Marine Biodiversity
https://doi.org/10.1007/s12526-018-0924-3

SENCKENBERG

SHORT COMMUNICATION

Between a rock and a hard place: combined effect of trampling and phototrophic shell-degrading endoliths in marine intertidal mussels

Katy R. Nicastro[1] · Christopher D. McQuaid[1] · Gerardo I. Zardi[2]

Received: 1 August 2018 / Revised: 21 September 2018 / Accepted: 27 September 2018

Abstract
Ecosystems often face a complex combination of natural and anthropogenic disturbances. The assessment of anthropogenic pressures and co-occurring natural stressors is crucial to our understanding of ecosystem structures, dynamics and their conservation. The present study provides the first experimental assessment of the combined effects of trampling (anthropogenic stressor) and endolithic induced erosion (natural stressor) on two coexisting intertidal mussel species, *Mytilus galloprovincialis* (de Lamarck, 1819) and *Perna perna* (Linnaeus, 1758). Mixed beds of the two species experiencing a wide range of endolithic parasitation were exposed to increasing intensities of human trampling. Our results clearly show that endolith-infested mussels are more vulnerable to trampling than non-infested individuals. At high trampling intensities, *P. perna* suffered significantly lower mortalities rates than *M. galloprovincialis*. More than 20% of large, infested *M. galloprovincialis* individuals were crushed even at low trampling intensities. Unexpectedly, mortalities rates decreased with mussel size, suggesting a sheltering role of larger conspecifics. Beyond ecological interest, such findings can provide critical experimental support for conservation and management actions.

Keywords Parasites · Anthropogenic stressor · *Mytilus galloprovincialis* · *Perna perna*

Introduction

Natural and anthropogenic disturbances play a determinant role in structuring ecosystems by influencing the abundance, distribution and diversity of species (e.g. Mayer-Pinto et al. 2015). Importantly, the response of ecosystems to human pressures can be partly affected by the natural disturbance conditions that drive their dynamics and structure (Marcogliese and Pietrock 2011). It has been suggested that severe and frequent natural disturbance may select for resistant species, thus enhancing ecosystems' resilience to additional human disturbance (Côté and Darling 2010). However, there is evidence that natural and anthropogenic disturbances may interact synergistically, resulting in higher vulnerability to human impacts in the presence of natural disturbance (Breitburg et al. 1998; Folt et al. 1999; Crain et al. 2008).

Coastal habitats are exposed to a multitude of human and natural disturbances (Sih et al. 2004). In particular, among anthropogenic disturbances, trampling due to recreational and harvesting uses is a serious source of disturbance that can cause significant environmental damage through direct or indirect effects on populations and communities (e.g. Rossi et al. 2007; Araújo et al. 2009; Micheli et al. 2016). The vulnerability to trampling largely depends on the structure of the local rocky shore habitat and communities, and on the intensity of human use (e.g. Keough and Quinn 1998; Araújo et al. 2009). Significant impacts of pedestrian traffic have been highlighted for the composition, percentage cover, diversity and biomass of several assemblages inhabiting distinct intertidal habitats (e.g. Milazzo et al. 2002, 2004; Araújo et al. 2009). In particular, intertidal rocky shores have been the focus of extensive studies reporting physical disturbance in the form of trampling of invertebrates, seagrass beds and algal canopies (Travaille et al. 2015). Despite numerous studies on the direct effects of trampling in intertidal habitats, the

Communicated by B. W. Hoeksema

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12526-018-0924-3) contains supplementary material, which is available to authorized users.

✉ Gerardo I. Zardi
zardi73@yahoo.it

[1] CCMAR-CIMAR – Associated Laboratory, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[2] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

Published online: 18 October 2018

question of whether the impact of trampling interacts with natural stressors has received limited attention.

In this study, we investigate the effects of the combination of trampling (anthropogenic stressor) and endolithic parasites (natural stressor) on two coexisting and competing intertidal mussel species, *Mytilus galloprovincialis* (de Lamarck, 1819) and *Perna perna* (Linnaeus, 1758). These two bioengineers form extensive beds, commonly dominating most of the rocky intertidal habitat along southern and northern African shores (McQuaid et al. 2015). Both *M. galloprovincialis* and *P. perna* are commonly parasitised by phototrophic shell-degrading endoliths (mainly cyanobacteria) that bore into their shells (Zardi et al. 2009). The erosion of the host's shell occurs through chemical dissolution, with carbonate ions released from calcite being converted into CO_2 that can then be used in photosynthesis (Garcia-Pichel et al. 2010). Many studies have reported negative, sub-lethal impacts of endolith infestation in bivalves (e.g. Kaehler and McQuaid 1999; Zardi et al. 2009; Marquet et al. 2013). The high energy demand for shell repair can affect the energetic budgeting at the expenses of growth, reproduction, byssal attachment strength and general conditions. Because of their boring activity, these parasites cause significant weakening of shell strength and, when infestation is particularly severe, endolithic activity can lead to shell breakage and be responsible for 50% of total mortality (Kaehler and McQuaid 1999; Zardi et al. 2009). Previous studies have shown that shell strength of both *M. galloprovincialis* and *P. perna* are significantly weakened by the presence of endoliths. However, *M. galloprovincialis* suffers a higher infestation incidence and damaging effect of endolithic excavation than *P. perna* across different habitats, probably due to a thinner and more fragile shell (Zardi et al. 2009). The shell is a vital protection against shell-crushing predators and the breaking action of waves (Kirk et al. 2007; Christensen et al. 2012), and there is evidence that mussels with weakened shells are more susceptible to both hydrodynamic stress of waves and predation (Beadman et al. 2003).

Marine calcifying organisms are globally affected by endolithic infestation (e.g. Bentis et al. 2000; Tribollet et al. 2009; Lourenço et al. 2017). In intertidal ecosystems, endolithic erosion has been reported in different environmental conditions, ranging from cold-temperate to sub-tropical/tropical (e.g. Stefaniak et al. 2005; Marquet et al. 2013; Lourenço et al. 2017). Given the ubiquitous nature of endolithic infestation and its predicted increase as a result of increasing ocean acidification and warming rates (Tribollet et al. 2009; Reyes-Nivia et al. 2013) understanding the positive and negative consequences of microbial endoliths has critical management and conservation relevance.

In this study, we investigate how endolith-induced shell erosion affects mussels' vulnerability to increasing intensities of trampling. Specifically, we hypothesise that (1) the impact of trampling will be significantly higher in infested mussels because of endolithic induced shell weakening, (2) the combined effect of endolith infestation and trampling will be less pronounced in *P. perna* than *M. galloprovincialis* due to its greater thickness of the shell and its lower propensity to endolithic infestation, and (3) smaller mussels will be more impacted than larger conspecifics due to their weaker shells.

Material and methods

The study site was exposed rocky shore on the south coast of South Africa and included two locations about 250 km apart (Schoenmakerskop 34.0110° S, 25.5918° E, Brenton-on-Sea 34.0707° S, 23.0265° E) at which quadrats (50×50 cm^2) were haphazardly placed in the mid-mussel zone in sun-exposed areas (as defined in Zardi et al. 2009) with 100% mussel cover, same wave exposure (i.e. same tidal height, lack of protecting structures in front of the area, same orientation towards the incoming waves, same shoreline angle). In each quadrat, the number of mussels belonging to each species (*Mytilus galloprovincialis* or *Perna perna*), size class (small 0–2, medium 2–4 or large 4–6 cm) and infestation category (non-infested to slightly infested A-B or infested to heavily infested C–E; see Fig. 1 in Zardi et al. 2009) was recorded. The quadrats were haphazardly assigned to each of the three trampling intensities ($n = 3$). To apply each intensity treatment, a ~ 75-kg person wearing soft-soled shoes (~ 253 cm^2) walked either 50, 100 or 150 steps (i.e. different quadrats for the different treatments), making sure to cover all areas of the quadrat. At the end of each treatment, the number with at least one crushed valve was counted. For each species, size and category the percentage of mussels out of the initial count with at least one crushed valve was used as a dependent factor in the data analysis.

To test for the effect of trampling intensity, a five-way PERMANOVA was performed with intensity (3 levels), species (2 levels), size (2 levels), and infestation (2 levels) as fixed factors, and site (2 levels) as a random factor. Prior analysis, data [Ln(X + 1)] were transformed to fulfil assumptions. Pair-wise a posteriori comparisons were used to further explore interactions of the main effects and a Bonferroni adjustment applied according to the number of tests performed. All analyses were run in PERMANOVA standalone software (Anderson 2005).

Results and discussion

The effect of trampling significantly increased with intensity, but not equally for all shell sizes, infestation category and mussel species (trampling × species × infestation category × sizes; $p = 0.02$; Table 1 in Supplementary Material; Fig. 1). There were no significant differences between species when individuals were not infested. However, trampling affected on average 16.6% of *M galloprovincialis* and only 4.5% of *P. perna* infested mussels. In particular, after Bonferroni's adjustment

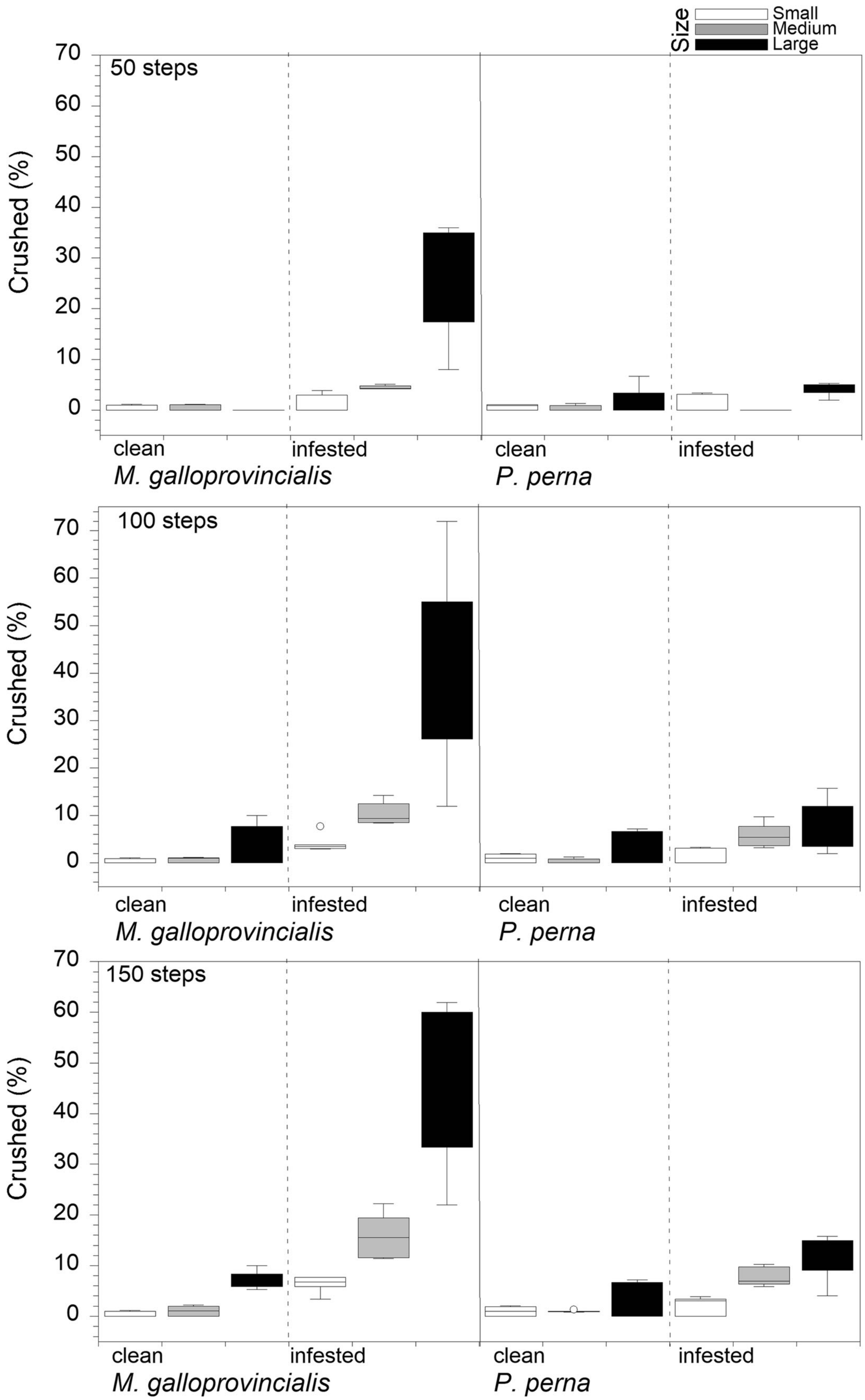

Fig. 1 Box plots of the trampling experiments. Extremities of the box represent standard deviation, and the whiskers stand for minimum and maximum observed values

lowered the 0.05 to 0.0028, infested *M. galloprovincialis* specimens crushed more than infested *P. perna* mussels of the same size, except for small (all trampling treatments) and medium (100 steps treatment) mussels where the number of crushed infested individuals did not differ between species.

Overall, infested mussels crushed more than clean mussels with the exception of small-size mussels at low intensity, medium-size *P. perna* at low intensity and large-size *P. perna* at all intensities.

Our results contribute to our still limited understanding of the combined effects of human and natural disturbances. Investigations of how anthropogenic pressure act with co-occurring natural disturbances are crucial to identify all multiple factors that shape ecosystems, and to design optimal management and conservation strategies (e.g. Micheli et al. 2016; Plicanti et al. 2016). This is the first study that examines how the impacts of trampling may vary with endolithic shell eroding activity. Our results supported our initial hypotheses by showing that endolith-infested mussels are more vulnerable to trampling than non-infested individuals and that, overall, infested *Mytilus galloprovincialis* was more affected than infested *Perna perna*, particularly for larger individuals. However, contrary to our expectations, smaller mussels suffered comparatively lower mortality rates. While a relatively low percentage of small individuals were crushed, the majority of these small mussels were found within the interstitial space between larger conspecifics and, therefore, they were not subjected to damage by trampling. This result supports previous studies in that it highlights the critical role of mussel beds as nursery habitats providing shelter from biotic and abiotic stress to early life stages and juveniles (e.g. Gosling 2008; McQuaid et al. 2015).

Intertidal mussels are commonly impacted by foot traffic. They are frequently collected for food and for use as bait by recreational fishers. In southern Californian, these human disturbances are contributing to mussel-bed declines in heavily visited shores (Murray et al. 1999; Smith and Murray 2005). The effect of pedestrian traffic on South African intertidal rocky shore communities has been previously assessed (Bally and Griffiths 1989); this study focused on the effect of different trampling regimes on several algae and invertebrates (mussels were not considered) at one wave-exposed rocky beach in the Cape Town area. Interestingly, results revealed low impact of pedestrian pressure, with wave action having a considerably greater importance to intertidal communities. Our results show that shell weakening induced by endolithic erosion can expose trampled mussels to extremely high mortality rates. Even low levels of trampling killed more than 20% of large infested *M. galloprovincialis*. Indirect effects of the trampling are also expected. Additional and substantial mussel cover loss occurs during the period following the trampling effect (Smith and Murray 2005), most likely due to the weakening of byssal-thread attachments between adjacent mussels, which increases their susceptibility to wave action (Denny 1987). Infested mussels have reduced attachment strength (Zardi et al. 2009) and the mortality following the impacts of crushing mussels underfoot is likely to be much higher for parasitized than for non-parasitized mussels.

The long-lasting effect of human pedestrian disturbance on intertidal bioengineers has been highlighted in previous studies. In the absence of additional human pedestrian disturbance, recovery of mussel beds and macroalga canopies occurs over time scales of few years (Micheli et al. 2016). However, effects on community cover and diversity can be still evident after 8 years (Schiel and Lilley 2011). Interestingly, recovery dynamics of populations and assemblages can be largely influenced by life history feature and several biotic (e.g. species interactions) and abiotic (e.g. disturbance regimes) processes (Hutchings and Reynolds 2004; Micheli et al. 2008). There is ample evidence that endoliths can significantly decrease the mussels' development of the reproductive tissue of mussels (Zardi et al. 2009). Additionally, mussels heavily infested with endoliths have lower attachment and shell strength, and are thus more vulnerable at sites where predation is intense or during periods of strong wave action (e.g. Marquet et al. 2013). Depending on the physical environment, differences in the degree of endolithic parasitation of coexisting species can regulate competitive balances (Zardi et al. 2009). Thus, our results suggest that endoliths, in addition to enhancing trampling disturbance, could potentially affect recovery rates by modulating the effects that environmental stress has on their host and species interaction.

The study of the combined effects of anthropogenic stressors and natural disturbances is not only of great ecological interest; it has broader implications for the conservation of biodiversity and can direct to more effective protection measures (Strain et al. 2014). Previous studies have suggested that functional management approaches for intertidal rocky shores experiencing high levels of disturbance should also include no-access reserves, rather than limitation of access (Micheli et al. 2016; Plicanti et al. 2016). Our findings are of particular concern considering the increasing evidence for incidence and impacts of endoliths in a variety of intertidal taxa and systems (e.g. Raghukumar et al. 1988; Hook and Golubic 1993; Webb and Korrubel 1994; Ćurin et al. 2014). Results presented here can be used for experimentally informed conservation approaches and can promote similar assessments and actions at sites affected by similar disturbances.

Acknowledgements Mussels were collected under permit number RES2014/12 issued by the Department of Agriculture and Fisheries to the Department of Zoology and Entomology at Rhodes University. We thank S. Donovan, G.C. Cadée and the Editor for their comments.

Funding This research was funded by projects UID/Multi/04326/2013, IF/01413/2014/CP1217/CT0004 from the Fundação para a Ciência e Tecnologia (FCT-MEC, Portugal) and further supported by the South

African Research Chairs Initiative (SARChI) of Department of Science and Technology (DST) and the National Research Foundation (NRF).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

References

Anderson MJ (2005) Permutational multivariate analysis of variance. Department of Statistics, University of Auckland, Auckland

Araújo R, Vaselli S, Almeida M, Serrão E, Sousa-Pinto I (2009) Effects of disturbance on marginal populations: human trampling on *Ascophyllum nodosum* assemblages at its southern distribution limit. Mar Ecol Prog Ser 378:81–92

Bally R, Griffiths CL (1989) Effects of human trampling on an exposed rocky shore. Int J Environ Stud 34:115–125

Beadman H, Caldow R, Kaiser M, Willows R (2003) How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. Mar Biol 142:487–494. https://doi.org/10.1007/s00227-002-0977-4

Bentis CJ, Kaufman L, Golubic S (2000) Endolithic fungi in reef-building corals (Order: Scleractinia) are common, cosmopolitan, and potentially pathogenic. Biol Bull 198:254–260

Breitburg DL, Baxter JW, Hatfield CA, Howarth RW, Jones CG, Lovett GM, Wigand C (1998) Understanding effects of multiple stressors: ideas and challenges. In: Pace ML, Groffman (eds) successes, limitations, and frontiers in ecosystem science. Springer, New York, pp 416–431

Christensen HT, Dolmer P, Petersen JK, Tørring D (2012) Comparative study of predatory responses in blue mussels (*Mytilus edulis* L.) produced in suspended long line cultures or collected from natural bottom mussel beds. Helgol Mar Res 66:1–9. https://doi.org/10.1007/s10152-010-0241-0

Côté IM, Darling ES (2010) Rethinking ecosystem resilience in the face of climate change. PLoS Biol 8:e1000438

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304–1315

Ćurin M, Peharda M, Calcinai B, Golubić S (2014) Incidence of damaging endolith infestation of the edible mytilid bivalve *Modiolus barbatus*. Mar Biol Res 10:179–189

de Lamarck JB (1819) Histoire naturelle des animaux sans vertèbres: précédée d'une introduction offrant la détermination des caractères essentiels de l'animal, sa distinction du végétal et des autres corps naturels, enfin, l'exposition des principes fondamentaux de la zoologie, vol 6. Verdière, Paris

Denny MW (1987) Lift as a mechanism of patch initiation in mussel beds. J Exp Mar Biol Ecol 113:231–245

Folt C, Chen C, Moore M, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44:864–877

Garcia-Pichel F, Ramírez-Reinat E, Gao Q (2010) Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca2+ transport. Proc Natl Acad Sci U S A 107:21749–21754. https://doi.org/10.1073/pnas.1011884108

Gosling E (2008) Bivalve molluscs: biology, ecology and culture. John Wiley & Sons, Hoboken

Hook JE, Golubic S (1993) Microbial shell destruction in deep-sea mussels, Florida Escarpment. Mar Ecol 14:81–89

Hutchings JA, Reynolds JD (2004) Marine fish population collapses: consequences for recovery and extinction risk. AIBS Bull 54:297–309

Kaehler S, McQuaid CD (1999) Lethal and sub-lethal effects of phototrophic endoliths attacking the shell of the intertidal mussel *Perna perna*. Mar Biol 135:497–503. https://doi.org/10.1007/s002270050650

Keough MJ, Quinn G (1998) Effects of periodic disturbances from trampling onrocky intertidal algal beds. Ecol Appl 8:141–161

Kirk M, Esler D, Boyd WS (2007) Morphology and density of mussels on natural and aquaculture structure habitats: implications for sea duck predators. Mar Ecol Prog Ser 346:179–187

Linnaeus C (1758) Systema naturae per regna tria naturae :secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis, vol 1. Impensis Direct. Laurentii Salvii, Holmiae

Lourenço CR, Nicastro KR, McQuaid CD, Sabour B, Zardi GI (2017) Latitudinal incidence of phototrophic shell-degrading endoliths and their effects on mussel bed microclimates. Mar Biol 164:129

Marcogliese DJ, Pietrock M (2011) Combined effects of parasites and contaminants on animal health: parasites do matter. Trends Parasitol 27:123–130

Marquet N, Nicastro KR, Gektidis M, McQuaid CD, Pearson GA, Serrão EA, Zardi GI (2013) Comparison of phototrophic shell-degrading endoliths in invasive and native populations of the intertidal mussel *Mytilus galloprovincialis*. Biol Invasions 15:1253–1272. https://doi.org/10.1007/s10530-012-0363-1

Mayer-Pinto M, Underwood AJ, Marzinelli EM (2015) The matrix influences direct and indirect effects of an anthropogenic disturbance on marine organisms. Environ Res 136:15–20

McQuaid CD, Porri F, Nicastro KR, Zardi GI (2015) Simple, scale-dependent patterns emerge from very complex effects: an example from the intertidal mussels *Mytilus galloprovincialis* and *Perna perna*. Oceanogr Mar Biol Annu Rev 53:127–156

Micheli F, Shelton AO, Bushinsky SM, Chiu AL, Haupt AJ, Heiman KW, Kappel CV, Lynch MC, Martone RG, Dunbar RB, Watanabe J (2008) Persistence of depleted abalones in marine reserves of central California. Biol Conserv 141:1078–1090

Micheli F, Heiman KW, Kappel CV, Martone RG, Sethi SA, Osio GC, Fraschetti S, Shelton AO, Tanner JM (2016) Combined impacts of natural and human disturbances on rocky shore communities. Ocean Coast Manag 126:42–50

Milazzo M, Chemello R, Badalamenti F, Camarda R, Riggio S (2002) The impact of human recreational activities in marine protected areas: what lessons should be learnt in the Mediterranean sea? Mar Ecol 23:280–290

Milazzo M, Badalamenti F, Riggio S, Chemello R (2004) Patterns of algal recovery and small-scale effects of canopy removal as a result of human trampling on a Mediterranean rocky shallow community. Biol Conserv 117(2):191–202

Plicanti A, Domínguez R, Dubois SF, Bertocci I (2016) Human impacts on biogenic habitats: effects of experimental trampling on *Sabellaria alveolata* (Linnaeus, 1767) reefs. J Exp Mar Biol Ecol 478:34–4

Raghukumar C, Pathak S, Chandramohan D (1988) Biodegradation of calcareous shells of the window-pane oyster by shell-boring cyanobacteria. In: Thompson MF, Sarojini R, Nagbhushanam R (eds) Marine Biodeterioration. Oxford & IBH Publishing, New Delhi

Reyes-Nivia C, Diaz-Pulido G, Kline D, Guldberg OH, Dove S (2013) Ocean acidification and warming scenarios increase microbioerosion of coral skeletons. Glob Chang Biol 19:1919–1929

Rossi F, Forster R, Montserrat F, Ponti M, Terlizzi A, Ysebaert T, Middelburg J (2007) Human trampling as short-term disturbance on intertidal mudflats: effects on macrofauna biodiversity and population dynamics of bivalves. Mar Biol 151:2077–2090

Schiel DR, Lilley SA (2011) Impacts and negative feedbacks in community recovery over eight years following removal of habitat-forming macroalgae. J Exp Mar Biol Ecol 407:108–115. https://doi.org/10.1016/j.jembe.2011.07.004

Sih A, Bell AM, Kerby JL (2004) Two stressors are far deadlier than one. Trends Ecol Evol 19:274–276

Smith JR, Murray SN (2005) The effects of experimental bait collection and trampling on a *Mytilus californianus* mussel bed in southern California. Mar Biol 147:699–706

Stefaniak LM, McAtee J, Shulman MJ (2005) The costs of being bored: effects of a clionid sponge on the gastropod *Littorina littorea* (L). J Exp Mar Biol Ecol 20:103–114

Strain EMA, Thomson RJ, Michel iF, Mancuso FP, Airoldi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. Glob Chang Biol 20:3300–3312. https://doi.org/10.1111/gcb.12619

Travaille K, Salinas-de-León P, Bell J (2015) Indication of visitor trampling impacts on intertidal seagrass beds in a New Zealand marine reserve. Ocean Coast Manag 114:145–150

Tribollet A, Godinot C, Atkinson M, Langdon C (2009) Effects of elevated pCO_2 on dissolution of coral carbonates by microbial euendoliths. Glob Biogeochem Cycles 23:3

Webb SC, Korrubel JL (1994) Shell weakening in marine mytilids attributable to blue-green alga, *Mastigocoleus* sp. (Nostochopsidaceae). J Shellfish Res 13:11–17

Zardi GI, Nicastro KR, McQuaid CD, Gektidis M (2009) Effects of endolithic parasitism on invasive and indigenous mussels in a variable physical environment. PLoS One 4:e6560