Title: Do low oxygen environments facilitate marine invasions? Relative tolerance of native and invasive species to low oxygen conditions

Running head: Marine invasive species in hypoxic habitats

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**Abstract**

Biological invasions are one of the biggest threats to global biodiversity. Marine artificial structures are proliferating worldwide and provide a haven for marine invasive species. Such structures disrupt local hydrodynamics, which can lead to the formation of oxygen-depleted microsites when the rate of oxygen consumption by fouling communities exceeds the rate of oxygen replenishment. The extent to which native fauna can cope with such low oxygen conditions, and whether invasive species, long associated with artificial structures in flow-restricted habitats, have adapted to these conditions remains unclear. We measured water flow oxygen availability in marinas and piers at the scales relevant to sessile marine invertebrates (mm). We then measured the capacity of invasive and native marine invertebrates to maintain metabolic rates under decreasing levels of oxygen using standard laboratory assays. We found that marinas reduce the water flow relative to piers, and that local oxygen levels can be as low as zero in these low flow conditions. We also found that for species with arborescent growth forms, invasive species can tolerate much lower levels of oxygen relative to native species. Integrating the field and laboratory data showed that up to 30% of available microhabitats within low flow environments are physiologically stressful for native species, while only 18% of the habitat is physiologically stressful for invasive species. These results suggest that invasive species have adapted to low oxygen habitats associated with manmade habitats, and artificial structures may be creating realized niche opportunities for invasive species.

**Introduction**

Biological invasions are considered, together with habitat destruction, to be one of the biggest threats to biodiversity around the world (Dafforn *et al.*, 2009a, Davis, 2003, Vitousek *et al.*, 1996). The damage caused by invasive species can have far-reaching consequences for biodiversity. Beyond the obvious damage to natural systems, invasive species can also negatively impact on human activities, increase disease proliferation, and damage agriculture (Mack *et al.*, 2000, Pimentel *et al.*, 2001). While some habitats are more susceptible than others, no habitat is immune to invasion (Shea & Chesson, 2002). The invasion process is complex, largely because invasion success is determined by both the characteristics of the potentially invaded habitat, the traits of the invasive species, and their interaction (Andow *et al.*, 1990, Arim *et al.*, 2006, van Kleunen *et al.*, 2010a, van Kleunen *et al.*, 2010b, Zhao & Feng, 2015). Several hypotheses have been put forward to explain the role of the environment in determining the likelihood of invasions. Some focus on the characteristics of the environment only, and others on species-habitat interactions; however most hypotheses invoke resource usage in one way or another (Davies *et al.*, 2005, Keane & Crawley, 2002, Shea & Chesson, 2002, Simberloff & Von Holle, 1999).

There are two broad classes of resource competition that can mediate invasion, and they relate to the type of competition that occurs. Invasion “from above” occurs when interference competition dominates, and species with large resource requirements are able to overcome limitations by seizing resources from established residents. On the other hand, invasion “from below” occurs when exploitative competition dominates, and species with lower resource requirements are more successful because they use scarce resources in a more efficient way (Crawley *et al.*, 1986, Ferguson *et al.*, 2013, Hart & Marshall, 2012, Persson, 1985).

Resource availability and usage are therefore key elements of biological invasion. Environments may create “niche opportunities” in terms of resources that stimulate or limit invasions (Chesson, 2000, Davies *et al.*, 2005). When exploitative competition is important, the species that comes to dominate the community may be the one that can persist under the lowest resource levels. Known as the R\* theory of competition, this theory predicts that when two or more species compete for the same resource, resources will be depleted and the species that has the lowest resource requirements (i.e. has the lowest R\* value) will continue to draw resources down beyond levels that other species can tolerate such that it will eventually displace all other species at equilibrium (Tilman, 2004). Successful invasive species may be more effective than native species at using resources. For example, the diatom *Didymosphenia geminata* is highly invasive and appears to have a low R\*, which allows it to outcompete native species in oligotrophic systems around the world (Cullis *et al.*, 2012, Sundareshwar *et al.*, 2011). Resource availability and usage are therefore key elements of biological invasion. Environments may create “niche opportunities” in terms of resources that stimulate or limit invasions (Chesson, 2000, Davies *et al.*, 2005).

In marine systems, there is a strong association between artificial hard structures and invasive species. Commercial marinas and other artificial structures such as pilings, pontoons and jetties are considered windows for biological invasion (Airoldi *et al.*, 2015, Bulleri & Airoldi, 2005, Dafforn *et al.*, 2009b, Glasby *et al.*, 2007, Ruiz *et al.*, 2009). Several hypotheses have been proposed for why artificial structures increase the likelihood of invasion. For example, propagule pressure is thought to be much higher around artificial structures; similarly, higher rates of pollution may facilitate invasion by pollution-tolerant invaders (Erfmeier *et al.*, 2013, Kinlan & Gaines, 2003, McKenzie *et al.*, 2012). In addition to these factors, artificial structures modify natural environments in ways that may facilitate invasion: by reducing wave exposure and water flow, they provide a sheltered habitat that nevertheless has abundant hard surfaces available for colonization (Bulleri & Airoldi, 2005, Clark & Johnston, 2005, Glasby *et al.*, 2007). We suspect that the reduction in water flow plays a key role in mediating the establishment of artificial structures by invasive species.

Water flow is an important driver of community structure and composition in marine systems (Lastra *et al.*, 2004, Palardy & Witman, 2011). Water flow influences the performance of sessile marine invertebrates as it affects the delivery of essential resources: food and oxygen (Gardella & Edmunds, 1999, Lastra *et al.*, 2004, Okamura, 1985, Shimeta & Jumars, 1991, Svensson & Marshall, 2015). The interface between the fluid and a solid surface creates a condition known as a boundary layer, the thickness of which depends mostly on the flow of water. At small scales (millimeters), habitats with complex topography, as in sessile invertebrate communities, generate a skimming flow that traps layers of water within the boundary layer; increasing the residence time of the water and reducing exchange (Koch & Gust, 1999). This boundary layer effect, in combination with the metabolic demands of the dense aggregation of sessile communities, can deplete oxygen levels in the water immediately surrounding benthic organisms (Ferguson *et al.*, 2013, Moore *et al.*, 1996). In some instances, oxygen levels can be so low that they fall below the physiological tolerance of some members of the communities (Ferguson *et al.*, 2013). Importantly, growth form seems to be a strong determinant of tolerance to low oxygen conditions: species that have a flat growth form have much better tolerances to lower oxygen levels than species that have an erect growth form (Ferguson *et al.*, 2013). Presumably these different tolerances reflect the fact that flat species are more likely to live entirely within the boundary layer, and are therefore more likely to experience low oxygen conditions, and have adapted accordingly. Because of the long association of invasive species with artificial structures, which reduce flow in the environment (Bulleri & Airoldi, 2005, Dafforn *et al.*, 2009b, Wilding, 2014), it seems likely that invasive species may have adapted to low oxygen conditions. In other words, invasive species may have a lower R\* for oxygen than native species in sessile marine invertebrate communities, but tests are lacking.

One way to estimate the R\* for oxygen is to determine the level at which rates of oxygen consumption (a proxy for aerobic rates of metabolism) begin to drop with oxygen levels; this level is known as the critical oxygen concentration () or critical oxygen pressure () (Hochachka & Somero, 2002, Portner & Grieshaber, 1993). Below that value, aerobic metabolic rate decreases, anaerobic mechanisms become more important, and conditions are considered physiologically stressful (Armstrong *et al.*, 2009, Hochachka & Somero, 2002, Portner & Grieshaber, 1993). In marine invertebrates, which are often neither strict oxy-conformers nor oxy-regulators, measuring is less straightforward (see Methods), because declining oxygen levels can cause substantial reductions in rates of oxygen consumption without necessarily causing increases in anaerobic metabolism (e.g. Portner et al 1985, Hardewig et al 1991). In *Sipunculus nudus*, for example, rates of oxygen consumption decline linearly with ambient oxygen down to an oxygen partial pressure () of ~5-10 kPa; the rate of oxygen consumption then exhibits an inflection at this , which is indicative of the commencement of anaerobic metabolism (Portner et al 1985, Hardewig et al 1991). Thus, even in the absence of anaerobic metabolism in these species, decreasing causes a reduction of aerobic metabolism, and the level to which metabolic rate declines with decreases in oxygen will estimate the capacity for maintaining aerobic biological processes in the face of low oxygen conditions. In terms of R\*, species that are able to maintain higher levels of aerobic metabolism under lower oxygen conditions will have a competitive advantage over those that exhibit reduced aerobic metabolism at relatively higher oxygen conditions.

Here, we measure water flow rates and oxygen availability at small scales across 5 artificial structures that vary from relatively high flow environments (piers) to relatively low flow environments (marinas). Then we measured the oxygen tolerances of a range sessile marine invertebrates that grow on artificial structures that are invasive and native to Australia.

We define invasive species an introduced, exotic, or non-indigenous species inhabiting an area outside of its current or historical range of distribution (Colautti & MacIsaac, 2004, Neill & Arim, 2011). In the present study, all of these invasive species are thought to have originated from outside of Australia. Because growth form strongly affects oxygen tolerance in this group (Ferguson *et al.*, 2013), we also measured tolerances across species with erect growth forms and flat growth forms. We then combine field data on oxygen availability and laboratory data on oxygen tolerance to estimate the proportion of microsites that are physiologically stressful for native versus invasive species.

**Materials and methods**

*Organism collection and the estimation of tolerance to low oxygen conditions*

When was possible, the organism collection was conducted in the same sites that we measured our field estimates, within Port Phillip Bay, Victoria Australia (see below). The animal collection sites were: Altona Pier (37° 52’ 23’’ S; 144° 49’ 49’’ E), Blairgowrie Yacht Squadron (38° 21’ 23” S; 144° 46’ 22” E), Portarlington pier (38° 6’ 40” S; 144° 39’ 9” E). Royal Brighton Yacht Club (37° 54’23” S; 144° 58’ 53” E), Royal Melbourne Yacht Squadron (St Kilda) (31° 51’ 45” S; 144° 57’ 51” E).

We collected specimens of larger species (e.g. solitary ascidians, arborescent bryozoans) by peeling adults from the floating pontoons. Smaller species (e.g. flat bryozoans and colonial ascidians) were collected from pre-roughened acetate sheets that had been deployed at field sites according to standard methods (Hart & Marshall, 2009), for two years prior to the experiment. The species used in these studies were classified according to their invasion status (i.e. native or invasive to Australia; Table 1) and their growth form (i.e. erect or flat; Table 1). We classed species as native or invasive based on classifications by the Australian government ([www.environment.gov.au](http://www.environment.gov.au)) and Australian museum records. Based on these criteria, the species classed as invasive are unlikely to have been anywhere in Australia before being transported by boats sometime in the last 200 years. Thus the invasive species as defined here are not a redistribution of endemic communities from elsewhere in Australia. All of the species came from the same study sites so as to prevent confounding site of origin effects. The organisms were transported to the lab in insulated aquaria with aerated seawater and acclimatized to laboratory conditions for 2 days in the dark at 19 ˚C.

We measured oxygen consumption using two different closed respirometry systems, depending on the size of the study organism (Ferguson *et al.*, 2013, Pettersen *et al.*, 2015). Larger organisms were measured using hermetic 1.8 L chambers with circulating water connected to a 4-channel Firesting fiber optic oxygen meter (Pyro Sciences, Aachen-Germany). Smaller organisms were cut from acetate sheets and placed in 25 ml vials mounted on a 24-channel sensor dish reader (Sensor Dish Reader SDR, PreSens, Regensburg- Germany). At least two issues must be kept in mind when using closed respirometry systems: First, measurements are made in decreasing oxygen levels, due to the use of oxygen by the animal, and so the duration of exposure to the experimental system is confounded with oxygen level. The use of organismal oxygen consumption to draw down the oxygen level in a sealed chamber also means that secondary metabolites associated with metabolic activity accumulate as oxygen levels decline. However we chose these systems because we aimed to measure oxygen consumption in an environment of decreasing oxygen, and because in our previous work with marine invertebrates we have successfully used closed respirometry systems to estimate rates of oxygen consumption and relate these to fitness, and to measure tolerances to low oxygen conditions and relate these to growth form (Ferguson *et al.*, 2013, Pettersen *et al.*, 2015). The chambers and vials were filled with micro-filtered (0.2 μm), sterilized seawater that had been kept at 19 ˚C with constant aeration for at least 24 hours prior to experiments. Rates of oxygen consumption (, ml h-1) were calculated as described in previous studies (Ferguson *et al.*, 2013, Pettersen *et al.*, 2015, White *et al.*, 2011). Dry mass was determined after the oxygen consumption trials by drying each organism in an oven at 60 ˚C for one week, then weighing each individual with a precision balance (Adventurer Pro OHAUS, New Jersey, USA) to the nearest milligram.

*Model*

In contrast to what is observed for most vertebrates, where a clear can be discerned (Marshall *et al.*, 2013), our data were curvilinear, such that there was no clear point where the organisms transitioned from a perfect oxyregulator to an oxyconformer (Fig. 1). Instead we fit a Michaelis-Menten function to our consumption data:

, (1)

where is an asymptotic , and is the value of where = . Importantly, in order to achieve model convergence, we employ a transformation to . For each individual, we standardise based on its maximum value, so all individuals present a relative bounded between zero and one. We note that this transformation implicitly assumes that is independent of body mass – this assumption has mixed support from other studies (for a detailed discussion of this issue, see Lease et al. 2012). Importantly however, while transformation affects the estimated , it does not affect our primary goal, which is to estimate for each species because it entails dividing the numerator in Equation (1) by an individual-specific constant (i.e. the maximum measures metabolic rate), hence , which is in the denominator, is not affected.

For the model, we fit equation (1) above in a Bayesian framework by calling *JAGS* version 4.2.0 from the R package *R2jags* version 0.05-6 (Su & Yajima, 2015) in order to derive posterior distributions and associated 95% credible intervals (CIs) for the fitted parameters, and . We allow and to vary randomly among species. Random effects were assumed to be normally distributed, with means of 0. Fitted parameters were assigned priors that were vague (i.e. locally uniform over the region supported by the likelihood) (Kruschke, 2014). The posterior distributions of model parameters were estimated using Markov chain Monte Carlo (MCMC) methods by constructing three chains of 1.5 106 steps each, including 7.5 105-step burn-in periods. Chains were thinned using a 375-step interval, so a total of 6,000 steps were retained to estimate posterior distributions (i.e. 3 (1.5 106 - 7.5 105)/375 = 6,000).

We use the species-specific estimates (n = 14) for obtained in *JAGS* in order to fit three separate ANOVA’s: one to test for differences in between species status (native and invasive), a second to test for differences in between species shape (erect and flat), and a third one to test for differences in between species status of erect shaped organisms (native and invasive). Ideally, a fairer test would be represented by a two-way ANOVA with a formal statistical interaction between status and shape. However, given that we only have 14 species in our dataset, doing so would most likely overfit the data (i.e. too many parameters to be estimated from few observations), so our approach is conservative. We fit these ANOVA’s for each one of the 6,000 MCMC parameter estimates in order to obtain a full ‘posterior distribution’ of differences in between categories (status or shape). Statistical significance is judged by the lack of overlap between the 95% credible intervals of such distributions.

Using the parameter estimates from the model above, for each species we first calculate at 100% ( and use the value of in which = as our proxy for . We have also estimated the average point at which different species start displaying signs of stress due to decreasing oxygen availability. To do so, for each species, we use the average species-specific parameters from our Bayesian model in order to calculate the value of air saturation, , in which = .

*Field estimates of water flow velocity and oxygen availability*

All flow and oxygen measurements were conducted at sites within Port Phillip Bay, Victoria Australia. Flow and oxygen measurements were done in 5 sites: Blairgowrie Yacht Squadron (38° 21’ 23” S; 144° 46’ 22” E), Royal Brighton Yacht Club (37° 54’23” S; 144° 58’ 53” E), Royal Melbourne Yacht Squadron (St Kilda) (31° 51’ 45” S; 144° 57’ 51” E), Queenscliff Harbour (38° 15’ 50” S; 144° 40’ 10” E) and Queenscliff Pier (38° 15’ 47.20” S; 144° 40’ 6.00” E). All sites other than Queenscliff pier are sheltered by a breakwall, floating pontoons, or both.

We were interested in flow velocities at the scales and microsites that were relevant to the study organisms – the conditions just a few millimeters above the organisms. As such, we needed to use a more old-fashioned but reliable approach to measuring local flow speeds (Vogel, 1994). To measure flow velocities adjacent to the study communities, we released 30 ml of milk among the sessile community and measured the distance travelled (cm) by the leading edge from the point-source 30 and 60 seconds following release. Three replicate measures at each sampling location where taken, from which we calculated an average flow velocity (cm s-1) for each site.

To measure oxygen content in the water adjacent to the sessile community, we used fiber optic sensors connected to a fiber optic oxygen meter (Firesting; Pyro Sciences, Aachen-Germany). The sensors, stainless tubes of 10 cm length with a sensor of 3 mm diameter on the tip, were calibrated using air saturated seawater (100% saturation) and seawater containing 2% sodium sulfite (0% saturation). After calibration the sensors were placed at 30 cm depth at ~1 mm distance from benthic communities growing on the surface of floating pontoons and piers – for detailed methods see Ferguson *et al*. (2013). For study sites within marinas, the oxygen availability from 12 regularly spaced sampling points was measured; four sampling points in the most sheltered zone of the marina, four in the most exposed zone and four in the middle of each marina. At each sampling point within each site, six replicate oxygen measures were taken. The duration of the samplings lasted until oxygen readings had stabilized after the disturbance of introducing the probe had dissipated (approximately 5 - 10 minutes). At pier sites, which were smaller than the marinas, we measured oxygen levels from three sampling points with equidistant locations (~ 15 m apart). To estimate temporal variability in flow and oxygen conditions at each site, we measured both flow velocities and oxygen levels on five noncontiguous days at each site yielding a total of 1530 measures of oxygen across all 5 sites.

**Results**

*Tolerance to low oxygen conditions*

Both the status of species (invasive and native) and growth form of species (erect or flat) influenced their tolerance to low oxygen conditions (i.e. , where = , Equation 1): invasive species tolerate oxygen levels that are ~1.7-fold lower than the critical values for native species (Fig. 2a) while maintaining similar normalised metabolic rates (average: 0.12 ml O2 h-1; 95% CI 0.09 – 0.16 for invasive species; average: 0.14; 95% CI 0.11 – 0.19 for native species; Supplementary material). Similarly flat species tolerate oxygen levels that are on average ~2.3-fold lower than the critical values for erect species (Fig. 2b). Unfortunately, the collection sites we used only had one native species with a flat growth form so we could not formally compare invasive and native species with that growth form. Consequently, when we consider just the erect form, for which there were both multiple invasive and native species in the dataset, erect invasive species could tolerate significantly lower oxygen levels than erect native species (Fig. 3).

*Field estimates of water flow and oxygen availability*

The sites with the lowest flow velocity were St. Kilda (1.4 ± 1.0 cm seg-1) and Brighton (1.5 ± 4.3 cm seg-1), followed by Queenscliff Harbor (3.5 ± 2.9 cm seg-1) and Blairgowrie (3.6 ± 2.9 cm seg-1). Queenscliff Pier had the highest flow compared to all other studies sites (19.0 ± 6.5 cm seg-1). The rank order of flow conditions at any one site corresponded roughly with mean local oxygen availability although this relationship was largely driven by 100% oxygen conditions at the site with the highest flow rates (Fig. 4). Microsites (i.e. samples) with high oxygen levels (% air saturation) were found at all sites (Fig. 4). St Kilda had the highest variation in oxygen availability and also had higher frequency of microsites with 0 % of oxygen (Table 2, Fig. 4). In contrast, Queenscliff Pier had the lowest variability in oxygen availability, and no microsite showed oxygen levels at 0 % (Table 2, Fig. 4).

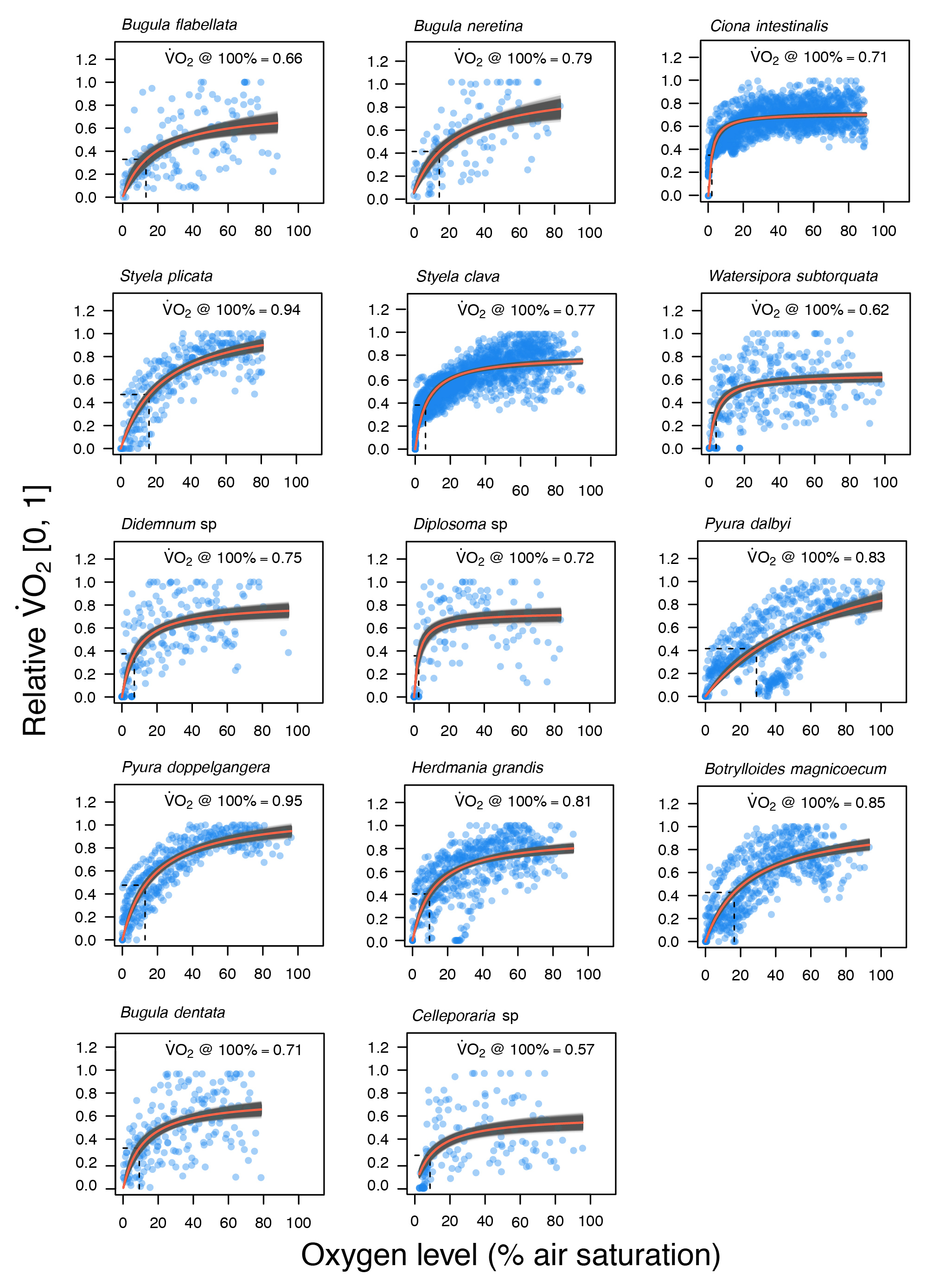
When we combined the estimates of oxygen availability with the estimates of tolerance to low oxygen, we found that 22–30% of microsites fell below the tolerances of native species in low flow sites (St Kilda and Brighton) but only 12–18% of microsites were below the tolerance of invasive species. At the site with the second highest flow, only between 11% and 18% of microsites were unavailable to invasive and native species respectively. At the site with the highest flow, all of the microsites were habitable to species of both status types.

**Table 1** Sessile species used in this study. Species are classified according to their status (Invasive or native) and their shape (Erect or flat), the average dry body mass (DBM; average ± S.D.) and the number of individuals analyzed (n).

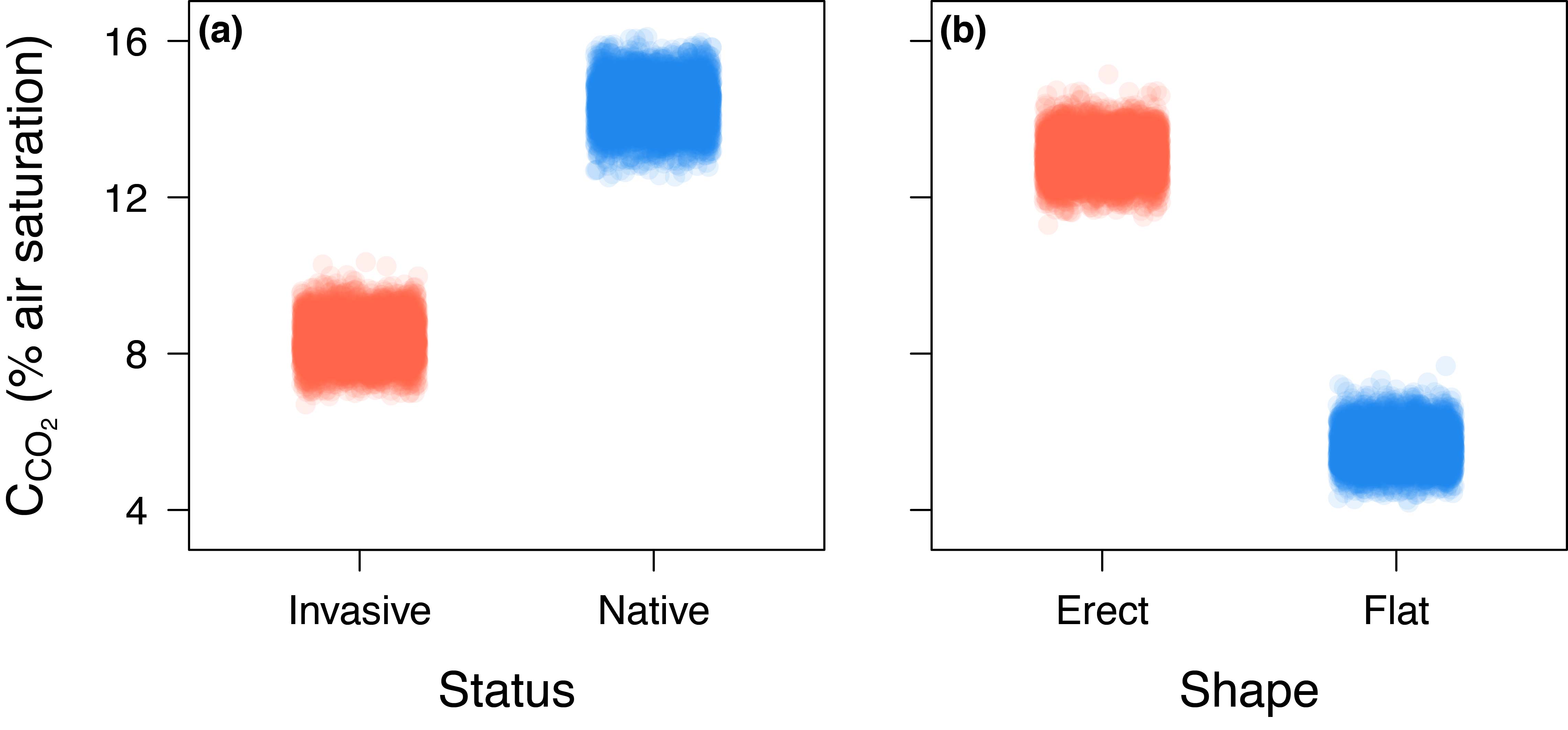
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Growth shape | Status | DBM (g ± S.D) | n |
| *Bugula flabellata* | Erect | Invasive | 0.03 (± 0.00) | 7 |
| *Bugula neretina* | Erect | Invasive | 0.06 (± 0.02) | 11 |
| *Ciona intestinalis* | Erect | Invasive | 0.22 (± 0.06) | 9 |
| *Styela plicata* | Erect | Invasive | 1.47 (± 0.40) | 8 |
| *Styela clava* | Erect | Invasive | 1.17 (± 0.30) | 17 |
| *Watersipora subtorquata* | Flat | Invasive | 0.08 (± 0.04) | 13 |
| *Didemnum sp* | Flat | Invasive | 0.19 (± 0.11) | 11 |
| *Diplosoma sp* | Flat | Invasive | 0.08 (± 0.03) | 9 |
| *Pyura dalbyi* | Erect | Native | 5.13 (± 1.10) | 9 |
| *Pyura doppelgangera* | Erect | Native | 4.67 (± 1.31) | 9 |
| *Herdmania grandis* | Erect | Native | 2.12 (± 0.90) | 13 |
| *Botrylloides magnicoecum* | Erect | Native | 0.70 (± 0.30) | 9 |
| *Bugula dentata* | Erect | Native | 0.05 (± 0.00) | 11 |
| *Celleporaria sp* | Flat | Native | 0.17 (± 0.06) | 7 |

**Table 2** Mean, standard deviation and range of oxygen levels.

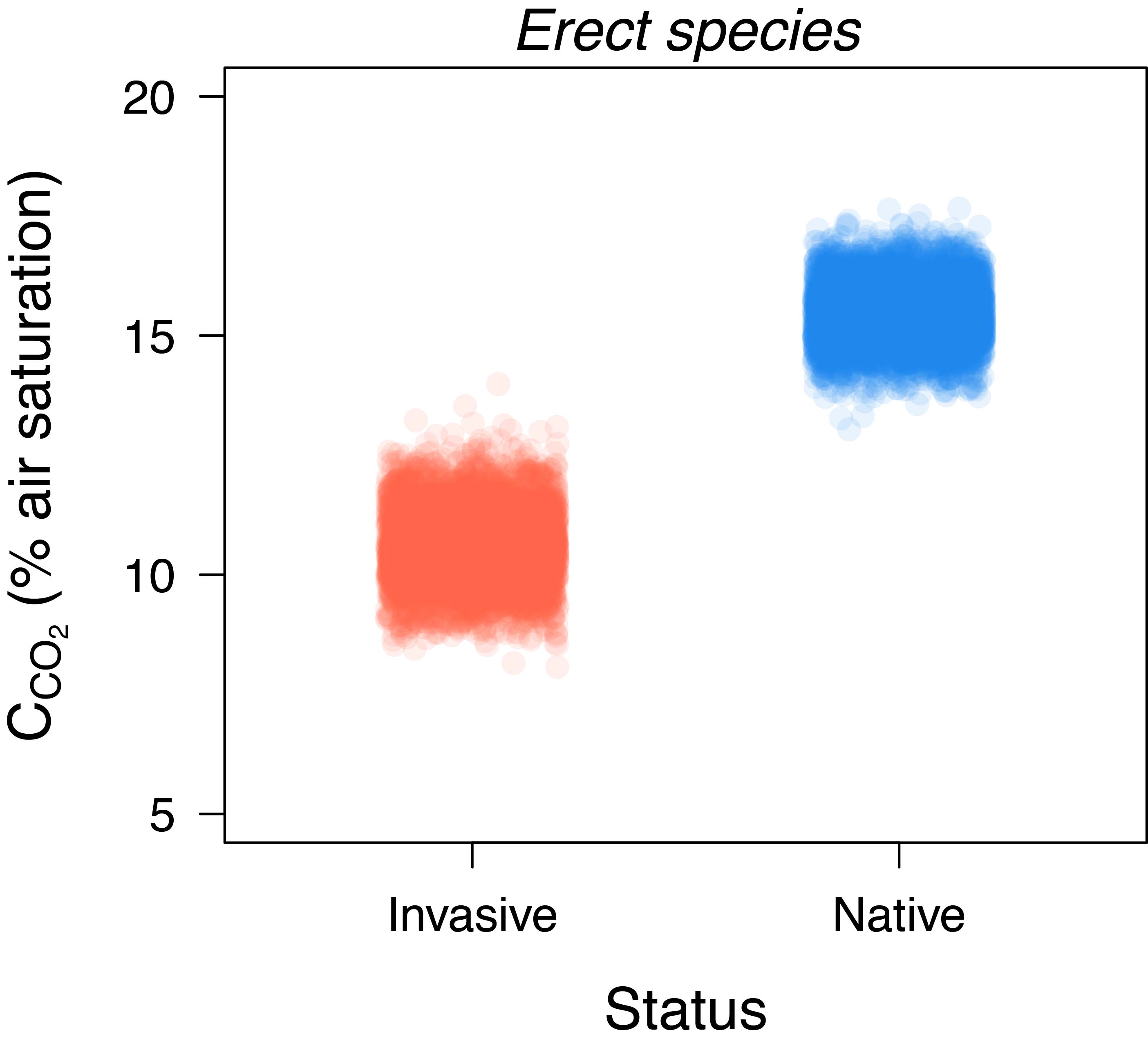
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Site | Mean | SD | Min | Max |
| Saint Kilda | 77.02 | 26.57 | 0.00 | 103.73 |
| Brighton | 80.09 | 21.48 | 0.00 | 111.66 |
| Queenscliff Harbour | 89.76 | 15.87 | 0.36 | 137.75 |
| Blairgowrie | 84.37 | 24.50 | 0.32 | 118.55 |
| Queenscliff Pier | 100.61 | 6.85 | 62.72 | 113.63 |



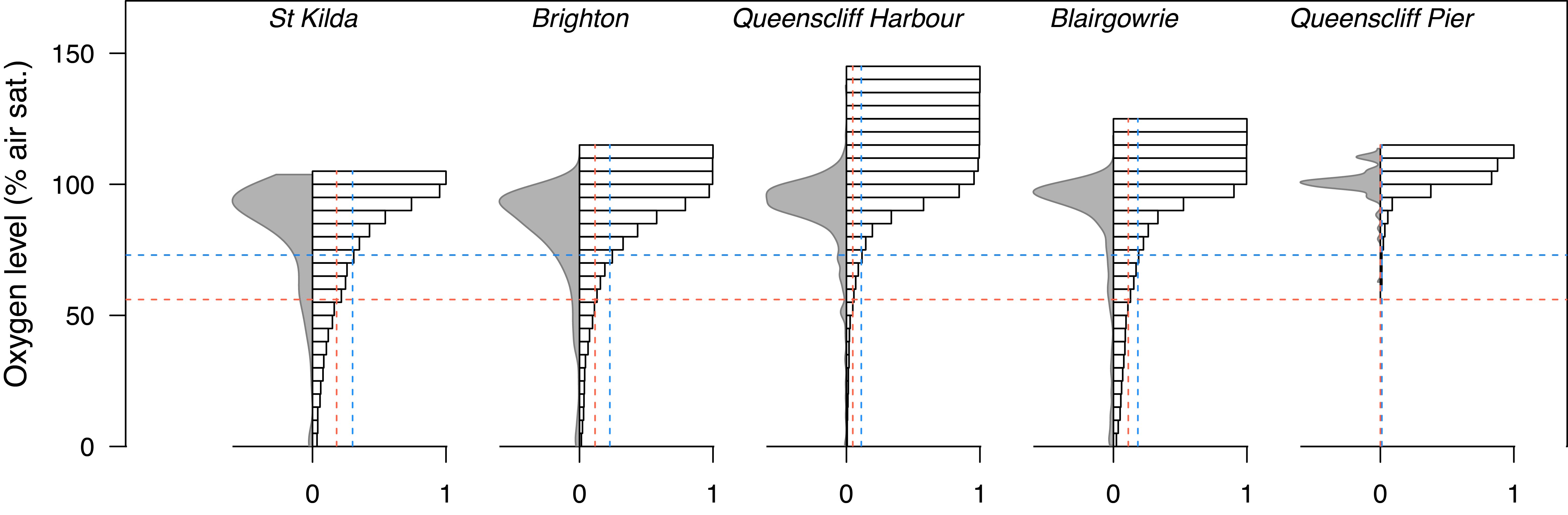
**Fig. 1** Plots of oxygen level (% air saturation) with relative respiration rate of each species measured (, 0-1). The intersection of dashed lines with x-axis shows the average calculated .



**Fig. 2** Differences in between: (a) species status (native and invasive), and (b) species shape (erect and flat). Each of the 6,000 circles for each category represents an average of estimated by an ANOVA using species-specific values of drawn from MCMC samples from a Michaelis-Menten function fitted in *JAGS*.

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**Fig. 3** Differences in of between status (native and invasive) for “erect shaped species” only. Each of the 6,000 circles for each category represents an average of estimated by an ANOVA using species-specific values of drawn from MCMC samples from a Michaelis-Menten function fitted in *JAGS*.

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**Fig. 4** Distribution of oxygen level across five different marine sites. Field sites are ordered according to their ranking of their average water speed, from slowest to fastest. Left side of each plot represent frequency distribution of oxygen. Right sides of the plots show cumulative density histograms of oxygen availability for each site. Vertical dashed lines indicate the level where the respiration rate of the animals start to decline (i.e. the value of air saturation in which oxygen consumption is 5% lower than that at 100% air saturation). The horizontal lines correspond to the percentage of microsites that represent physiology stress due to oxygen limitation for native and invasive species form each site. Blue lines are for invasive organisms and red for natives.

**Discussion**

We find that human-made structures, particularly marinas, cause reductions in local availability of oxygen in marine environments, and in some cases, push oxygen levels below the tolerance of the species that could live there, particularly native species. Environments with higher water flow provide almost exclusively normoxic microsites with low spatial and temporal variation in oxygen levels. On the other hand, low flow environments show higher variation in oxygen levels in both space and time. Flat species have greater tolerance to hypoxic conditions than erect species, a result that echoes previous studies in warm water sessile marine invertebrate communities (Ferguson *et al.*, 2013). Most importantly, we found that invasive species can tolerate lower oxygen levels than native species – invasive species could maintain a at oxygen levels that were ~1.7-fold lower than those of native species. Based on the values reported here, we calculated that in some sites, up to 30% of the microsites are physiologically stressful for native species.

Previous studies have recorded broad scale reductions in oxygen levels in low flow marinas (Stammerjohn *et al.*, 1991), but few have explored oxygen levels at the scales that are likely to be relevant to organisms. Our approach is likely to slightly overestimate oxygen availability in the field. We measured oxygen during daylight hours and in regions that were exposed to ambient light. Oxygenation of the boundary layer from photosynthesis by micro-phyto-benthos will therefore increase local oxygen levels during the day relative to those same areas at night (for analogous effects in tide pools, coral reefs and other low flow systems see: (Dodds *et al.*, 2007, Kinsey & Kinsey, 1967, Nilsson & Ostlund-Nilsson, 2004, Osinga *et al.*, 1999). We found generally higher estimates of oxygen availability in Port Phillip Bay relative to a similar study in a marina in subtropical Australia (Ferguson *et al.*, 2013). The subtropical site had similar or higher flow rates than the sites we measured, so differences in flow are unlikely to explain the observed difference in oxygen availability. We suspect that the higher temperature at the subtropical site (25 °C there versus 19 °C during our study) increased the metabolic demands of the local community, leading to lower oxygen levels overall. An important next step would be to determine whether oxygen availability covaries with seasonal changes in temperature at the study sites of the present study. Interestingly, even with differences in mean oxygen availability at the subtropical site and the St Kilda site, we find a similar percentage of habitat is predicted to be physiologically stressful to that found in the previous study (Ferguson *et al.*, 2013).

It’s important to note that our use of = as our proxy for is arbitrary. We cannot be sure that this proxy represents the oxygen level at which the contribution of aerobic metabolism begins; identifying the oxygen level at which this transition occurs, and comparing this value among native and invasive species would be a valuable avenue for future work. However, as a first approach, measure that we employ in the present study represents a good estimation of how metabolic processes are depressed by hypoxia. Perhaps most reassuringly, the estimates we made, and the patterns we observe, are similar to those using more traditional estimators in marine organisms (Ferguson *et al.*, 2013, Nilsson & Ostlund-Nilsson, 2004). Thus we believe the reported values to provide a fairly good indication of hypoxia resistance that makes no strong assumptions about the shape of the relationship between oxygen levels and metabolism. In organism such as fishes and mammals, which are generally strong oxyregulators, the can be found in a straightforward way, as they keep their oxygen consumption constant until they are exposed to a critical oxygen level (Portner & Grieshaber, 1993). However, the animals we measured ranged on the continuum between oxyconformation and oxyregulation, which is perhaps unsurprising given that our study included animals from many different phyla. We suggest that for more details about differences between oxyconformity and oxyregulation read Magnums & Vanwinkl (1973) and Portner & Grieshaber (1993). Oxyconformers rely on anaerobiosis at very low oxygen levels (Portner & Grieshaber, 1993), hence the functional groups measured here may be considered hypoxia tolerant, as they were able to withstand oxygen levels under around 1.8 mg l-1, ~ 25 % air saturation at 19 °C. Flat organisms, however, were able to withstand more extreme hypoxic conditions, and overall had lower values than erect species. Flat species are prone to live in low oxygen environments, as the boundary layers where they live are highly likely to be oxygen depleted (Ferguson *et al.*, 2013, Shashar *et al.*, 1993). On the other hand, at least the adult stages of erect species may not need to adapt to extreme hypoxic environments, as they can grow beyond the limits of the boundary layer and access more oxygenated water.

Invasive species presented a lower than natives. Moreover, we also found that erect-invasive organisms had lower values than erect-natives. Because we only had one native-flat species in our data set, we could not formally compare native and invasive flat species. However, we note that the flat-invasive organisms had the lowest across all functional groups, and could withstand extremely hypoxic levels (~ 5 % air saturation). Within the context of R\* theory, species with low (or should be better competitors than species with higher because they can maintain aerobic metabolism at relatively higher rates in hypoxic conditions. It has also been demonstrated that species with low can diminish the oxygen in the areas immediately surrounding them, leaving little oxygen available for other species (Ferguson *et al.*, 2013). It therefore seems that exploitative competition for oxygen has the potential to play an important role in marine invasions.

Across a range of taxa and systems, invasive species tend to have characteristics that make them more resistant to stressful conditions than native species (Lejeusne *et al.*, 2014, van Kleunen *et al.*, 2010b, Zerebecki & Sorte, 2011). Some studies suggest that invasive species are evolving to tolerate anthropogenic perturbations. For example, heavy metals, antifouling agents and other pollutants are selective pressures that favor invasive organisms, and studies of invasive species have shown that resistance to pollutants can be heritable (Floerl & Inglis, 2005, Levinton *et al.*, 2003, McKenzie *et al.*, 2011, Piola *et al.*, 2009). Tolerance to lower oxygen levels has evolved independently many times, as a response to environments where hypoxic conditions or strong fluctuations in oxygen availability dominate (Hochachka & Lutz, 2001, Mandic *et al.*, 2009, Nilsson & Ostlund-Nilsson, 2004). Usually the physiological threshold of a species matches the minimum oxygen level of its environment; therefore hypoxia tolerance is an important trait that can determine the distribution and abundance of organisms (Lagos *et al.*, 2011, Stillman & Somero, 1996, Verberk *et al.*, 2011). We do not know if resistance to hypoxia in invasive organisms is a heritable trait, or whether this trait is an example of phenotypic plasticity. However, the reduction of oxygen levels associated with artificial structures may be acting as a selection pressure that favors invasive organisms. We would therefore suggest that species that are already tolerant to low oxygen conditions might be more likely to become invasive if translocated by humans.

Our results suggest that artificial structures provide windows for invasion via mediation of water flow. As lowering water flow increases the prevalence of hypoxic and anoxic microsites it is likely that a higher proportion of such habitats are hostile to native species while still allowing invasive species to function normally. From a management perspective, artificial structures that maintain water flow rates that result in the adequate replenishment of oxygen at local scales might be more effective at promoting the proliferation of native species and discouraging invasion.

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**Data accessibility**

All data and R code (analyses, figures and tables) can be downloaded from a GitHub repository (<https://github.com/dbarneche/vo2Inverts>), which will be made publicly available upon publication.

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