ExMarine: An experimental field mesocosm system to study multiple-stressor impacts on rock pool biodiversity

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Abstract

Biodiversity loss due to increasing anthropogenic activities is one of the biggest threats to humanity. Understanding the impacts of multiple-stressors on ecosystems and biodiversity is therefore an urgent task. Shore ecosystems are especially valuable, as they harbour a high biodiversity and provide important ecosystems services. Until now, experimental approaches addressing multiple-stressor impacts on these ecosystems have been rare and mostly run with a limited number of replicates and under non-natural conditions. Here, an experimental field mesocosm system that allows studying multiple-stressor impacts on rock pool biodiversity is proposed. The *ExMarine* mesocosm system is composed of 64 experimental rock pool mesocosms in a fully randomised block design, which allows studying multiple-stressor impacts under highly standardised conditions. Water is taken directly from the sea, allowing biota to immigrate and emigrate freely. Water flow into the mesocosms can be regulated and it is possible to simulate disturbance through waves during high tide. The system can help to understand the impacts of multiple stressors on biodiversity, to monitor ecosystem health and to plan measures preventing the further loss of biodiversity.

Introduction

The ongoing loss of biodiversity is one of the biggest threats to humanity (Rockström et al. 2009). As biodiversity provides food resources (Bommarco et al. 2013) and important ecosystem services such as water purification (see e.g. Edwards & Abivardi 1998; Vörösmarty et al. 2010) and carbon pulldown (see e.g. Hügler & Sievert 2011; Gilroy et al. 2014), societies worldwide rely on intact ecosystems and their biodiversity. However, anthropogenic activities such as intensified land use, industrialisation and overexploitation of natural resources have been shown to impact all levels of biodiversity from genes to ecosystems (Steffen et al. 2015). These multiple anthropogenic stressors can interact in different ways, sometimes leading to "ecological surprises" and impacting ecosystems and biodiversity in unexpected ways (Piggott et al. 2015a; Jackson et al. 2016). Therefore, an in-depth understanding of complex multiple-stressor interactions is urgently needed in order to prevent the further loss of biodiversity and ecosystems functions. To gain this understanding, studying ecosystems that harbour a high biodiversity is especially promising. Studies have addressed the impacts of multiple stressors on different ecosystems, from terrestrial (e.g. Luers et al. 2003; Davalos et al. 2014) to freshwater (e.g. Piggott et al. 2015b; Macher et al. 2016) and marine (e.g. Ban et al. 2014; Ellis et al. 2015). One marine ecosystem that is especially suitable for studying multiple-stressor effects is the rocky shore. Rocky shores are highly dynamic areas, affected by both sea and land, and harbour a high number of habitats and species communities along a relatively narrow strip (Levinton 1995). In addition, rocky shores are of significant economic value in many parts of the world, as a large number of edible species (such as crabs and seaweeds) are harvested in this ecosystem (e.g. Duran & Castilla 1989). One feature of rocky shores is the formation of rock or tide pools in the intertidal zone. The intertidal zone is mostly submerged at high tides and during storms, but can be exposed to wind, freshwater influx and extreme temperatures during low tide. Due to these conditions, rock pools are considered extreme habitats (Knox 2000). Rock pools have been studied intensively due to their accessibility, their diverse and ecologically interesting species communities (e.g. Connell 1961, Oksanen et al. 2002; Vanschoenwinkel et al. 2007; Brendonck et al. 2015) and due to the fact that they can be used as natural mesocosms in experimental studies (e.g. Dethier 1984, Romanuk et al. 2009). Therefore, they seem perfectly suitable for studies on multiple-stressor impacts on biodiversity and ecosystem functions. However, experimental studies on rock pools to date have been mostly conducted either in natural rock pools, which vary in size, depth, position relative to the shore and which therefore do not allow conducting experiments under strictly controlled conditions (e.g.

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Dethier 1984; Romanuk et al. 2009) or in mostly artificial pools, which suffer from drawbacks such as closed community structure or lack of water influx from the sea (e.g. Sloman et al. 2008). Some studies have used intermediate designs, e.g. by building artificial pools close to the shoreline that are allowed to flood regularly (e.g. Nielsen 2001, Romanuk 2009), but that do not allow a manipulation of physicochemical parameters or addition of stressors throughout the time of the experiment. In all studies, the number of replicates has been rather low. None of the existing systems allows manipulating physicochemical parameters in a highly replicated mesocosms system under natural conditions. Here, an experimental system that allows studying the impacts of multiple stressors on rock pools under highly realistic and standardised conditions and with a high number of replicates in a fully randomised block design is proposed. The system is based on the *ExStream* field mesocosm system, which was first used by Lange et al. (2011) to study freshwater species communities under multiple stressors and which has since been successfully used in several large experiments (e.g. Piggott et al. 2012, Elbrecht et al. 2016). The here-proposed system is called *ExMarine* (Experimental Marine Rock Pool Mesocosm System).

Material and Methods

As described in Lange et al. (2011) and Piggott et al. (2015b), the experimental system consists of mesocosms that are placed on a two-storey scaffold next to the studied habitat, therefore allowing studying the ecosystem of interest under natural conditions. For the *ExMarine* system, the scaffold is set up next to an area of shore with rock pools. A pump inlet with filters to prevent the intake of coarse material is placed in the intertidal zone. The pump transports water to the scaffold during high tides only in order to simulate the fluctuating supply of water during high and low tides. Pumps can either be switched on and off manually or activated via a programmed timer. If required, the pump can also run and supply the mesocosms with water at all times if placed in deeper water below the intertidal zone. It is also possible to run the pumps at longer intervals to allow desiccation of the rock pool mesocosms. The water pumped from the seas is distributed into eight header tanks placed on the second-storey platform of the scaffold. Each header tank then distributes the water into eight rock pool mesocosms via gravity (Figure 1). The flow rate can be controlled via valves, which are installed upstream of every channel. When water is supplied to the mesocosms during high tide, the excess water leaves the mesocosms via an outlet. During low tides, when the pump is not working, the water level in the mesocosm falls below the outflow level. In total, the *ExMarine* system consists of 64 rock pool mesocosms. Prior to an experiment, the mesocosms can be filled with substratum and can be stocked with biota from the surrounding rock pools. Throughout the experiment, small biota (mainly microbes, algae and invertebrates) get pumped into the experimental system directly from the sea together with the water for the mesocosms. They can then settle in the mesocosms or leave them through the outlet during simulated high tide phases. The *ExMarine* system can be built in two different ways. One possibility is to build it so that water is directly pumped into the header tanks, from where it is distributed to the mesocosms via gravity (Figure 1, a + b). This setup does allow simulation of high tide and low tide phases, but not of varying water influx during high tide, which is typical for rock pools on the intertidal zone. Therefore, it is also possible to build the setup with water scoops, which are located next to the header tanks (Figure 1, c + d). During high tide, water is pumped into the water scoops, gradually filling them. Thereby, the water scoops become heavier and finally tilt, releasing the water into the header tanks in a squall. From the header tanks, the squall is distributed to the mesocosms, simulating the varying influx of water and the turbulences caused by waves. After the completion of the experimental phase, the community in each channel is assessed either by morphological identification or molecular methods such as barcoding (Hebert et al. 2003), metabarcoding (see e.g. Taberlet et al. 2012; Leray & Knowlton 2015), metagenomics (see e.g. Riesenfeld et al. 2004; Crampton-Platt et al. 2015) or metatransciptomics (see e.g. Shi et al. 2009; Marchetti et al. 2012) in order to assess the impacts of the stressors on community structure, species diversity, genetic diversity and ecosystem functions.

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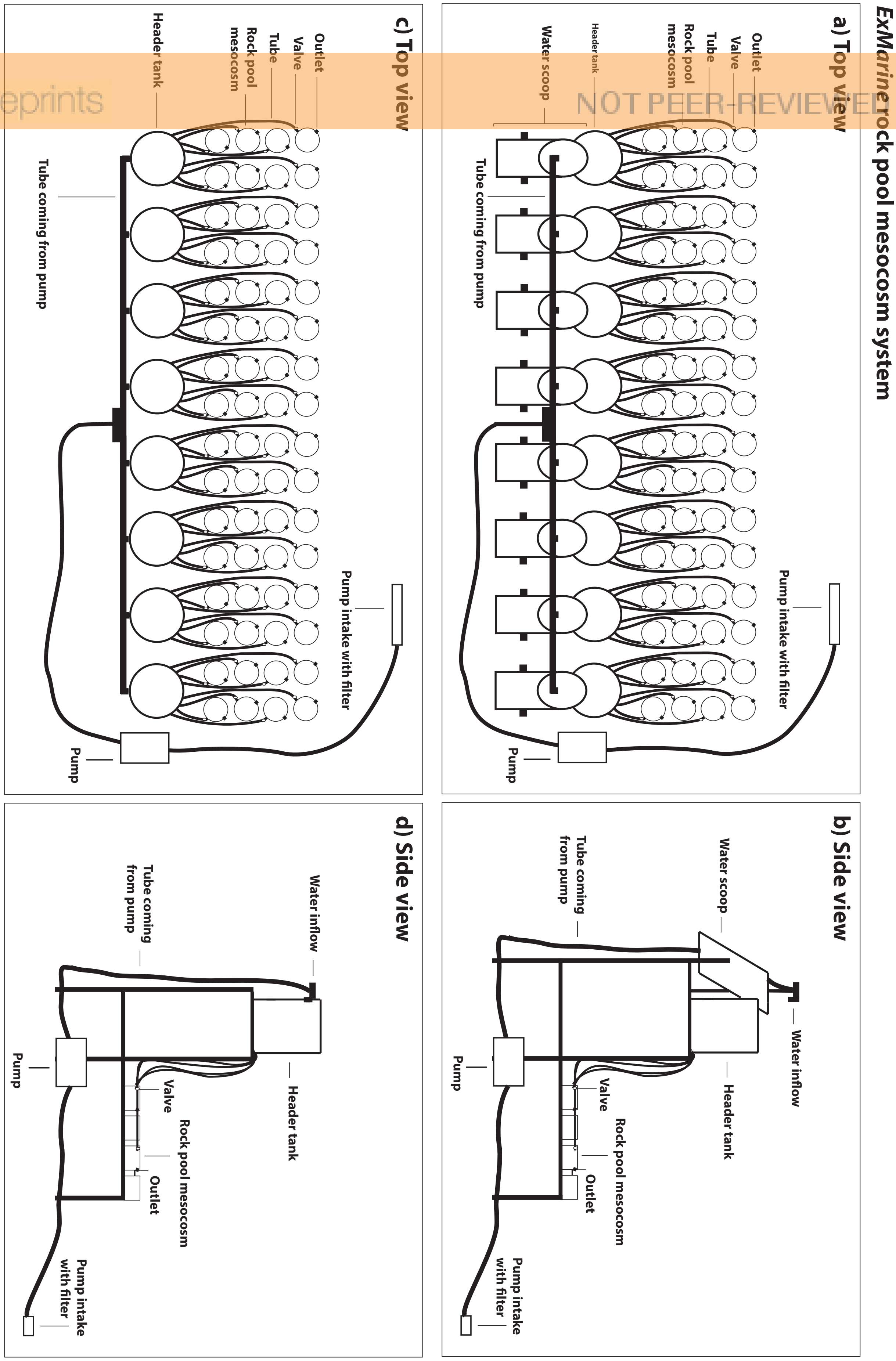

Figure 1: *ExMarine* rock pool mesocosm system. a) Top view; system with water scoops installed. b) Side view; system with water scoops installed. c) Top view; system without water scoops. d) Side view; system without water scoops.

Discussion

The here-proposed *ExMarine* field mesocosm system allows studying the impacts of multiple stressors on biodiversity in rock pools, bringing together the statistical power of highly replicated experimental approaches and the benefits of a real-world setting with free immigration and emigration of biota. This approach has been shown to be highly effective in freshwater ecosystems (e.g. Piggott et al. 2015b) and is novel for the study of biodiversity in shore ecosystems. Stressors can be applied to channels via drippers connected to pumps (e.g. nutrients, see e.g. Elbrecht et al. 2016) or added manually (e.g. sediment). Existing experimental rock pool mesocosm systems mostly suffer from drawbacks such as low number of replicates, non-realistic settings, closed communities or (in case of artificial pools build on the shore) the problem that stressors cannot be applied throughout the whole experimental phase due to waves washing them out. In contrast, the *ExMarine* mesocosm systems offers a solution to these drawbacks. Due to the highly replicated and randomised block design (eight blocks with eight channels each), three stressors can be tested alone and in combination. Together with a control treatment, each stressor and stressor combination can be tested in eight replicates (one per block). Two stressors plus control can be tested in 16 replicates each (two per block). The system can also be enhanced and run with 128 or more channels, or downscaled and run with 32 channels. The great advantage of this experimental system over existing systems is the fact that it is run with water directly from the natural habitat, that specimens can colonise the mesocosms, and that they can leave the experimental system should the conditions in the mesocosms be unfavourable. It has to be acknowledged, however, that pumping of water directly from the sea prevents larger organisms from entering the mesocosms, making it impossible for large predators such as fish to impact on the community in the mesocosms. Also, disturbance through waves might be stronger in real rock pools. However, the experiments using the *ExStream* stream mesocosm system have shown that results are meaningful and the community is similar to that in the natural environment. Therefore, it can be concluded that the *ExMarine* rock pool mesocosms work equally well and can give insights into the studied ecosystem, allowing to study the ecosystem under highly realistic conditions. Further, since rock pools are extreme, often shallow and small habitats, the mesocosms are expected to accurately depict this habitat. Therefore, the community found in the mesocosms is expected to closely resemble the one in a real rock pool, a fact that can and should be verified by sampling nearby natural rock pools. Overall, the *ExMarine* system can help to deepen the understanding of how multiple stressors impact on an important and highly diverse ecosystem. The experimental system can be used to monitor ecosystem health and the results could be helpful when planning conservation measures.

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