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Functional responses of intertidal bivalves to repeated sub-lethal, physical disturbances

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ABSTRACT

In coastal habitats, physical disturbances of benthic organisms can be caused by natural events like wave-born objects and human activity like trampling, and these disturbances can be sub-lethal (e.g., resulting in the organism's displacement). We know little of how sessile organisms respond to physical disturbances such as displacements. Using *Mytilaster minimus*, a mussel that is native to the Mediterranean Sea, we tested how byssus production and oxygen uptake rates changed in response to different frequencies of disturbance events (10–60 events h^{-1}). Mussels increased oxygen uptake rates but not byssus production with increasing disturbance frequencies (50–60 events h^{-1}). Our results show that sub-lethal, physical disturbances can cause increased physiological rates in mussels if disturbances repeat rapidly. Therefore, sub-lethal, physical disturbances can have negative consequences for benthic organisms even if they do not cause immediate damage or mortality.

1. Introduction

An ecological disturbance is a temporary event that interferes with the normal structure and function of an ecosystem on some level of organization (Sousa, 1979). In the most extreme cases, a disturbance results in the death and removal of organisms from a habitat, which in turn creates new space in the habitat that is available for colonization by other organisms (Sousa, 1979; Gunderson et al., 2016). On the other hand, sub-lethal disturbances can affect organisms through damage or displacement, or by disrupting the organisms' normal behaviors (e.g., Paine, 1974). In rocky intertidal zones, sessile organisms frequently experience sub-lethal, physical disturbances in the form of displacements, or movements. For example, the substratum can become unstable and overturn (Wright and Shanks, 1995), hydrodynamic forces or projectiles can partially dislodge the organism (Shanks and Wright, 1986), and predators can move the organism during attempted predation events (Giacoletti et al., 2016). Despite the common nature of sub-lethal, physical disturbances in the intertidal zone, little is known about how these events affect sessile organisms (e.g., Waller et al., 1999; Lacoste et al., 2002).

Mytilid mussels are sessile organisms that are the dominant habitat-formers and competitors for space in the rocky intertidal zone (Paine, 1974). Mussels avoid dislodgement from the shore by living in dense aggregations and producing collagenous byssal threads that anchor themselves to the substratum (Bell and Gosline, 1996), yet dislodgement and displacement still occur (Mendez et al., 2018). A mussel's response to dislodgement and displacement can involve energetically unfavorable components (Carrington et al., 2015). First, the mussel strengthens its attachment to the substratum by increasing the number or strength of byssal threads. Attaching new byssal threads can happen quickly (< 10 min; Wright and Shanks, 1995), and the strength of byssal threads can change with the local hydrodynamic conditions (Bell and Gosline, 1996). While strong byssal threads can minimize the mussel's risk of dislodgement (Schneider et al., 2005), producing byssus requires a large energy expenditure that can reduce the mussel's capacity for reproduction (Babarro and Carrington, 2013). Second, mechanical stimuli from displacements can prompt the mussel to close its valves, inherently preventing it from filter-feeding and taking in energy (Rovero et al., 1999). Valve closure can also be followed by a decrease in the mussel's metabolism that helps the mussel cope with the low oxygen conditions that can develop within their shells, as well as missed feeding opportunities (Widdows and Shick, 1985). However, mechanical stimuli that occur while the valves are closed (e.g., predation attempts) can prompt the mussel to increase its metabolism as part of a stress response (Rovero et al., 1999). Upon recovery and opening its valves, the mussel can show large increases in oxygen uptake rates – well above baseline values – that are associated with reoxygenation of the hemolymph and recovery from a long period of reduced oxygen

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uptake (Shick et al., 1988). In total, a mussel's response to physical disturbance is energetically unfavorable: closing the valves and ceasing energy intake, but then producing byssus to secure attachment to the substratum. It is unknown how these combined responses affect the mussel's physiological recovery from disturbance, especially with prolonged or repeated disturbances.

Here, we examined how the intertidal mussel *Mytilaster minimus* responded (i.e., byssus production, oxygen uptake rates) to disturbances (i.e., series of displacements) that were repeated at different frequencies. *M. minimus* is a dominant habitat-forming and space-occupying organism on rocky shores in the Mediterranean Sea (Sarà and De Pirro, 2011; Morton and Puljas, 2018) where disturbances occur from all of the aforementioned natural sources, as well as anthropogenic sources like boat wake, trampling by humans, or handling by humans (Waller et al., 1999; Mendez et al., 2018). Our specific goal was to test the hypotheses that the frequency of disturbance events affects the duration and magnitude of the mussel's response to the disturbances. We predicted that rapidly repeating disturbance events would prompt *M. minimus* to (1) increase byssus production during the disturbances because some Mytilids are known to produce byssus after physical disturbances (Wright and Shanks, 1995), and (2) increase oxygen uptake rates after the disturbances because some Mytilids respond to unfavorable environmental conditions by closing their valves, suppressing oxygen uptake rates, and then exhibiting elevated oxygen uptake rates during recovery (Widdows and Shick, 1985). We further predicted that disturbances would only affect the mussel's response when the disturbances occurred above a certain frequency (i.e., the critical disturbance frequency), or when the time between disturbances was shorter than the time needed for the mussel to recover from an individual disturbance, and that the time to recover would increase with disturbance frequency. Identifying this critical frequency can help to predict the responses of *M. minimus* and similar organisms to regularly occurring disturbances, like displacements, in their environment, especially for mussels in areas that are heavily impacted by human activity. Furthermore, understanding the mussel's functional responses to repeated, but short-term and sub-lethal disturbances can provide information about how longer bouts of repeated, sub-lethal disturbances will affect mussels at the individual- and population-levels (e.g., Buckley et al., 2001).

2. Methods

2.1. Collection

Mytilaster minimus were collected from the vermetid reefs located in Capo Gallo and Isola delle Femmine Marine Protected Area (MPA) close to Palermo, Italy (38°11′53"N; 13°14′36"E) in July 2014 and transported to the laboratory at the University of Palermo. Mussels were haphazardly collected over a distance of approximately 10 m, so all mussels originated from a similar wave exposure. Adult *M. minimus* can have shells up to 16 mm long (Morton and Puljas, 2018), and only adults near this upper size limit were collected. Water at the collection site had a salinity of 37 ppt and temperature of 25 °C (http://www.mareografico.it/). After collection, mussels were held in aerated seawater (salinity = 37 ppt, temperature = 25 °C) between 2 and 3 days prior to experimentation.

2.2. Simulating disturbance

Individual *M. minimus* were scrubbed clean of epibionts, existing byssal threads were cut away from the mussels, and the mussels were placed in separate 1.5 mL microcentrifuge tubes with each mussel's posterior end facing upward. The diameter of the tubes closely matched the diameter of the mussels, stabilizing the mussels such that they did not move within the tubes during the experiments. The small size of the microcentrifuge tubes also limited the motion of water relative to the mussels when the tubes were moved, minimizing hydrodynamic signals from the experiment that the mussels might perceive (i.e., reducing hydrodynamic signals was important for measuring the mussels' responses to the movement of their bodies rather than the mussels' responses to the mechanical effects of water motion). The microcentrifuge tubes were filled completely with well-oxygenated seawater from the holding tank and then sealed. A custom-built apparatus was used to produce displacements. The apparatus was an Arduino-controlled stepper motor that oscillated a stage about the horizontal axis. For each trial, ten mussels in their microcentrifuge tubes were placed on the oscillating stage, 2 cm from the axis of rotation so that all mussels received the same displacements (see Supplementary Material). For a control group (i.e., receiving no displacements), ten mussels were placed in individual microcentrifuge tubes on a stationary stage next to the oscillating stage. The control group's tubes were filled with well-oxygenated water prior to being sealed. Mussels were allowed to recover from handling for 15 min before the experiments.

For each displacement the apparatus oscillated the mussels between the angles of −45° and +45° relative to the horizon with a period of 2 s (i.e., the mussels were not completely inverted) (see Supplementary Material). A disturbance event consisted of 30 displacements, lasting a total of 60 s. The time between disturbance events was varied to produce different disturbance frequencies. We subjected mussels to 10, 20, 30, 40, 50 and 60 disturbance events h^{-1} and used a random number generator to determine the order of the experiments. Each experiment lasted 1 h. Table 1 lists the length of rest periods between disturbance events for each frequency, and Fig. 1 shows a 10 min window of the disturbance regimes. Displacements were designed to mimic the general movement that mussels can experience when attached to an unstable substratum, or when dislodged from the substratum, in the back-and-forth water motion of waves, or when moved by the actions of another organism (e.g., predation attempts, trampling) (Wright and Shanks, 1995; Giacoletti et al., 2016). Additionally, displacements were designed to isolate the movement of disturbance events, rather than impact forces or compressive forces that might be exerted on the mussel during certain types of disturbance events (e.g., Burnett and Belk, 2018). The angles used encompass the back-and-forth movement that an unstable substratum can exhibit without inverting in wave-driven flow (Wright and Shanks, 1995). The disturbance frequencies used encompass the range of disturbance frequencies that can occur in nature from normal ocean waves, increased wave action from boat wake (e.g., several events h^{-1} in areas with heavy traffic; Demes et al., 2012), and trampling by humans (e.g., dozens of events h^{-1} depending on foot traffic; Brosnan and Crumrine, 1994). The duration of each event (1 min) was selected to represent a typical bout of disturbance caused by a passing boat or a group of humans walking along the shore (see above references).

2.3. Response to disturbance

After mussels experienced one hour of disturbance events, we measured their oxygen uptake rates to quantify their stress responses to the disturbances. Here we use a broad definition of "stress" to encompass the physiological consequences of responses to disturbance (e.g., closure of valves, reduced oxygen uptake, production of byssus)

Table 1
Disturbance frequencies and the rest periods between disturbance events.

Disturbance frequency (events h^{-1})	Rest period between disturbances (s)
10	300
20	120
30	60
40	30
50	12
60	0

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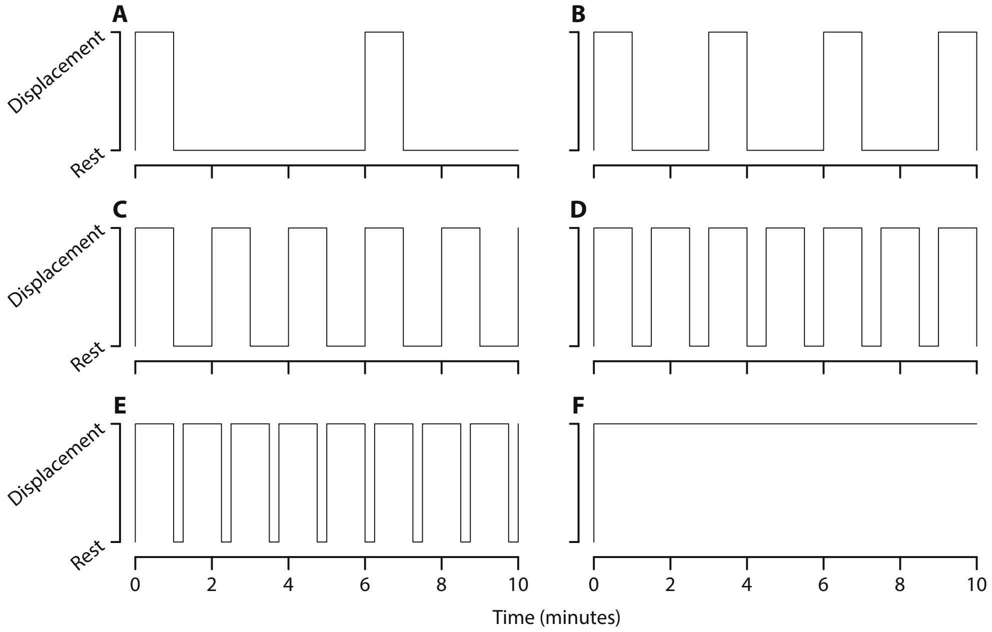

Fig. 1. Disturbance regimes for the first 10 min of each treatment. The frequencies of disturbance events were 10, 20, 30, 40, 50, 60 events h^{-1} for panels A-F, respectively.

that mussels exhibit (Babarro and Carrington, 2013). Following each disturbance regime, four mussels from each experimental and control group were selected with a random number generator. These mussels were removed from their microcentrifuge tubes and placed into individual respiration chambers. In each chamber, the mussel rested atop plastic mesh near the top of the chamber while a magnetic stir bar circulated water below. Oxygen uptake rates were measured over 1 h with FireStingO2 fiber-optic oxygen probes (Pyro Science, Aachen, Germany) and the manufacturer-provided software. The remaining six mussels of each group were left in their microcentrifuge tubes, and the byssal threads produced during the experiment (including acclimation and disturbance periods) were counted under a dissecting microscope. We did not count the byssal threads produced by mussels that were used for respirometry measurements because doing so would have caused additional disturbances to the mussels, like those used in the experiment. After the oxygen uptake measurements and the byssus counts, the soft tissues of each mussel were removed and weighed to the nearest 0.001 g using an analytical balance. Oxygen uptake rates were normalized by the wet weight of the mussels.

Oxygen uptake rates and byssus production were each compared between treatment and control groups of each disturbance frequency using Mann-Whitney U tests. To test whether oxygen uptake rates changed over the course of the recovery period, we divided the recovery period into 4-min intervals, calculated the oxygen uptake rate in that interval, and used Mann-Whitney U tests to compare those values between the treatment and control groups of each disturbance frequency. Last, because producing byssus requires energy, we tested whether there was a linear correlation between the median oxygen uptake rates and median byssus produced in the treatment and control groups. Median values were used because data were not normally distributed within every group. All statistical calculations were done in R Statistical Software version 3.5.0 (R Core Team, 2018).

3. Results

Mytilaster minimus used in the experiment weighed 0.024 ± 0.012 g (wet weight; mean ± SD). After one hour of a disturbance regime, mussels showed elevated oxygen uptake rates relative to controls for disturbance frequencies above 40 events h^{-1} (Fig. 2A, Mann-Whitney U tests, $p < 0.05$). In each disturbance frequency, comparisons of oxygen uptake rates in 4-min intervals showed results similar to the oxygen uptake rates calculated for the entire recovery period (Mann-Whitney U tests, $p = 0.05$ for significance). Thus, we were not able to quantify recovery times for the treatments (i.e., when oxygen uptake rates treatment groups matched those of control groups).

Byssus production was similar between the treatment and control groups of each disturbance frequency (Mann-Whitney U tests, $p > 0.05$; Fig. 2B), except in the 50 events h^{-1} treatment, where mussels in the treatment group produced more byssus than mussels in the control groups ($p = 0.034$). Not all mussels produced byssus: in each group, between 33% and 67% of the mussels produced byssus. For example, in the group that experienced constant disturbance (60 events h^{-1}), two mussels produced byssus and secured themselves to the walls of the microcentrifuge tubes.

We tested for a correlation between oxygen uptake rates and byssus production of the treatment and control groups. In each group, byssus production was not correlated with oxygen uptake rates (linear regression, $p > 0.05$). Additionally, oxygen uptake rates and byssus production of each group were not correlated to the order of measurements (Spearman's Rank-Order Correlations, $p > 0.05$) or the time of measurements (Pearson's Product-Moment Correlations, $p > 0.05$).

4. Discussion

We exposed *Mytilaster minimus* to sub-lethal, physical disturbances repeated at different frequencies, and we found that the mussel showed an elevated metabolic response to disturbances occurring more than 40 times h^{-1}. Based on these data, we hypothesize that when disturbances occurred at a slow frequency, the mussel could have recovered from each disturbance (e.g., reoxygenated its hemolymph) during the long rest period between disturbances (Fig. 2A). We further hypothesize that when disturbances occurred at a fast frequency, the mussel may not have completely recovered from each disturbance during the short rest periods before needing to close its valves again, so that after 1 h the mussel needed a large amount of oxygen to recover (Fig. 2A). The oxygen uptake rates of control mussels reported here were within the range of rates reported for *M. minimus* measured under undisturbed, standard laboratory conditions and under different concentrations of

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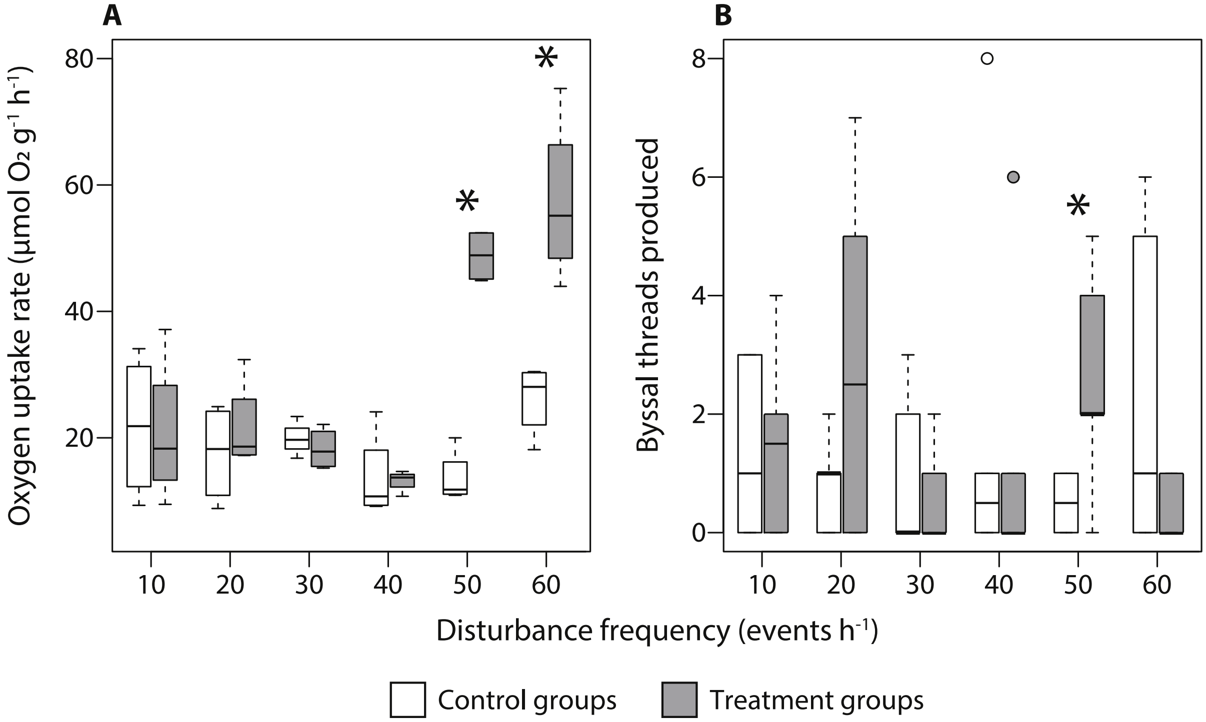

Fig. 2. (A) Oxygen uptake rates of mussels following 1 h of disturbance events and (B) byssal threads produced during the disturbance events. White boxes are control groups and gray boxes are treatment groups. Asterisks show significant differences between control and treatment groups (Mann-Whitney U tests, $p < 0.05$). Boxes indicate the quartiles around the median, error bars show the most extreme data point that is no more than 1.5 times the interquartile range from the box, and circles indicate values beyond 1.5 times the interquartile range from the box. Sample sizes for each group in (A) = 4, except for the control group with 30 events h^{-1} (n = 3). Sample sizes for each group in (B) = 6, except for the control group with 10 events h^{-1} (n = 5).

biologically-derived pesticides (Manachini et al., 2013). The disturbances used in this experiment mimicked disturbances that repeat over long time scales in nature (> 1 h), like waves crashing on the shore. While the results shown here indicate that 1 h of sub-lethal disturbance can increase metabolic rates in *M. minimus* during a 1-h recovery period, longer periods of sub-lethal disturbance and elevated metabolic rates could have additional negative consequences for the mussel such as reduced capacity for growth, reproduction, and somatic maintenance, reduced immune function, and even mortality (Lacoste et al., 2002; Sebens et al., 2018).

Byssus production was highly variable, and although mussels produced byssal threads in each treatment, byssus production differed only at a single, intermediate disturbance frequency (50 events h^{-1}; Fig. 2B). To our knowledge, there are no other records of byssus production for *M. minimus* with which we can compare our results. Rapidly producing byssal threads after a disturbance or dislodgement event can help the mussel remain attached to the substratum, and in nature, this could allow the mussel to survive subsequent disturbances (Schneider et al., 2005). However, unstable substrata, predators, or aerial exposure can continue to prevent the mussel from opening its valves and producing byssus after a disturbance event (Widdows and Shick, 1985; Wright and Shanks, 1995; Rovero et al., 1999).

Oxygen uptake rates and byssus production were not linearly correlated across all treatments for either the control group or the treatment group of mussels. Variation in oxygen uptake rates and byssus production in the control group may have been due to factors other than the timing of measurements, such as vibrations felt by the mussels through the laboratory bench from the experimental apparatus. For the treatment group, the increased oxygen uptake rates in the 50 events h^{-1} treatment may have been linked to the increased byssus production observed for that treatment. We hypothesize that the nearly constant displacements of the 50 events h^{-1} treatment, with only 12 s between disturbance events, still allowed the mussel to open its valves and anchor itself between disturbances, rather than closing its valves and waiting for the disturbances to end. Additionally, we hypothesize that the increased oxygen uptake rates of the 50 events h^{-1} treatment were due to the physiological stress of the mussel closing its valves for a large proportion of the 1 h period while also dealing with the energetic cost of producing byssus. Further studies investigating disturbance frequencies in the 40–60 events h^{-1} range could test these hypotheses. In the other groups, the elevated oxygen uptake rates of *M. minimus* were likely a result of other responses to disturbances, such as closed valves and/or elevated heartbeat rate (Rovero et al., 1999), which did not manifest into an increased post-treatment oxygen uptake rate until the time between disturbances was shorter than the time needed to fully recover from each disturbance. Biochemical assays could further reveal the mussel's physiological response to different disturbance frequencies (e.g., Widdows and Shick, 1985). In conclusion, the physiological response of *M. minimus* to sub-lethal disturbances was likely separate from the mussel's attempt to anchor itself, but the disturbances only led to elevated metabolic rates when the disturbances occurred in rapid succession. These results indicate that sub-lethal disturbances are an important component of the physical environment for benthic organisms.

Sub-lethal, physical disturbances of benthic organisms will likely increase in frequency as human activity increases in coastal areas (Davenport and Davenport, 2006; Mendez et al., 2018) and as storm activity increases in severity and frequency due to climate change (e.g., Cai et al., 2014). Here we observed elevated metabolic rates when disturbances occurred more than 40 times h^{-1}, and this value is within the range of disturbance frequencies produced by anthropogenic activities such as boat wake and trampling (e.g., Brosnan and Crumrine, 1994; Whitfield and Becker, 2014). Many studies have detailed the negative effects of anthropogenic disturbances on benthic organisms, with a focus on the community compositions and presence/absence of focal organisms (e.g., Reyes-Martínez et al., 2015). In particular,

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trampling has been shown to reduce the cover and density of intertidal mussels, either by dislodging or crushing the mussels (Smith and Murray, 2005; Mendez et al., 2018), and these effects can be pronounced for large mussels or mussels whose shells are weakened (e.g., by boring organisms; Nicastro et al., 2018). Sub-lethal disturbances from the hydrodynamic forces of ocean waves will likely become more prevalent in the future as storms become larger and more frequent, suggesting that intertidal mussels will experience more energetic stress with negative impacts on their reproductive output and population stability (e.g., Sebens et al., 2018), although mussels could also be killed immediately if the storms and accompanying hydrodynamic forces are excessive. Overall, many of the studies examining the effects of sub-lethal disturbance on benthic organisms are conducted under field conditions where disturbances may have complex temporal patterns, multiple sources, and varying magnitudes (e.g., Reyes-Martínez et al., 2015), and to our knowledge, there have been no side-by-side comparisons of the functional consequences for the different types of sub-lethal disturbances (e.g., physical displacement, compression) that benthic organisms can experience. As a result, we know little about the immediate effects of sub-lethal disturbances on individuals. The results of the present study show that these types of disturbances can affect individual organisms in ways that would be overlooked if we only examined the most drastic outcomes of death and dislodgement.

Disturbances in nature can vary in many ways, including duration, magnitude, and frequency, and they can come from a variety of sources (Miller et al., 2011). For instance, disturbances can co-occur with other stressors (e.g., waves from boat wake can occur with engine noise and motor oil pollution) (Whitfield and Becker, 2014) and their effects on organisms can depend on the state of the organisms (e.g., parasitized) (Macleod and Poulin, 2016). While we showed that disturbance frequency can affect the metabolism of *M. minimus*, there are many variables that remain to be tested in order for us to have a complete understanding how *M. minimus* and similar organisms are affected by disturbance events associated with the natural environment and anthropogenic activities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.04.008.

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