{ x } 60(t_1 - t_0)^{-1}$$

where the C_{t0} is oxygen concentration at the beginning of the measurement, C_{t1} is the oxygen concentration at the end of the measurement, and Vol_r is the volume of water in the respirometric chamber. After RR measurement, each animal was killed by gentle freezing and dissected. The shell was separated from the body tissue to calculate their individual dry weights (after drying 24 h at 95 °C in the oven) and to standardize RR to body weight.

2.2.2. Clearance rate

Clearance rate is defined as the volume of water cleared of suspended particles per hour, and is determined by measuring the exponential decline of suspended algal cells added to filtered seawater in a closed system such as a 1 l glass beaker over a period of 2 h (Sarà et al., 2008). Specimens of *M. galloprovincialis* were gently placed into separate beakers, each containing 1l of filtered seawater and a magnetic stirrer bar placed on the opposite side of each individual, in order to avoid physical disturbance. In order to allow mussels to open their valves and start the filtration activity, they were given 15 min before the addition of food (~ 25,000 *I. galbana* cells ml^{-1}, representative of natural concentrations) thus avoiding pseudofaeces production and the inhibition of CR, as suggested by Widdows and Staff (2006). Two control beakers, containing only sea water and algal cells acted as a control, ensuring no significant decline in cell concentration over the entire experimental period. Five minutes after food was added an aliquot of 20 ml was collected from the center of each beaker using a large syringe with a rubber tube at 30 min. Intervals over a 2 h period. The decline in algal cell concentration was monitored using a Coulter Counter (Beckman Coulter© Model Z2), fitted with a 100 μm aperture tube and set to count particles between 2 and 6.5 μm. CR was calculated from the Coughlan (1969) equation:

$$CR\ (l\ h^{-1}) = Vol \text{ x } (C1 \blacksquare C2)/\text{time interval (h)},$$

where Vol is the volume of water (l), and C1 and C2 are the initial and final cell concentrations at each time increment. The maximum clearance rate of each mussel was then calculated, based on a period of two consecutive time increments (i.e., 30 mins), during which the decline in cell concentration was the greatest. After CR measurement, each specimen was processed as described above for RR measurements to standardize CR to body weight.

2.2.3. Gills histological analysis and injury scoring

Samples were processed using standard formalin-fixed paraffin embedded tissue histology procedures, involving a sequence of dehydration in ethanol, consecutive xylene baths and inclusion in paraffin wax. Tissues were mounted in histology blocks and 4 μm-thick sections were stained using Haematoxylin and Eosin (H&E) for histopathological analysis. Samples were analysed using a light microscope (Nikon eclipse e200, Nikon Instruments). The analysis involved the evaluation of several histological features (enlarged central vessel, ECV; loss of cilia, LC; haemocyte infiltration, HI; hypertrophy of goblet cells, HGC; and melanin/lipofuscin deposits, MLD) using the following scoring system according to the affected gill tissues area: 0 = less than 10% of gill tissues affected; 1 = 10–25% of gill tissues affected, 2 = 25–50% of gill tissues affected and 3 = more than 50% of gill tissue impacted (adapted from Pagano et al., 2016).

2.3. Index creation and statistical analysis

2.3.1. Effect size index

To measure the effect of single or multiple stressors on treated organisms' traits with respect to controls, the effect size index was applied (Hedges and Olkin, 1985). The 5 replicates for each treatment and monitoring time were identified by a numerical id by assigning a value from 1 to 5. Once the individual RR from single stressor experiments were measured, an effect size index "m" was calculated using the following formula:

$$m = \frac{RR\ PEN_{i,t}}{RR\ NoPEN_{i,t}}$$

where "m" is the effect size index, RR PEN $_{i,t}$ and RR NoPEN $_{i,t}$ are the oxygen consumption rates of the i-th specimens at a given sampling time "t", treated (PEN) and not treated (NoPEN). At each time interval, RR of each treated specimen was divided by RR of each untreated specimen (1:1; 1:2; 1:3, 1:4; 1:5, 2:1, …, 5:3; 5:4; 5:5), obtaining at the end of the processing 25 "m" values per monitoring period. The effect size "m" can have values ranging from 0 to infinite. When it has a value of 1 this indicates that there is no difference between treated and control groups; the closer it is to 0 the higher is the oxygen consumption of the control compared to the treated group, while values greater than one indicates that the treated group has a higher rate of oxygen consumption than the control.

For both MS experiments the same procedure was followed. A first set of "m" was calculated as:

$$m = \frac{RR\ PEN28_{i,t}}{RR\ PEN23_{i,t}}$$

where "m" is the effect size index for the combination of two stressors (PEN and HighTemp), RR PEN23 $_{i,t}$ and RR PEN28 $_{i,t}$ are the oxygen consumption rates of the i-th specimens at a given sampling time "t", at low temperature (PEN) and at high temperature (PEN + HighTemp).

The effect of hypoxia has also been calculated as:

$$m = \frac{RR\ PEN28\ HYP_{i,t}}{RR\ PEN28_{i,t}}$$

where "m" is the effect size index for the combination of the three stressors (*P. disticha*, High Temperature and Hypoxia), RR PEN28 i,t and RR PEN28 $Hyp_{i,t}$ are the oxygen consumption rates of the i-th specimens at a given sampling time "t", at high temperature (PEN + HighTemp) and at high temperature and hypoxia (PEN + HighTemp + Hyp).

CR's effect size was calculated following the same approach as for RR data, for both the single stress experiment (PEN vs NoPEN), and the two MS experiments ("PEN + HighTemp" and "PEN + HighTemp + Hyp").

2.3.2. Statistical analysis

To test for significant differences in respiration and clearance rates over time, regression analysis was performed, using the effect size "m" as a respondent variable and the time elapsed since the end of exposure to the stressor(s) as the independent variable. Both generalized linear models (GLM) and generalized additive models (GAM) were tested, assigning a Gamma-type error family distribution, since the response variable can only take positive values. The best model was then selected by comparing the AIC values and the normality of the residuals, as well as by confirming the absence of particular trends in the residuals. All the statistical analyses were performed using R software (R Core Team, 2021).

3. Results

Exposure to *P. disticha* fragments significantly impacted both mussels' RR and CR (Table 1, Fig. 1). Treated individuals showed low

Table 1
Results from Generalized Additive Model (GAM) analysis for each experimental trial (single and multiple stressor experiments) where: "PEN" vs "NoPEN" means contact/no contact with *Pennaria disticha* cnidocytes at natural environmental temperature, 23 °C; "PEN + HighTemp" refers to exposure to *P. disticha* at a high temperature of 28 °C; "PEN + HighTemp + Hyp" includes the addition of hypoxia along with *Pennaria* and high temperature conditions.

Single stressor			
"PEN" vs "NoPEN"	Df	F_{value}	p_{value}
RR	2.820	5.5	0.004
CR	2.894	8.27	< 0.001

Multiple stressor			
"PEN + HighTemp" vs "PEN"	Df	F_{value}	p_{value}
RR	2.283	8.398	< 0.001
CR	2.956	35.75	< 0.001

"PEN + HighTemp + Hyp" vs "PEN + HighTemp"	Df	F_{value}	p_{value}
RR	1.538	7.258	0.005
CR	2.722	6.103	0.004

metabolic response and high filtering activity (with respect to controls) immediately after the period of contact with the hydroid fragments. An accelerated metabolism (increasing RR) was recorded during the following 12 h, then a decrease in the RR values (from 12 h to 30 h), followed by a new rise in oxygen consumption until the buffering of the "Pennaria effect" (treat = ctrl) after 48 h. CR showed the opposite trend; a rapid decrease and consecutive increase in the filtering activity was observed over the 30 h period following the end of the exposure to the hydroid, buffering the effect of the stressor at the end of the monitoring period.

Histological analysis showed a significant impact from contact with cnidarian stinging cells on the mussels' gill epithelium (Fig. 2). Observations of samples revealed multifocal degeneration of the gill lamellae in treated organisms. Moreover, increasing values in ECV were observed over time, peaking at 48 h after the end of exposure to *Pennaria* stinging cells. Also, marked cilia loss was recorded immediately after the end of the biotic stressor exposure and an apparent recovery during the following days was observed. The other three features (HI, HGC and MLD) investigated, did not show differences with respect to controls or over time.

Regarding MS experiments, the combined stress from *Pennaria* exposure and increasing temperature conditions significantly impacted both RR and CR (Table 1, Fig. 3). Results from organisms' responses to combined stressors revealed filtering activity decreased remarkably within the first 12 h after the end of exposure to the stressors. Values reached and exceeded those of the controls after 30 h, maintaining slightly higher CR values during the rest of monitoring period (F_{value} = 35.75, Df = 2.95, $P_{value} < 0.001$). Overall, mussels' RR was higher with respect to control buffering at the end of the monitoring period (F_{value} = 8.39, Df = 2.28, $P_{value} < 0.001$).

Equally, the combination of cnidarian contact, acute high temperature and hypoxic conditions significantly affected RR and CR (Table 1, Fig. 4). Treated organisms presented high RR values at the start of monitoring but suffered a metabolic depression over time (F_{value} = 7.25, Df = 1.53, P_{value} = 0.005). Filtering activity maintained low values in exposed individuals for almost 24 h, then experienced a sharp increase followed by a decrease in the last part of the curve (F_{value} = 6.10, Df = 2.72, P_{value} = 0.004).

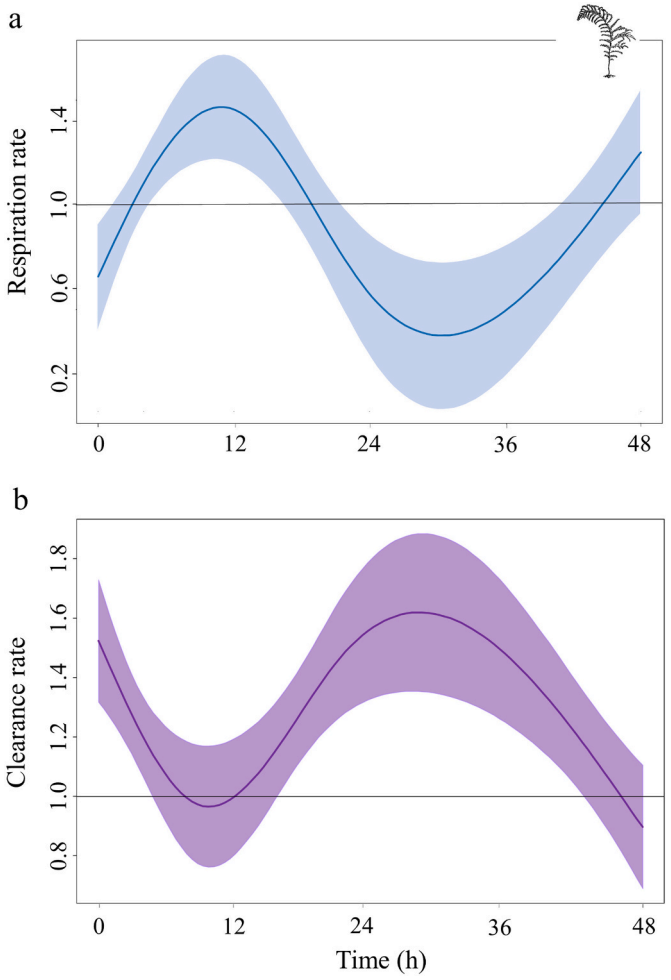

Fig. 1. Respiration rate (RR) (a) and clearance rate (CR) (b) of individuals exposed to *P. disticha* (Single stressor: PEN vs NoPEN treatments). Horizontal line represents the buffer condition where controls and treated organisms present the same values of RR and CR. Values above the line means that treated mussels experienced higher respiration or clearance rates with respect to controls; while values below the line indicates that controls showed higher rates.

4. Discussion

Results from multiple stressor experiments such as this one, are essential to aid our understanding of stressors' combined impacts and interaction, generating more realistic scenarios that will improve our comprehension of global climatic change impacts on marine ecosystems and ecosystem-dependent anthropogenic activities (Weber et al., 2020). Here, we proposed an integrated approach to investigate farmed mussels' functional traits when exposed to combined biotic and abiotic stressors that can occur during routine cleaning processes in marine aquaculture. Results showed that the short-term exposure to different stressor combinations stimulates organisms' prompt responses, influencing metabolism and filter feeding performance, as well as the ability to recover and the temporal dynamics after the end of the disturbance event. Exposure to stinging foulers may elicit metabolic compensation mechanisms represented by oscillating RR and increasing CR values in treated mussels, that reach a compensation level after 48 h. Morphological analysis on gill epithelium showed typical primary responses to acute injury characterized by vascular alterations (the enlargement of the central vessel) and necrosis of tissue involving the loss of cilia. These are potential markers of the first stages of gill damage in mussels, with significant increased vascularization (as demonstrated by the high ECV values) after the loss of cilia from gills filaments (Pagano et al., 2016).

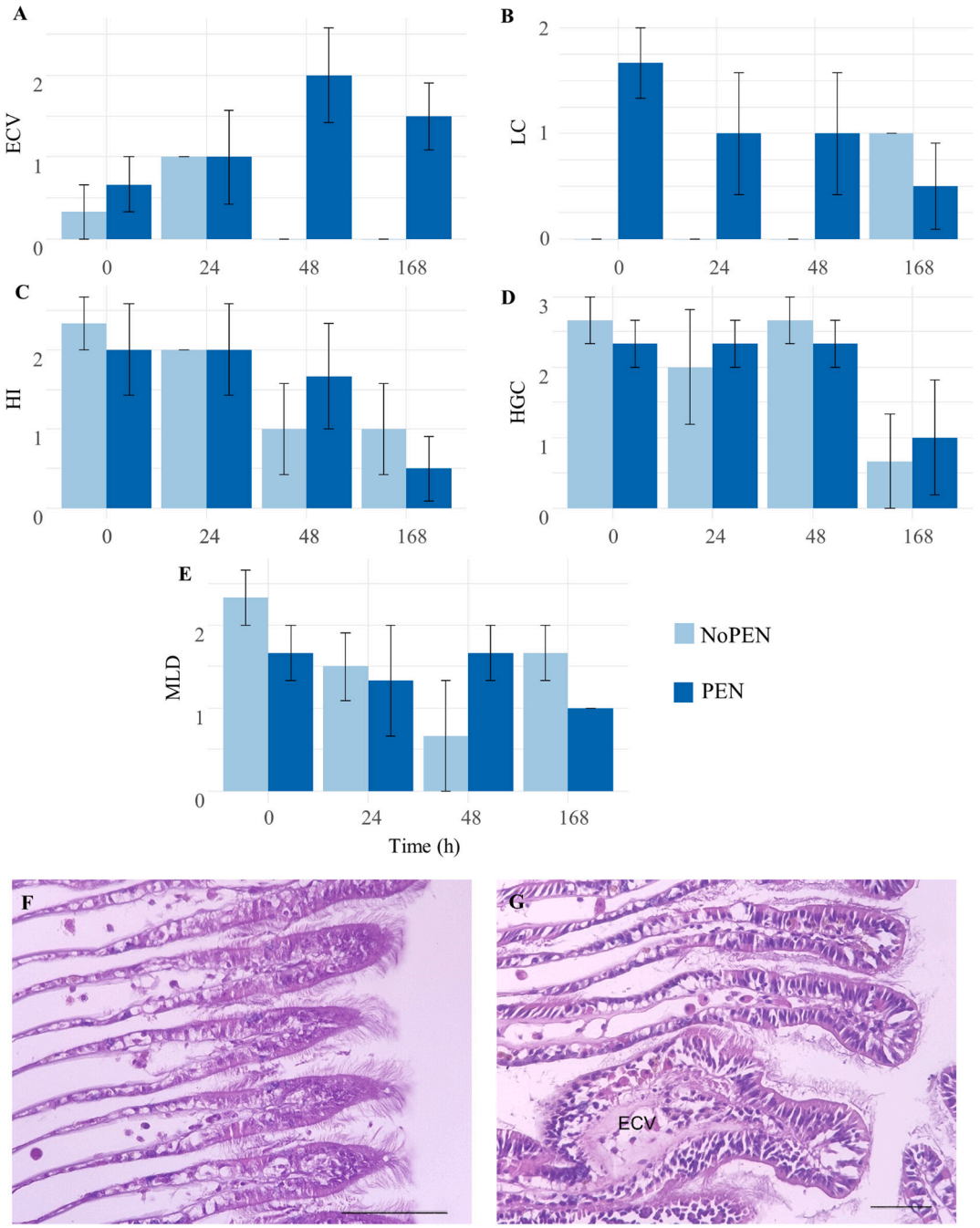

Fig. 2. Morpho-histological analysis on gill lamellae. A to E: gill injury scoring for each investigated feature on mussel gill epithelium: enlarged central vessel (ECV), loss of cilia (LC), haemocyte infiltrates (HI), hypertrophy of goblet cells (HGC), melanin/lipofuscin deposit (MLD). Graphics legend: PEN refers to contact with *P. disticha*, while NoPEN refers to controls. F and G: High power photographs of non-treated gills (F) and Pennaria-exposed gills (G). Vascular disturbaces (ECV) and cilia loss is noticeable. Stain: haematoxylin & eosin. Scale bar 50 μm.

The loss of cilia could be considered as the start of gill exfoliation, that if severe could lead to feeding difficulties and breathing issues, compromising individuals' fitness (Auffret, 1988; Carballeira et al., 2011). Differences in the levels of HI, HGC and MLD were less evident between treated and control organisms, even if signs of advance inflammatory responses were observed in some samples, presenting huge deposits of lipofuscin and hemocyte infiltrates (De Vico and Carella, 2012). Moreover, multifocal degeneration of several lamellae was observed during the analysis, preventing a complete scoring evaluation.

Global warming is a highly relevant stressor in aquatic ecosystems as temperature is a key factor regulating all biological processes (Hoegh-Guldberg and Bruno, 2010; Kooijman, 2010; Yvon-Durocher et al., 2012). Increasing temperature usually leads to increasing oxygen demand and metabolic and ingestion rates in ectotherms (Anestis et al., 2010; Brown et al., 2004; Parisi et al., 2021; Pörtner and Farrell, 2008; Steeves et al., 2018). Previous studies on *M. galloprovincialis'* - one of the most physiologically plastic *Mytilus* species (Gosling, 2022) - response to thermal stressors have shown that the species did not exhibit a physiological response to increasing temperatures (Braby and Somero, 2006; Collins et al., 2020). Nevertheless, the joint action of increasing temperature and *Pennaria* treatment may present a potential synergistic effect on mussels' metabolism by increasing oxygen consumption rate to maintain aerobic metabolism. In fact, mussels' respiration rate affected by *P. disticha* significantly increased up to 80% when considering global

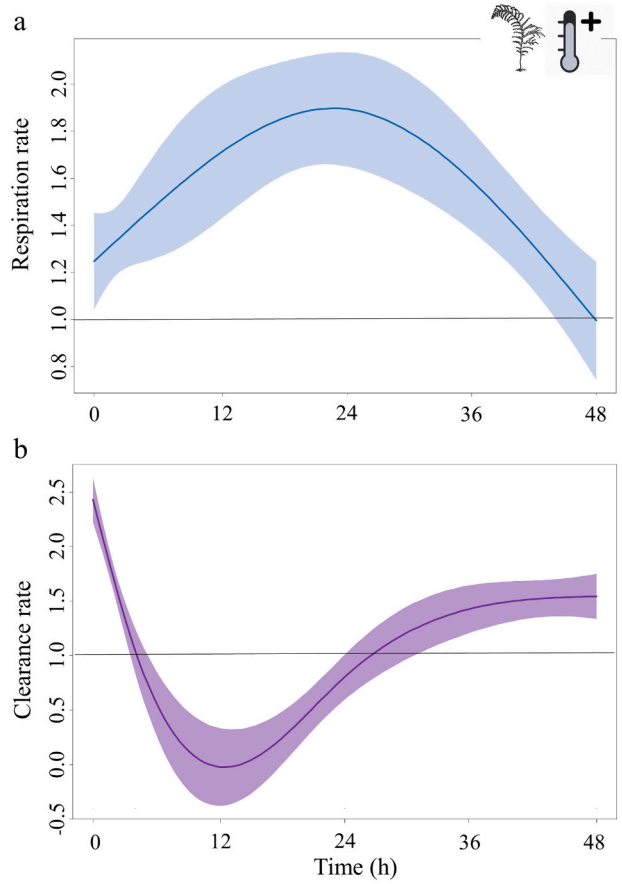

Fig. 3. Respiration rate (RR) (a) and clearance rate (CR) (b) of individuals exposed *P. disticha* and high temperature (Multiple stressor: PEN + HTemp). Horizontal line represents the buffer condition where controls and treated organisms present the same values or RR and CR. Values above the line means that treated mussels experienced higher respiration or clearance rates with respect to controls; while values below the line indicate that controls showed higher rates.

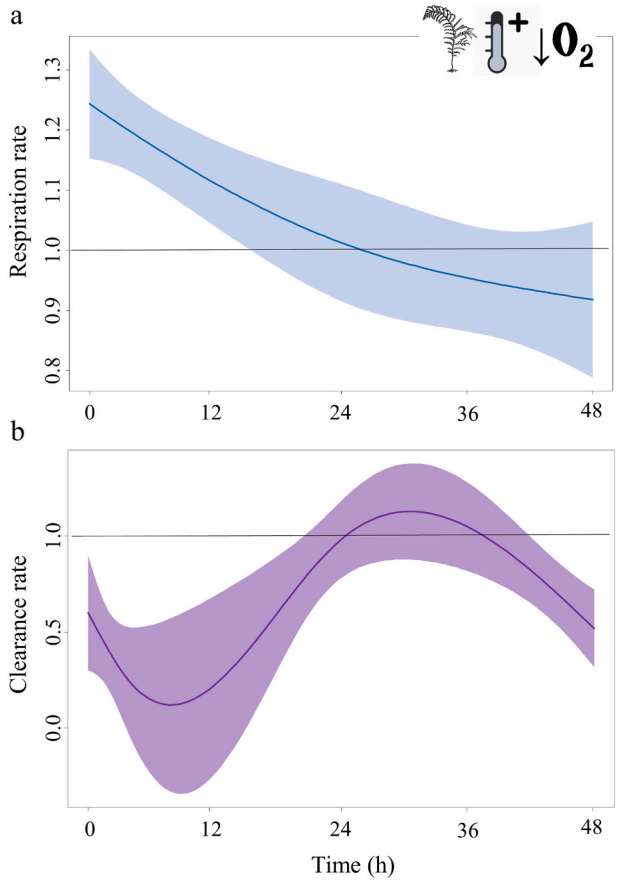

Fig. 4. Respiration rate (RR) (a) and clearance rate (CR) (b) of individuals exposed to contact with *P. disticha* and high temperature and hypoxia conditions (Multiple stressor: PEN + HighTemp + Hyp). Horizontal line represents the buffer condition where controls and treated organisms present the same values or RR and CR. Values above the line means that treated mussels experienced higher respiration or clearance rates with respect to controls; while values below the line indicate that controls showed higher rates.

warming scenarios with respect to the current climatic situation (as shown in Fig. 3). At the same time, the combined effect of increasing temperature and contact with stinging foulers may exert an antagonist action on feeding performance, through the immediate and complete inhibition of feeding (− 90%) that tends to rise after 24 h maintaining slightly higher clearance rates at 48 h. The opposite patterns, between respiration and clearance rates, revealed in experimental organisms when exposed to single (*Pennaria*) and multiple (*Pennaria* plus high temperature) treatments, could reflect the individual's low ability to cope with the stressful conditions, switching from one compensatory mechanism to another to overcome the changing environment.

Elevated surface temperature usually leads to water column stratification, that together with the decline in oxygen solubility (inherent to increasing temperature; Verberk et al., 2011) would exacerbate the problem caused by increased respiratory activity, potentially triggering hypoxic events. Low levels of dissolved oxygen have been associated with impaired metabolism and feeding performance in bivalves by reducing shell growth and survival (Gu and Jun, 2018; Gu et al., 2019; Leung and Cheung, 2018). Exposure to detrimental conditions caused a metabolic depression in treated mussels, causing a 10% reduction in respiration rate at the end of the monitoring period (48 h). This condition may stimulate a switch from aerobic to anaerobic metabolism (Anestis et al., 2007), as has been previously reported for the species when exposed to thermal stressors, keeping the valves closed and experiencing a decrease in metabolic rate, to potentially limit the mussel's depletion of oxygen in the mantle tissue (Anestis et al., 2010; Artigaud et al., 2014). Equally, feeding performance seems impaired, presenting overall low feeding rates during the experiment.

Multiple stressors action may also affect species thermal tolerance by reducing the width of their thermal window (Pörtner et al., 2017; Sokolova et al., 2012). Jansen et al. (2009) reported that *M. galloprovincialis'* upper thermal limit was between 27 °C and 30 °C. In the MS conditions presented here, an environmental temperature of 28 °C (which is a common value present during late summer periods in the southern Mediterranean coasts that can last several days and even weeks), may represent a temperature value slightly above the upper species' thermal tolerance threshold and may entail significant impacts on mussels' growth and survival (sensu, Kooijman, 2010). Even if our experimental approach is based on short-term exposure events, the cumulative action of biotic and abiotic stressors provoked a performance impairment in experimental organisms and potentially delayed or inhibited their ability to recover. These results are particularly concerning since future scenarios of climate change and ocean warming foresee increasing gelatinous organisms' populations as a potential driver of negative impacts on fishery and aquaculture (Barange et al., 2018; Boero et al., 2016).

Despite the significant role of all the tested stressors, temperature was the most influential source of disturbance on organisms' metabolic and filtering ability, being responsible for the highest differences in respiration and clearance rates during the experiments (80% and - 90% respectively, Fig. 3). Longer or more frequent exposure to these stressful

situations may have devastating effects on farmed organisms' fitness or natural population dynamics, therefore more research focused on stressors properties and their combined impact is needed to understand the species ability and mechanisms to cope with stressful conditions. In the current and future contexts of CC, the duration and frequency of stressors affecting natural ecosystems functioning and human economic activities may increase, and the multiple interactions as well as the sequence of exposure to these stressors may jeopardize the resilience ability of ecosystems and production activities (Jackson et al., 2021; Sarà et al., 2021). Improving our understanding of the cumulative impacts of multiple stressors is critical for ecosystem conservation and effective management of economic activities (Orr et al., 2020).

CRediT authorship contribution statement

Mar Bosch-Belmar: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Antonio Giacoletti:** Investigation, Formal analysis, Writing – review & editing. **Chiara Giommi:** Investigation, Writing – review & editing. **Albert Girons:** Investigation, Writing – review & editing. **Giacomo Milisenda:** Conceptualization, Investigation, Formal analysis, Resources, Supervision, Writing – review & editing. **Gianluca Sarà:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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