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

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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. 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 and 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 water flow relative to piers, and that local oxygen levels can be zero in low flow conditions. We also found that for species with erect 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 same 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 niche opportunities for invasive species.

KEYWORDS
artificial structures, exploitative competition, invasions, low flow, low oxygen, marinas, nonindigenous species, sessile organisms

1 INTRODUCTION

Biological invasions, together with habitat destruction, are considered to be the biggest threats to biodiversity around the world (Dafforn, Glasby, & Johnston, 2009; Davis, 2003; Vitousek, Dantonio, Loope, & Westbrooks, 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 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, Kareiva, Levin, & Okubo, 1990; Arim, Abades, Neill, Lima, & Marquet, 2006; Van Kleunen, Dawson, Schlaepfer, Jeschke, & Fischer, 2010; Van Kleunen, Weber, & Fischer, 2010; 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, White, & Marshall, 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 at using resources than native species. 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).

In marine systems, there is a strong association between artificial hard structures and invasive species. Marinas and other artificial structures such as pilings, pontoons, and jetties are considered windows for biological invasion (Airoldi, Turon, Perkol-Finkel, & Rius, 2015; Bulleri & Airoldi, 2005; Dafforn, Johnston, & Glasby, 2009; Glasby, Connell, Holloway, & Hewitt, 2007; Ruiz, Freestone, Fofonoff, & Simkanin, 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, Hantsch, & Bruel-heide, 2013; Kinlan & Gaines, 2003; Mckenzie, Brooks, & Johnston, 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 the 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 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, Neckles, & Orth, 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 more likely to experience low oxygen conditions, so have adapted accordingly. Because of the long association of invasive species with artificial structures, which reduce flow in the environment (Bulleri & Airoldi, 2005; Dafforn, Johnson, et al., 2009; Wilding, 2014), we hypothesize that invasive species 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 often known as the critical oxygen concentration (\(C_{O_2}^{*}\)) or critical oxygen pressure (\(P_{O_2}^{*}\)) (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, Webb, Darwent, & Beckett, 2009; Hochachka & Somero, 2002; Portner & Grieshaber, 1993). In marine invertebrates, which are often neither strict oxy-conformers nor oxy-regulators, measuring \(C_{O_2}^{*}\) 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., Hardewig, Addlnk, Greishaber, Portner, & Van Den Thillart, 1991; Portner, Heisler, & Greishaber, 1985). In *Sipunculus nudus*, for example, rates of oxygen consumption decline linearly with ambient oxygen down to an oxygen partial pressure (\(P_{O_2}\)) of ~5-10 kPa; the rate of oxygen consumption then exhibits an inflection at this \(P_{O_2}\), which is indicative of the commencement of anaerobic metabolism (Hardewig et al., 1991; Portner et al., 1985). Thus, even in the absence of anaerobic metabolism in these species, decreasing \(P_{O_2}\) causes a reduction of aerobic metabolism. The level to which metabolic rate declines with
decreases in oxygen provides an indication of 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 should 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 five 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 hypothesize that low flow environments have lower oxygen availability than high flow environments and that invasive species can tolerate lower oxygen levels than native species.

We define invasive species as introduced, exotic, or nonindigenous species inhabiting an area outside of its natural or historical range of distribution (Colautti & MacIsaac, 2004; Neill & Arim, 2011). In this 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 vs. invasive species.

2 | MATERIALS AND METHODS

2.1 | Organism collection and the estimation of tolerance to low oxygen conditions

Where possible, we collected organisms from the same sites as those where we made our estimates of oxygen availability, 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 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 preroughened 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 classified species as native or invasive based on classifications by the Australian government (www.environment.gov.au) and Australian museum records. Based on these criteria, the species classified as invasive are unlikely to have been anywhere in Australia before being transported by boats sometime in the last 200 years and 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 laboratory in insulated aquaria with aerated seawater and acclimatized to laboratory conditions for two 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, White, & Marshall, 2015). Larger organisms were measured in hermetic 1.8 L chambers with recirculating water connected to a four-channel Firesting O2 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 sought 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, White, & Marshall, 2016; Pettersen et al., 2015). The chambers and vials were filled with microfiltered (0.2 μm), sterilized seawater that had been kept at 19°C with constant aeration for at least 24 hr prior to experiments. Rates of oxygen consumption (VO2,

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth shape</th>
<th>Status</th>
<th>DBM (g ± SD)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bugula flabellata</td>
<td>Erect</td>
<td>Invasive</td>
<td>0.03 (±0.01)</td>
<td>7</td>
</tr>
<tr>
<td>Bugula neritina</td>
<td>Erect</td>
<td>Invasive</td>
<td>0.07 (±0.03)</td>
<td>11</td>
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<tr>
<td>Ciona intestinalis</td>
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<td>Invasive</td>
<td>0.22 (±0.07)</td>
<td>9</td>
</tr>
<tr>
<td>Styela plicata</td>
<td>Erect</td>
<td>Invasive</td>
<td>1.48 (±0.40)</td>
<td>8</td>
</tr>
<tr>
<td>Styela clava</td>
<td>Erect</td>
<td>Invasive</td>
<td>1.17 (±0.30)</td>
<td>17</td>
</tr>
<tr>
<td>Watersipora subtortuquata</td>
<td>Flat</td>
<td>Invasive</td>
<td>0.09 (±0.04)</td>
<td>13</td>
</tr>
<tr>
<td>Didemnum sp.</td>
<td>Flat</td>
<td>Invasive</td>
<td>0.19 (±0.11)</td>
<td>11</td>
</tr>
<tr>
<td>Diplosoma sp.</td>
<td>Flat</td>
<td>Invasive</td>
<td>0.08 (±0.04)</td>
<td>9</td>
</tr>
<tr>
<td>Pyura dalbyi</td>
<td>Erect</td>
<td>Native</td>
<td>5.13 (±1.10)</td>
<td>9</td>
</tr>
<tr>
<td>Pyura doppelgangeria</td>
<td>Erect</td>
<td>Native</td>
<td>4.67 (±1.31)</td>
<td>9</td>
</tr>
<tr>
<td>Herdmania grandis</td>
<td>Erect</td>
<td>Native</td>
<td>2.13 (±0.90)</td>
<td>13</td>
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<tr>
<td>Botrylloides magnicoecum</td>
<td>Erect</td>
<td>Native</td>
<td>0.70 (±0.31)</td>
<td>9</td>
</tr>
<tr>
<td>Bugula dentata</td>
<td>Erect</td>
<td>Native</td>
<td>0.05 (±0.01)</td>
<td>11</td>
</tr>
<tr>
<td>Celleporaria sp.</td>
<td>Flat</td>
<td>Native</td>
<td>0.17 (±0.06)</td>
<td>7</td>
</tr>
</tbody>
</table>
ml hr⁻¹) were calculated as described in previous studies (Ferguson et al., 2013; Pettersen et al., 2015; White, Kearney, Matthews, Kooljman, & Marshall, 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 OHNAUS, Pine Brook, NJ, USA) to the nearest milligram.

2.2 Model

In contrast to what is observed for most vertebrates, where a clear CCO₂ can be discerned (Marshall, Bode, & White, 2013), our VO₂ data were curvilinear, such that there was no clear point where the organisms transitioned from a perfect oxyregulator to an oxyconformer (Figure 1). Instead we fit a Michaelis–Menten function to our VO₂ consumption data:

\[
\text{VO}_2 = \frac{\text{VO}_2\text{max} \times \text{CO}_2}{C_{\text{SO}_2/\text{VO}_2} + \text{CO}_2}
\]  

(1)

where \(\text{VO}_2\text{max}\) is an asymptotic \(\text{VO}_2\), and \(C_{\text{SO}_2/\text{VO}_2}\) is the value of \(\text{CO}_2\) where \(\text{VO}_2 = \text{VO}_2\text{max}/2\). Importantly, to achieve model convergence, we employ a transformation to \(\text{VO}_2\). For each individual, we standardize \(\text{VO}_2\) based on its maximum value, so all individuals present a relative \(\text{VO}_2\) bounded between zero and one. We note that this transformation implicitly assumes that \(C_{\text{SO}_2/\text{VO}_2}\) is independent of body mass – this assumption has mixed support from other studies (for a detailed discussion of this issue, see Lease, Klok, Kaiser, & Harrison, 2012). Importantantly, however, while transformation affects the estimated \(\text{VO}_2\text{max}\), it does not affect our primary goal, which is to estimate \(C_{\text{SO}_2/\text{VO}_2}\) 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 \(C_{\text{SO}_2/\text{VO}_2}\), which is in the denominator, is not affected.

For the \(C_{\text{SO}_2/\text{VO}_2}\) model, we fit Equation (1) above in a Bayesian framework by calling JAGS version 4.2.0 from the R package rjags version 0.05-6 (Su & Yajima, 2015) in order to derive posterior distributions and associated 95% credible intervals (CIs) for the fitted parameters, \(\text{VO}_2\text{max}\) and \(C_{\text{SO}_2/\text{VO}_2}\). We allow \(\text{VO}_2\text{max}\) and \(C_{\text{SO}_2/\text{VO}_2}\) 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 \(\times 10^6\) steps each, including 7.5 \(\times 10^5\)-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 \(\times (1.5 \times 10^6-7.5 \times 10^5)/375 = 6,000)\).

We use the species-specific estimates \((n = 14\); Figure 1\) for \(C_{\text{SO}_2/\text{VO}_2}\) obtained in JAGS in order to fit three separate ANOVA’s: one to test for differences in \(C_{\text{SO}_2/\text{VO}_2}\) between species status (native and invasive), a second test to test for differences in \(C_{\text{SO}_2/\text{VO}_2}\) between species shape (erect and flat), and a third one to test for differences in \(C_{\text{SO}_2/\text{VO}_2}\) between species status and shape. However, given that we only have 14 species in our data set, 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 to obtain a full “posterior distribution” of differences in \(C_{\text{SO}_2/\text{VO}_2}\) 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 \(\text{VO}_2\) at 100% CO₂ (\(\text{VO}_2\text{100}\)) and use the value of CO₂ in which \(\text{VO}_2 = \text{VO}_2\text{100}/2\) as our proxy for CO₂. 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 to calculate the value of air saturation, CO₂, in which \(\text{VO}_2 = \text{VO}_2\text{100} \times 0.95\).

2.3 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 taken 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 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
FIGURE 1  Plots of oxygen level (% air saturation) with relative respiration rate of each species measured ($\dot{V}O_2$, 0–1). The intersection of dashed lines (highlighted with an arrow) with x-axis shows the average calculated $C_{CO_2}$.
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 min). At pier sites, which were generally 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 five sites.

3 | RESULTS

3.1 | 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., $C_{CO_2}^O$, $C_{O_2}$ where $VO_2 = \frac{VO_2}{V_{O_2}^{100}}$ / 2, Equation 1): invasive species tolerate oxygen levels that are ~1.7-fold lower than the critical values for native species (Figure 2) while maintaining similar normalized metabolic rates (average: 0.12 ml O2 hr⁻¹; 95% CI 0.09–0.16 for invasive species; average: 0.14; 95% CI 0.11–0.19 for native species; Appendix S1). Similarly, flat species tolerate oxygen levels that are on average ~2.3-fold lower than the critical values for erect species (Figure 2). 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 data set; erect invasive species could tolerate significantly lower oxygen levels than erect native species (Figure 2).

3.2 | 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 (Figure 3). Microsites (i.e., samples) with high oxygen levels (% air saturation) were found at all sites (Figure 3). St Kilda had the highest variation in oxygen availability and also had higher frequency of microsites with 0% of oxygen (Table 2, Figure 3). In contrast, Queenscliff Pier had the lowest variability in oxygen availability, and no microsite showed oxygen levels at 0% (Table 2, Figure 3).

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 (Figure 3). 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.

4 | DISCUSSION

We find that humanmade structures, particularly marinas, cause reductions in the local availability of oxygen in marine environments, and in some cases, push oxygen levels below the tolerance of (particularly native) species that could otherwise live there. Environments with higher water flow provide almost exclusively normoxic

![FIGURE 2 Differences in $C_{CO_2}$ between: (a) species status (native and invasive), (b) species shape (erect and flat), and (c) between natives and invasive species for erect species only. Each of the 6,000 circles for each category represents an average of $C_{CO_2}$ estimated by an ANOVA using species-specific values of $C_{CO_2}$ drawn from MCMC samples from a Michaelis–Menten function fitted in JAGS.](image-url)
Based on the gen levels that were found generally higher estimates of oxygen availability in Port Phillip (Ostlund-Nilsson, 2004; Osinga, Tramper, & Wijffels, 1999). We robustly recorded reductions in 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 microphytobenthos 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, Roberts, Taylor, & Marubini, 2007; Kinsey & Kinsey, 1967; Nilsson & Ostlund-Nilsson, 2004; Oisinga, Tramper, & Wijffels, 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 vs. 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 is important to note that our use of $V\text{O}_2 = \frac{V\text{O}_2_{20\text{C}}}{2}$ as our proxy for $C_{\text{O}_2}$ 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 preliminary measure, our approach provides 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 $C_{\text{O}_2}$ 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. Based on this metric, the functional groups measured here 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 Mangum and Vanwinkle (1973) and Portner and Grieshaber (1993).

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** 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 side 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.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
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<tbody>
<tr>
<td>Saint Kilda</td>
<td>77.02</td>
<td>26.57</td>
<td>0.00</td>
<td>103.73</td>
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<tr>
<td>Brighton</td>
<td>80.09</td>
<td>21.48</td>
<td>0.00</td>
<td>111.66</td>
</tr>
<tr>
<td>Queenscliff Harbour</td>
<td>89.76</td>
<td>15.87</td>
<td>0.36</td>
<td>137.75</td>
</tr>
<tr>
<td>Blairgowrie</td>
<td>84.37</td>
<td>24.50</td>
<td>0.32</td>
<td>118.55</td>
</tr>
<tr>
<td>Queenscliff Pier</td>
<td>100.61</td>
<td>6.85</td>
<td>62.72</td>
<td>113.63</td>
</tr>
</tbody>
</table>

Previous studies have recorded broad scale reductions in oxygen levels in low flow marinas (Stammerjohn, Smith, Boynton, & Kemp, 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 microphytobenthos 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, Roberts, Taylor, & Marubini, 2007; Kinsey & Kinsey, 1967; Nilsson & Ostlund-Nilsson, 2004; Oisinga, Tramper, & Wijffels, 1999). We found generally higher estimates of oxygen availability in Port Phillip...
Oxyconformers rely on anaerobiosis at very low oxygen levels (Porter & 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 \(C_{\text{CC}}\) 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, Cohen, & Loya, 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 \(C_{\text{CC}}\) than natives. Moreover, we also found that erect-invasive organisms had lower \(C_{\text{CC}}\) 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 \(C_{\text{CC}}\) across all functional groups, and could withstand extremely hypoxic levels (~5% air saturation). Within the context of \(R^*\) theory, species with low \(C_{\text{CC}}\) (or \(P_{\text{CO}_2}\)) should be better competitors than species with higher \(C_{\text{CC}}\) because they can maintain aerobic metabolism at relatively higher rates in hypoxic conditions. It has also been demonstrated that species with low \(C_{\text{CC}}\) 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, Latchere, Petit, Rico, & Green, 2014; Van Kleunen, Weber, et al., 2010; Zerebecki & Sorte, 2011). Across our pool of species, their life histories are very similar such that it is difficult to discern covariance between invasion status and life history. Similarly, we wondered whether original habitat may have played a role in shaping hypoxia tolerance. For example, estuaries are more likely to experience oxygen fluctuations so may select for species with lower \(C_{\text{CC}}\) because they can maintain aerobic metabolism at relatively higher rates in hypoxic conditions. It has also been demonstrated that species with low \(C_{\text{CC}}\) 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.

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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/vo2Inve)

REFERENCES


Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.