Role of oxygen in shaping invertebrates sessile communities

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B (Honors)

Thesis submitted for the degree of *Doctor of Philosophy*
Monash University
November 2016
School of Biological Sciences

Abstract

Resources, and their use by organisms shape populations and communities. Oxygen is a key resource that may play an important role in marine communities. When low oxygen conditions are a risk, mobile organisms can always avoid the physiological stress through movement. Sessile organisms, however, must either actively avoid low oxygen environments during the mobile larval stage, or forever tolerate it at as adult. In the first two chapters, I use the marine invertebrate *Bugula neritina* as a model species to determine whether larvae can detect and avoid low oxygen conditions during habitat selection. Then I investigate whether larvae are capable of integrating information about oxygen levels in the water and biofilm characteristics to select settlement habitats with optimal oxygen levels. The results indicate that larvae respond to both cues in a hierarchical way. First they choose habitats according to the oxygen levels in the water, and then by oxygenation history of the biofilm. Next, I explore the role of artificial structures in altering local oxygen levels. Artificial structures create perfect niche opportunities that select invasive species and jeopardize natives by disrupting natural flows, and decreasing local oxygen levels. In the third and fourth chapter I measured oxygen availability and water flow at the micro-scale level (millimeters) in marinas and piers in Port Phillip Bay, Australia. I also collected individuals of 14 species to determine their metabolic rates and their tolerance to hypoxia. Species were classified according to their body shape (erect or flat) and invasive status (native or invasive). I found that invasive species and flat species are able to resist lower oxygen conditions more than natives and erect shaped organisms. Small invasive species have higher metabolic rates than natives with the same size, but both groups have similar metabolic rates at bigger body masses. Flat species have lower metabolic rates than erect species in every measured size. I found that oxygen availability was correlated with flow rates in marinas and piers. Low flow environments create more hypoxic and anoxic microenvironments that are likely to be physiologically stressful to native species in particular. Highly modified marinas with low flow conditions may therefore generate conditions that are conducive to the proliferation of invasive species.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: 

Print Name: Marcelo Eduardo Lagos Oróstica

Date: 02-Nov-2016

Thesis including published works General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes two original papers published in peer-reviewed journals. The core theme of the thesis is how oxygen can shape invertebrates sessile communities. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the school of Biological sciences under the supervision of Professor Dustin Marshall and Professor Craig White as co-advisor.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 1 and 2 my contribution to the work involved the following:

Thesis chapter	**Publication Title**	**Status**	**Nature and % of student contribution**	**Co-author names Nature and % of Co-author's contribution**	**Co-authors, Monash student Y/N*-**
1	Avoiding low oxygen environments: oxytaxis as a mechanism of habitat selection in a marine invertebrate	Published	Data collection, experiment design, data analysis and writing 70%.	1)Dustin Marshall: data analysis, experimental design and input into manuscript 20% 2)Craig White: experimental design input into manuscript 10%	N
2	Biofilm history and oxygen availability interact to affect habitat selection in a marine invertebrate	Published	Data collection, experiment design, data analysis and writing 70%	1)Dustin Marshall: data analysis, experimental design and input into manuscript 20% 2)Craig White: experimental design input into manuscript 10%	N

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date: 02-Nov-2016**

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student and co-authors' contributions to this work.

Main Supervisor signature: [redacted] **Date: 02-Nov-2016**

Acknowledgments

"This is a journey of four years that began in a distant place.
Then, crossing the oceans of time I arrived to a magical land.
I found a witch, a master
and a group of friends that helped me in my quest.
I entered in to the cave to face myself.
At the end, I came back
to finish…
and defeat the monster"

I want to thank many people that were involved in some way, in the development of my research and my life during these years in Oz. Firstly, I want to thank my supervisor Professor Dustin Marshall who did the possible, and maybe the impossible, to help me and guide me during my research. To my co-advisor, Professor Craig White, for all his help and support. To my lab mates: Mattew Thompson , Annie Guillaume, Henry Wootton, Robin Svensson, Rolanda O'connor, Keyne Monro, Diego Barneche, Martino Malerba, Chun-Yi "Ted" Chang, Evatt Chirgwin, Karin Svanfeld , Belinda Comerford for all their support in the field, writing, statistics and fun times; and especially Amanda Pettersen and Hayley Cameron for all their help proofing the drafts of my papers. To Fiona Hibbert for all her assistance. To my housemates: Manoj Kamalanathan , Mattia Pierangellini, Nataly Hidalgo and Bertrand Gauffre for all the funny times. To my friend Stefanie Rog for her company and motivation during the time we spent writing together. To Simone Scotti Requena, for being my "alter-ego" in this and all the other realities. Finally, I want to dedicate this thesis to my parents that are far away, in the last place in the world.

Contents

GENERAL INTRODUCTION

The availability of resources and how organisms interact to fulfill their needs can shape communities at all levels (Shea and Chesson 2002). In marine systems, there has been a strong tradition of focusing on interactions for the resources of food and space (Paine 1966, Jackson 1977, Svensson and Marshall 2015). Competition theory, keystone species, alternative stable states and the role of predation in shaping marine communities all focus on either food or space as key limiting resources in marine systems (Connell 1961, Sutherland 1974, Buss 1990). While these resources are undoubtedly important, the almost exclusive focus that marine biologists have maintained on food and space have meant that other, potentially equally important resources have received much less attention – chief among those is oxygen.

Oxygen is a key resource for aerobic organisms. Differences in environmental oxygen concentration and pressure affects the life of organisms at every development stage (Baker and Mann 1992, Seymou and White 2006, Ahn and Burne 2007, Moran and Woods 2007, Verberk et al. 2011); and limits natural boundaries of distribution and influence the evolutionary direction of species (Stillman 2000, Dzal et al. 2015). Remarkably, with only few exceptions, the role of oxygen shaping communities has not been well explored (Altieri and Witman 2006, Ferguson et al. 2013). Perhaps this is because for terrestrial habitats oxygen is not really a limiting resource, with a few exceptions in soils and high altitude habitats (Ludemann et al. 2000, Jacobsen 2008). However, in aquatic systems oxygen is a resource that can be limited spatially and temporally. The availability of oxygen and the abilities of the animals to extract it depends of several physicochemical factors. The oxygen capacitance of water is 30 times lower than air, and diminishes with increases in temperature and salinity. Gas molecules can diffuse up to 10,000 times more rapidly in air than in water. In addition, oxygen diffusion decreases with increases in the distance between the hemolymph and respiratory surface that is in contact with the environment (Cameron 1986, Verberk et al. 2011).

Some aquatic systems have naturally low oxygen levels. Oxygen minimums zones are a good example of large-scale areas of low oxygen (Morrison et al. 1999, Fuenzalida et al. 2009). Unfortunately, human modifications of natural systems over the last 50 years have increased the number of hypoxic and anoxic habitats. At larger scales, processes such as eutrophication are leading to mass mortalities that can modify and destroy marine communities. Such eutrophication events have been shown to affect not only biodiversity, but also human economies and subsistence (Altieri and Witman 2006, Altieri 2008, Diaz and Rosenberg 2008). These decreases in oxygen levels over large spatial scales are one of the more obvious consequences of human impacts on

oxygen levels in aquatic systems - less understood are the impacts of humans on oxygen levels at smaller scales.

Oxygen levels in aquatic habitats vary naturally at small spatial scales. Habitats with low flow such as pools and coral outcrops also show significant variation in oxygen level (Kinsey and Kinsey 1967, Osinga et al. 1999, Nilsson and Ostlund-Nilsson 2004, Dodds et al. 2007). Artificial structures such as marinas, piers and pontoons in coastal areas also reduce the water flow and initial studies suggest that these habitats have unusually low oxygen levels (Ferguson et al. 2013). The combined effect of low flow, complex topography and oxygen consumption of the dense fouling communities can lead to the formation of anoxic and hypoxic microhabitats (Vogel 1994, Ferguson et al. 2013, Wilding 2014). While the idea that artificial marine structures tend to have lower oxygen levels is increasingly well recognised, the impacts of lower oxygen on communities living on these structure have not been explored (Verberk et al. 2011).

Different species have developed several physiological, morphological and behavioral strategies to cope the stress of hypoxia (Breitburg 1994, Stillman and Somero 1996, Lagos et al. 2011). The strategies that animals use to deal with physiologic stress are related with their style of life. Mobile species can, in some instances, avoid stress by moving to another place. However, for sessile or with low mobility organisms, the only option they have is to either avoid the low oxygen area at colonization or tolerate the low oxygen conditions (Craig et al. 2005, Altieri 2006, Ludsin et al. 2009, Ferguson et al. 2013). The limits of tolerance increase through different mechanisms. The most common are related changes in blood chemistry (hemocyanin-oxygen affinities), variations in metabolic activities, strong regulation of acid-base balance and modification of respiratory structures (Truchot 1980, Lallier and Truchot 1989, Dahlhoff et al. 2002, Vargas et al. 2010). For sessile species, the only mobile moment in their lives is during larvae stage, and only during this stage they can potentially avoid the physiologic stress of low oxygen conditions. Whether the larvae of sessile organisms can detect and avoid low oxygen conditions remains largely unknown.

In this thesis I explored how oxygen can shape marine sessile communities. In the first two chapters I measured pre-settlement behaviour and larval avoidance of hypoxic microenvironments, and in the next two, the oxygen physiology of adults. For sessile organisms, the larvae stage is the only in their lifecycle with mobility; therefore, the ability of larvae to choose where to settle is fundamental for the organism to maximize their chances of survival (Doyle 1974, Doyle 1975, Rodriguez et al. 1993). More generally, larval settlement choices can fundamentally alter community assembly, and the distribution and abundance of species in marine systems (Gaines and Roughgarden 1985). Habitat selection involves the interaction between behavioral constraints, larvae age and environmental cues (Burgess et al. 2009). Some of the important larval settlement cues are light intensity and quality, biofilm composition, the presence of adults (both conspecific

and competitors) (Grosberg 1981, Jensen 1989, Mundy and Babcock 1998, Thiyagarajan 2010, Cheung et al. 2014). To my knowledge, few studies have explored how larvae of sessile organisms can use oxygen availability as a cue for habitat selection.

The second chapter is focused on the interaction between larvae behavior and biofilm history. In sessile communities, oxygen availability can vary over very small spatial and temporal scales (Vogel 1994, Gardella and Edmunds 1999, Ferguson et al. 2013). Therefore, the capacity of larvae to select an enduringly suitable habitat will be undermined if the flow conditions, or any other factor that can modify the oxygen levels, change over short time periods. For this reason, larvae must integrate different cues that can give them an indication of what is happening now, and what happened within the habitat in the past, to increase the probability of making more accurate predictions about the quality of the habitat in the future. Bacterial biofilms (both presence and composition), have been described as two of the most important factors that can affect larvae settlement (Dahms et al. 2004, Dobretsov and Qian 2006, Bao et al. 2007). Larvae can recognize and discriminate among biofilms of differing age, composition and density and use this information as cues for habitat selection (Wieczorek and Todd 1997, Bao et al. 2007, Cheung et al. 2014). Larvae of sessile organisms prefer to settle on surfaces that were generated in high oxygen conditions, suggesting that the oxygenation history of bacterial biofilms alters larval settlement preferences (Cheung et al. 2014). If the oxygen conditions in the water change, it can be expected that composition of the biofilms generated will change as well. In this way, biofilms can provide an integrated account of previous oxygen conditions in that particular microenvironment. My aim in this chapter was to understand how larvae can integrate short-term (oxygen availibility in the water) and longer-term (biofilm oxygenation history) cues to select a suitable habitat.

While the first two chapters focus on larvae, the next two chapters focus on adult marine invertebrates. Specifically, I explore how tolerance to low oxygen conditions and oxygen usage varies with growth form and invasion status in sessile marine communities. Virtually every marine environment that has been modified by humans has been invaded by non-indigenous species. Artificial structures such as marinas, piers and pontoons are particularly invaded as they modify the natural conditions of habitats, which jeopardize native fauna, provide free space and spread invasions (Bulleri and Airoldi 2005, Dafforn et al. 2009). However, the probability of invasions will depend of the interaction between habitats conditions and particular features of the invasive species (Andow et al. 1990, Arim et al. 2006, van Kleunen et al. 2010a, van Kleunen et al. 2010b, Zhao and Feng 2015).

When environmental resources are limited, competition is the most common response of organisms (Connell 1961, Buss 1979). In this way, if we think about oxygen as a resource, species that use it will compete for it when scares (Ferguson et al. 2013). In a competition scenario, the

lowest amount of any particular resource that that is required for the species (R*) is critic. R* theory predicts that when two species compete for the same resource, the species with the lowest requirement will be the winner (Tilman 1982, Tilman 2004). In marine sessile communities, the growth form of the organisms is linked to competitive ability for oxygen. For example, flat species have a lower R* of oxygen requirements and it is anticipated that they are competitively dominant over erect shaped organisms, in terms of the use of oxygen (Ferguson et al. 2013). In this chapter I had two aims: first, to quantify how much the flow conditions can affect the oxygen availability in marine artificial structures; second, to determine if sessile invasive species have a lower requirement for oxygen than natives. Having a low R* for oxygen can be one of the key features that explains the success of invasive species, as they are associated to environments that can be physiologically stressful for natives.

Understanding the energetic requirements of different groups is not trivial. How metabolic metabolic rate scales with body mass is related with other traits that can explain important ecological questions. General consensus indicates a metabolic scaling exponent of 2/3 or 3/4 for living organism (Farrell-Gray and Gotelli 2005, Glazier 2005, Glazier 2010). However, groups with different life histories may have different metabolic rates. For example, organisms with high metabolic rates are associated with high reproduction, fast growths, quick reproduction and fast life histories (McMahon 2002, Burton et al. 2011, Pettersen et al. 2016). These "weedy/r-selected" characteristics are typical of most invasive species (Sakai et al. 2001). Nevertheless, it has been hypothesized that invasive species could have either relatively higher or relatively lower metabolic rates than native species. For example can be sited the case of the invasive shrimp *Palaemon macrodactylus* has a lower metabolism than its native counterpart *P. longirostris,* and the brown trout *Salmo trutta* that has an elevated metabolic rate (McMahon 2002, Alvarez and Nicieza 2005, Gonzalez-Ortegon et al. 2010). However, different hypothesis must be interpreted within the context of factors as level of perturbations in the environment, successional stage in the communities and the peace of life of the species. The final chapter of this thesis focuses on, how growth form and invasion status affect the relationship between body mass and metabolic rate in sessile marine invertebrates from a range of phyla.

In the first two chapters I used the arborescent bryozoan *Bugula neritina* as model species. Some characteristics make this species a good model for studying larval settlement behaviour. They can easily found on submerged hard substrates at shallow depth. *B. neritina* release non-feeding larvae, that are fully competent to settle immediately upon release (Doyle 1974). I did a series of experiments to test if larvae behaviour and habitat selection change in normoxy and hypoxic conditions of the environment, and biofilm history. In the chapter three I measured the relationship between flow and oxygen variability in the field. In the chapter four I measured physiologic

tolerance and metabolic rate of 14 marine sessile species, categorized according to their growth shape (erect-flat) and invasion status (invasive-native).

Declaration for Thesis Chapter 1

Declaration by candidate

In the case of Chapter 1, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Development of key ideas, data collection, experiment design, data analysis and writing	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dustin Marshall	Assistance in data analysis, experimental design and input into manuscript	20
Craig White	Experimental design, input into manuscript	10

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 02-Nov- 2016
Main Supervisor's Signature	[redacted]	Date: 02-Nov- 2016

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER I

Avoiding low oxygen environments: oxytaxis as a mechanism of habitat selection in a marine invertebrate

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This chapter is currently published in the journal:

Marine Ecology Progress Series (2015), Vol. 540: 99–107, 2015.

doi: 10.3354/meps11509

ABSTRACT: Oxygen poor habitats are increasingly common in aquatic environments. Human activities are accelerating the spread of oxygen-poor environments, yet the way in which most animals avoid low oxygen conditions remains poorly resolved. For organisms with a sessile or sedentary adult phase, habitat selection is crucial and many organisms show sophisticated responses to various habitat cues during colonization. Whether oxygen availability serves as such a cue is unknown, yet increasingly it seems that oxygen is an essential limiting resource in some systems. In a series of experiments, we manipulated oxygen levels during dispersal and colonization in the larvae of the model marine invertebrate *Bugula neritina* in the laboratory. We found that larvae reduce the time used in habitat exploration and delay colonization in the presence of lower oxygen levels. We also found that larvae avoid hypoxic water (positive oxytaxis) – the first such demonstration for marine larvae. All of these behaviours may decrease the likelihood of colonizing low oxygen habitats in nature. Our results suggest that marine invertebrate larvae can use oxygen availability as a cue for habitat selection.
Keywords: *Bugula neritina*, Colonization, Dispersal, Hypoxia, Larval behaviour.

INTRODUCTION

Oxygen is a critical resource for most organisms. If oxygen falls below a minimum threshold of tolerance, physiological and behavioral responses are induced that can determine the distribution and abundance of organisms at many spatial scales (Diaz and Rosenberg 1995, Jacobsen 2008, Vargas et al. 2010, Long et al. 2014). At smaller spatial scales for example, oxygen availability can have an effect on physiology and morphology of intertidal organism, and therefore limit their vertical distribution (Burggren and McMahon 1981, Stillman and Somero 1996, Lagos et al. 2011, Lucon-Xiccato et al. 2014). While at larger scales, species richness can show altitudinal clines with decreasing partial pressures of oxygen (Jacobsen 2008, Dubay and Witt 2014).

Increasingly, it appears that many human activities are causing the proliferation of low oxygen environments in aquatic systems. Eutrophication is generating extended hypoxic environments that can lead to the formation of "dead zones" (Diaz 2001). At large scales, dead zones represent a collapse of aquatic ecosystems, with mass mortality events that can extirpate local communities and the ecosystem services they provide (Altieri and Witman 2006, Diaz and Rosenberg 2008). While naturally-occurring regions of low oxygen are common, such regions have been growing in prevalence and size for over 50 years due to anthropogenic influences, and hypoxic environments now represent a major threat to marine systems worldwide (Diaz and Rosenberg 1995, Diaz 2001) An understanding of how organisms cope with or avoid low oxygen environments has therefore taken on renewed importance and urgency.

Human activities can cause low oxygen environments over larger spatial scales, (e.g. kilometers; (Joyce 2000, Diaz and Rosenberg 2008) but they can also influence smaller scale variation in oxygen availability. Habitat modifications by humans can result in low oxygen environments in marine coastal areas, at the small spatial scale where larval settlement and habitat selection occurs (Ferguson et al. 2013, Wilding 2014). Structures such as piers, marinas and docks are designed to reduce water movement so that boats can be docked and boarded safely. These human-made structures are typically colonized by a specialized guild of sessile marine invertebrates known sometimes as a 'fouling community'. Such assemblages can be dense, occupying all of the available human-made surfaces, and biomasses can exceed 1 kilogram per square meter of area. Fouling communities are often topographically complex and can increase the thickness of the boundary layer as well as inducing skimming flow, which further reduces the flow of water adjacent to the community (Vogel 1994). The combination of exceedingly low-flow environments and the metabolic demands of dense fouling communities mean that oxygen levels adjacent to these communities often fall to less than half of air saturation, and can sometimes even be zero (Vogel 1994, Ferguson et al. 2013). In effect, docks, marinas and piers, which increasingly make up a

significant proportion of the world's coastlines (Duarte et al. 2013), are generating oxygen-limited microenvironments that are much more rare in unmodified habitats with higher flows.

In natural habitats, low flow conditions are commonly associated with soft bottom communities. In the absence of scouring flows, most upward-facing hard surfaces will accumulate a coating of sediment. However, human-made structures are unusual in that they provide both low-flow conditions and hard surfaces that face sideways and downwards, thereby avoiding sedimentation (Glasby et al. 2007, Ruiz et al. 2009, Duarte et al. 2013, Ferguson et al. 2013). This unique oxygen-limited habitat presents a significant challenge to the organisms that live there: species must either cope with or avoid these low oxygen conditions.

Many species either avoid low oxygen conditions or express traits that allow them to cope with such conditions. Mobile animals, such as fishes and copepods, have the opportunity to swim vertically in the water column to find more oxygen-rich environments (Palomares-Garcia et al. 2013); however, benthic animals, especially sessile organisms, are less able to avoid the stressful conditions of low oxygen environments as adults. Several species cope with low oxygen environments by expressing phenotypes that either reduce oxygen demand or increase oxygen uptake. Some species reduce their metabolic rate (Dahlhoff et al. 2002), others increase gill surface areas, blood pigment concentrations and oxygen affinities (Truchot 1980, Lallier and Truchot 1989, Sollid et al. 2003, Nilsson et al. 2012). The mechanism used will depend in part on oxygen demands of the organism, habitat characteristics, and the frequency and magnitude of the hypoxic conditions (Stillman and Somero 1996, Altieri and Witman 2006). All of these coping strategies have their limits when oxygen levels fall below some minimum level, and organisms will still experience the negative effects of hypoxia when oxygen levels are sufficiently low.

For sessile organisms, the mobile larval phase may be the only chance that individuals have to avoid low oxygen environments; in this way, habitat selection (i.e., settlement) is critical for their fitness. There is extensive literature on the factors affecting larval habitat selection, including the presence of competitors (Grosberg 1981, Bullard et al. 2004), pollution (Hunte and Wittenberg 1992, Knott et al. 2009), light intensity and quality (Mundy and Babcock 1998), chemical cues (Thiyagarajan 2010), biofilm composition (Wieczorek and Todd 1997, Cheung et al. 2014, Whalan and Webster 2014), the presence of conspecific adults (Jensen 1989, Gebauer et al. 2011), and properties of the substratum (Roberts et al. 1991, Tapella et al. 2012). Larvae may use such cues as indicators of habitat quality in order to maximize their chance of surviving to post-settlement (Raimondi and Keough 1990, Donahue 2006). While oxygen availability would seem to be another important cue for habitat selection, to our knowledge, no study has examined whether larvae avoid microhabitats that are low in oxygen.

In a series of laboratory experiments, we examined larval responses to differences in the availability of dissolved oxygen. Specifically, we measured the proportion of time that larvae spent swimming versus exploring surfaces and the time taken to settle in different levels of oxygen, as well as the oxygen preference of larvae in water under a gradient of oxygen saturation. We focused on the larvae of the bryozoan *Bugula neritina* (Linnaeus, 1758) as it is a model for studies of marine larval behavior and this species is known to a have relatively poor tolerance for low oxygen conditions (Ferguson et al. 2013). Our aim is to understand how oxygen levels within the water column influence behavior and habitat selection patterns.

MATERIALS AND METHODS

Study species and general methods

Bugula neritina (hereafter referred to by its genus name) is a ubiquitous arborescent bryozoan that inhabits submerged hard substrates at shallow depths. It occurs on artificial structures around the world and is a common member of the epibenthic community on human-made structures in Australia. *Bugula* release non-feeding larvae that are competent to settle immediately upon release. These characteristics have long made it a model species for studying larval settlement behavior (Doyle 1974). We collected mature colonies of *Bugula* from floating piers located at Royal Brighton Yacht Club, Port Phillip Bay, Victoria, Australia (37° 54'25'' S 144° 58'52'' E) during the austral spring of 2013. We used standard methods (Strathmann 1987) to collect larvae. Briefly, colonies were maintained in constant darkness for two days in aquaria with constantly aerated seawater maintained at a stable temperature (~19.5 °C). To collect larvae, we exposed 10 colonies to bright light in each experimental run for up to one hour. Colonies began to release larvae within 10 min. Larvae were pipetted from the aquaria and placed in the experimental chamber assigned to each experiment. Because *Bugula* larvae prefer to settle on a roughened surface, all of the acetate sheets, Petrie dishes, and eppendorf tubes used as experimental settlement surfaces were sanded before use (Marshall and Keough 2003). To further encourage settlement, surfaces were maintained in aquaria with constantly aerated seawater for one week prior to the experiment, to develop a biofilm (Wieczorek and Todd 1997, Rius et al. 2009). In each experiment we used 0.10 μm filtered seawater at a temperature of ~19.5 °C. The oxygen saturation of the water (% saturation) was manipulated by bubbling nitrogen gas, and monitored using fiber optical sensors connected to a Firesting O_2 fiber optic oxygen meter.

Swimming and exploring behavior

We tested the effect of oxygen saturation on larval behavior using a standard behavioral assay (Burgess et al. 2009). Briefly, *Bugula* larvae show clearly recognizable behaviors that can be

characterized as ‘swimming’ and ‘exploring’ (Burgess et al. 2009). Swimming behavior is identified as erratic and quick movement through the water column, and exploring behavior represents a fine-scale searching behavior that occurs prior to settlement, identified by adoption of a stationary position or slow movement on the settlement surface, and includes characteristic spinning and crawling behavior (Walters et al. 1999). We randomly selected individual larvae from the pool of 10 colonies, and pipetted individuals into a petri dish (3.5 cm diameter, 1.1 cm depth) containing 10 mL of seawater with an oxygen saturation of either 100% saturation (the ‘high’ treatment) or 25% saturation (the ‘low’ treatment). These levels were established prior to the addition of the larvae in to the petri dish. During the experiments we didn’t add air or nitrogen to avoid any effect of the bubbles on larvae; however preliminary test showed that the water can show a small fluctuation of no more that 5% saturation during the time necessary for our experiments. Immediately after placing the larvae in the experimental treatment, individual larvae were observed under a microscope for five minutes, and the amount of time spent exploring settlement surfaces and the amount of time spent swimming in the water column were recorded. Previous studies have shown that larval age affects larval behavior (Burgess et al. 2009), so we also tested the effects of larval age, using larvae that were 0 and 60 minutes old. In total, we measured the behavior of 40 larvae (10 per age class and seawater treatment combination) across three experimental runs.

Settlement time

To measure the settlement time of *Bugula* under different oxygen levels, randomly selected individual larvae were collected from a pool of 10 colonies and then pipetted into eppendorf tubes filled with seawater (1.5 mL). Again, the oxygen saturation of the seawater in the tubes was manipulated to be either high or low (same levels as the previous experiment) prior to the addition of larvae. Then, we monitored individual tubes continuously and recorded the time until the settlement occurred in each larva. Settlement in *Bugula* is straightforward to observe as larvae attach permanently to the substrate. We measured 10 larvae per treatment across two experimental runs.

Habitat selection: association and settlement

We created a larval settlement choice chamber that contained a longitudinal oxygen gradient. For these experiments, we used two acrylic chambers 80 × 15 × 15 cm, filled with 0.10 μm -filtered seawater at ~19.5 °C. At one end of the chamber we bubbled compressed air, and at the other end we bubbled nitrogen gas. The turbulence created by the bubbles at both ends was attenuated by screens of 100 μm mesh, located at a distance of 10 cm from each end of the chamber. A stable oxygen gradient formed within one hour of the commencement of bubbling, with typical oxygen

levels between 25 and 80 % saturation (See Figure 1 for an example of the experimental oxygen gradient). Ten experimental runs were conducted in each of the follow experiments, two each day for 5 days, alternating the oxygen-nitrogen orientation in each chamber and, as *Bugula* larvae exhibit phototaxis, covering the chambers to keep them in constant darkness to exclude potentially confounding effects of light (Wendt & Woollacott 1999).

To measure larval association with different levels of oxygen, we pipetted larvae across 5 locations (20 larvae per location) at 10 cm intervals along the chamber (100 larvae in total). Thirty minutes after larvae had been introduced, acrylic separators were introduced into the chamber 10 cm apart, to isolate the larvae and keep them in the position they were located; after this, larvae were collected using funnels and counted. The number of larvae that were in each section was then recorded.

To measure settlement, we repeated the same protocol as above but also covered the inner surface of the chamber with a contiguous piece of acetate sheet that had been pre-roughened and then pipetted larvae into the chamber as described above. Thirty minutes after larvae had been introduced, the acetate sheet was retrieved and the position of each settled larvae was measured. We then repeated this experiment with a longer duration, measuring the larval settlement 60 minutes after release into the chamber.

Statistical analyses

The effects of oxygen saturation and larval age on larval behavior and larval settlement time were tested with a mixed model GLM. Oxygen level and age were fixed factors; experimental run (day) was a random factor. Non-significant interactions including the random factor were eliminated from the model (Quinn and Keough 2002). All ANOVA were done using the statistical software Systat ver. 13. Association and settlement selection data were analyzed with χ^2 tests using Microsoft excel V 14.1.0. Alpha value was set at 0.05 in each test.

RESULTS

Oxygen saturation affected the behavior of *Bugula neritina* larvae. In the swimming and exploring behaviour experiment, the larvae explored potential settlement surfaces significantly less when oxygen levels were low (Fig. 2). The effect of oxygen level was consistent regardless of larval age and experimental run (Table 1A). Also, we found significant differences in the settlement time experiment (Table 1B); here, *Bugula* larvae delayed their settlement in the presence of low oxygen (Fig. 3).

In the habitat selection experiment we found that *Bugula neritina* larvae tended to avoid swimming in water with low oxygen levels (χ^2=4.55, p= *0.03*). Unsettled larvae were strongly

associated with high oxygen levels (50-65 and 65-80 % saturation; Fig. 4). The oxygen preference of swimming larvae was not reflected in their settlement. We detected no settlement preference between low and high oxygen levels. This result was consistent when settlement was assessed 30 minutes and 60 minutes after larvae were placed in the experimental chambers (30 min: χ^2=0.16, p=0.68; 60 min: χ^2=0.32, p=0.56).

DISCUSSION

Low oxygen conditions have always been a feature of aquatic systems, but they are increasing in prevalence and severity (Diaz 2001, Diaz and Rosenberg 2008). We found evidence that an organism that occurs within a habitat where low oxygen conditions occur shows a variety of behaviours to avoid low oxygen during colonization. *Bugula* larvae delayed settlement and spent more time swimming than exploring settlement surfaces in low oxygen conditions, both behaviours that are likely to increase the chance that larvae escape low oxygen conditions. We also found evidence for oxytaxis – larvae preferentially associated with higher oxygen conditions more than low oxygen conditions when given a choice. To our knowledge, few studies have demonstrated oxytaxis in adults metazoans and none in larvae, though it is common in unicellular organism such as bacteria and microalgae (Hillesdon and Pedley 1996, Porterfield 1997, Lorz 2010, Yazdi and Ardekani 2012, Broszeit et al. 2013), and we eagerly await other studies.

During dispersal and habitat selection, marine invertebrate larvae are neither entirely passive nor completely in control of their movement and destination, instead, they exert important though limited influence on both (Walters et al. 1999). By changing their position in the water column and accepting or rejecting habitat, marine larvae settle non-randomly and even counter to prevailing hydrodynamics (Vogel 1994). In our study, in the presence of low oxygen, larvae swam upwards, engaged in less exploratory behavior on the benthos and avoided settlement. In the field, these behaviors would increase the probability that larvae settle in higher oxygen conditions. Previous studies have shown that oxygen availability varies over very small spatial scales (Ferguson et al. 2013); even slight water movement might therefore be sufficient to move a larva the necessary short distance to a new location with possibly higher oxygen conditions, if the larva refuses to settle and swims upward.

It could be argued that changes in larval behavior and settlement patters could be the results of a direct effect of low oxygen on metabolism, causing reductions of metabolic rate. But is important to consider that *Bugula* larvae are negatively buoyant and any metabolic depression would be evidenced as more exploring behavior; however, we see the opposite effect in the behaviour trials. Our results show more activity in low oxygen, with larvae swimming rather than exploring; therefore metabolic suppression is not an explanation. If metabolic rates are maintained

at low oxygen tension, the differences in behavior and settlement rates are likely to be mediated by processes other than metabolic rate per se (e.g., oxygen sensing).

The avoidance of low oxygen conditions, while presumably beneficial (see below), is not without costs. In species with nonfeeding larvae such as *Bugula*, delaying settlement carries significant fitness costs – energy expended on swimming cannot be used for post-metamorphic growth and even brief extensions of the larval period can therefore reduce post-metamorphic performance (Wendt and Woollacott 1999, Marshall et al. 2003, Elkin and Marshall 2007, Burgess et al. 2009). Furthermore, while estimates of planktonic mortality are rare, it seems likely that these rates are high so delaying settlement will increase the risk of mortality. If the low-oxygen avoidance behavior is adaptive, then the costs imposed by delaying settlement must be balanced by other benefits, presumably a higher likelihood of settling in conditions that are more conducive to post-metamorphic success.

There are two broad classes of likely benefits for sessile species to avoiding low oxygen conditions. First, aerobic respiration requires a considerable amount of oxygen, but gives a great quantity of energy per mole of substrate and does not produce toxic byproducts. However, low oxygen environments cannot supply the high amount of oxygen required to maintain aerobic metabolism. While the low oxygen levels we used in our experiments (20% saturation) exceed the minimum level recorded for this species at another location, metabolic functions can still be more costly at this level, as oxygen levels below the organism requirements can have a negative effect on enzymatic activity, feeding activity and changes in metabolic rate that can lead to behavioral changes of organisms (Stillman and Somero 1996, Haye and Ojeda 1998, Dahlhoff et al. 2002, Lagos et al. 2011).

Second, locally low oxygen levels may act as a proxy for the intensity of local competition and the availability of food at a given settlement location. Such cues are vital because the larval stage is the last and only chance to avoid bad locations for sessile species. In the habitats that *Bugula* occupies, in the water column and on settlement surfaces that are not adjacent to the benthic community, the oxygen availability exceeds 95% saturation (Ferguson et al. 2013). Oxygen levels fall to low levels adjacent to respiring organisms and under exceptionally low flows within the boundary layer of a topographically complex benthic community – producing a low quality microenvironment. Given that oxygen can serve as a proxy for multiple types of poor environment, using oxygen as a cue for habitat choice is an ingenious solution to the challenges that marine invertebrate larvae face. Marine larvae must make rapid decisions about the long-term suitability of settlement sites using limited sensory capabilities. Studies have shown that larvae can avoid settling near residents that are superior competitors, though no study has determined the mechanism by which species recognize residents (Grosberg 1981, Young and Chia 1981, Rius et al. 2009). Upon

reflection, the idea that a simple larva can recognize a whole host of different species and classify each according to its competitive ability seems unlikely. Instead, we propose that larvae simply use the local availability of oxygen as a proxy for the presence of competitors, and the particular characteristics they have, such as metabolic rate and therefore their size and food requirements. Previously, it has been shown that superior spatial competitors cause a greater reduction in local oxygen levels than inferior spatial competitors (Ferguson et al. 2013). Thus, oxygen availability at the micro-scale may be a reliable indicator of the presence of competitors and the high availability of oxygen indicates the absence of strong competitors. In other words, by detecting oxygen levels larvae may be able to avoid competitors without having to recognize and classify each competitor. To test this hypothesis, an experiment that orthogonally manipulates competitor presence and absence and oxygen availability is required: if larvae settle in the presence of a superior competitor under high oxygen conditions, then our hypothesis is supported. Regardless of why larvae avoid low oxygen, it is clear that oxygen availability should be added to the canon of cues that marine larvae use to select settlement sites.

Despite finding evidence for the avoidance of low oxygen during the larval phase, we found no differences in the number of larvae that settled in high and low oxygen environments. This apparent paradox may be due to the nature of our experiment. *Bugula* larvae have the tendency to swim and select to be associated to high oxygen water. However the final settlement is a mechanism regulated by several factors, some of them unknown. Previous studies suggest that *Bugula* larvae avoid settlement in low flow water (Walters et al. 1999). In our settlement choice assays, water movement was nonexistent such that larvae were not carried efficiently, affecting the results of such settlement assays. Perhaps more importantly, the settlement surfaces that we offered larvae were not allowed to accumulate a biofilm under the different oxygen conditions. A recent publication indicates that biofilm density can be modified by the oxygen level in the water, altering the recruitment patterns of larvae (Cheung et al. 2014), although the settlement is affect is not completely clear yet. Specifically for *Bugula*, preliminary work (Lagos unpublished data) indicates that different biofilms generated under high and low oxygen conditions affect larvae settlement. As such, the settlement behavior we observed might be driven by the fact that we created an artificial scenario where water oxygen levels were not reflected by biofilms on the settlement surfaces.

Alternatively, the low overall settlement rates in the chamber experiment may explain the lack of an effect of oxygen on settlement. Only about 35% of larvae settled, even after 60 minutes and it may be that the settlement surfaces in this experiment were so unappealing that only the smallest, most ‘desperate’ subset of larvae settled and they settled indiscriminately. Previous work (Gribben et al. 2006, Marshall and Keough 2003) shows that smaller, older *Bugula* larvae tend to settle indiscriminately and that settlement inhibitors are less effective on these larvae

Considering that low oxygen environments are spreading through the world, our research may be important under the frame of invasion ecology. Recent literature suggest that human-made structures may be creating habitats that are more conducive to invasive species that have long associations with low oxygen environments and artificial structures (Glasby et al. 2007, Dafforn et al. 2009, Ruiz et al. 2009, van Kleunen et al. 2010). We focused on *Bugula neritina* because is an ubiquitous marine invasive species with a long history of association with low flow, low oxygen habitats because we thought it would be the most likely to have evolved responses to low oxygen conditions. An important next step will be to explore whether these larval behaviours are present in native species that may be less commonly associated with low oxygen environments.

Acknowledgements. The authors thank Royal Brighton Yacht Club for access to their field site. Amanda Pettersen for her help in the field and lab work and comments on the final version of the manuscript, and Henry Wootton, Hayley Cameron, Keyne Monro and Chun-Yi Chang for their comments and help in the elaboration of the draft of this paper. C. R. W. and D. J. M. are supported by grants from the Australian Research Council. M.E.L. is supported by grants from Conicyt Becas-Chile Scholarship.

Table 1. ANOVA for effect of oxygen level, day, age and factor interactions on; a) Exploring time and, b) Settlement time of *Bugula neritina* larvae.

	Source	df	MS	*F*	*P*
A)	Exploring time				
	Oxygen saturation	1	10,115.58	13.49	0.001
	Day	2	1,217.50	1.62	0.212
	Age	1	6.97	0.01	0.924
	Age*Oxygen saturation	1	557.26	0.74	0.394
	Error	34	749.52		
B)	Settlement time				
	Oxygen saturation	1	351.649	33.59	<0.001
	Day	1	80.089	7.65	0.009
	Day*Oxygen saturation	1	58.564	6.00	0.247
	Error	36	10.468		

Note: Both models were edited and reduced after testing for nonsignificant interactions between random and fixed factors.

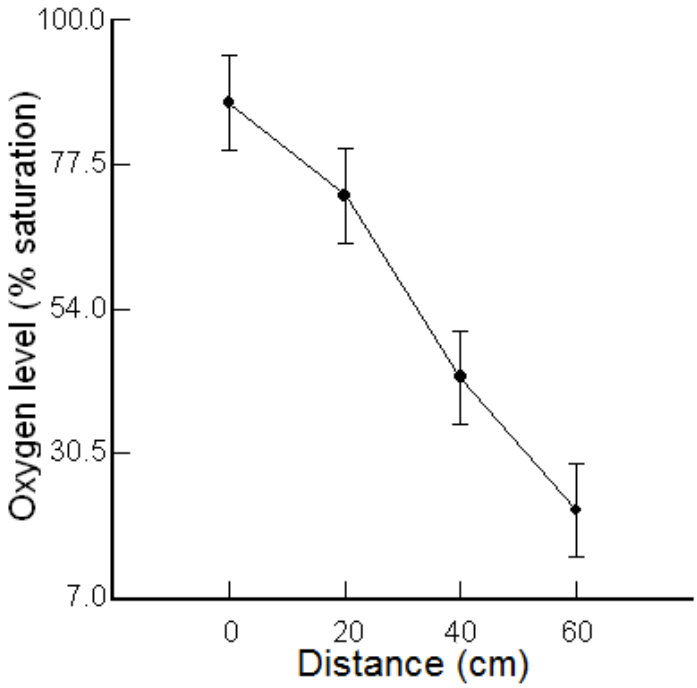

Figure 1. Measured mean oxygen levels (% saturation) in our test chambers used for examining the effect of oxygen availability on the distribution and settlement of *Bugula neritina* larvae (n=10) (± S.E).

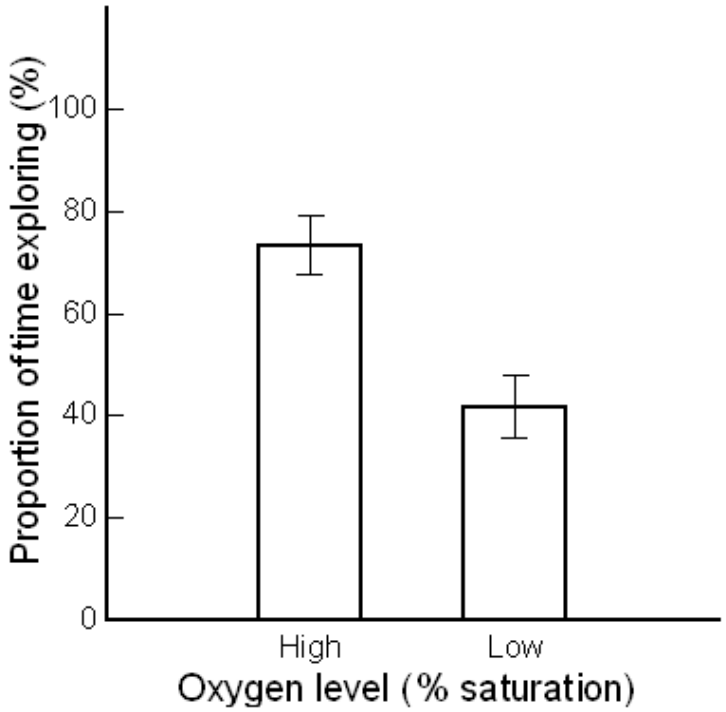

Figure 2. Exploring time of *Bugula neritina* larvae at low and high levels of oxygen saturation (25% and 100%, respectively). Bars represent means of the percentage time of the total used exploring (± SE).

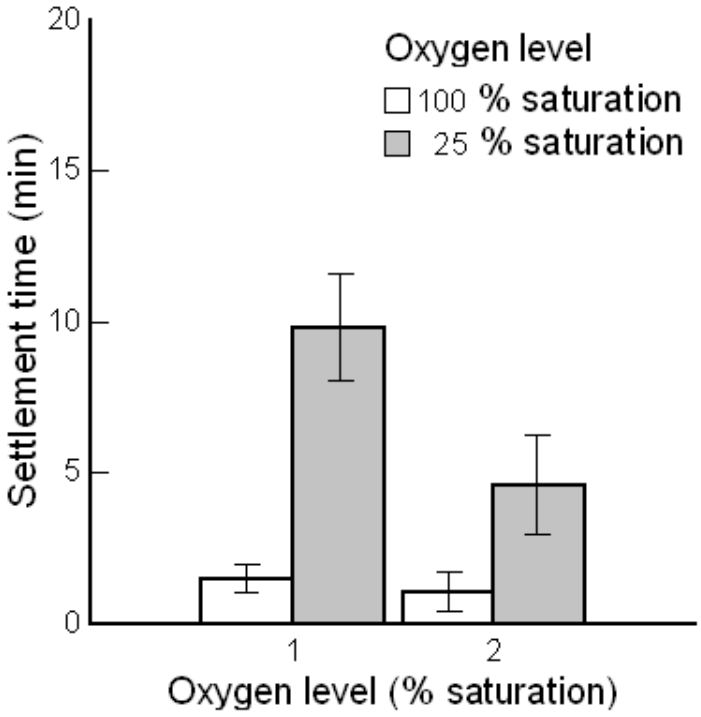

Figure 3. Settlement time of *Bugula neritina* larvae on two experimental days at low oxygen levels (25% saturation, grey bars) and high oxygen levels (100% saturation). Bars represent means (± SE).

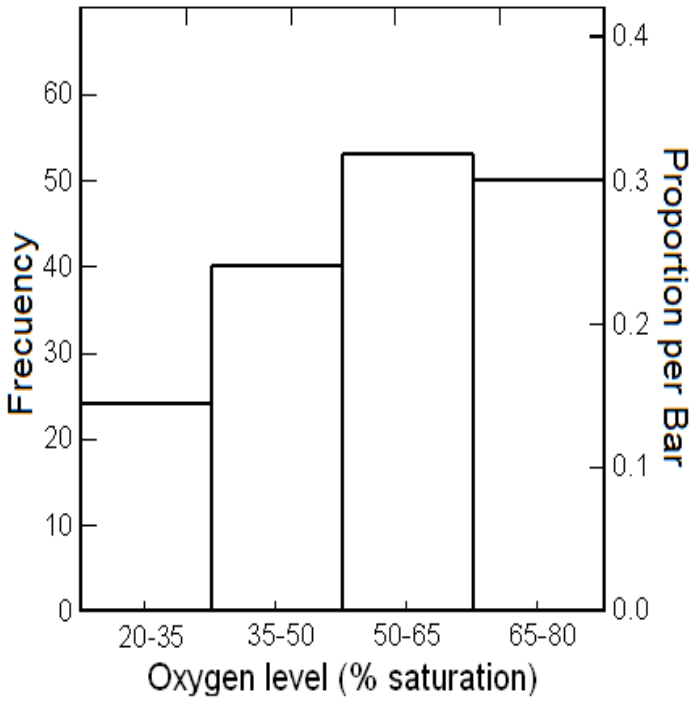

Figure 4. Histogram of association of *Bugula neritina* larvae with water across an oxygen gradient (% saturation). Bars show the frequency of larvae in each oxygen category, and the proportion of each category in the total.

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Declaration for Thesis Chapter 2

Declaration by candidate

In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Development of key ideas, data collection, experiment design, data analysis and writing	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dustin Marshall	Assistance in data analysis, experimental design and input into manuscript	20
Craig White	Experimental design, input into manuscript	10

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature [redacted] Date: 02-Nov- 2016

Main Supervisor's Signature [redacted] Date: 02-Nov- 2016

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER II

Biofilm history and oxygen availability interact to affect habitat selection in a marine invertebrate

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This chapter is currently published in the journal:

Biofouling (2016),Vol 32 (6), 645–655

dx.doi.org/10.1080/08927014.2016.1178725

ABSTRACT: In marine systems, oxygen availability varies at small temporal and spatial scales, such that current oxygen levels may not reflect conditions of the past. Different studies have shown that marine invertebrate larvae can select settlement sites based on local oxygen levels and oxygenation history of the biofilm, but no study has examined the interaction of both. The influence of normoxic and hypoxic water and oxygenation history of biofilms on pre-settlement behavior and settlement of the bryozoan Bugula neritina was tested. Larvae used cues in a hierarchical way: the oxygen levels in the water prime larvae to respond, the response to different biofilms is contingent on oxygen levels in the water. When oxygen levels varied throughout biofilm formation, larvae responded differently depending on the history of the biofilm. It appears that B. neritina larvae integrate cues about current and historical oxygen levels to select the appropriate microhabitat and maximize their fitness.
Keywords: Bryozoans, hypoxia, normoxia, settlement, larvae, behavior.

INTRODUCTION

Settlement, the process by which larvae change from a pelagic lifestyle to a benthic one, is a crucial moment in the lives of sessile invertebrates (Rodriguez et al. 1993). The settlement process of larvae begins with selection of a suitable surface, which can be characterized by specific pre-settlement behaviors, and finishes with attachment to a substratum and subsequent metamorphosis into the adult body plan (Rodriguez et al. 1993, Walters et al. 1999, Burgess et al. 2009). The consequences of settlement choice can be extreme because where a larva settles determines its chances of survival and reproduction. Accordingly, larvae use multiple cues to determine whether a potential settlement surface is likely to be conducive to post-settlement success (Morse 1991, Gebauer et al. 2011, Lagos et al. 2015).

A number of cues can induce settlement in aquatic larvae, and some cues appear to act as indicators of habitat quality. Larvae that settle in response to favorable environmental cues maximize their chance of surviving to post-settlement and adult life-stages (Raimondi and Keough 1990, Donahue 2006). Cues used by larvae include: the presence of relatively stronger competitors (Grosberg 1981, Bullard et al. 2004), epifaunal assemblages (Todd and Keough 1994), presence of conspecific adults (Jensen 1989, Osman and Whitlatch 1995, Gebauer et al. 2011), surface topography, complexity and composition (Roberts et al. 1991, Tapella et al. 2012, Whalan et al. 2015), light intensity and quality (Mundy and Babcock 1998), chemical cues (Morse 1991, Thiyagarajan 2010, Tebben et al. 2015), and biofilms that can induce or inhibit larvae settlement (Wieczorek and Todd 1997, Sneed et al. 2014, Whalan and Webster 2014). All of these cues are thought to reflect the likelihood that a settlement surface will support post-settlement survival, growth and reproduction. More recently, local oxygen availability has also been shown to affect larval settlement behavior (Wieczorek and Todd 1997) Lagos et al. 2015).

In sessile marine invertebrate communities, the availability of dissolved oxygen can be highly variable at small spatial scales (Ferguson et al. 2013). Topographic complexity, thickness of the boundary layer, skimming flow and oxygen consumption of organisms that occupy an area can change microenvironments from hyperoxic to hypoxic over a few hours and a few centimeters (Vogel 1994, Gardella and Edmunds 1999, Ferguson et al. 2013). Recent studies show that when settling, marine invertebrate larvae avoid microhabitats that are low in oxygen via two mechanisms. First, larvae will delay settlement and spend less time exploring settlement surfaces when dissolved oxygen in the water column is relatively low (Lagos et al. 2015). Second, larvae have lower recruitment on surfaces that were colonized by bacteria under low dissolved oxygen conditions, and prefer surfaces colonized by bacteria under high oxygen conditions (Cheung et al. 2014). Both of these cues provide different information about the local habitat (Lagos et al. 2015). Dissolved oxygen in the water column provides instantaneous information about current oxygen levels, but

provides less information about past conditions (of even a few minutes earlier). Biofilms, on the other hand, might provide integrated information about historical oxygen levels, but are less likely to reflect current dissolved oxygen conditions as biofilm communities take hours to days to grow (Rao 2003, Shikuma and Hadfield 2005).

While oxygen levels in the water column and biofilm oxygen history have been explored separately, no study has yet examined how larvae integrate these two environmental cues, which are both likely to provide information about local conditions on very different time scales. The aim of this article is to understand how the interaction between oxygen availability and biofilm composition influence the settlement of larvae of sessile marine species. First the effects of biofilm history on larval settlement in the field were examined, after which interactions between dissolved oxygen levels and biofilm oxygen history on larval behavior in the laboratory were explored.

The bryozoan *Bugula neritina* (Linnaeus, 1758) was chosen as a model system as this species is commonly used in studies of larvae behavior (Wendt 1998, Walters et al. 1999, Burgess et al. 2009), the effects of mono and multi-species biofilms as a stimulator and inhibitor on larval settlement and post-settlement success (Bryan et al. 1997, Dobretsov and Qian 2006)

, and the low tolerance of adults colonies under oxygen-deprived conditions (Ferguson et al. 2013). Here, the settlement response of larvae of the bryozoan *B. neritina* to different dissolved oxygen conditions as well as biofilms with different compositions were examined. Furthermore, as oxygen levels are unlikely to be constant in time, the effects of variability in oxygen history of biofilms on larval settlement were also examined.

MATERIALS AND METHODS

Biofilm generation

All settlement surfaces used in this study, including acetate sheets, centrifuge tubes, Petri dishes and PVC plates, were sanded prior to use as previous studies have demonstrated that *Bugula* larvae prefer to settle on roughened surfaces (Marshall and Keough 2003). Biofilm was generated by maintaining the settlement surfaces in aquaria with non-filtered natural seawater, collected from Port Phillip Bay, Victoria, Australia, at ~19.5 °C for 5 days (Wieczorek and Todd 1997). Two different treatments of biofilms were generated: low oxygen levels (25 % air saturation) and high oxygen levels (100 % air saturation). For the low oxygen treatment the oxygen content of the seawater was manipulated during the 5 days period of biofilm generation, by bubbling nitrogen gas and air. High oxygen treatments were maintained by aerating aquaria with air only. Monitoring showed that oxygen fluctuations within the treatments were no more than 5 % air saturation different to the targeted levels throughout the experimental period. The oxygen levels were

measured using fiber optic sensors connected to a Firesting O_2 fiber optic oxygen meter (Pyro Sciences, Aachen-Germany) (Lagos et al. 2015).

Field experiment – the effect of biofilm history on community assembly

Initially the effects of biofilm type on larval settlement were tested for members of the local fouling community in the field. For each experimental run, four PVC panels (60 x 60 cm) were deployed at Royal Brighton Yacht Club marina in Port Phillip Bay, Victoria, Australia (37° 54'25'' S 144° 58'52'' E) during the Austral spring of 2014. The marina (approximately 150 m by 350 m; average depth is 3 m) provides a sheltered area that is protected by a breakwater, and floating pontoons that extend throughout the site action created by waves and wind. Each panel held eight PVC plates (10 x 10 cm) with low oxygen biofilm history (LB) treatment and eight plates with high oxygen biofilm history (HB). The plates were randomly distributed on each panel. Panels were then outplanted in the field and hung from the floating dock 1.5 meters below the water surface for a period of 7 hours (from 5am to 12pm). After this time, the plates were then returned to the laboratory at Monash University, Clayton, and immediately examined under the microscope to measure diversity (species richness) of invertebrate settlers. This procedure was repeated 12 times, with an interval of two days between each run.

Study species – laboratory experiments

The model species, *Bugula neritina* (hereafter *Bugula*) is a marine bryozoan which inhabits submerged hard substrates, including artificial structures at shallow depths worldwide. *Bugula* release non-feeding larvae that are fully competent to settle immediately upon release, and therefore serve as model species for studying larval settlement behavior (Doyle 1974). Standard methods (Strathmann 1987) were used to collect colonies from floating pontoons located at Royal Brighton Yacht Club , Port Phillip Bay, Victoria, Australia (37° 54'25'' S 144° 58'52'' E) during the Austral summer and spring of 2013 and 2014. Colonies were then immediately transported to the laboratory in insulated aquaria with constant aeration (~ 30 min journey). Colonies were maintained in darkness within aquaria with constantly aerated seawater at a stable temperature (~19.5 °C) for two days prior to experiments. For each experimental run, larvae were collected by exposing 10 mature colonies to bright light for up to one hour. Upon release, the spawned larvae were immediately collected using a pipette and assigned to an experimental treatment (0 min old larvae). Experiments with 60 min old larvae were kept in a shaker to inhibit settlement for 60 min and then placed in the experimental chamber.

Pre-settlement behavior, oxygen levels and biofilm history

Interactive effects of oxygen level in the water, the oxygenation history of the biofilm and larval age on larval behavior were investigated with a standard assay (Burgess et al. 2009). *Bugula* larvae generally exhibit behaviors that can be characterized as either 'swimming' or 'exploring' (Burgess et al. 2009). Swimming behavior involves erratic and quick movements through the water column, whereas exploring behavior involves fine-scale searching that occurs prior to settlement and can be recognized by the adoption of a stationary position or slow movement on the settlement surface and includes characteristic spinning and crawling behavior (Walters et al. 1999). Larvae were spawned from 10 colonies, of which 5 larvae were then randomly selected and pipetted into each of the prepared biofilm Petri dishes (3.5 cm diameter, 1.1cm depth) containing 10 ml of seawater with an oxygen level of 100% air saturation (high oxygen treatment) or 25% air saturation (low oxygen treatment). There were two types of Petri dishes: the low oxygen treatment dishes were biofilmed in water with oxygen levels at 25 % air saturation, and the high oxygen treatment dishes were biofilmed with water at 100% air saturation. Immediately after assigning a larva to an experimental treatment, the individual was observed under a microscope for five minutes to measure the amount of time the larva engaged in swimming or exploring behavior, using a stopwatch. Previous studies have shown that larval age affects behavior (Burgess et al. 2009), so the effects of larval age on exhibited behavior were also tested using larvae that were either 0 or 60 minutes old. We considered 0 min old larvae as those that were immediately pipetted in to the experimental surface after spawning from the colony. In total, the behavior of 80 larvae were measured across three experimental runs (10 in each of the 2 biofilms types x 2 oxygen levels x 2 larval ages).

Larval settlement, oxygen levels and biofilm history

To examine the effect of biofilm history on larval settlement, two series of experiments were made. The aim was to make the difference between the situations were larvae are forced to settle (time trial experiment) in biofilms with high or low oxygen history, or when larvae have the chance to choose were to settle (settlement selection experiment). Petri dishes (3.5 cm diameter, 1.1cm depth) that were previously biofilmed at high (100% air saturation) and low (25% air saturation) oxygen levels (HB and LB respectively) were used. Five larvae were pipetted into each Petri dish containing 10 ml of seawater at either 100% or 25% air saturation. The total number of larvae settled in each experimental run after 60 min were measured. This experiment was repeated 40 times in total, with 10 larvae in each biofilm type at one of two oxygen levels.

As an extension of the previous experiment that demonstrated either the ability or inability for a larva to select where to settle, the capacity of *Bugula* larva to select settlement surface according

to biofilm composition was measured. A settlement selection experiment was established in the laboratory by lining one half of the bottom of an acrylic aquaria (17 x17 x 25 cm) with acetate that had been pre-conditioned in low oxygen seawater (25% air saturation; LB), and the other half was lined with acetate previously exposed to high oxygen seawater (100% air saturation; HB). Replicate aquaria were then assigned to seawater at either high (100%) or low (25%) air saturation. Ten larvae were transferred into each aquarium and measured the number of larvae that had chosen to settle on each settlement surface type (low and high oxygen biofilms) after 60 mins. This procedure was repeated 10 times for each oxygen level treatment.

Effect of variation in biofilm history on larval settlement

The ability of biofilm composition to provide cues of historical oxygen levels for settling *Bugula* larvae was investigated. Petri dishes were biofilmed (3.5 cm diameter, 1.1cm depth) under a series of sequenced oxygen level combinations of water at low (L) and high (H) oxygen conditions (25 and 100% air saturation respectively). Prior to the experiment, plates were immersed for five days in either low (L) or high (H) oxygen conditions (100 and 25 % air saturation, respectively). The plates were then switched to either high or low oxygen conditions for a further five days before a final switch for another five days, where some remained in the same treatment. This resulted in a total immersion time of 15 days, where the combination of treatments with "early, mid and late biofilm" were: HHH, HHL, HLH, HLL, LHH, LHL, LLH and LLL. At the end of the biofilming process, five larvae were pipetted into each Petri dish containing 10 ml of seawater with one of two oxygen level treatments (100 % and 25% air saturation). The total number of larvae that settled in each experimental run after 60 min was measured. This protocol was repeated 10 times for the eight-biofilm history treatments at two oxygen levels.

Statistical analysis

The effect of fouling community richness on field settlement was analyzed using a linear mixed model, where biofilm was a fixed factor, and run and panel were random factors. The effect of biofilm history on the swimming/exploring settlement behavior of *Bugula* larvae were analyzed using a General Linear Model, where the response variable was the proportion of the larvae that settled; oxygen level, age of the larvae and biofilm were fixed factors and experimental run (day) was a random factor. Any non-significant interactions involving the random factor were eliminated from the model (Quinn and Keough 2002). Tukey multiple comparisons were used to identify differences between treatments. The settlement selection experiment was analyzed with a χ^2 test. All ANOVA analyses were conducted with the statistical software Systat ver. 13. Settlement

selection data were analyzed with χ^2 tests using Microsoft excel V 14.1.0. Alpha values were set at p = 0.05 for each test.

RESULTS

Field experiment

Nine different species of settlers were found: one barnacle, one sponge, the bryozoans *Watersipora subtorquata*, *Bugula flabellata*, *Bugula neritina* and *Celleporella sp*, and three tunicates, *Diplosoma* sp (two different species) and *Botryllus* sp. Biofilm type did not influence species richness in a simple way; instead biofilm interacted with both run (time) and panel (space) effects (Time x Panel x Treatment: $\chi^2 = 38.89$, d.f. = 1, P <0.001). Generally, higher species richness occurred on plates with biofilms that had a high oxygen history, though this pattern reversed for some panels and days (Figure 1).

Pre-settlement behavior, oxygen levels and biofilm history

No effect of experimental run (day) was found on any interactions with age ($F_{2,57} = 1.070$, P= 0.350), oxygen ($F_{2,57} = 0.206$, P=0.814) or biofilm, ($F_{1,57} = 1.615$, P= 0.533), and the overall interaction among these factors ($F_{2.57} = 0.149$, P = 0.862), so the model was reduced.

No significant effect of biofilm and its interactions was found on larval behavior (Table 1A). However there was a significant effect of the interaction between oxygen levels in the water and larval age (Table 1A). In the high oxygen treatment, the amount of time larvae spent exploring was similar between the 0 and 60 min old larvae (Tukey P=0.684). However, in the low oxygen treatment there was a reduction in the exploration time of larvae exposed to low oxygen conditions at both ages; younger larvae spent the shortest time in the exploration phase (Tukey P<0.05), but the magnitude of the reduction in exploration time was greater for young larvae than older ones (Figure 2).

Larval settlement, oxygen levels and biofilm history

Oxygen levels in the water and biofilm oxygen history interacted to affect larval settlement (Table 1B). In low oxygen water, biofilm history had no effect on the proportion of larvae that settled, but in high oxygen water there was a significant effect of biofilm history, where the lowest settlement occurred on low oxygen history biofilms (Tukey >0.05; Figure 3A).

The larval settlement selection experiment showed that both, biofilm history and oxygen levels in the water also affected larval choice (Interaction: χ^2=6.707, p=0.009). Larvae had similar proportions of settlement on both biofilms when immersed in high oxygen water. The lowest

settlement was in the low oxygen water treatment combined with the low oxygen biofilm history (Figure 3B).

Effect of variation in biofilm history on larval settlement

Larval settlement was affected by the entire history of the biofilm, not just the most recent oxygen history of the biofilm (Table 1C). For larvae exposed to high oxygen water, the effects of biofilm history were cumulative and apparently dose-dependent: larvae had lowest settlement on biofilms that had little experience of higher oxygen levels and the highest settlement when biofilms had the longest history with higher oxygen levels (Figure 4A). In contrast, larvae in low oxygen water showed no clear settlement patterns with regards to biofilm history (Figure 4B).

There was a significant effect of the early history of biofilms on settlement (Table 1C), with larvae settling in a lower proportion on the biofilms with low oxygen history (Figure 5). An interaction between oxygen levels in the water and late biofilm history was found (Table 1C), with settlement being lowest for the low oxygen water with low-oxygen biofilm histories compared to all other treatments (Tukey $P < 0.05$; Figure 6). There was also a significant interaction between mid- and late-biofilm history (Table 1C); settlement on late history biofilms was affected by the mid history biofilm, where settlement was higher when mid-history biofilms were generated in high oxygen conditions (Tukey $P < 0.05$; Figure 7).

A significant interaction between biofilm history and oxygen content in the water was observed (Table 1C). In high oxygen water, larvae displayed higher settlement on biofilms that started and finished with high oxygen conditions. Settlement proportion decreased progressively through the lower oxygen level treatments. The lowest proportion of larval settlement occurred on biofilms that started and finished in low oxygen conditions (Figure 8)(Tukey < 0.05) There was no significant difference among the biofilm groups immersed in the water with low oxygen content (Tukey > 0.05).

DISCUSSION

Bugula neritina alter their settlement behaviour according to both local oxygen levels in the water column and the oxygen history of the biofilm they are presented with at settlement, and these factors interact in a hierarchical way. Oxygen concentration in the water column appears to be the primary cue for habitat selection; larvae in high oxygen conditions have a tendency to actively reduce swimming time and spend more time exploring settlement surfaces at fine scales. The response of *Bugula* larvae to biofilm history depended on the concentration of oxygen in the water, suggesting that larvae integrate information from both benthic and pelagic conditions. Larvae in water with high oxygen content avoid settling on biofilms that have a long history of immersion in

low oxygen water, but the oxygen history of biofilms is less important when the oxygen content of water is low.

Biofilm oxygen history affected settlement in the field, albeit in complex ways. The effect of biofilm history in the field varied in time and space – high oxygen biofilms mostly enhanced settlement rates but on occasions decreased settlement. This temporal and spatial variation in the effect of biofilm history on settlement may come from spatial and temporal heterogeneity in larval supply – on some days, in some places, very different fouling communities may have 'sampled' on the settlement plates (Boucher et al. 1987, Aiken and Navarrete 2011)**.** For example, some species (e.g. colonial ascidians) seemed to prefer biofilms that were formed under low oxygen conditions and on days when colonial ascidian larvae were more common settlers, biofilms that had experienced lower oxygen levels had greater settlement. In contrast, most other species favoured biofilms that had experienced higher oxygen levels and so when colonial ascidian settlers were relatively rare, high oxygen biofilms had the most settlement. These variable effects of biofilm history in the field warrant further exploration, but for now at least, it is clear that larval settlement is affected by biofilm oxygenation history. Recently, Cheung et al. (2014) showed strong effects of biofilm oxygenation history on recruitment (sensu Keough and Downes 1982); the present results suggest that these effects on recruitment are driven by settlement rather than differential post-settlement survival.

Larvae are capable of distinguishing between settlement locations through their response to chemical cues (Dobretsov and Qian 2006). The mechanisms behind this have previously been described for benthic organisms, where active organic compounds synthesised by bacteria stimulate larval settlement and subsequent metamorphosis (Hadfield 2011, Sneed et al. 2014). The magnitude of the response shown by larvae was found to be related to the concentration of the organic compounds, which are in turn dependent on the density and composition of the biofilm. Hypoxia is known to reduce biofilm densities and can alter the composition of the bacterial community (Cheung et al. 2014), and therefore the amount of organic compounds.

It has been suggested that larvae show increased interaction with mature biofilms, as mature biofilms provide an indication of extended periods of immersion (Hadfield 2011). Biofilms with higher densities provide larvae with a cue of environmental stability. If more micro-organisms are attached to a substrate, there is greater accumulation of metabolic products and nutrients which allows for greater growth kinetics within the biofilm (Caldwell and Lawrence 1986, Jeffrey and Paul 1986). However, the historical process involved in biofilm formation must also be considered. While it is true that in the present experiments larvae generally showed lower settlement on hypoxic biofilms, in water with high oxygen content larvae avoided settlement on biofilms that had been generated mostly with low oxygen water. In other words, a biofilm that has been immersed in high

oxygen conditions for 100% of the time, and therefore has a high oxygen biofilm history, will receive higher settlement than a biofilm that is generated with hypoxic water or alternating normoxic-hypoxic conditions. Also, settlement is influenced by point of origin of the biofilm. When biofilm originates in low oxygen conditions, settlement by larvae is reduced regardless of its subsequent exposure to higher oxygen levels. To explain this apparent memory of the biofilm, it is necessary to consider that colonisation history can change the interactions among species (Sutherland 1974, Robinson and Edgemon 1988). It would be expected that different biofilm communities with similar histories could have the same composition and structure. However stochastic variation of the environment, in this case changes in water oxygenation, influence the colonisation process during earlier stages of ecological succession, and are therefore unpredictable at times (Vellend et al. 2014). Biofilms with different histories will result in biofilms with different final compositions, and it appears that larvae react differently depending on this composition.

Bugula larvae can select their habitat using oxygen levels in the water column as a proxy for habitat quality when presented with a choice of settlement options (Lagos et al. 2015), but the present results suggest that this ability changes if settlement is forced (i.e. a larva is presented only one settlement option). In the settlement experiment in the present study, larvae rejected settlement sites on hypoxic biofilms with high oxygen levels, but when they were allowed to choose the settlement surface, they rejected the most stressful conditions (that is, hypoxic biofilm and hypoxic water). The drivers of these effects are unknown, but it is anticipated that there are negative effects of settlement in environments with low oxygen conditions, such as is the case with some larvae of serpulid polychaetes that suffer alterations in their development and reduced survivorship (Shin et al. 2013). Alternatively, low oxygen conditions indicate a superior competitor and larvae are seeking to avoid such microsites.

Compared with younger larvae, older larvae have a longer exploration time in water with low oxygen content. This suggests that younger larvae can perceive the quality of the habitat more efficiently, and that habitat selectivity diminishes with age (Knight-Jones 1953, Toonen and Pawlik 2001, Gribben et al. 2006). Therefore, the probability that a younger larva will leave a given settlement location and search for a new one is higher when using oxygen as cue. *Bugula* larvae are lecithotrophic (non-feeding) and their energy reserves decrease with age. Extra time spent swimming in search of suitable habitats therefore reduces larval energy reserves. As such, larvae become increasingly desperate to settle as they age, and therefore increasingly willing to accept poorer quality settlement sites, regardless of environmental cues (Marshall and Keough 2003). The cost of delayed larval settlement can have short or long term impacts. For example, previous experiments on the ascidian *Diplosoma listerianu* has shown reduced growth rates and development of small feeding zooids when settlement is delayed (Marshall et al. 2003). Similarly, in some

barnacles and *Bugula,* development and survival from the juvenile stage to maturity is also negatively affected by delayed settlement (Pechenik et al. 1993, Wendt 1998). It must be considered that environmental perturbations by human actions can change natural conditions, decrease fauna diversity and increase the number of habitats with low oxygenation (Ruiz et al. 2009, Ferguson et al. 2013, Wilding 2014). Based on theoretical models (Burgess et al. 2009) that consider how habitat availability and larval behaviour can affect post-settlement fitness, it is expected that with an increase in the number of low oxygen microenvironments, larvae will encounter fewer suitable habitats, and therefore spend more time swimming. Since non-feeding larvae cannot search indefinitely, they become increasingly desperate to settle and indiscriminately choose settlement habitats (Knight-Jones 1953, Toonen and Pawlik 2001). Settling in sub-optimal (low oxygen) habitats could therefore result in diminished outcomes for settlers such as alteration in the short-term extracellular homeostasis, in the acid base balance, death and may ultimately, shift the structure of benthic marine communities (Henry and Wheatly 1992, Cancino et al. 2000, Gribben et al. 2006, Elkin and Marshall 2007, Burgess et al. 2009, Gebauer et al. 2011).

Seawater oxygen concentrations provides larvae with an important initial indication of the suitability of an environment as a settlement site, while biofilms provide evidence of both past and present environmental oxygen conditions. In encrusting communities, water conditions at microscales can vary spatially and temporally and can show strong variations in oxygen availability (Ferguson et al. 2013). The high complexity of the topography of these environments, together with daily variations of the tide, direction and wind speed, as well as oxygen consumption by organisms, can all generate high variability in flow and oxygen availability at microscale levels across short time spans (Vogel 1994). Therefore at small-scales, oxygen availability can provide a useful indication of habitat quality to settling larvae. As oxygen conditions are likely to change rapidly over time, biofilms may provide a longer-term cue regarding the history and stability of the environment. We suggest that biofilm industries test antifouling approaches that incorporate waterborne oxygen conditions, since our results clearly demonstrate that low oxygen conditions (both waterborne and biofilm histories) reduce larval settlement.

In conclusion, *Bugula neritina* larvae can select habitats according to oxygen levels and biofilm composition. Biofilms appear to be a secondary cue, while larvae appear to respond most strongly to oxygen levels in the water column. It still remains to be demonstrated empirically how successional changes in biofilm composition are affected by variation in environmental variables such as oxygen content, and how this variation may influence settlements patterns, and therefore the structure of communities.

Acknowledgements. C.R.W. and D.J.M. are supported by grants from the Australian Research Council. M.E.L. is supported by grants from Conicyt Becas-Chile Scholarship. The authors thank the Royal Brighton Yacht Club (Australia) for access to the field site. Amanda Pettersen, Henry Wootton, Hayley Cameron and Annie Guillaume are thanked for their comments on the final version of the manuscript.

Table 1: ANOVA for effect of oxygen level in the water (OL), run, age, biofilm and factor interactions on A) Pre-settlement behavior (exploring time), B) Settlement of *Bugula neritina* larvae on biofilms with histories of low and high oxygen immersion C) Settlement of *Bugula neritina* larvae on biofilms with variations in oxygenation history.

Note: Model A) was reduced after testing for non-significant interactions between random and fixed factors.

	Source	SS	df	MS	*F*	*P*
A)	Exploring time					
	Age	171.991	1	171.991	0.256	0.614
	Run	1,551.75	2	775.875	1.155	0.321
	OL	20,298.01	1	20,298.01	30.229	<0.001
	Biofilm	317.206	1	317.206	0.472	0.494
	OL*Age	2,907.67	1	2,907.67	4.33	0.041
	Biofilm*Age	283.881	1	283.881	0.423	0.518
	Biofilm*OL	0.055	1	0.055	0	0.993
	Error	47,674.56	71	671.473		
B)	Settlement					
	Biofilm	0.676	1	0.676	14.282	0.001
	OL	0.016	1	0.016	0.338	0.565
	OL*Biofilm	0.484	1	0.484	10.225	0.003
	Error	1.704	36	0.047		
C)	Biofilm History					
	OL	0.009	1	0.009	0.154	0.695
	Early	0.361	1	0.361	6.19	0.014
	Mid	0.144	1	0.144	2.469	0.118
	Late	0.121	1	0.121	2.075	0.152
	Early*OL	0.144	1	0.144	2.469	0.118
	Mid*OL	0.001	1	0.001	0.017	0.896
	Late*OL	0.324	1	0.324	5.556	0.020
	Mid*Early	0.025	1	0.025	0.429	0.514
	Late*Early	0.196	1	0.196	3.361	0.069
	Late*Mid	0.441	1	0.441	7.562	0.007
	Mid*Early*OL	0.004	1	0.004	0.069	0.794
	Late*Early*OL	0.225	1	0.225	3.858	0.051
	Late*Mid*Early	0.144	1	0.144	2.469	0.118
	Late*Mid*Early*OL	0.025	1	0.025	0.429	0.514
	Error	8.456	145	0.058		

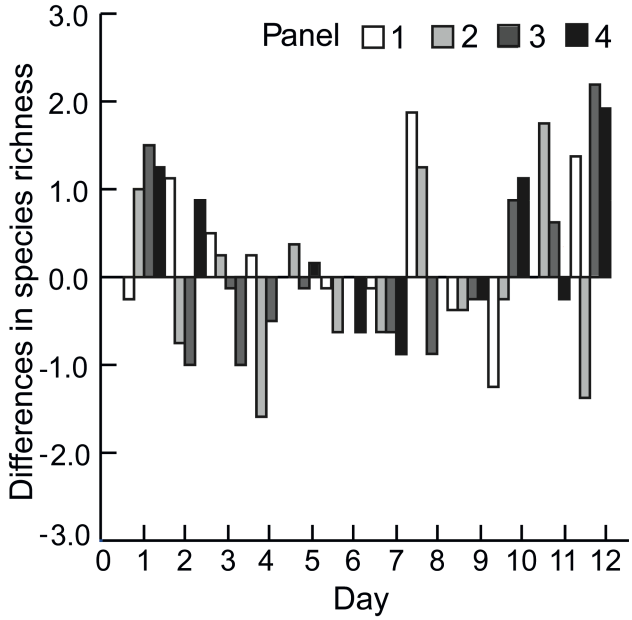

Figure 1: Spatial (panel) and temporal (day) comparison in species richness. Bars represent the difference between the average of species richness that settled on two different biofilms. Biofilms were generated with an oxygenation history of high (HB) and low (LB) oxygen levels (100 and 25 % air saturation).

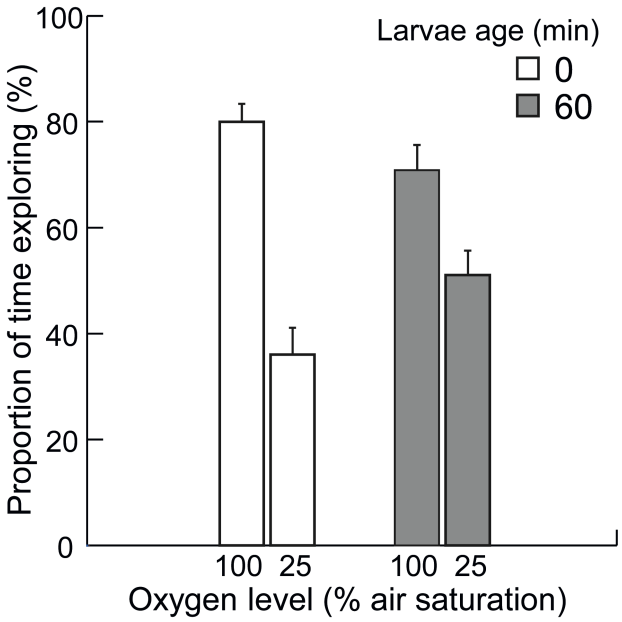

Figure 2: Proportion of time that *Bugula neritina* larvae spent exploring settlement surfaces at two different ages: 0 min old (white bars) and 60 min old (grey bars), in water at low oxygen and high oxygen levels (25 and 100 % air saturation). Bars represent means (± SE).

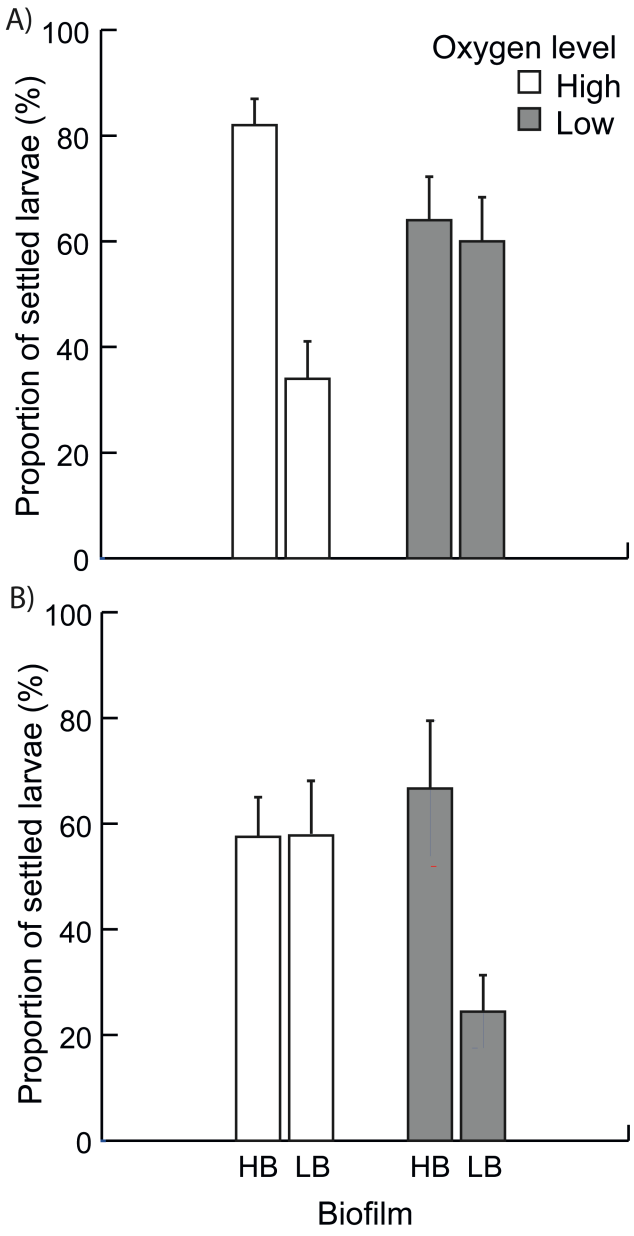

Figure 3: Proportion of *Bugula neritina* larvae settled on biofilms generated at high (HB) and low (LB) oxygen levels (100 and 25 % air saturation). A) Time trial experiment: settlement of larvae in each biofilm after 60 min of water immersion. B) Larval settlement selection experiment: settling of larvae that selected each side of the experimental surface, half of which had biofilms generated at high (HB) and low (LB) oxygen conditions. Both experiments were performed in water at high (100% air saturation, white bars) and low (25 % air saturation, grey bars) oxygen levels. Bars represent means (± SE).

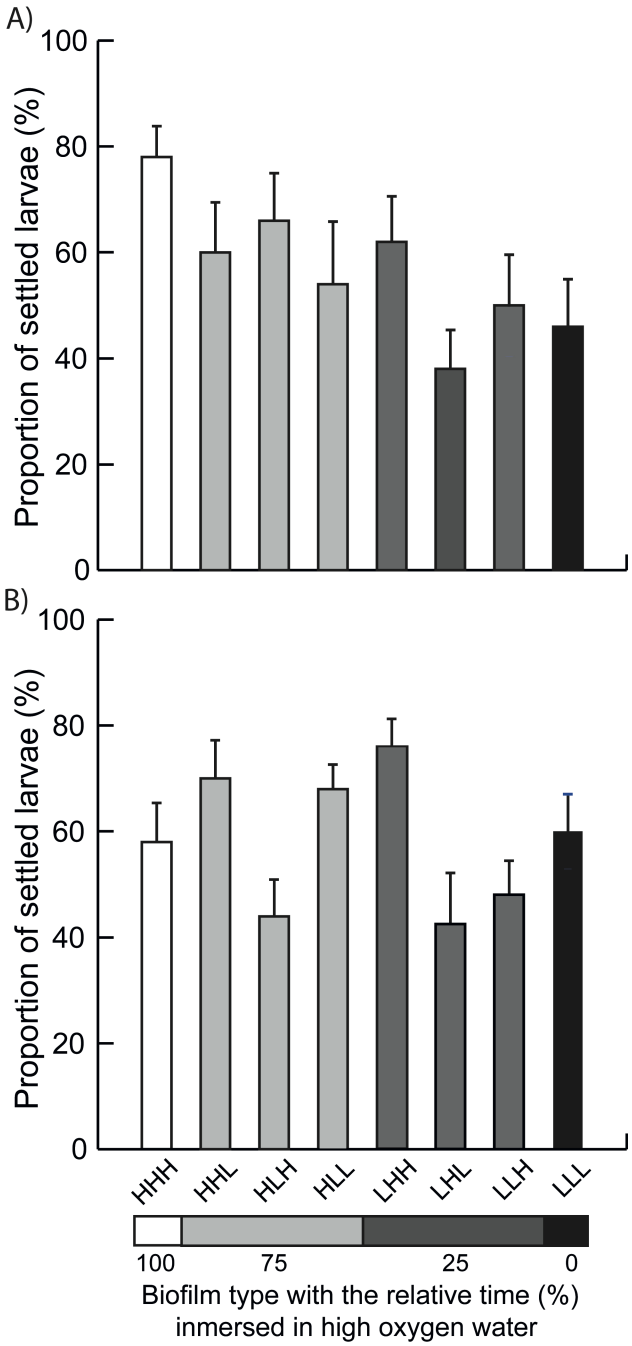

Figure 4: Proportion of larvae settled in biofilms with eight different oxygenation histories. Experiments were performed in water with oxygen 100 % air saturation (A) and 25 % air saturation (B). The gradient horizontal bar represents the persistence of submersion at high oxygen condition during the generation of each biofilm. Bars represent means (± SE).

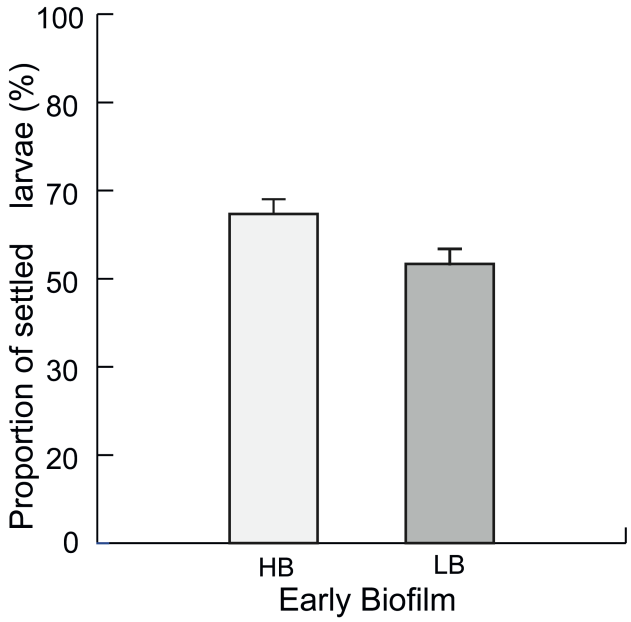

Figure 5: Effect of early stage of biofilms history on settlement of *Bugula neritina* larvae settled in biofilms generated with low (LB) and high (HB) oxygen levels (25 and 100 % air saturation). Bars represent means (± SE).

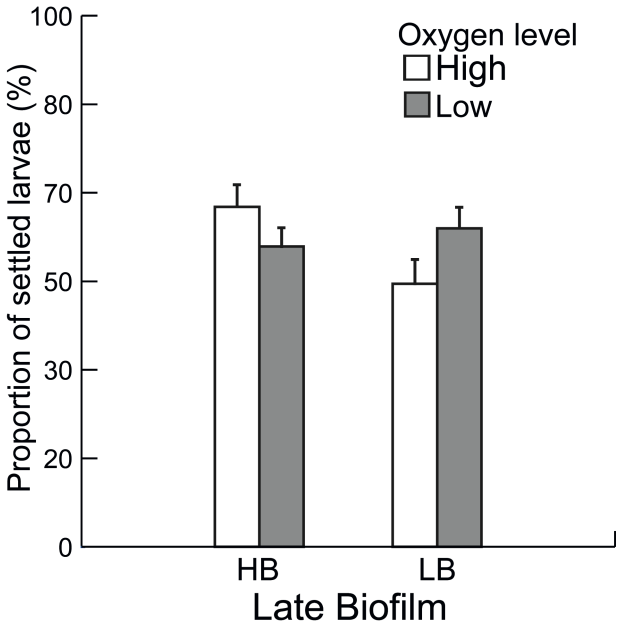

Figure 6: Effect of the interaction between late stage of biofilms histories with oxygen levels in the water on settlement of *Bugula neritina* larvae. Biofilms were generated with low (LB) and high (HB) oxygen levels (25% and 100% air saturation), and the oxygen levels in the water were high (100 % air saturation) and low (25 % air saturation). Bars represent means (± SE).

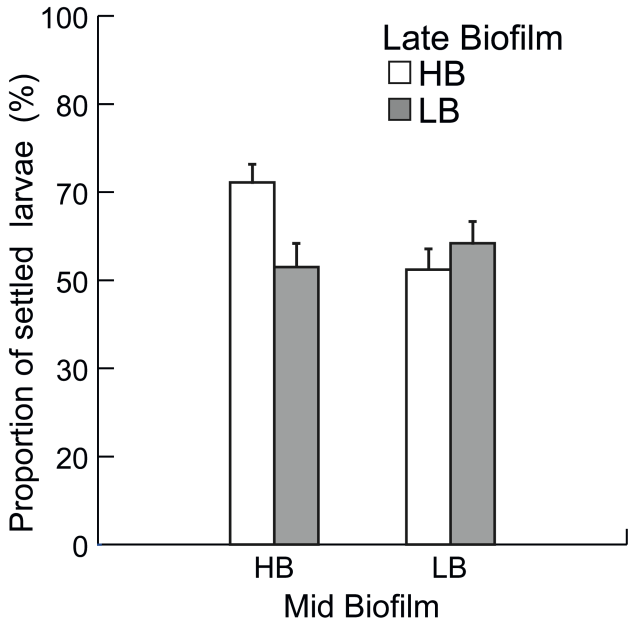

Figure 7: Effect of the interaction between mid and late stages of biofilms histories generated with low (LB) and high (HB) oxygen levels (0% and 100% air saturation) on settlement of *Bugula neritina* larvae. Bars represent means (± SE).

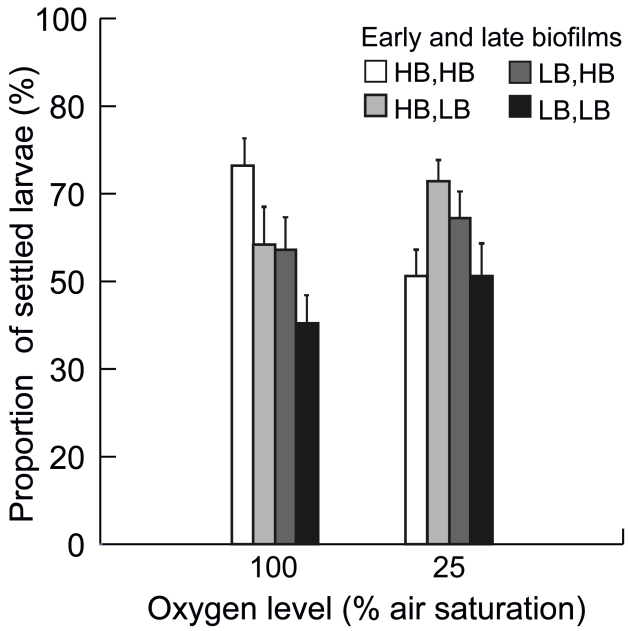

Figure 8: Effect of the interaction between oxygen levels of the water with early and late stages of biofilms histories generated with low (LB) and high (HB) oxygen levels (0% and 100% air saturation respectively) on settlement of *Bugula neritina* larvae. Bars represent means (± SE).

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Declaration for Thesis Chapter 3

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Development of key ideas, data collection, experiment design, data analysis and writing	65

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dustin Marshall	Assistance in data analysis, experimental design and input into manuscript	20
Craig White	Experimental design, input into manuscript	10
Diego Barneche	Data analysis	5

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 02-Nov- 2016
Main Supervisor's Signature	[redacted]	Date: 02-Nov- 2016

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER III

Do low oxygen environments facilitate invasion?
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 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, can resist these conditions remains unclear. I measured water flow oxygen availability in marinas and piers at the scales relevant to sessile marine invertebrates (mm). I then measured the capacity of invasive and native marine invertebrates to maintain metabolic rates under decreasing levels of oxygen using standard laboratory assays. I 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. I also found that invasive species with erect growth forms, can tolerate much lower levels of oxygen relative their native counterparts. Integrating the field and laboratory data showed that in sites with low flow, up to 30% of available microhabitats are physiologically stressful for native species, given the high amount of anoxic and hypoxic microsites. While only 18% of the habitat is physiologically stressful for invasive species. These results suggest that invasive species are well adapted to low oxygen habitats associated with manmade habitats, and artificial structures may be creating realized niche opportunities for invasive species.
Keywords: Non indigenous species, sessile communities, low flow, exploitative competition.

INTRODUCTION

Biological invasions are considered, together with habitat destruction, to be one of the biggest threats to biodiversity around the world (Vitousek et al. 1996, Davis 2003, Dafforn et al. 2009a). 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 and 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 and 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 (Simberloff and Von Holle 1999, Keane and Crawley 2002, Shea and Chesson 2002, Davies et al. 2005).

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 (Persson 1985, Crawley et al. 1986, Hart and Marshall 2012, Ferguson et al. 2013).

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 (i.e. has the lowest R* value, sensu Tilman 2004), and displace all other species at equilibrium (Tilman 2004). Therefore, 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 (Sundareshwar et al. 2011, Cullis et al. 2012).

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 (Bulleri and Airoldi 2005, Glasby et al. 2007, Dafforn et al. 2009b, Ruiz et al. 2009, Airoldi et al. 2015). 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 (Kinlan and Gaines 2003, McKenzie et al. 2012, Erfmeier et al. 2013). 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 and Airoldi 2005, Clark and Johnston 2005, Glasby et al. 2007). I 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 and Witman 2011). Water flow influences the performance of sessile marine invertebrates as it affects the delivery of essential resources: food and oxygen (Okamura 1985, Shimeta and Jumars 1991, Gardella and Edmunds 1999, Lastra et al. 2004, Svensson and 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 and 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 (Moore et al. 1996, Ferguson et al. 2013). 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. Probably, invasive species have adapted accordingly because of the long association of invasive species with artificial structures, which reduce flow in the environment (Bulleri and Airoldi 2005, Dafforn et al. 2009b, Wilding 2014). It seems likely that invasive species may be more resistant to low oxygen conditions, as they are for other measured trades (Lenz et al. 2011) . 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 – i.e. the oxygen level at which an individual becomes an oxyconformer (Portner and Grieshaber 1993). In mammals, which are strong oxyregulators, this level is known as the critical oxygen concentration

($C_{C_{O_2}}$) or critical oxygen pressure ($P_{C_{O_2}}$); below that value, aerobic metabolic rate decreases, anaerobic mechanisms become more important, and conditions are considered physiologically stressful (Hochachka and Somero 2002, Armstrong et al. 2009). In marine invertebrates, which are often neither strict oxy-conformers nor oxy-regulators, measuring $C_{C_{O_2}}$ is less straightforward (see Methods), but the level at 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, I 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 I measured the oxygen tolerances of a range of invasive and native sessile marine invertebrates that grow on artificial structures in these environments. Because growth form strongly affects oxygen tolerance in this group (Ferguson et al. 2013), I also measured tolerances across species with erect growth forms and flat growth forms. I 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 I measured our field estimates, within sites with a high diversity of flow conditions in 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) and Royal Melbourne Yacht Squadron (St Kilda) (31° 51’ 45” S; 144° 57’ 51” E).

I 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 and Marshall 2009), for two years prior to the experiment. The species used in these studies were classified according to their status (i.e. native or invasive to Australia; Table 1) and their growth form (i.e. erect or flat; Table 1). 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.

I 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 O_2 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, Aachen- Germany). Solitarian organisms were measured individually, colonial were measured the whole colony and treated as individuals during the statistical tests. These systems were chosen because they do not consume oxygen, and have accurately used to estimate oxygen consumption for marine invertebrates in previous studies (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 ($\dot{V}O_2$, ml h^{-1}) were calculated as :

$$VO_2 = -1[(m_a - m_c)/100)] \times V \times \beta O_2 \quad (1)$$

Where the Rate of oxygen consumption (VO_2, ml h^{-1}) was calculated from the slope of the line relating oxygen saturation and time for the vials containing organisms (m_a, % air saturation h^{-1}), the equivalent slope for the control vials (m_c, % air saturation h^{-1}), the oxygen capacitance of air-saturated seawater (βO_2, 5.3 ml l^{-1} at 19.0 °C; Cameron 1986) and the volume of the vial minus the volume of the organism and acetate (when they have it), (White et al. 2011, Ferguson et al. 2013, Pettersen et al. 2015). 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 C_{CO_2} can be discerned (Marshall et al. 2013), my $\dot{V}O_2$ data were curvilinear, such that there was no clear point where the organisms transitioned from a perfect oxyregulator to an oxyconformer (Fig. 1). Instead I fit a Michaelis-Menten function to our $\dot{V}O_2$ consumption data:

$$\dot{V}O_2 = \frac{\dot{V}O_{2max} \times CO_2}{C_{50\%\dot{V}O_{2max}} + CO_2}, \quad (2)$$

where $\dot{V}O_{2_{max}}$ is an asymptotic $\dot{V}O_2$, and $C_{50\%\dot{V}O_{2_{max}}}$ is the value of CO_2 where $\dot{V}O_2 = \dot{V}O_{2_{max}}/2$. Importantly, in order to achieve model convergence, I employ a transformation to $\dot{V}O_2$. For each individual, I standardized $\dot{V}O_2$ based on its maximum value, so all individuals present a relative $\dot{V}O_2$ bounded between zero and one. I note that this transformation implicitly assumes that $C_{50\%\dot{V}O_{2_{max}}}$ is independent of body mass. While this transformation affects the estimated $\dot{V}O_{2_{max}}$, it does not affect our primary goal, which is to estimate $C_{50\%\dot{V}O_{2_{max}}}$ for each species because it entails adding a constant to the numerator in Equation (1), hence $C_{50\%\dot{V}O_2}$, which is in the denominator, is not affected.

The typical parametric approach would have lead to get a non-representative sampling size for the statistical analysis, resulting in only one $C_{50\%\dot{V}O_{2_{max}}}$ value per species. To avoid this problem, the $C_{50\%\dot{V}O_2}$ model was obtained fitting equation (2) 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% confidence intervals (CIs) for the fitted parameters, $\dot{V}O_{2_{max}}$ and $C_{50\%\dot{V}O_2}$. I allow $\dot{V}O_{2_{max}}$ and $C_{50\%\dot{V}O_2}$ 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 × 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).

I use the species-specific estimates (n = 14) for $C_{50\%\dot{V}O_2}$ obtained in *JAGS* in order to fit three separate ANOVA's: one to test for differences in $C_{50\%\dot{V}O_2}$ between species status (native and invasive), a second to test for differences in $C_{50\%\dot{V}O_2}$ between species shape (erect and flat), and a third one to test for differences in $C_{50\%\dot{V}O_2}$ 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 I 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. I 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 $C_{50\%\dot{V}O_2}$ 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 I first calculate $\dot{V}O_2$ at 100% CO_2 ($\dot{V}O_{2_{100\%}}$) and use the value of CO_2 in which $\dot{V}O_2 = \dot{V}O_{2_{100\%}}/2$ as our proxy for $C_{C_{O_2}}$. I

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, I use the average species-specific parameters from our Bayesian model in order to calculate the value of air saturation, CO_2, in which $\dot{V}O_2 = \dot{V}O_{2_{100\%}} \times 0.95$.

Field estimates of water flow velocity and oxygen availability

I was interested in how flow velocities can shape the oxygen availability at the scales and microsites that were relevant to the study organisms – the conditions just a few millimeters above the organism. 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.

To measure flow velocities adjacent to the study communities I needed to use a more old-fashioned but reliable approach to measuring local flow speeds (Vogel 1994). I released 30 ml of milk on the surface, among the sessile community and measured the distance travelled (cm) by the leading edge from the point-source 30 and 60 seconds following release. The contrast between the color of the milk and water made visually, following the changes of the milk stain and measuring it. Three replicate measures at each sampling location where taken, from which I calculated an average flow velocity (cm s^{-1}) for each site.

To measure oxygen content in the water adjacent to the sessile community, I 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, I 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, I 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. $C_{C_{O_2}}$, CO_2 where $\dot{V}O_2 = \dot{V}O_{2_{100\%}}/2$, Equation 1): invasive species tolerate oxygen levels that are ~1.7-fold lower than the critical values for native species (Fig. 2a); 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 I used only had one native species with a flat growth form so I could not formally compare invasive and native species with that growth form. Consequently, when was 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 hypoxic or anoxic levels oxygen levels(Table 2, Fig. 4).

When I combined the estimates of oxygen availability with the estimates of tolerance to low oxygen, I 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.

DISCUSSION

I find that human-made structures, particularly marinas, cause reductions in local availability of oxygen in marine environments. In some cases, artificial structures 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, I found that invasive species can tolerate lower oxygen levels than native species – invasive species could maintain a $\dot{V}O_{2_{100\%}}/2$ at oxygen levels that were ~1.7-fold lower than those of native species. Based on the $C_{C_{O_2}}$ values reported here, I 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. I 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: (Kinsey and Kinsey 1967, Osinga et al. 1999, Nilsson and Ostlund-Nilsson 2004, Dodds et al. 2007). I 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 I measured, so differences in flow are unlikely to explain the observed differences in oxygen availability. I 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, I find a similar percentage of habitat is predicted to be physiologically stressful to that found in the previous study (Ferguson et al. 2013).

The $C_{C_{O_2}}$ values reported here are similar to those found for other sessile marine organisms and fishes (Nilsson and Ostlund-Nilsson 2004, Ferguson et al. 2013). Therefore, I believe the reported $C_{C_{O_2}}$ values to provide a fairly good indication of hypoxia resistance. 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 $C_{C_{O_2}}$ 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 (Shashar et al. 1993, Ferguson et al. 2013). 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. However, as a juvenile they will entirely live within the boundary layer, given their small size, changes through ontogeny of oxygen tolerance would be expected.

Invasive species presented a lower $C_{C_{O_2}}$ than natives. Moreover, I also found that erect-invasive organisms had lower $C_{C_{O_2}}$ values than erect-natives. Because I only had one native-flat species in our data set, I could not formally compare native and invasive flat species. However, I note that the flat-invasive organisms had the lowest $C_{C_{O_2}}$ across all functional groups, and could withstand extremely hypoxic levels (~ 5 % air saturation). Within the context of R* theory, species with low $C_{C_{O_2}}$ (or $P_{C_{O_2}}$) should be better competitors than species with higher $C_{C_{O_2}}$ because they can maintain aerobic metabolism at relatively higher rates in hypoxic conditions. It has also been demonstrated that species with low $C_{C_{O_2}}$ 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 (van Kleunen et al. 2010b, Zerebecki and Sorte 2011, Lejeusne et al. 2014). 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 (Levinton et al. 2003, Floerl and Inglis 2005, Piola et al. 2009, McKenzie et al. 2011). 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 and Lutz 2001, Nilsson and Ostlund-Nilsson 2004, Mandic et al. 2009). Usually the physiological thresholds of the species matches the minimum oxygen level of the environment, therefore hypoxia tolerance is an important trait that can determine the distribution and abundance of organisms (Stillman and Somero 1996, Lagos et al. 2011, Verberk et al. 2011). I 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. I would therefore suggest that species that are already tolerant to low oxygen conditions might be more likely to become invasive if translocated by humans.

An interesting potential work would be study species diversity in artificial structures and see if there is any relationship with flow and oxygen conditions. Trough field observations I can tell that St Kilda and Brighton, which are the places with more hypoxic microsites, present a low diversity and a relative higher amount of invasive species than the other sites. Contrastingly Blairgowrie and Queenscliff , which are sites with few or zero hypoxic microsites are highly diverse and dense; also they present a high amount of macroalgies that could be rising up the levels of oxygen. Of course, these only field observations that need to be formally tested.

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.

Acknowledgments. The authors thank to Blairgowrie Yacht Squadron, Royal Brighton Yacht Club, Royal Melbourne Yacht Squadron and Queenscliff Harbour for access to the field site. We thank Amanda Pettersen and Hayley Cameron for their help in the elaboration of the draft of this paper, Martino Malerba for statistical advice, Mattia Pierangelini, Camila Arnes and Yussi Palacios for their help in the field. C.R.W. and D.J.M. were supported by grants from the Australian Research Council. M.E.L. is supported by grants from Conicyt Becas-Chile Scholarship. D.R.B. is funded by the Monash University Centre for Geometric Biology.

Table 1. Sessile species used in this study. Species are classified according to their status (Invasive or native) and their shape (Erect or flat) and the number of individuals analyzed (n).

Species	Growth shape	Status	n
Styela plicata	Erect	Invasive	8
Styela clava	Erect	Invasive	17
Ciona intestinalis	Erect	Invasive	9
Pyura dalbyi	Erect	Native	9
Pyura doppelgangera	Erect	Native	9
Herdmania grandis	Erect	Native	13
Botrylloides magnicoecum	Erect	Native	9
Bugula dentata	Erect	Native	11
Bugula neretina	Erect	Invasive	11
Bugula flabellata	Erect	Invasive	7
Watersipora subtorquata	Flat	Invasive	13
Didemnum sp.	Flat	Invasive	11
Celleporaria sp.	Flat	Native	7
Diplosoma sp.	Flat	Invasive	9

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

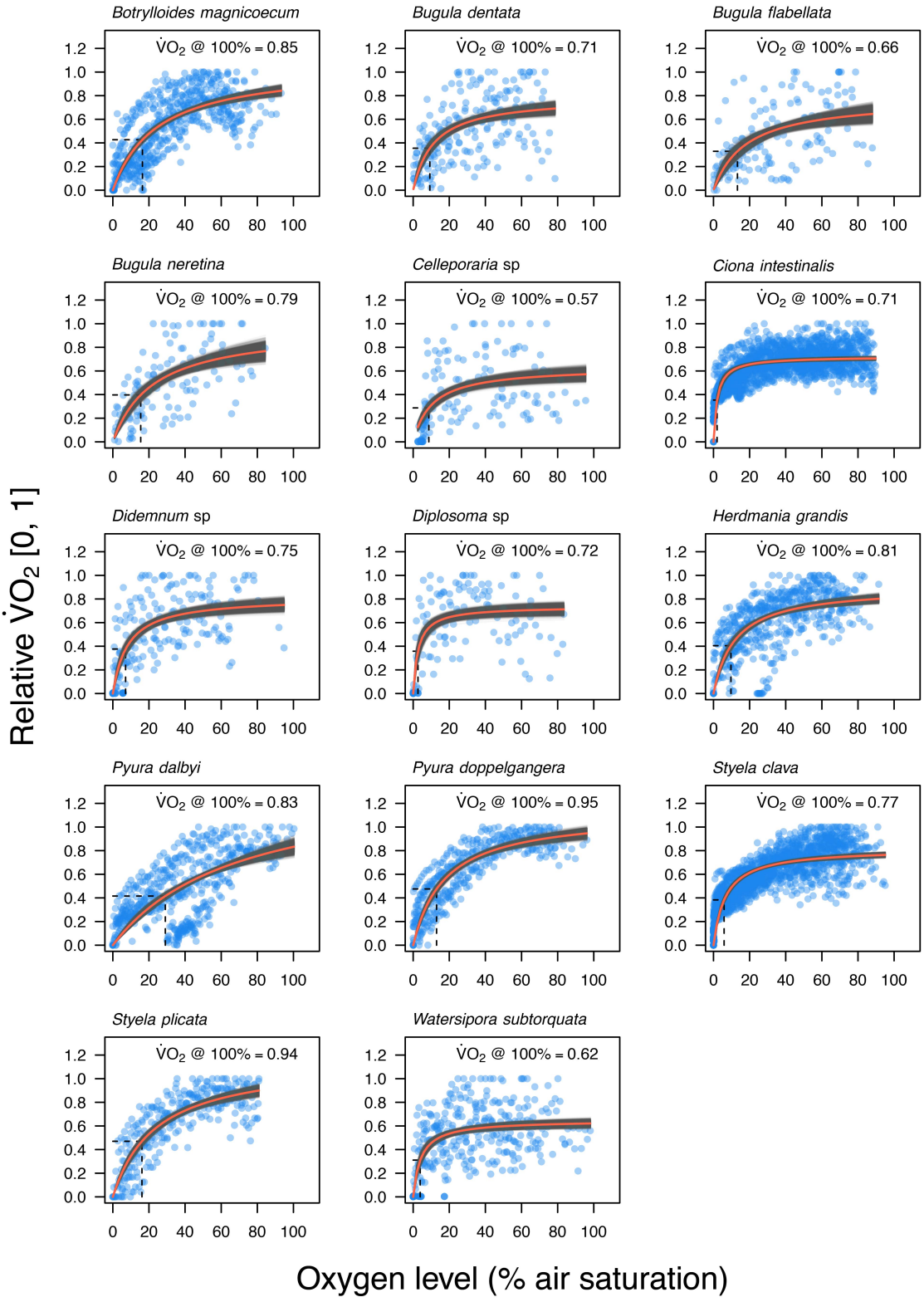

Figure 1. Plots of oxygen level (% air saturation) with relative respiration rate of each species measured ($\dot{V}O_2$, 0-1). The intersection of dashed lines with x-axis shows the average calculated $C_{C_{O_2}}$.

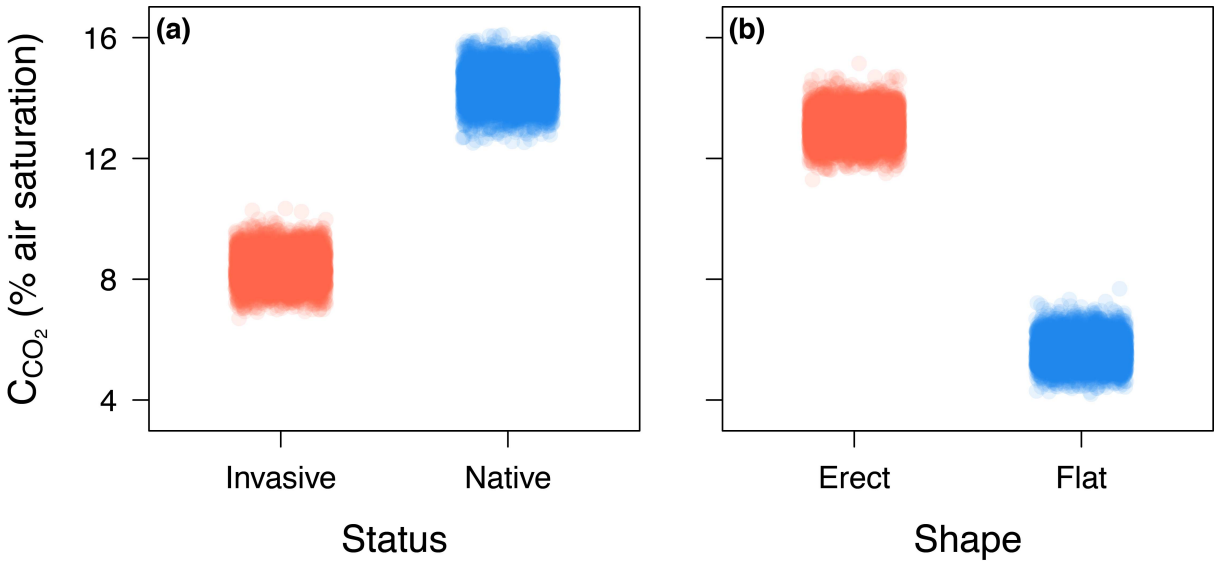

Figure 2. Differences in $C_{C_{O_2}}$ between: (a) species status (native and invasive), and (b) species shape (erect and flat). Each of the 6,000 circles for each category represents an average of $C_{C_{O_2}}$ estimated by an ANOVA using species-specific values of $C_{C_{O_2}}$ drawn from MCMC samples from a Michaelis-Menten function fitted in *JAGS*.

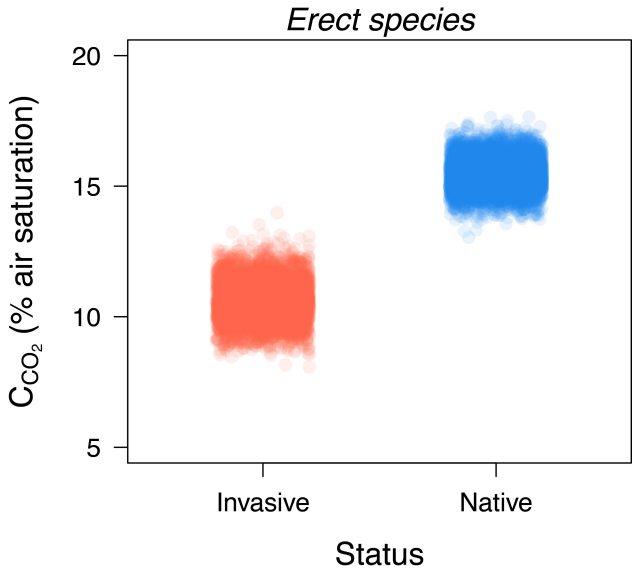

Figure 3. Differences in of $C_{C_{O_2}}$ between status (native and invasive) for "erect shaped species" only. Each of the 6,000 circles for each category represents an average of $C_{C_{O_2}}$ estimated by an ANOVA using species-specific values of $C_{C_{O_2}}$ drawn from MCMC samples from a Michaelis-Menten function fitted in *JAGS*.

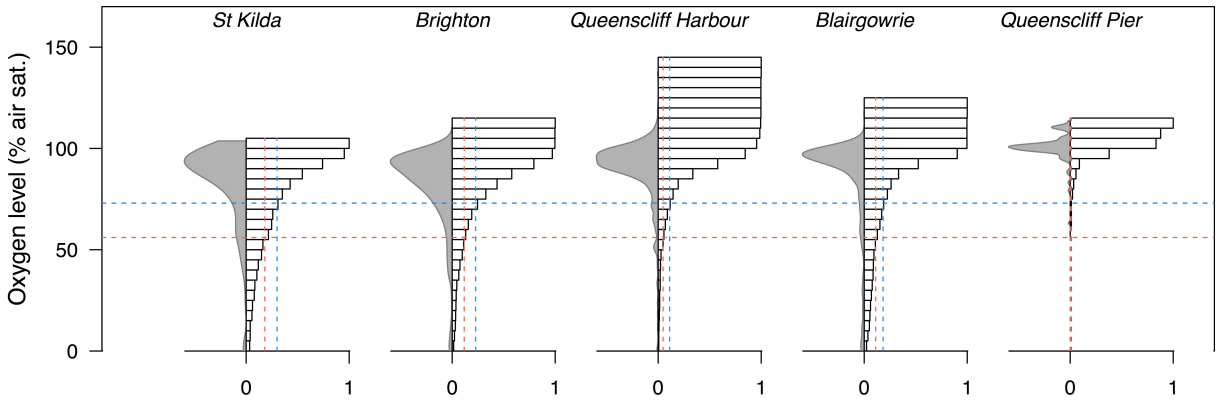

Figure 4. Distribution of oxygen level across five different marine sites. Field sites are ordered according to their ranking of their average water speed, from slowest to fastest. Left side of each plot represent frequency distribution of oxygen. Right sides of the plots show cumulative density histograms 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.

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Declaration for Thesis Chapter 4

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Development of key ideas, data collection, experiment design, data analysis and writing	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dustin Marshall	Assistance in data analysis, experimental design and input into manuscript	20
Craig White	Experimental design, input into manuscript	10

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 02-Nov- 2016
Main Supervisor's Signature	[redacted]	Date: 02-Nov- 2016

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER IV

Metabolic rate depends on body mass, growth form and invasion status in sessile marine invertebrates

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ABSTRACT: Invasive organisms often share characteristics that make them successful in novel habitats. Traits such as rapid growth and short generation times are classic ‘weedy’ phenotypes, such that invasive species often have r-selected rather than k-selected life histories. Given that invasive species often display ‘fast’ life histories, invasive species may have relatively higher metabolic rates but systematic tests across taxa are lacking. Here I studied metabolic-scaling relationships across 14 sessile invasive and native marine invertebrates. I also investigated the influence of growth form (erect versus flat species) on the metabolic rate of these species, since growth form can also affect metabolic rate. I found an effect of growth form on the relationship between body mass and metabolic rate: flat organisms generally have lower metabolic rates than erect organisms, and this difference is accentuated at higher body masses. For species with an erect growth form, I found an effect of invasive status on how metabolism scales with body mass. Invasive organisms have higher metabolic rates for lower body masses, but there is little difference in metabolic rate for invasive and native species at higher body masses. Given that smaller-bodied invasive organisms are typically early-successional, ‘fugitive’ species, a higher metabolic rate may allow a faster pace of life, enhancing their capacity to invade and reproduce in newly created disturbed habitats.
Keywords: Energy consumption, life history, sessile organisms, invasion ecology, artificial structures, pace of life, metabolic scaling.

INTRODUCTION

The probability of biological invasions is influenced by the characteristics of the potentially invaded habitat, the traits of the invasive species and the interaction between these two factors (Andow et al. 1990, Arim et al. 2006, van Kleunen et al. 2010a, van Kleunen et al. 2010b, Zhao and Feng 2015). Invasive organisms often share common characteristics that make them successful invaders: relative to native species, they generally have more rapid growth, reproduce sooner and therefore have shorter generation times (Ehrlich 1986, Lodge 1993, van Kleunen et al. 2010a, Matzek 2012, Lejeusne et al. 2014). Together, these traits make for the classic 'weedy' phenotype, whereby invasive species are thought to have r-selected rather than k-selected life histories (Ehrlich 1986, Sakai et al. 2001, McMahon 2002). That invasive species tend to have faster life histories than noninvasive species has long been recognized, but the underlying physiological drivers of faster, invasive life histories are poorly understood. Measures of metabolic rate integrate the costs associated with a range of organismal functions, including the maintenance of homeostasis, feeding and digestion, growth and reproduction. Metabolic rate is therefore a likely driver of differences in life history among invasive and native species.

Several lines of evidence suggest that systematic differences in the metabolic rates of native and invasive species are likely. Generally, metabolic rate is thought to covary with key life history traits, which together determine the pace of the life history (Burton et al. 2011). For example, according to the 'increased intake' hypothesis, higher metabolic rates are correlated with faster growth and greater movement (Boratynski and Koteja 2010, Burton et al. 2011). Similarly, early theories about the 'rate of living' assumed a negative relationship between lifespan and metabolic rate (Pearl 1928; Schmidt-Nielsen 1984). A more recent study showed that individuals with higher metabolic rates grow faster but reproduce and died sooner in the field (Pettersen et al. 2016). I might therefore expect invasive species to have higher metabolic rates than natives. On the other hand, the 'compensation hypothesis' predicts that lower metabolic rates allow for the reallocation of more energy to growth and reproduction, and increasing both may also facilitate invasion (Nilsson 2002, Burton et al. 2011). In support of the compensation hypothesis, lower metabolic rates were associated with higher growth rates in fishes in the field (Alvarez and Nicieza 2005, Norin and Malte 2011). Thus, general theory makes conflicting predictions about the association between metabolic rate, the pace of life, life history and invasions (Burton et al. 2011).

Despite the ambivalence of general theory regarding invasion and metabolism, a higher metabolic rate has repeatedly been invoked as a key trait for successful invaders (Maazouzi et al. 2011). For example, is has been suggested that invasive freshwater clams are successful invaders in part because they have higher metabolic rates than native species occupying the same niche, allowing them to burrow and filter food more rapidly (McMahon 2002). Nevertheless, others have

argued that invasive organisms have lower metabolic rates than their native congeners (Gonzalez-Ortegon et al. 2010, Lejeusne et al. 2014). Thus, there are conflicting accounts about how metabolic rate varies systematically between native and invasive species. Most studies have been restricted to only one or two species, hampering our ability to generalize about whether metabolic rate is related to invasion success.

To better understand the relationship between metabolic rates and invasion success, systematic comparisons of the metabolic rates of native and invasive species from the same system are necessary, ideally involving species within the same trophic guild but across wide taxonomic ranges. Here, I tested how metabolic rate differs between invasive and native species, using a metabolic scaling approach. I estimated the rate of oxygen consumption in 14 species of marine sessile invertebrate from a range of taxa that varied in whether they were native or invasive. Because metabolic rate scales strongly with body mass, I measured metabolic rates across a range of body masses.

In addition to examining the effect of invasion status on metabolic rate, I also examined growth form because there is a link between the growth form and the oxygen requirements of sessile marine invertebrates (Ferguson, White & Marshall 2013). Species with a flatter, two dimensional growth form (e.g. sponges, encrusting bryozoans and many colonial ascidians) can cope with lower oxygen levels than species with an erect, three dimensional growth form (e.g. tubeworms, solitary ascidians, arborescent bryozoans; Ferguson *et al*. 2013). Presumably these differences in tolerance to low oxygen conditions reflect the fact that flat species live entirely within the oxygen depleted boundary layer while erect species may project out of it (Ferguson *et al*. 2013). It is unclear whether metabolic rates also covary with growth form, though theory predicts that there should be systematic differences in the relationship between body mass and metabolic rate for 2-d and 3-d organisms (White et al. 2011).

MATERIALS AND METHODS

Organism collection and maintenance

Animals were collected from sites within Port Phillip Bay, Victoria Australia: 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) and Royal Melbourne Yacht Squadron (St Kilda) (31° 51’ 45” S; 144° 57’ 51” E). While large species (e.g. solitary ascidians), were collected by scraping organism from the floating pontoons, smaller species (e.g. bryozoans and colonial ascidians) were cut from pre-roughened acetate sheets collected from the field. Acetate sheets had been deployed for two years on PVC panels, hanging from floating pontoons at 1.5 m depth. The organisms were transported to the lab in

insulated aquaria with aerated seawater to be acclimatized to laboratory conditions for 2 days in the dark at 19 °C.

Measurement of metabolic rate

Rate of oxygen consumption, a widely used proxy for metabolic rate (Lighton 2008), was measured using two different closed respirometry systems based on the size of the species (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 O_2 fiber optic oxygen meter (Pyro Sciences, Aachen-Germany). Each experimental run of 12 chambers included 4 chambers containing water but no animals to act as controls for background rates of oxygen consumption. For smaller organisms, sections of the acetate bearing whole colonies and placed in 25 ml vials were mounted on a 24-channel sensor dish reader (Sensor Dish Reader SDR, PreSens, Aachen, Germany). Each experimental run of 24 vials included 4 vials containing only water as controls. The experimental chambers were filled with previously micro-filtered (0.2 μm) and pasteurized seawater that had been kept at 19 ° C with constant aeration for at least 24 hours prior to experiments to allow water temperature to stabilize. Measurements were conducted in darkness for a period of 1 to 10 hours; the duration of measurement depended on the rate of oxygen consumption of each species.

Rate of oxygen consumption (VO_2, ml h^{-1}) was calculated from the slope of the line relating oxygen saturation and time for the vials containing organisms (m_a, % air saturation h^{-1}), the equivalent slope for the control vials (m_c, % air saturation h^{-1}), the oxygen capacitance of air-saturated seawater (βO_2, 5.3 ml l^{-1} at 19.0 °C; Cameron 1986) and the volume of the vial, minus the volume of the organism and acetate (when relevant) using the following equation (White et al. 2011; Ferguson et al. 2013; Pettersen et al. 2015):

$$(1) \qquad VO_2 = -1[(m_a - m_c)/100)] \times V \times \beta O_2.$$

After the oxygen measurements, the organisms were placed in an oven for one week at 60º C to obtain dry body mass. The species used were classified according to their status (i.e. native or invasive; Table1) and their growth form (i.e. erect or flat; Table 1).

Statistical tests

How metabolic rate scales with body mass is usually mathematically described by the power function $Y = aM^b$, where Y is the VO_2, a is the scaling factor, M is the body mass of the organism and b is the scaling exponent (White & Seymour 2011; Pettersen et al. 2015).

All oxygen consumption data (VO_2, ml h^{-1}) and dry body mass (M, g) were log–log transformed. Mixed-effects ANCOVA was used to test whether the relationship between mass and metabolic rate is affected by species identity, growth form and native-invasive status of erect

organisms and with dry body mass as covariate. Because there was not sufficient replication at the level of species, I could not test for a mass x growth form x invasion status 3-way interaction, instead, I could only test for mass x growth form and mass x status. I performed an additional test of the effect of invasion status, just within species with an erect growth, because in this growth form, I had sufficient replication.

Where evidence for different relationships between mass and metabolic rate was found among species or functional groups, I then used linear regression to calculate coefficient and scaling exponent values for $\log_{10}$-transformed data for each species or functional group according to the equation:

$$(2) \qquad \log_{10} VO_2 = b \times \log_{10} M + log_{10}\, a.$$

Wald tests were done to test whether the scaling exponent different significantly from 0, and from 1. All ANCOVA and Walden tests were done using the statistical software Systat ver.13. (Systat, Cranes Software International, Bangalore, India)

RESULTS

The relationship between body mass and rate of oxygen consumption (VO_2) differed significantly among species (Table 2a). However, due to the high variation in VO_2 within species, I found significant relationships between mass and VO_2 in only 7 of the 14 measured species (Table 3). Relatively higher respiration rates were found in *Bugula flabellata,* with values up to 3 fold higher than *Bugula neritina* - the species that showed lowest oxygen consumption (Table 3, Fig 1) - even though both species are invasive and have an erect growth form.

I detected a significant effect of invasion status and growth form on the relationship between oxygen consumption and body mass (Table 2b). Erect organisms showed a higher scaling exponent than flat organisms, 0.679 versus 0,406 (Table 3, Fig 2). Within erect-shaped organisms, invasion status was found to have a significant effect on the VO_2-body mass relationship (Table 2c). Native species had a higher scaling exponent than invasive species, 0.784 versus 0.658 found in invasive species. However, in invasive species, the coefficient "a" was higher than in natives, 0.294 versus 0.184 (Table 3, Fig 3). Unfortunately, due to logistical limitations of the study, I only measured VO_2 in one species within the native-flat group; therefore I could not include flat shapes in the model to be formally tested. Based on the measurements however, I observed that flat-invasive species had the lowest scaling exponent of all the functional groups (Table 3).

DISCUSSION

The relationship between body mass and metabolic rate depends on both growth form and invasion status in the sessile marine invertebrates I studied. Species with a flat growth form showed a shallower scaling relationship than species with an erect growth form (Fig. 2). The scaling exponent for the relationship between mass and metabolic rate was higher in native species than invasive species, but invasive species had an overall higher metabolic rate (higher intercept) than natives (Fig. 3). Overall, metabolic rates of invasive species (with erect growth forms) were much higher than those of native species at lower body masses, but largely equivalent high body masses.

Growth form affects the scaling relationship between body mass and metabolic rate in ways anticipated by theory. So far, several models have attempted to estimate the differences in energy expenditure between 2-d and 3-d organisms. The "fractal geometry model" predicts a scaling exponent of 2/3 for 2-d organisms and 3/4 for 3-d organisms (West et al. 1999), however recent modifications of fractal geometry predict wider ranges of scaling exponents (Enquist et al. 2007, Kolokotrones et al. 2010). Previous studies have shown the dynamic energy budget theory (DEB) proved to be a good predictor of metabolic scaling, as empirical data fits with the predictions of a scaling exponent of 0.5 for two-dimensional species (White et al. 2011). I found that the scaling exponent of flat and erect sessile species to be significantly different with 'b' equal to 0.40 and 0.67 for flat and erect species respectively. As the coefficient 'a' is lower in flat-shaped species, their overall metabolic rate is lower than in erect organisms for any measured size. The difference between both groups increases considerably more at bigger body masses.

A potential explanation of the relatively higher metabolism of erect-shaped species over flat-shaped species, could be that oxygen limitation occurs in flat species more than in erect species due to higher amounts of self-shading. DEB theory predicts that in flat colonies metabolic rates of the edges are higher than in the center (White *et al.* 2011). While the edges must fulfill the metabolic cost of maintenance and colony growth, the zooids in the center only require energy for maintenance (White et al. 2011). The growth pattern of the 2-d flat organisms may enable lower energetic demands than erect shaped organisms, which grow three-dimensionally and have less self-shading. In solitary, erect organisms such as large ascidians, the use of a muscular system in order to move the water through the body and extract oxygen incurs a higher energetic cost, however oxygen intake is more efficient (Kumpp 1984).

Alternatively, the differences in oxygen consumption that I observed across different masses may be driven by methodology. Recall that I used a still water approach for smaller species and a re-circulating approach for larger species. It seems likely that self-shading and oxygen limitation at a very small scale is more likely in a still water system relative to a re-circulating system. I

found that metabolic rates between invasive and native species were most different for small-bodied species – but these species were also studied in still water. It is possible, that the apparent difference in metabolic rate between invasive and native species arises, because invasive species are better able to maintain higher metabolic rates under local oxygen limitation than native species. Indeed, in a separate study I found that invasive species are better able to tolerate low oxygen conditions than native species (Lagos et al. 2016. unpublished). The methodological differences I used between larger and smaller body sizes were unavoidable due to practical constraints so it is unfortunate I cannot disentangle the relative roles of different methods and body size. Regardless of this limitation, my primary observation that metabolic rates differ among native and invasive species remains unaffected, it is the status x mass interaction that may be driven by the methodological differences.

High metabolic rates are sometimes associated with faster growth and earlier onset of reproduction (Pettersen *et al*. 2016). Such "r- selected" or "weedy" phenotypes can be especially important for species living in communities where the sessile style of life is dominant. When animals are sessile and small, susceptibility to predation and being outcompeted is often high (Paine 1974, Jackson 1979, Buss 1980). The characteristics associated with erect organisms allow them to quickly reach a size refuge in order to avoid predation and being outcompeted, while allowing for fast regeneration of lost body parts. Smaller invasive individuals clearly have higher metabolic rates than their native counterpart, at least under our experimental conditions. It is likely that the relatively higher metabolic rate of small invasive organisms is one of the causes of their invasibility. Nevertheless, it is unlikely that small invasive species can compete against adults or larger-sized natives, especially in communities that are reaching equilibrium (MacArthur 1970, Shea and Chesson 2002). However, as early successional species they can quickly reproduce after settlement and can be highly successful colonizing disturbed habitats or environments with vacant patches (Sakai et al. 2001, Ghermandi et al. 2004).

My results indicate that body shape affects the relationship between metabolic rate and body mass of sessile species. At least in erect shaped species, the comparative metabolic advantage of small invasive may provide an advantage over their native counterparts. However, it still remains to be seen how metabolic rate changes through different ontogenetic stages and how it could be linked with the ecology of invasion.

Acknowledgements. The authors thank to Blairgowrie Yacht Squadron, Royal Brighton Yacht Club and Royal Melbourne Yacht Squadron for access to the field site. To Amanda Pettersen and Hayley Cameron for their extensive help in the preparation of the draft of this paper. C.R.W. and

D.J.M. are supported by grants from the Australian Research Council. M.E.L. is supported by grants from Conicyt Becas-Chile Scholarship.

Table 1. Sessile species used and the sample size (n). Species are classified according to their status (invasive or native) and they grow shape (erect or flat).

Species	Shape	Status	n
Styela plicata	Erect	Invasive	11
Styela clava	Erect	Invasive	15
Ciona intestinalis	Erect	Invasive	9
Pyura dalbyi	Erect	Native	9
Pyura doppelgangera	Erect	Native	9
Herdmania grandis	Erect	Native	15
Botrylloides magnicoecum	Erect	Native	20
Bugula dentata	Erect	Native	17
Bugula neretina	Erect	Invasive	15
Bugula flabellata	Erect	Invasive	11
Watersipora subtorquata	Flat	Invasive	14
Didemnum sp	Flat	Invasive	13
Diplosoma sp	Flat	Invasive	10
Celleporaria sp	Flat	Native	10

Table 2. ANCOVAs for effect of $\log_{10}$ oxygen consumption and $\log_{10}$ body dry mass of sessile organisms in: A) individual species, B) growth shape (flat-erect) and C) Status (invasive-native) of erect organisms.

	Source	SS	df	MS	F	*p*
A)	$\log_{10}$ mass	1.136	1	1.136	49.503	< 0.01
	Species	1.613	13	0.124	5.406	< 0.01
	Species × $\log_{10}$ mass	0.589	13	0.045	1.973	0.027
	Error	3.42	149	0.023		
B)	$\log_{10}$ mass	3.86	1	3.86	86.854	< 0.01
	Shape	1.155	1	1.155	25.984	< 0.01
	Shape × $\log_{10}$ mass	0.244	1	0.244	5.494	0.02
	Error	7.732	174	0.044		
C)	$\log_{10}$ mass	32.088	1	32.088	1,021.55	< 0.01
	Status	0.39	1	0.390	12.492	0.01
	Status × $\log_{10}$ mass	0.355	1	0.355	11.306	0.01
	Error	3.989	127	0.031		

Table 3. Summary of scaling exponents (*b*) (± SE) and coefficients for metabolic rate and mass (*a*) of invasive and native sessile species, using a log–log transformed linear relationship, where: $\log_{10} VO_2 = b \times \log_{10} dry\ mass + a$. Data are in groups of individual species, growth form and the interaction between body shape and species (erect-flat, invasive-native), that presented significant regressions.

Species	*(a)*	*(b)*	*p-value* $b \neq 0$	*p-value* $b \neq 1$	R^2
Styela plicata	0.185	1.100 (±0.132)	0.234	<0.01	0.885
Pyura dalbyi	0.124	0.833 (±0.174)	0.185	0.002	0.766
Herdamania grandis	0.202	0.964 (±0.314)	0.457	0.009	0.420
Botrylloides magnicoecum	0.327	1.122 (±0.309)	0.349	0.002	0.422
Bugula neritina	0.220	0.582 (±0.170)	0.014	0.005	0.473
Bugula flabelata	0.127	1.588 (±0.334)	0.056	0.001	0.715
Celleporaria sp	0.194	1.071 (±0.431)	0.437	0.046	0.410
Erect organisms	0.237	0.679 (±0.024)	<0.01	<0.01	0.861
Flat organisms	0.058	0.406 (±0.121)	<0.01	<0.01	0.199
Invasives-flat	0.046	0.312 (±0.136)	<0.01	0.027	0.132
Natives-erect	0.184	0.784 (±0.031)	<0.01	<0.01	0.941
Invasive-erect	0.294	0.658 (±0.025)	<0.01	<0.01	0.919

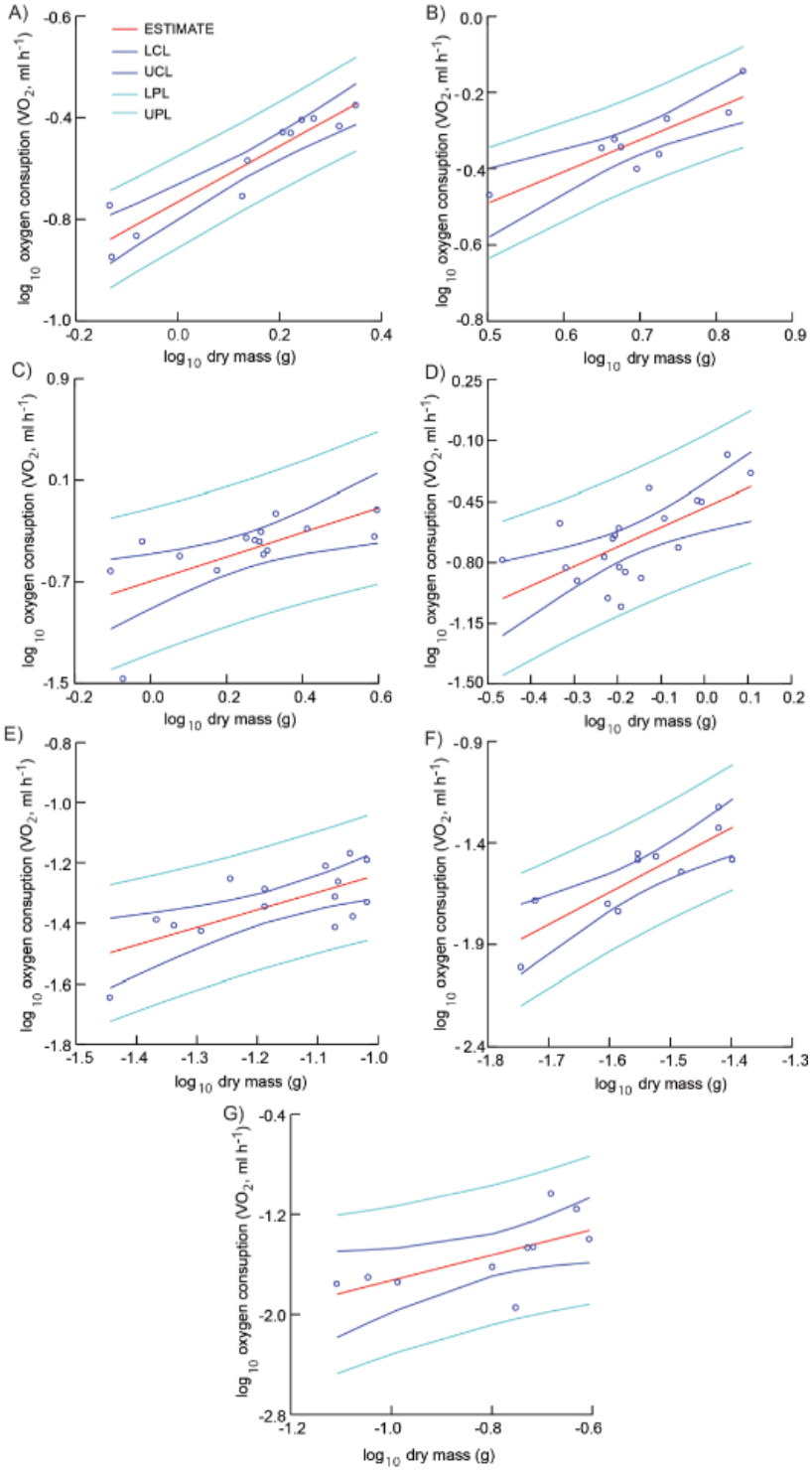

Figure 1: Linear regressions for the relationship between $\log_{10}$ dry body mass (g) and $\log_{10}$ oxygen consumption (VO_2, ml h^{-1}) on individual sessile marine species. A) *Styela plicata*, B) *Pyura dalbyi*, C) *Herdamania grandis*, D) *Botrylloides magnicoecum*, E) *Bugula neritina*, F) *Bugula flabellata and* G) *Celleporaria sp.* Blue lines represent the prediction intervals and the pale blue lines represent the confidence intervals for the regression lines.

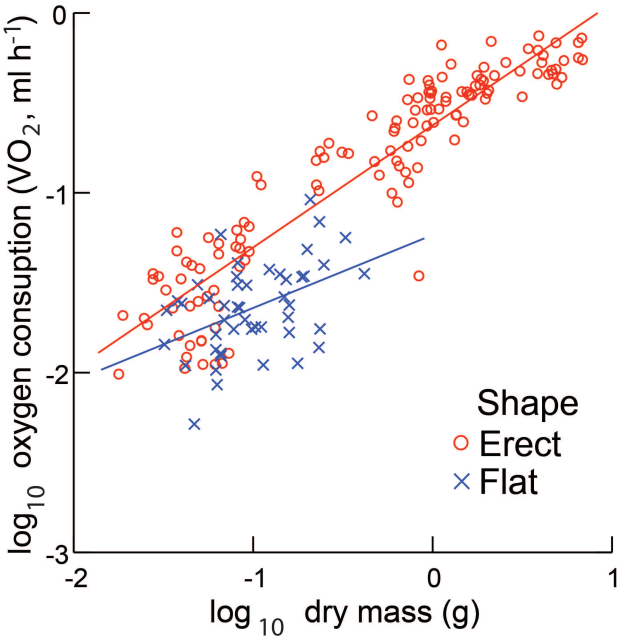

Figure 2: Linear regressions for the relationship between $\log_{10}$ dry body mass (g) and $\log_{10}$ of oxygen consumption (VO_2, ml h^{-1}) of sessile organisms by growth shape (flat-erect).

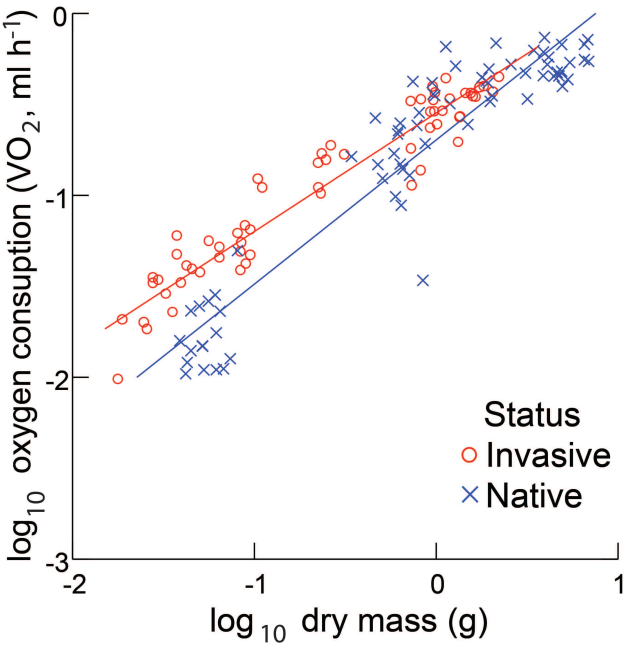

Figure 3: Linear regressions for the relationship between $\log_{10}$ dry body mass (g) and $\log_{10}$ oxygen consumption (VO_2, ml h^{-1}) of erect organisms by status (invasive-native).

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GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

Today, it is impossible to exclude the impacts of humans from discussions about ecology. Human activities have altered nature for thousands of years but during the last 50 years, human impacts have increased to worrying levels (Diaz and Rosenberg 2008). Marine habitats, especially costal habitats are one of the most threatened. Such habitat modifications can facilitate the spread of non-native species, and can jeopardize native fauna around the world (Vitousek et al. 1996, Pimentel et al. 2001, Arim et al. 2006). Artificial structures, which are highly associated with invasive species, are becoming more abundant every year. For example, in some places in Europe artificial structures occupy 60% of the coast; and are projected to increase in future decades (Schubchard et al. 1984). In this thesis, I have explored the impacts artificial structures have on oxygen levels and how marine organisms in these habitats exist within such habitats. Below I outline some of the new questions arising from my research that I did not have the opportunity to explore.

There is an inverse relationship between the variation in water flow and oxygen levels within benthic habitats on artificial structures. At the marina which had the lowest flow conditions, I found that 30 % of the microsites measured contained oxygen levels below the physiological limits of native species. My field sampling methodology can be considered a conservative measure of oxygen availability, since all measurements were conducted during the day when algae are photosynthetically active. I show that even under the best conditions, there is limitation of oxygen in low flow systems, therefore in worse case scenarios the amount of microsites that represent physiologic stress for natives can potentially be higher. Future research should consider measuring oxygen in marinas with lower flow conditions than those measured here – my study sites were not the most modified in the study area, they were just more easily accessed. Furthermore, future studies should also aim to characterize fluctuations in oxygen availability across larger temporal scales, include changes in oxygen levels throughout the night in the absence of photosynthetic activity. Additionally, as the physicochemical conditions of the water change with temperature, the oxygen variability at different latitudes should be measured.

Oxygen availability altered larval colonization behaviors in manifold ways. Larval behavior depends on larval age, oxygen levels in the water and the oxygenation history of the biofilm. As was seen in chapter one and two, in low oxygen conditions, larvae typically delay settlement, spend less time exploring settlement surfaces and increase their time swimming in the water column. All these behaviors indicate that larvae can discriminate so as to increase their chances of settling in habitats with favorable oxygen levels. I speculate that this strategy may also act as a tool for larvae to avoid settlement next to superior competitors. Previous research has shown that aggressive species can inhibit larval settlement, and that competitively dominant adult organisms can deplete

surrounding oxygen levels (Grosberg 1981, Ferguson et al. 2013). Therefore, it is reasonable to suggest that larvae may use microsite oxygen availability as clue to avoid settling next to superior competitors. Future studies that orthogonally manipulate oxygen levels and the presence of competitors to assess larval settlement behavior are required to test this hypothesis.

Biofilm characteristics also affected larval settlement, but it seems that this effect is secondary to the role of oxygen levels in the water. When larvae are in low oxygen conditions, they preferentially settle on biofilms that represent habitats with high and stable oxygen levels. When oxygen levels within the water column are low, however, larvae did not show a consistent settlement response to biofilms of different oxygen histories. This ability to integrate different cues for identification of high quality habitats are likely more related to decisions about the future development in the adult stage, rather than the physiological thresholds of the larvae. As can be seen in chapter three and previous literature, erect organisms such as *Bugula neritina* have low tolerance to hypoxic conditions when compared with flat or encrusting species (Ferguson et al. 2013). Therefore, the selection of favorable habitats made for the larvae, is fundamental for the development of the colony. It would be interesting to test the physiological tolerance of *Bugula neritina* settlers (as opposed to adults c.f. Chapter 3) to low oxygen conditions. *Bugula* and other erect shaped organism live within the hypoxic conditions of the boundary layer during earlier developmental stages. Therefore, it may be that throughout ontogeny, such species display a shift in hypoxia tolerance, such that early stages of erect species may display similar oxygen tolerances to the encrusting species with which they share the same spatial niche.

The high amount of hypoxic and anoxic microsites offered in marinas can also alter community structure. *Bugula* larvae delay settlement when they cannot find a suitable habitat. Many marine invertebrates, including *Bugula*, have non-feeding larvae, and therefore limited resources to sustain their time in the plankton (Jaeckle 1994). As such, delaying settlement depletes resources that could otherwise be used for growth and development after settlement. This can have at least two negative consequences: 1. Delayed settlement means larvae have less energy for the earliest post-metamorphic stages of development, and 2. The ability of larvae to interpret cues is less efficient with increasing larval age (as seen in Chapter 2). Here, I have addressed whether larvae can settle in low oxygen environments and whether low oxygen conditions discourage larval settlement directly through changes in larval behavior, and indirectly through cues about habitat quality through biofilm characteristics. However, is still not known whether oxygen availability can influence survival and performance after settlement. This question should be tested.

Physiological tolerance to low oxygen conditions ($C_{50\%VO_2}$) and metabolic rate change across body shapes (flat versus erect) and species status (invasive versus native). The differences in tolerance thresholds and metabolic rates found between flat and erect shaped organism were found

to be associated with their life histories. Flat species exhibited the lowest oxygen requirements ($C_{50\%VO_2}$), which may be due to adaptation associated with horizontal growth, close to hypoxic layers that are in contact with the surfaces (Vogel 1994). Low metabolic rates found in this group are often found to be correlated with slow growth and reduced levels of reproduction. However, these characteristics of a slow pace of life may provide flat species with a competitive advantage over erect-shaped organisms, particularly during the early life stages when they share the same spatial niche. For erect shaped species, having a lower resistance to hypoxia is partly compensated for by a high metabolic rate. The high levels of oxygen consumption may provide the option to allocate energy towards faster growth and reproduction. A fast pace of life may allow for rapid growth away from the limits of the boundary layer and environments with high competition by flat species. It would be interesting for future studies to explore whether there are differences in the physiological tolerances and metabolic rates among solitary and colonial species. The body structure of colonial species may increase synchronization among zooids, which is likely to serve an evolutionary advantage if increases in filtration performance enhance respiratory performance.

Invasive species were found to resist lower oxygen levels. Artificial structures such as pier and pylons provide low oxygen environments that serve to benefit invasive species relative to native fauna. In environments with oxygen limitation, species that can tolerate low levels of oxygen can competitively displace species with higher oxygen demands. Species with the ability to colonize these low oxygen environments are likely to deplete oxygen to a level at which species with higher oxygen requirements cannot occupy (Ferguson et al. 2013). It can therefore be concluded that native species suffer the negative consequences of low oxygen conditions offered by the artificial structures and the habitat modifications produced by invasive organisms. As demonstrated in Chapter Four, invasive species of small size have higher metabolic rates than natives of the same size; these r-selected species with high metabolic rates and fast growth and reproduction are the first colonizing environments that suffered strong perturbations or when free spaces are introduced to the habitat. Such fugitive species occupy the available resource and modify their environment in a way that allows for the introduction of a second wave of invasions (Sakai et al. 2001). Habitat modifications and the introduction of invasive species can present high levels of disturbance, not only for sessile native organisms, but to the species that interact with them also invasions (Sakai et al. 2001, Ghermandi et al. 2004, Neill and Arim 2011). Many pelagic and benthic species rely on sessile communities as a source of food and refuge. Factors that affect the abundance of architect species are therefore highly relevant for community dynamics. Personal observations indicate that environments with higher amount of hypoxic microsites are less divers and have a high prevalence of invasive species, comparing with less perturbed sites. A natural extension of this work would be

test if the perturbations generated by low flow and high amount of hypoxic microsites covariates with changes of local diversity.

Human activity is affecting marine environments. Artificial structures such as marinas can deplete oxygen levels beyond the limits of which native species can tolerate. Larval behaviour is strongly affected by oxygen levels; factors that reduce oxygen in the plankton reduce their ability to colonize an environment, while adult stages are shown to be physiologically affected. Invasive species have a competitive advantage in low-oxygen artificial environments, which consequently affects community structure dynamics. These findings provide valuable insight into the effects of oxygen depletion on individual recruitment into populations and as forecast species assemblage in marine environments, which will enable further research in the field of community ecology and invasion biology. Lastly, it is anticipated that this work will help to inform the management strategies of costal habitats around the world. The knowledge generated in this thesis can be used for the planification of new artificial structures in coastal areas. I suggest that natural flows should not be diminished beyond the strictly necessary and avoid complete disruption of natural flows. Also it should be considered to incorporate airstream systems, specially in structures, such as marinas were organic matter from the boats is introduced directly in to the water. Physicochemical properties of the water change with temperature, therefore is necessary test the changes of oxygen in different aquatic environments around the world to prevent the spread of invasive organisms and to protect native biodiversity.

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