Resource acquisition and allocation in a colonial marine invertebrate

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Master of Science

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Abstract

Modularity is widespread across taxa, and include higher plants, fungi, and colonial marine invertebrates. Compared to unitary organisms, the life histories of modular organisms present complications, as it can occur at both organismal, and at modular levels. For all modular groups, our understanding of how life history strategies have formed and developed with the use of modular traits is unclear. Out of all modular groups, however, modularity is best studied in plants. In plants, modules act as a way to optimise the resource economy of the whole organism, through resource recycling involving partial senescence. Depending on resource availabilities, plants experience different strategies regarding module longevity and growth. Though modular species have almost identical life histories, the theory developed for plants has not been conveyed to other modular groups, like colonial marine invertebrates. Through the four data chapters of my thesis, I aim to explore the life history consequences of modularity in a colonial marine invertebrate, and how traits at various stages of the life history differ with variation in resource availability. When examining my study species, I include theory and comparative studies from the literature on modular plants.

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.

Karin Svanfeldt, 24 Marsh 2017
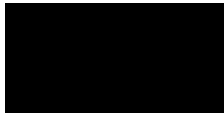
Publications during enrolment

Peer-reviewed publications and unpublished data chapters included in thesis

Svanfeldt, K., Monro, K., & Marshall, D. J. (2016). Dispersal duration mediates selection on offspring size. *Oikos*. DOI: 10.1111/oik.03604

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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 and two unpublished chapters. The ideas, development and writing up of all the chapters in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of Professor Dustin J Marshall and Dr. Keyne Monro. (The inclusion of co-authors reflects the fact that the work came from active collaborations between researchers, and acknowledges input into team-based research.) In the case of chapter 2, & 3, my contribution to the work involved the following:

Thesis Chapter	Publication title	Status	Nature and % of student contribution	Co-author names Nature and % of contribution
Chapter 2	Dispersal duration mediates selection on offspring size	Accepted	85%	Keyne Monro (5%), Dustin Marshall (10%)
Chapter 3	Field manipulations of resources mediate the transition from intraspecific competition to facilitation	Accepted	85%	Keyne Monro (5%), Dustin Marshall (10%)

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

Student signature: ███ **Date: 14/03/2017**

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: **Date: 24/0//2017**

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Conference titles – oral presentations

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Chapter one

Introduction

Life history variation

Life history theory holds that an individual's total lifetime fitness is the net product of trade-offs between many traits (Van Noordwijk and de Jong 1986; Stearns 1989). The possible combinations of trade-offs lead to a vast number of life history strategies, and these vary both within and across populations, species, and individuals (Stearns 1992; Tuttle 2003; Blanck and Lamouroux 2007). Selection for certain life history combinations often depends on environmental conditions, such as temperature or resource availability, such that strategies that are favorable in one environment are unfavorable in another environment (Richard et al. 2000; Le Lann et al. 2011; Guillaume et al. 2015). Whereas life history strategies of unitary organisms are highly diverse, they follow a linear sequence of events: growth, reproduction, and death. In comparison, the life histories of modular, or colonial, organisms, like higher plants and sessile colonial invertebrates, are more complicated.

Life histories of modular species

Modular organisms comprise of architectural systems of repeated, multicellular modules (Harper and White 1974, Harper 1977, White 1979, 1984, Monro and Poore, 2004). Compared to cells, tissues or organs of unitary organisms, the modules of modular organisms are semi-autonomous, as they are connected and share signals and resources but still have the ability to survive, reproduce and die more-or-less independently, and are not reliant on each

other for vital functioning (Tuomi and Vuorisalo 1989)(Tuomi et al 1983, Watson and Casper 1984, Gill 1986). The technical term for a module with this semi-independence is 'ramet', where all ramets with the same genetic identity are collectively termed a 'genet'. The semi-autonomous nature of ramets facilitates indeterminate growth of modular organisms, where fragmentation or physical damage in some part of the organism is not detrimental for the genet as a whole (Lirman 2000). Numerous characteristics unite modular organisms, such as the potential for indefinite growth (Winston 2010), the occurrence of both sexual and asexual reproduction, a sessile adult life phase with a dispersing offspring phase (Orive 2001), and resource recycling through partial senescence (Hamilton et al. 1987; Thomas 1994).

Module longevity and energy recycling in higher plants

Of all organismal groups where modularity occurs, plants are the best studied. In plants, both theory and empiricism show that modular plant traits, including module longevity, act to optimise the whole-plant resource economy (Kikuzawa and Ackerly 1999; Wright et al. 2004). This is best seen in the turnover of leaves. Though leaves themselves are not full modules in an autonomous sense, they acquire energy to support their own function, but also the function of the entire organism through energy relocation. With time, older leaves accumulate damage, are shaded and become less effective. In order for ineffective leaves not to become a liability to the genet, they senesce and their resources are resorbed and redistributed through the plant to younger, more effective modules (Thomas and Stoddart 1980; Smart 1994).

In environments with varying resource availability or competition, the optimal timing of leaf senescence will vary (Monk 1966). For example, it has been empirically shown that in high nutrient conditions, plants reduce leaf longevity as a response to an increase in whole-plant growth rate (Grime and Hodgson 1987). If nutrient availability is high, plants will compete

for the resource of sunlight. In these conditions, fast growth will be favoured. In these instances, fast growth prevents costs of 'self-shading' (when younger leaves limit sunlight for older leaves) and increases the plant's canopy area, allowing better ability to out-shade its neighbours. In environments with low resource availability, plants show various strategies that affect leaf longevity. Most commonly, leaf longevity increases as a response of nutrient retention in the plant (see Aerts 1999).

Predicting leaf longevity

Many general models predict the longevity of leaves (Hikosaka 2005). Most models are based on optimal net nutrient (nitrate) gain for the entire plant, and consist of three simplified parameters, the maximum daily photosynthesis capacity of a leaf, the time until leaf production is 0 (the age at which it stops photosynthesising), and the construction cost of a leaf (Kikuzawa 1991b). The model predicts that when time until leaf production is 0 and leaf construction cost increases, leaf longevity will increase. It also predicts that when the daily photosynthesis capacity of a leaf increases, the leaf longevity will decrease. This model has been empirically validated for a wide range of plant species, and is generally accepted across higher plant groups (Ackerly and Bazzaz 1995; Ackerly 1999; Kikuzawa and Ackerly 1999; Wright et al. 2004). However, there are no formal theories predicting module longevity in other modular organisms, like colonial animals.

Clonal animals (such as corals, bryozoans, sponges and colonial ascidians) share similar life histories to those of plants, where semi-independent ramets form colonies or genets (Hughes 2005). Rather than leaves, the modules of colonial animals are various forms of zooids that perform a range of functions within the genet. Like plants, many colonial animals also experience zooid senescence, which does not always affect genet mortality. Though

senescence in some colonial animals is a fixed intrinsic trait (Rinkevich et al. 1992), other

species show plasticity in zooid longevity (Harvell and Grosberg 1988). For example, zooids

of the marine bryozoan *Electra pilosa* have shorter longevity in high resource environments

(Bayer et al. 1994), and zooids of the marine bryozoan *Watersipora subtorquata* show

different turnover rates at varying depths (Lange et al. 2016). However, both manipulative

studies and formal theories on the role of modularity in shaping the resource economy in

colonial animals are exceedingly rare.

Thesis Aim

Through the four data chapters of my thesis, I explore the effects of resource acquisition and

allocation through the life history of a colonial marine invertebrate. The goal of my research

was to improve our understanding of how selection acts on life history traits of colonial

organisms and how variation in resources mediates the strength and direction of this

selection.

Study species – Watersipora subtorquata

The encrusting colonial marine bryozoan, *Watersipora subtorquata* is a common member of

the sessile shallow water marine community found in Port Phillip Bay, and also throughout

most of the Australian coast and temperate shallow waters world-wide (Marshall and Keough

2009). New *Watersipora* colonies are initialised by sexual reproduction, where free

swimming lecithotrophic larvae are released into the water column, until they find a suitable

settlement surface. On experimental substrata, settlement is facilitated by the presence of

biofilms, which are chemically detected by the larvae that will hover over potential settlement

areas before committing to settle. The larvae take several hours to settle (Marshall and

Keough 2004). After settlement, the larvae experience metamorphosis, forming the first

feeding module of the colony, the ancestrular zooid. Zooids feed using filtering siphons, the

lophophores. Since the colonies are sessile, they are dependent on the local food particle

concentration and flow rate for feeding success. Asexual reproduction initiates via budding,

with circular bands of feeding and sexually reproducing zooids growing outwards from the

ancestrula. Growth occurs mainly in zooids on the outer margin of the colony, where each

zooid produces one to three buds at a single occasion throughout its lifetime. Feeding, and

later sexual reproduction, then occurs in circular bands closer towards the colony centre.

Resources accumulated from feeding zooids are shared throughout the colony via porous cell

walls (Banta 1969). With time, the centre of the colony, starting from the ancestrula, will

begin to senesce and form a grey inner circle of dead zooids. As for all modular organisms,

Watersipora experiences life history both at the level of the colony (genet) and at the level of

each individual zooid. As the colony grows, the circle of senesced zooids will expand (Hart

and Keough 2009; Marshall and Monro 2013). Within the life history of the colony, each

zooid will go through its own life history, where each zooid will be formed, and go through

both asexual and sexual reproduction. When a zooid starts to decline, the majority of energy

of the zooid will be reabsorbed by the colony and the zooid will effectively senesce and die.

Eventually, the entire colony will mature and sexual reproduction will initiate. With time, the

whole colony will also senesce and die.

Chapter 2, Resource investment in offspring and cost of dispersal

The sessile adult life phase of *Watersipora,* and other modular organisms, makes the larvae

phase a crucial stage for dispersal and habitat selection (Massot et al. 2002; Stamps et al.

2005; Bonte et al. 2012). For marine invertebrates in particular, there are high costs of

dispersal (Woollacott et al. 1989; Marshall et al. 2003b; Burgess 2011) and calamitous

consequences for accepting a low quality colonisation site. Whereas dispersal for most plants

is often a passive process, many marine invertebrate larvae have active dispersal phases that

often burn limited resources. Whereas the larvae of some marine invertebrates are able to feed throughout the dispersal phase, many colonial marine invertebrates, like *Watersipora*, have a nonfeeding larvae phase, where the dispersing juveniles are completely reliant on maternal investment in offspring energy reserves. The general assumption in offspring size theory is that bigger offspring have more energy, and therefore perform better than smaller offspring (Smith and Fretwell 1974; McGinley et al. 1987). For mothers, however, there is a trade-off between size and number of offspring produced (Smith and Fretwell 1974; Lloyd 1987). The general predictions are that for mothers in poor environments, it is beneficial to produce many small offspring (to increase the chance of success), whereas in good environments, it is beneficial to produce fewer, large, offspring. In nature, environmental conditions are likely to alter the strength and direction the selection for offspring size. In Chapter 2, I estimated the strength and direction of selection on offspring size for *Watersipora* larvae in varying conditions in the field. In one treatment, larvae were allowed to settle immediately after they were released. In the other treatment, larvae were forced to experience an extended dispersal period, diminishing their energy reserves, before they were allowed to settle.

Chapter 3, Resource availability and conspecific density

Though the offspring phase allows dispersal for modular organisms, there are still consequences of the sessile adult phase. Because of immobility once settled, the colony is unable to move away from undesirable neighbours, and the life histories of modular organisms are therefore highly dependent on the dynamics of their immediate neighbours, where neighbour density affects individual performance (Watkinson 1980; Damuth 1981; Lawton 1989; Gaston and Blackburn 2008; Silvertown and Charlesworth 2009). The interactions between neighbours can have both positive and negative influences on organismal fitness (Dickie et al. 2005; Leslie 2005a; García-Cervigón et al. 2013). If

neighbours are reliant on the same resources, like nutrients or sunlight for plants, or space,

food or oxygen for colonial animals, neighbours are likely to compete for these resources and

in doing so, reduce each other's fitness (Antonovics and Levin 1980; Wilson Jr 1983; Webb

and Peart 1999). Still, there are numerous studies when positive neighbour interactions have

been observed (Dickie et al. 2005; Leslie 2005a; García-Cervigón et al. 2013). A formal

theory predicting the direction of density dependent interactions is the stress gradient

hypothesis (SGH). The SGH predicts that if environmental conditions are good, sessile

neighbours will compete for resources, and hence show negative density dependence. In

harsh environments, SGH predicts that neighbours will facilitate the stress for one another,

and therefore result positive density dependence (Bertness and Callaway 1994). In reviewing

the SGH, Maestre et al. (2009) states that SGH may not hold if the stressor is the same as the

limiting resource. In Chapter 3, I hypothesised that by adding resources to a sessile modular

system (*Watersipora*), competition would decrease and facilitation would dominate the

density dependent interactions. I tested this hypothesis by conducting a field experiment

where I manipulated the resource availability over a natural gradient of conspecific neighbour

densities.

Chapter 4, Selection for module longevity in varying resource conditions

Another key to optimise the life strategies of modular organisms, is that whereas life history

occurs for the whole organism (genet), each module also experience its own life history,

separated from, but at the same time within the whole organism (Harvell and Grosberg 1988).

This allows for adaptive strategies using resource recycling through partial senescence

(Hamilton et al. 1987; Thomas 1994), where selection can act on traits both at organismal and

at modular levels to maximise organismal fitness (Monro & Poore 2004). As previously

mentioned, how module longevity alters resource economics for the whole organism is well

studied in plants (Kikuzawa and Ackerly 1999; Wright et al. 2004), however, studies

investigating selection on module longevity in colonial a or other modular species are rare.

Therefore, in Chapter 4, I performed a selection analysis on *Watersipora*, where I estimated

whether selection acted on zooid longevity and other zooid traits, and whether it varied with

varying resource availabilities.

Chapter 5, Predicting zooid longevity in varying resource conditions

The general theory of how resource availabilities affect selection on module longevity in

plants is modelled as a function of maximal nutrient (nitrogen) gain (Kikuzawa 1991b). The

model predicts that the optimal leaf longevity increases with time until the photosynthetic

rate of a leaf is 0, and the construction cost of a leaf. The model also predicts that zooid

longevity decreases when the maximum daily photosynthetic rate of a leaf increases. In

Chapter 5, I translated the parameters of the optimal leaf longevity model to parameters

suitable for the zooids of sessile marine invertebrates, in order to determine if the model

could be applied to other modular organisms. In laboratory trials, I measured zooid traits

separately under varying resource conditions, in order to apply the plant model to predict

zooid longevity. By comparing my predictions to my field observations (from Chapter 4), I

estimated the applicability of the leaf longevity formula on zooids of sessile marine

invertebrates.

Chapter two

Dispersal duration mediates selection on offspring size

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Abstract

Offspring size varies at all levels of organisation, among species, mothers and clutches. This variation is thought to be the result of a trade-off between offspring quality and quantity, where larger offspring perform better but are more costly to produce. Local environmental conditions alter the benefits of increased offspring size and thereby mediate selection on this trait. For sessile organisms, dispersal is a crucial part of the offspring phase, and in animals, bigger offspring tend to better endure longer dispersal distances than smaller offspring because they have more energy. Theory predicts that increasing distances between suitable habitats strengthens selection for larger offspring. We manipulated the dispersal duration of offspring of different sizes in the bryozoan *Watersipora subtorquata* and then examined the relationship between offspring size and post-metamorphic performance in the field. We found that selection on offspring size is altered by larval experience. Larger offspring had higher post-settlement performance if the larval period was short but, contrary to current theory, performed worse when the larval period was extended. The reversal of the relationship between offspring size and performance by extending the larval phase in *Watersipora* may be due to the way in which offspring size affects growth in this species. Regardless of the mechanism, it appears that experiences in one life-history stage alter selection on offspring size in another stage, even when they occupy identical habitats as adults.

Introduction

Offspring size is a highly variable trait that has pervasive consequences across the life history (Bagenal 1969; Stearns 1992; Thorson 1950). Variation in offspring size occurs at all levels of organisation: among species, but also within species, mothers and clutches (Marshall and Keough 2008; Williams 1994). The size of individual propagules is often linked to fitness - generally, larger offspring perform better than smaller offspring (McGinley et al. 1987; Smith and Fretwell 1974). However, it can be more costly to produce larger offspring, therefore mothers must trade off the size and number of offspring that they produce, from few larger offspring to many smaller offspring (Marshall and Uller 2007; Smith and Fretwell 1974; Vance 1973b).

While many studies support the assumption that larger offspring perform better, an increasing number of studies have shown that the relationship between offspring size and performance is highly context-dependent. Environmental variation has the potential to alter both the strength and direction of the offspring size-fitness relationship (Bashey 2006; Benton et al. 2005; Kaplan 1992; Marshall et al. 2006; Sams et al. 2015). If large, costly offspring and small, less-costly offspring have similar levels of fitness in a given environment, selection should favour mothers producing many small offspring. Conversely, if smaller offspring perform poorly relative to larger offspring in a given environment, mothers benefit from producing fewer, larger offspring (Geritz 1995; Geritz et al. 1999). A generally-held hypothesis is therefore that the offspring size-fitness relationship is determined by local environmental conditions (Allen et al. 2008; Fox 1997). Even slight changes in these conditions could alter the offspring size-fitness relationship (Bashey 2006; Gagliano et al. 2007; Marshall and Keough 2008), and studies have shown that mothers can alter offspring size in response to changes in the environment (Allen et al. 2008; Bertram and Strathmann 1998; Fox 1997; Marshall et al. 2000). While the role of environmental variation in altering the offspring size-performance function is well recognised,

the potential for other factors to alter this key relationship remains unexplored. Most

importantly, the role of dispersal in altering the offspring size-performance relationship is

largely unresolved.

For sessile species, a crucial function of the offspring phase is dispersal (Bonte et al. 2012;

Massot et al. 2002; Stamps et al. 2005). Dispersal for most plant species is a passive process

where offspring are dispersed by the wind. In general, smaller seeds have greater dispersal

potential, due to increased aerodynamics (Howe and Smallwood 1982; Levin and Muller-

Landau 2000). Furthermore, producing smaller seeds presumably allows mothers to produce

more seeds, and through sheer weight of numbers, disperse further and to more sites (Geritz

1995). In contrast to terrestrial plants, dispersal for most sessile marine invertebrates can be

particularly costly (Burgess 2011; Marshall et al. 2003b; Woollacott et al. 1989). For species

that have a nonfeeding larval phase, as is the case for more than 50% of coastal marine

invertebrates (Marshall et al. 2012), the larval phase incurs energetic costs as well as an

elevated risk of mortality. Nonfeeding larvae are dependent on maternally invested energy

reserves until they complete metamorphosis, and across a wide range of phyla, nonfeeding

individuals that experience an extended larval period have poorer performance after

metamorphosis, likely caused by depletions in their energy stores (Bennett and Marshall 2005;

Elkin and Marshall 2007; Marshall and Keough 2003; Pechenik 2006; Pechenik et al. 1998).

Generally, larger larvae disperse for longer and are thought to be better able to cope with

extensions of the larval period (Marshall and Steinberg 2015, Burgess et al., Marshall and

Keough 2003, Marshall and Dias 2010, Pettersen et al. 2015). Thus, it is clear that offspring

size can affect dispersal in marine invertebrates (Marshall and Keough 2003). What is less clear

is whether dispersal can affect selection on offspring size.

 We have good reasons to suspect that dispersal will alter selection on offspring size. For

 instance, if larger offspring are better able to disperse, then greater distances between

suitable habitats should favour the production of larger offspring (Burgess et al. 2013).

Furthermore, recent physiological studies suggest that larger offspring are better able to withstand the energetic stress of a prolonged dispersal phase because they have lower mass-specific energy requirements than smaller offspring (Pettersen et al. 2015). Though scarce, studies of offspring size, dispersal and performance suggest that dispersal can alter selection on offspring size. In a relatively recent study, Burgess et al (2013) predicted that mothers producing many small offspring should be favoured if the distances between suitable habitats were short, but increasing habitat spacing causes changes in selection to favour larger offspring. In the same study, this theory was empirically tested and supporting evidence was found on the bryozoan *Bugula neritina*, where the post settlement survival of smaller offspring decreased compared to bigger offspring if the dispersal period was extended. Whereas Burgess et al (2013) conclude that offspring size provisioning impact the potential for dispersal, they also acknowledge that for a more general understanding of the offspring size and habitat spacing relationship, further investigations in additional species are needed. Here, we experimentally prolonged the length of the dispersal phase of offspring of known size in the encrusting bryozoan, *Watersipora subtorquata*, and then monitored the performance of these individuals in the field, from settling to late life adult stages. We monitored performance as post-metamorphic survival, area and senescence of each individual for three months in the field.

Methods

Study species

Watersipora subtorquata is an encrusting, bright red bryozoan found in shallow waters of temperate and subtropical regions, and is an invasive, but now common member of the sessile marine communities of the south coast of Australia (Marshall and Keough 2009). Like all bryozoans, *Watersipora* forms colonies by asexual budding. Dispersal and new settlement is

initialized by sexual reproduction where nonfeeding, free-swimming larvae are released into the water column. After dispersal, permanent attachment to suitable surfaces usually takes place within a few hours after release provided suitable settlement surfaces are available, but the larval period can extend for as long as 15 hours (Marshall and Keough 2003; Marshall and Keough 2004). During dispersal, there are two typical larvae behaviours: passive surface exploring and active swimming (Burgess et al. 2009). Whilst actively swimming, it is likely that the energy expenditure of larvae is excessively higher than in the more passive behaviour, exploring, as shown empirically for other marine invertebrate larvae (Bennett and Marshall 2005). Once settled, the individual metamorphoses (an energetically expensive process; Pettersen et al. 2015), and the first zooid, the ancestrula, begins to feed. Colony growth occurs via asexual budding of daughter zooids. The initial budding rate is offspring size-dependent, where bigger settlers faster produce more buds (Marshall and Keough 2004). With time, older zooids at the centre of the colony lose their red hue, senesce and die, while growth and reproduction continue in younger zooids at the colony margin (Hart and Keough 2009; Marshall and Monro 2013).

Species collection and spawning

The study was conducted from autumn to spring 2013 at Royal Brighton Yacht Club, Port Phillip Bay, Australia (37°54'29.9"S 144°58'52.4"E).We collected *Watersipora* colonies from St Kilda Pier (37°51'50.3"S 144°57'56.7"E), which is situated within Port Phillip Bay, and is located about 4.5 km from our field site at the Royal Brighton Yacht Club. We brought the colonies to the laboratory within insulated aquaria, and kept them in constant darkness for two days before exposing them to light to induce them to spawn brooded larvae (Marshall and Keough 2004). We collected and pooled spawned larvae from all collected colonies, and either allowed the larvae to settle immediately following release (undelayed treatment), or delayed their settlement while keeping the larvae actively swimming for 2 hours (delayed treatment) representing a moderate dispersal stress. For the delayed treatment, larvae were placed in

sample jars with filtered seawater under constant light exposure and placed on a mechanical

roller, which gently rolled the jars and forced larvae to continue swimming. Throughout the

spawning period (~30min), we took care to allocate larvae to both treatments continuously. The

delay method resulted in ~20% mortality. After the appropriate larval duration (0 and 2 hours

for undelayed and delayed treatments, respectively), we settled the larvae in seawater droplets

on pre-roughened PVC plates (10x10cm). All larvae were allowed approximately three hours in

darkness to settle within seawater drops before unsettled larvae were discarded. Whereas

settlement success for delayed larvae was nearly 90%, non-delayed larvae settled with a success

of 60%. This pattern matches expectations regarding the 'desperate larvae' hypothesis for non-

feeding larvae- younger larvae tend to reject low quality settlement sites while older larvae

settle more indiscriminately (see Marshall and Keough 2003). Post-settlement, our experimental

setup consisted of in total 90 undelayed settlers and 86 settlers that had experienced an extended

larval phase. Offspring size was estimated from measurements of the first zooids (ancestrulae),

where the average of offspring size in this study measured $0.22mm^2$ (SD=0.06). In comparison,

Watersipora offspring in a study by Marshall and Keoug (2003) measured on average $0.17mm^2$.

Deployment and monitoring

We deployed the experiments by haphazardly allocating the PVC plates to PVC backing panels

and submerging panels at ~1.5m depth at our field site, Royal Brighton Yacht Club. In total, the

experiment consisted of 4 panels, each holding 16 plates, with one *Watersipora* settler per plate.

Though the final numbers of each treatment varied slightly across each run (due to the varying

pre-settlement survival and settlement success), we took care to allocate settlers from both

treatments as evenly as possible on each backing panel. We photographed all plates weekly in

the field, and from the photos, survival, colony area and area of senescence were measured in

ImageJ software (available at http:// imagej.net). Survival was measured as presence or absence

of vivid (red) zooids, colony area, and senescence as proportional area of grey zooids that had

lost their species characteristic red colour (Marshall and Monro 2013). To keep our field

conditions realistic, we let sessile communities from natural colonisation form on the plates

around the *Watersipora* colonies during the course of the experiment. Nevertheless, we could

track our focal colonies, initially from pen circles around them and later on from their positions

on the plates. We replicated the experiment three times, which we refer to here as runs. The

difference in initiation time between each run was one week, and in total, 176 individuals with

known size and known larval experience were deployed, whereof 91 experienced no delay in

metamorphosis and 85 experienced a delay of 2 hours before metamorphosis could initiate.

Data analysis

Throughout the three replicate runs of our experiment, we appeared to have a higher

representation of larger larvae in the delayed treatment compared to the undelayed treatment.

There are both biological and statistical implications of having a different covariate range

represented. From a statistical perspective, it may be inappropriate to compare slopes across the

entire covariate range when one treatment is not represented across that range. We believe that

the most conservative approach in our case is to truncate the data so that the ranges overlap. We

did so excluding all offspring smaller than 0.136 mm^2, and re-ran all of our analysis. Truncating

the data did not affect the directions or significance of our results, and therefore we present the

un-manipulated data set including all offspring sizes. All our analyses were run in SAS® 9.4

software and individual settlers were the units of replication.

To examine the effect of delayed settlement on survival, we used repeated logistic binary

regression. Offspring size, delay-treatment and time were modelled as fixed effects and panel

was modelled as random. When running our model through repeated measures, all of our time

points in the same data set would not converge. Instead, we ran the data from early stage

colonies and late stage colonies separately. Therefore, the model was repeated through time for

early stage colonies (week 2, 3, 4 and 5 in the field) and late stage colonies (week 10, 11, 12

and 13 in the field). To reduce the full model to the simplest model that best fit our data, we

performed log-likelihood ratio tests. However, we had difficulties in fitting time and the

interactions with time which led to abnormalities in our reduction of singled-out main effects.

To include all main effects, we therefore instead present the F-test of our analysis on survival.

To examine the effect of offspring size and delay treatment on colony area, we used repeated

linear mixed-effect model (fitted using ML). For the mixed model, offspring size, delay-

treatment and time were modelled as fixed effects (continuous, categorical and categorical

respectively). Panel was modelled as random effect. As with our survival analysis, our model

would not converge when fitted through all time points. Instead, early (week 2, 3, 4 and 5) and

late (week 10, 11, 12 and 13) stage colonies were analysed separately. As the random effect was

simply an experimental convenience, we reduced the models according to Quinn and Keough

(2002), using likelihood ratio tests and then tested each parameter separately in the finalised

model by reducing them one by one and then adding them back to the model

The effects of offspring size, delay treatment and time on percentage of colony senescence

were analysed on late stage colonies (week 10, 11, 12 and 13 in the field) using linear mixed

model (fitted through ML). Offspring size, delay treatment and time were modelled as fixed

effects and panel was modelled as random. Once again, the random effect was simply

experimental conveniences and the model was reduced according to Quinn and Keough

(2002) using likelihood ratio tests. The effects of each parameter in the final model was then

tested separately by reducing them on by one and then adding them back to the model.

Results

As mentioned in our methods section, we observed a difference in settlement success between

our two delay treatments, where delayed larvae settled at a higher rate (about 90%) than

undelayed larvae (about 60%). Our delay treatment also resulted in some mortality (about

20%). Whereas it is very unlikely that sizes between the larvae assigned to each treatment

would have varied (since they were collected simultaneously from the same cohorts), we did have offspring size differences between our treatments after settlement, with a higher representation of smaller larvae in our non-delayed treatment.

We found that in the treatment where larvae were not delayed, survival in the early stage weeks (week 2 to week 5 in the field) was higher for larger than for smaller larvae. In the treatment where metamorphosis was delayed, there was no apparent relationship between offspring size and early stage colony survival (Table 1. Fig. 1). However, in the delayed treatment, there was also a lack in representation of smaller offspring, possibly due to the loss of larvae prior to settling. For later stage colonies (week 10-13 in the field), we found no effects of delay treatment, offspring size or time on survival (Table 2).

Colony area in our early stage colonies (week 2-5 in the field) increased with offspring size if metamorphosis had not been delayed. If metamorphosis had been delayed, there was initially, at week 2 and 3, no difference in colony area depending on offspring size. At week 4 and 5 for colonies from delayed offspring, colony area decreased with offspring size (Table 3. Fig. 2). For early stage colonies, we also found a significant blocking effect by panel. At late stages of colony development (week 10, 11, 12 and 13 in the field), the effect of offspring size and delay treatment on colony area had faded and we found no significant effect of these effects at this stage. Throughout the late life development though, colonies that were delayed as offspring were larger as adults than colonies that were not delayed as offspring (Table 4. Fig. 3).

Neither offspring size nor delay treatment had any effect on colony senescence at our late stage colonies (week 10 to 13 in the field). What we found was merely that the percentage of senesced zooids within colonies increased slightly over time and had a significant blocking effect by panel (Table 5).

Discussion

While it has long been recognised that selection on offspring size is context-dependent (Bashey 2006; Benton et al. 2005; Kaplan 1992; Marshall et al. 2006; Sams et al. 2015), few studies have shown that events in one life-history stage alter selection in another stage (Burgess et al. 2013; Crean et al. 2011). We show that the length of the dispersal period prior to metamorphosis alters selection on offspring size, even if individuals experience identical conditions as adults. Our results suggest that not only does the habitat in which individuals find themselves affect selection on offspring size, but also the experience of offspring prior to habitat selection. This serves to be an emerging pattern in marine systems, where there is a link between the length and nature of the dispersal period, and subsequent dynamics of the adult phase (Marshall and Morgan 2011; Shima and Swearer 2010).

We found that the relationship between offspring size and fitness depends on offspring experience. Whereas survival and colony area for early stage adults increased with offspring size if the dispersal phase was short, colonies that had experienced a longer dispersal phase showed no relationship between offspring size and survival. More surprisingly, we found that for colonies that had experienced a longer dispersal phase, their size early in adult stage actually decreased with increased offspring size. Also, at later life stages, colonies that experienced delayed metamorphosis as offspring had larger areas than colonies that had experienced no delay. It is worth nothing, though, that our dispersal duration of two hours only shows the impacts of mild dispersal stress in *Watersipora*, and that both shorter and longer dispersal durations may have affected the offspring differently.

Burgess et al (2013) found that when the dispersal period of a bryozoan with a similar life history, *Bugula neritina,* is extended, there is selection for larger offspring size. Burgess's findings have intuitive appeal for three reasons: dispersal is costly for nonfeeding offspring

(Bennett and Marshall 2005; Strathmann 1985), larger offspring are released with more energy, and larger offspring use proportionally less energy during dispersal than smaller offspring (Pettersen et al. 2015). These three criteria also apply to our study species, *Watersipora*, but we found the reverse pattern to Burgess: colonies that were larger as offspring perform initially worse when they experience an extended dispersal period.

We were also slightly surprised that neither offspring size nor delay treatment had any effect on colony senescence. In other senescing species, such as plants, senescence acts as an energetic budgeting system, where the organism by reabsorption have the ability to redistribute energy and nutrients from ineffective modules in order to enhance performance in vivid and more useful units (Aerts 1996; Eckstein et al. 1999; Vitousek 1982).We suspected that if the initial energy and/or energy expenditure would affect the adult colony area, it would also show as differences in colony senescence as faster growing colonies, according to studies on plants (Ryser 1996), would have higher turnover rates and thus more senescence. Little is still known about why and how marine invertebrates senesce. That we found no evidence for a link between experience in the larval phase and senescence, even though larval experience affected colony area, may not necessarily mean that senescence is acting very differently in marine invertebrates than in plants. After all, our results showed effects on early stage adult area of colonies, but these effects seemed to have faded over time and disappeared in later stage colonies. Since senescence does not occur until later life stages, potential effects of our treatments that could have affected senescence may therefore no longer be noticeable for our *Watersipora* colonies. More interesting though, were the effects of offspring size and dispersal duration on colony survival and area- that a prolonged dispersal phase should favour smaller offspring sizes. There could be a few reasons for our unexpected findings, and we will discuss them each separately.

First, in our two delay treatments, we had differences both in settlement success and in the distribution of offspring sizes. Since the larvae assigned to each treatment were pooled cohorts

from the same mothers, it is very unlikely that the offspring size differed by chance alone.

Rather, we suspect that we differentially 'lost' smaller larvae in the delay treatment both during

the treatment and at settlement. Previous studies show that smaller *Watersipora* larvae tend to

settle indiscriminately sooner than larger larvae (Marshall and Keough 2003). In addition,

larger larvae in our treatment that were not delayed may have rejected the settlement substrate,

since larger *Watersipora* larvae tend to remain choosier longer before settlement (Marshall and

Keough 2003). Regardless, whether the size difference between our two treatments depended

on selection for large delayed larvae or small non-delayed larvae, similar processes will operate

under field conditions. From a biological perspective, our results also imply that the smaller

larvae that did persist through the delay process are a non-random subset- perhaps of higher

quality in dimensions other than offspring size. In contrast, the larger larvae did not appear to

go through this selection for other quality axis. That stressful conditions during the dispersal

phase can lead to selection for higher performing offspring has also been shown in previous

studies (Hamilton et al. 2008), and may explain some of the counter-intuitive findings in our

delay treatment where smaller offspring appeared to better cope with stress, and also that

colonies that were delayed as offspring performed better later in life than colonies that had not

been delayed.

 The poor growth of larger offspring in our delay treatment may also be the result of early

growth patterns of *Watersipora.* Previous studies show that the initial growth strategy of

Watersipora depends on offspring size (Marshall and Keough 2004). Following metamorphosis

into the first feeding zooid (the ancestrula), *Watersipora* grows by asexual budding of new

daughter zooids. These daughter zooids take days to begin feeding, and as such, they are

initially dependent on resources supplied by the ancestrula. Ancestrulae originating from larger

offspring tend to bud sooner after metamorphosis and produce more buds than ancestrulae from

smaller offspring (Marshall and Keough 2004). This has also been observed in other marine

invertebrate species (Marshall et al. 2003a). Thus, young colonies that come from larger

offspring have a lower feeding-zooid: developing-zooid ratio and therefore higher energy demands than young colonies from smaller larvae. Initial differences in budding rate of different sized offspring can have persistent effects on colony size throughout the life-history (Marshall and Keough 2008), which potentially explains the faster growth of *Watersipora* colonies from larger offspring relative to smaller offspring. Presumably, ancestrulae from larger offspring can sustain a higher initial budding rate because they have more resources than smaller offspring. However, if the larval period is extended and larval resources are depleted, then the low ratio of feeding zooids versus developing zooids that is typical of colonies from larger offspring may not be sustainable, such that performance is greatly reduced. Studies show that larval swimming does not change the relationship between offspring size and budding rate; larger offspring invariably produce ancestrulae with higher budding rates than smaller offspring (Marshall and Keough 2004; Marshall and Keough 2006). In other words, larger offspring create early colonies that depend heavily on initial resources to supply a larger number of developing zooids. However, if larval resources are depleted by extending the larval period (i.e. swimming time), then a higher budding rate may become unsustainable, resulting in lower colony performance overall.

Another explanation for why extending the dispersal period changes the relationship between offspring size and post-metamorphic performance is that offspring size also affects larval energy budgets in *Watersipora*. Larger larvae tend to engage in active swimming more than smaller larvae, whereas smaller larvae spend 500% more time exploring settlement surfaces than swimming in the water column (Burgess et al. 2009). Furthermore, as larval duration increases, larger larvae tend to maintain active swimming for longer than smaller larvae (Burgess et al. 2009). Given that *Watersipora* larvae are negatively buoyant and must actively avoid sinking, larger larvae that engage in active swimming for longer than smaller larvae are likely to consume more energy. Unfortunately, metabolic rates in *Watersipora* larvae have only been estimated for very young larvae (Pettersen et al. 2015). We currently have no estimates

for how delayed settlements may alter the metabolic rate of larvae, but this is an important next step to completely understand the connection between the pre- and post-metamorphic life stages for *Watersipora*.

Regardless of the mechanism driving the change in relationship between offspring size and performance, the fact that dispersal duration alters the offspring size-performance relationship has implications for selection on offspring size in this system. As noted earlier, selection on offspring size is driven by the relative benefits of producing larger offspring that perform better, or producing smaller, more numerous offspring (Smith and Fretwell 1974; Vance 1973a). If different offspring sizes perform equally well, selection favours mothers that produce smaller offspring, since they then can produce more of them (Geritz 1995; Geritz et al. 1999). We found that post-settlement, larger offspring perform initially worse than smaller offspring when the dispersal period is extended. Therefore, this presents a double cost in producing larger offspring if they must disperse for extended periods, since they are more expensive to make and have lower post-metamorphic fitness. Increasing distances between suitable habitat patches, which requires increased dispersal from the natal environment, should therefore select for the production of smaller offspring in *Watersipora*. However, we also found that smaller offspring have higher early life mortality, and that very small offspring sizes may not last during dispersal. With these findings, we have shown that the length of the dispersal period prior to metamorphosis alters the phenotypic selection on offspring size, even if individuals experience the same environment as adults. While it has long been recognised that selection on offspring size is context-dependent, it is rarely shown that events in one life-history stage alter selection in another stage of the life-history.

Dispersal for sessile species is ubiquitous but costly (Baguette and Van Dyck 2007; Bélichon et al. 1996; Bonte et al. 2012). That dispersal processes impose strong selection on life histories is clear – though it is less clear whether dispersal per se is the target of selection or the product of

selection on other traits. In this study, we show that dispersal has manifold consequences for selection on the key trait of offspring size. Factors that extend or reduce the dispersal phase will alter selection on offspring size with cascading and pervasive consequences for fitness throughout the life history. Our findings contradict previous studies that showed extending the dispersal period creates stronger selection for larger offspring – instead we find ambivalent effects. If we are to make broader generalisations about how dispersal mediates selection on offspring size, then further tests of how dispersal affects the relationship between offspring size and fitness are necessary.

Tables

Table 1. Effects of fixed effects and interactions in the final model (from full model reduction of best fit) on colony survival of early stage colones from week 2 to week 5 in the field, showing a significant effect of the interaction of offspring size and delay treatment on colony survival at this life history stage.

Colony survival, test of fixed effects, early stage colonies

Effect	df	Chi	F	Pr > ChiSq	Pr > F
offspring size	1	1.9	1.9	0.17	0.17
delay	1	2.6	2.6	0.10	0.10
time	3	15.6	5.2	**0.00**	**0.00**
offspring size*delay	1	4.3	4.3	**0.04**	**0.04**

Table 2. Effects of fixed effects and interactions in the final model (from full model

reduction of best fit) on colony survival of late stage colones from week 10 to week 13 in the

field, showing no significant effects on colony survival at this life history stage.

Colony survival, test of fixed effects, late stage colonies

Effect	df	Chi	F	Pr > ChiSq	Pr > F
offspring size	1	3.4	3.4	0.07	0.07
delay	1	0.3	0.3	0.62	0.62
time	3	6.9	2.3	0.07	0.07
offspring size*delay	1	0.4	0.4	0.53	0.53

Table 3. Effects of fixed and random effects and their interactions in the final model (from full model reduction of best fit) on colony area of early stage colonies from week 2 to week 5 in the field, showing a significant effect of panel and the interaction of offspring size and delay treatment on colony survival at this life history stage.

Colony area early weeks			
Term tested	Chi	df	p
panel	6.4	1	**0.01**
offspring size*delay	8.3	1	**0.00**
delay*time	3.9	3	0.27
offspring size	18.8	1	**0.00**
delay	4.8	1	**0.03**
time	138	3	**0.00**

Table 4. Effects of fixed effects and interactions on colony area of late stage colones from week 10 to week 13 in the field, showing a significant effect of the interaction of delay treatment and time on colony area at this life history stage.

Colony area late weeks

Term tested	Chi	df	p
delay*time	7.8	3	**0.05**
offspring size	0.3	1	0.58
delay	7.6	1	**0.01**
time	90	1	**0.00**

Table 5. Effects of fixed effects and interactions on colony senescence of early stage colones from week 2 to week 5 in the field, showing a significant effect panel and time on colony senescence percentage at this life history stage.

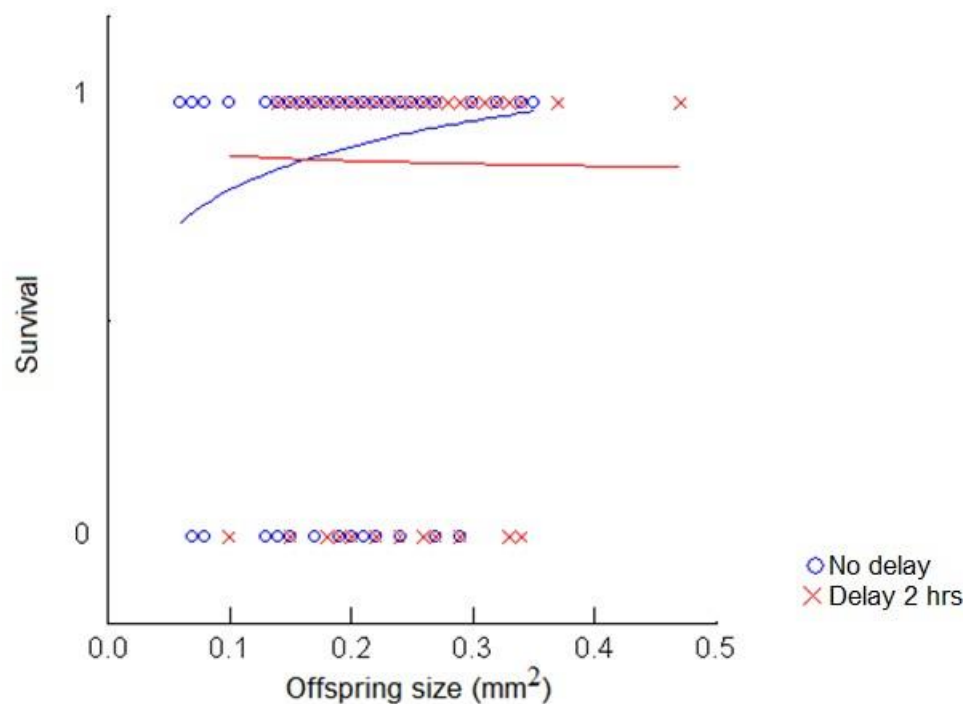| Colony senescence %, late weeks				
Term removed	-2LL	Chi	df	p
panel	2183.0	23.0	3	**0.00**
offspring size*delay*time	2161.9	2.0	3	0.57
offspring size*delay	2162.2	0.3	1	0.58
offspring size*time	2164.9	2.7	1	0.10
delay*time	2166.2	1.3	3	0.73
offspring size	2167.1	0.9	1	0.34
delay	2167.1	0.0	1	1.00
time	2176.0	8.9	3	**0.03**

Figures

Figure 1. Effects of offspring size and delay treatment on survival for early stage colonies, week 2 to 5 in the field, where 1 represents colonies alive and 0 represents dead colonies. Each dot represents a single colony, where non-delayed colonies are shown in blue and delayed colonies are shown in red. Whereas colony survival for early stage colonies increases with offspring size uin the non delayed treatment, there is no apparent difference in survivala of early stage colonies in the delayed treadment.
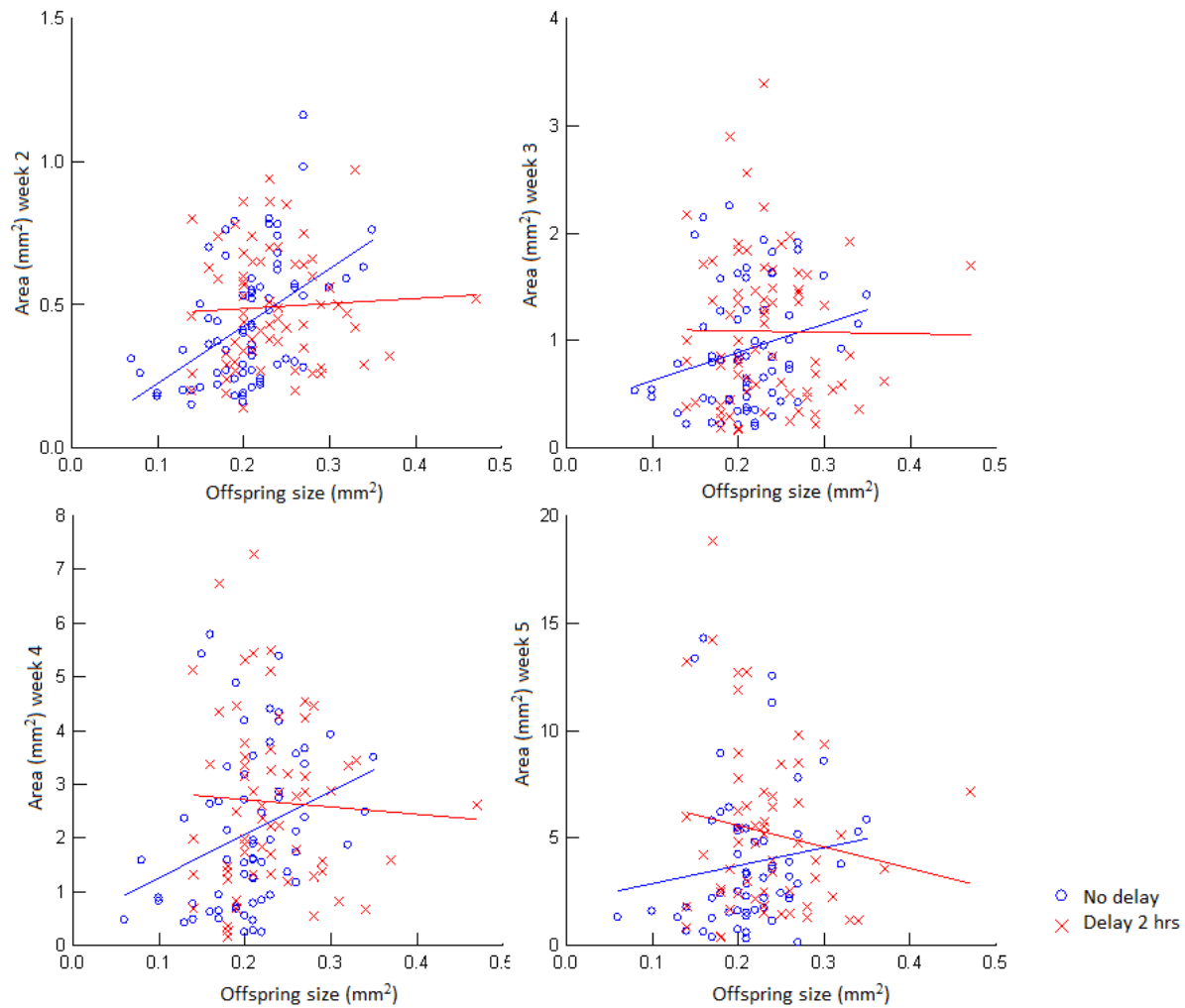
Figure 2. Effects of offspring size and delay treatment on area for early stage colonies at week 2, 3, 4 and 5 in the field. Each dot represents a single colony, where non-delayed colonies are shown in blue and delayed colonies are shown in red. Wheras colony area increased with offspring size at all weeks in the non-delayed treatment, colony area at week 2 and week 3 showed no difference due to initial offspring size in the delay treatment. At week 4 nd week 5 in the delay treatment, colony area decreased with offspring size.
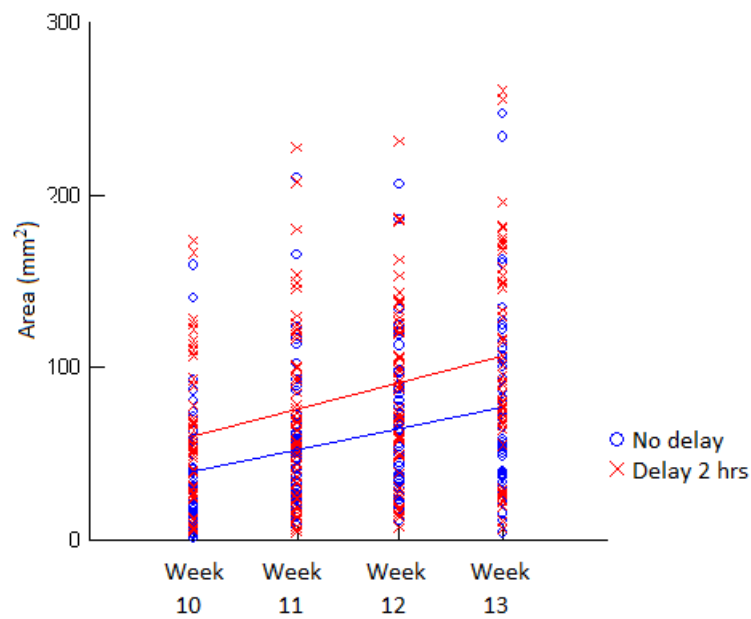
Figure 3. Effects of delay treatment over time on area for late stage colonies at week 10, 11, 12 and 13 in the field. Each dot represents a single colony, where non-delayed colonies are shown in blue and delayed colonies are shown in red. For all weeks, colony area was greater for colonies in the delay treatment than colonies in the non-delay treatment.

Chapter three

Field manipulations of resources mediate the transition from intraspecific competition to facilitation

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Abstract

1. Population density affects individual performance, though its effects are often mixed. For sessile species, increases in population density typically reduce performance. Still, cases of positive density dependence do occur in sessile systems and demand explanation. The stress gradient hypothesis (SGH) predicts that under stressful conditions, positive effects of facilitation may outweigh the negative effects of competition.

2. While some elements of the SGH are well studied, its potential to explain intraspecific facilitation has received little attention. Further, there have been questions regarding whether the SGH holds if the stressor is a resource. Most studies of interactions between the environment and intraspecific facilitation have relied on natural environmental gradients; manipulative studies are much rarer.

3. To test the effects of intraspecific density and resources, we manipulated resource availability over natural population densities for the marine bryozoan *Watersipora subtorquata*.

4. We found negative effects of density on colony performance in low resource environments, but mainly positive density-dependence in high resource environments. By adding resources, competition effects were reduced and the positive effects of facilitation were revealed.

5. Our results suggest that resource availability mediates the relative strength of competition and facilitation in our system. We also suggest that intraspecific facilitation is more common than may be appreciated and that environmental variation may mediate the balance between negative and positive density-dependence.

Introduction

A fundamental tenet of ecology is that population density affects individual performance (Damuth 1981; Gaston and Blackburn 2008; Lawton 1989; Silvertown and Charlesworth 2009; Watkinson 1980). For sessile species, density-dependent effects are generally expected to be negative, with conspecific neighbours reducing each other's fitness as they compete for the same resource(s) (Antonovics and Levin 1980; Webb and Peart 1999; Wilson Jr 1983). The negative effects of competition are thought to dominate density-dependent interactions across most taxa in sessile communities, but an increasing number of studies also find positive effects of increasing density (Dickie et al. 2005; García-Cervigón et al. 2013; Leslie 2005a). Positive density-dependence can arise from facilitation, whereby interactions between individuals benefit one or both of the contributors and are harmful to neither (Bruno et al. 2003; Callaway 2007; Stachowicz 2001).

While the underlying mechanisms may be diverse, increasingly it seems that many systems experience some influence of both positive and negative density-dependence. For example, in birds, the local density of conspecific neighbours can either increase or decrease offspring survival, via increased chick protection from neighbouring parental birds, or chick mortality due to attacks by conspecific neighbours, respectively. If predation pressure is high, the benefits of high conspecific density outweighs the risks, and hence alters the density relationship between density dependence and fitness (Ashbrook *et al*. 2010). Similarly in tropical tree communities, an increased density of interspecific species can increase the benefits of conspecifics, by decreasing the spread of species-specific herbivores and pests (Halton 2003). In many systems, however, the relative roles of facilitation and competition have traditionally been thought to be mediated by environmental stress.

The idea that environmental stress alters the balance between facilitation and competition has a long history in ecology, and continues to generate debate. An influential idea in this debate is

the stress gradient hypothesis, SGH (Bertness and Callaway 1994), first developed for plants.

The SGH predicts that the nature of interactions between organisms depends on environmental

stress and consumer pressure (for reviews see Brooker et al. 2008; Callaway 2007), and most

discussion and tests of the SGH have focused on interspecific interactions. In benign

environments, the SGH maintains that the dominant force is competition for resources (Connell

and Slatyer 1977) but in high stress environments, the benefits of facilitation from habitat

amelioration or resource enrichment outweigh the negative effects of competition (Holzapfel

and Mahall 1999; Maestre et al. 2003).

While the SGH was initially formulated for interspecific interactions (Bertness and Callaway

1994; Callaway and Walker 1997), its predictions are equally relevant to intraspecific

interactions among plants (García-Cervigón et al. 2013; Holzapfel and Mahall 1999; Silvertown

and Charlesworth 2009; Soliveres et al. 2010; Sthultz et al. 2007; Watkinson 1980) and sessile

invertebrates (Bertness 1989; Leslie 2005b). For example, 'nurse' plants can facilitate the

establishment of conspecific seedlings during stressful periods (Dickie et al. 2005; Kitzberger et

al. 2000), and barnacle populations can experience either positive- or negative density-

dependence across the intertidal zone depending on stress levels (Leslie 2005a).

While most SGH studies have focused on non-resource related stressors such as temperature or

soil structure (Callaway and Walker 1997; Miriti 2006; Wang et al. 2008), Maestre et al. (2009)

refined the SGH to include variation in interactions when the stressor is a resource such as

water, oxygen or food. Their predictions regarding resource-related stressors suggest that for

facilitation to occur, neighbours must directly increase the availability of the resource.

Okamura (1988) showed that for the encrusting bryozoan *Electra pilosa*, feeding success was

increased in high interspecific densities in environments with high flow. If the flow was low,

however, interspecific competition decreased the feeding success. The results from Okamura's

study stand out from other studies exploring facilitation in the way that they explore resource

availability (flow rate) rather than non-resource related stress and density-dependence (Fajardo

and McIntire 2011; Goldenheim et al. 2008; Leslie 2005a; McIntire and Fajardo 2011).

Important gaps in our understanding of how the environment alters the strength and direction of density-dependence remain. First, few field studies have directly manipulated environmental conditions: rather, natural environmental gradients have been used to explore how the environment affects intraspecific density-dependence (but see Hart and Marshall 2012). Second, it is unclear whether the SGH applies to intraspecific interactions when the stressor is resource-based (Maestre et al. 2009). Here, in a field experiment, we manipulated resource availability and density for the marine encrusting bryozoan, *Watersipora subtorquata*, (henceforth referred to as *Watersipora*). Previous studies on this species have shown that neighbouring conspecifics compete for waterborne resources and typically display negative density-dependence (Hart and Marshall 2012). The general assumption for sessile marine species such as *Watersipora*, is that neighbouring individuals compete almost solely for space (Bertness 1989; Connell 1961; Smally 1984). However, recent studies show that even when space is not limiting, sessile individuals compete via non-contact competition for both waterborne food and oxygen (Ferguson et al. 2013; Kim and Lasker 1997; Okamura 1988; Svensson and Marshall 2015; Thompson et al. 2015; Wildish and Kristmanson 2005). Thus, resource availability for sessile marine species is strongly determined by local flow regimes (Leichter and Witman 1997; Lesser et al. 1994).

In this study, rather than indirectly testing resource effects on density-dependence along a natural gradient, we orthogonally manipulated two key resources (food and flow) directly. Specifically, we added food to the direct surroundings of colonies growing in various densities using a newly developed experimental technique (Svensson and Marshall 2015), and we obstructed the flow in the direct surroundings of the colonies by baffling the water around our experimental populations, and tested how these manipulations affected density-dependent performance in the field. We used two levels of each resource manipulation (rather than a

gradient) because we sought to explore extreme differences in resource availability as a necessary first step to examining functional responses in density dependence. In contrast to the classic predictions of the SGH, where high stress environments amplifies the role of facilitation, we hypothesised that with resource availability as stressor, reducing stress (by adding resources) would increase the visible effects of facilitation by reducing the effects of competition. We predicted that when resources were scarce, the interactions between neighbouring individuals would be dominated by competition, and density dependence would be negative. If resources were abundant, however, we expected the effects of competition to diminish. If competition is ameliorated by resource addition, then we expected one of three possibilities: i) reduced negative density dependence such that increasing conspecific density reduced performance less strongly in high resource conditions; ii) the removal of density dependence effects under high resource conditions; or iii) a positive relationship between density and performance under higher resource conditions because facilitative effects were unmasked by the addition of resources.

Methods

Study species and site

Our trials were carried out during summer at Royal Brighton Yacht Club, Port Phillip Bay, Australia (37°54'29.9"S 144°58'52.4"E). Water flow within the marina is around 1cm/sec, and supports a diverse benthic marine sessile community. We chose *Watersipora* as our study organism because it is an easily-accessible species with a relatively well-known and trackable life history. *Watersipora* is a bright red, colonial filter feeder commonly found year-round on hard substrates in the marina. *Watersipora* is a cosmopolitan invader and is considered invasive in most sheltered subtidal areas around temperate regions of the world, including the southern coast of Australia (Hewitt et al. 2004). New *Watersipora* colonies are formed by sexual reproduction and the subsequent release of free-swimming larvae into the water column. Once settled, each larva undergoes metamorphosis and forms the first feeding unit of the colony, the

ancestrula zooid. Circular bands of feeding zooids (each capable of sexual reproduction upon maturity) then bud outwards from the ancestrula to form the colony. The colony has a shared resource economy, whereby energy from food consumption is distributed through porous cell walls between zooids. New growth occurs at the colony margin. With time, the zooids in the centre of the colony, starting from the ancestrula, lose colour and irreversibly senesce. Zooid senescence is visible as the appearance of a grey inner circle of dead zooids that expands as the colony grows (Hart and Keough 2009; Marshall and Monro 2013). It is debatable if new growth zones can form inwards once senesced zooids are lost due to fragmentation (however this was not observed in our study). In our system, despite being a common member of early successional assemblages, *Watersipora* is a subordinate competitor for space, often overgrown by other encrusting species such as colonial ascidians and sponges. Nevertheless *Watersipora* persists (albeit at much smaller sizes) in quite late-stage communities.

Manipulating resource availability

Watersipora is sessile and filters resources such as food (plankton) and oxygen from the surrounding water. The availability of food and other resources therefore depends on a number of factors: the direct density of resources in the water, the flow rate (more resources per time unit pass feeding structures in higher than lower flow rates), the density of competing neighbours, and the effect of self-shading (the blocking effect of resource capture to zooids at the centre of the colony by zooids growing on the margins) related to colony size. Therefore, we manipulated food availability and flow rate in the field for experimental populations of *Watersipora* colonies at different densities and monitored the growth and senescence of a single, focal colony within each experimental population. We had two levels for each factor: additional food or no additional food, and un-obstructed or obstructed flow. We then crossed the two resource levels over a range of naturally occurring conspecific densities.

To test the effects of food and flow alterations on density in the field, we varied all three factors

in an orthogonal design. PVC backing panels, each holding eight 10x10cm PVC settling plates with pre-roughened acetate sheets, were horizontally submerged using ropes and wire with the settling plates facing down at 1.5m depth along the protected side of a wave-breaking floating attenuator in the field. To manipulate food, we used a technique developed by Svensson and Marshall (2015). Slow-releasing food blocks (30ml) were created by mixing Reef Phytoplankton (Seachem, Madison, Georgia, USA) and NutraPlus Reef Feed (Nutra-Kol, Mullaloo, Australia) with Gyprock dental plaster (CSR, North Ryde BC, Australia) on a ratio of 7:7:6 respectively. Manipulating food in this manner increases the growth of some sessile marine invertebrates and can reduce the intensity of intraspecific competition (Svensson and Marshall 2015). We made controls by replacing the commercial feed preparations with water (as per Svensson and Marshall 2015). Both food blocks and control blocks were replaced fortnightly. On average, we released $4.6 \times 10^5 \pm 4.9 \times 10^4$ additional food particles (plankton cells) per food block to the direct close environment of our colonies. To manipulate flow, 10x10x5cm open-ended PVC boxes surrounded the settlement plates. With the openings facing downwards, the boxes efficiently obstructed the directional flow rate without further affecting access to the surrounding water. Controls for the flow manipulation were open plates, where natural conditions of the site exposed the plates to slow (1cm/sec) and mainly directional flow from the in- and out-lets of currents through the constructions of the marina. These constructions, in combination with the depth at which we hung the experimental panels, minimized exposure to wave turbulence for plates in both flow treatments. What we created were extremes in environmental conditions that overlap with those experienced naturally by *Waterspora*. In Port Phillip Bay, the natural flow rate at our field site varies from 0.1 - 2.5 cm/sec (Lagos *et al.* 2016), and food availability (habitat quality) varies strongly with depth (Lange, Monro & Marshall, 2016).

Field set-up

We created four different treatments: high food - high flow, high food - low flow, low food -

high flow, and low food - low flow, with two replicates of each treatment on each panel (8

plates per panel, spread across 20 panels). This resulted in 40 replicates of plates per treatment.

Cages attached to the plates held either slow-releasing food blocks (high food) or control plaster

blocks (low food). Within each food treatment, half of the plates received the low flow

treatment and half received the high flow treatment. In total, we deployed two runs with ten

panels per run (20 panels in total with 8 plates per panel), and with one week's time difference

between each run. Before deployment, we used natural variation in the settlement densities of

Watersipora settlers by leaving empty settlement plates in the field for one week. A variety of

juvenile invertebrates settled on each plate, so all but *Watersipora* settlers were scraped off.

This resulted in a range of densities from one to nine settlers per plate. The plates were

thereafter haphazardly allocated to our treatments, and thus our initial densities varied

independently of our treatments. By creating our experimental populations before we assigned

them to treatments, we effectively controlled for confounding effects of potential differences in

initial size or settling density across our treatments – on average all conspecific densities and

initial sizes were equal among treatments. Once the experimental populations were established

and allocated to their environmental treatments, all new settlers (*Watersipora* or otherwise)

were removed weekly. In each experimental population, one *Watersipora* was haphazardly

designated as the focal colony and the rest were designated non-focal colonies. We took

photographs of the focal colony weekly for 13 weeks, and size was estimated using ImageJ

software (available at http://imagej.net). For each focal colony we made weekly estimates of

colony size (colony area) and the percentage of the colony that had undergone senescence as

per established methods for this species (see Marshall & Monro 2013 for details). Additionally,

we aimed to estimate fecundity by monitoring the production of ovicells; however our colonies

did not reach sexual maturity during the experimental period. We also monitored the survival of

the non-focal colonies to determine whether survival of non-focal colonies differed across

treatments over time.

To estimate the effects of food availability, flow rate, intraspecific density and time on absolute colony area and senescence, we used repeated linear mixed-effect models (fitted using ML) from week 1 to week 11 for our estimates of colony area, and from week 5 to week 11 for our estimates of senesced area percentage (excluding week 3 and week 10 for both colony area and the senescence data sets where measurements were missing). For the parameter 'density', we used estimates of colony numbers per plate at week 0 (before the plates were assigned to the treatments). Given that densities at this stage were randomly assigned to our treatments, we believe this is the most appropriate predictor to use in the analysis. However, to account for mortality amongst colonies on the same plate over time, we performed a mixed model F-test to estimate the potential effects of initial density, food, flow, time and their interactions on the survival of non-focal colonies over time (we found no effects, see results). The number of non-focal colonies over time was only estimated at weeks 2, 6, 8 and 11 and therefore, our repeated measure for this test is limited to those time points. A previous study on *Watersipora*, showed that analysing relative growth rates and the absolute size of colonies yield qualitatively similar results. Since we had no initial size differences in our settlers among treatments, and because absolute size is a better measure of fitness, we therefore used absolute size as performance measure for colony area, which we present in our results section. Importantly, we also analysed relative growth and found qualitatively similar results (unpublished analyses). In all of our models, food, flow, density and time were modelled as fixed effects (categorical, categorical, continuous and continuous, respectively) and panel was modelled as random effect. Since run only had two levels, it was excluded from the analysis (any differences among runs, however, were accounted for by panel effects). To be able to analyse the effects on our response variables over time, we standardised the variation of our predictors (survival, senescence and colony area) for each week to 1 (mean=0, std=1). As the random effects were simply experimental conveniences, we reduced the models according to Quinn and Keough (2002) to test for random slopes, using standard likelihood-ratio tests based on chi-square distributions. We performed

model reductions in a hierarchical fashion, where higher order interactions were removed prior to lower order interactions. In this way, all interactions were evaluated before the main effects were tested. The response variables were mean colony size and senescence per plate, as these were the units of replication and the scale at which the treatments were applied. All analyses were run in SAS 9.4 software, Inc.

Results

We found no effects of initial density, food, flow or density on non-focal survival over time (see supplimentary materials, Table 3). However, we did see an effect of time (week) on non-focal survival; mortality increased with time. In addition, we found that the effects on both colony size and senscence in all environments varied by panel (spatial arrangement, Fig. 1), but these spatial effects did not interact with our treatments of interest.

We found an interaction between food, flow and density on colony area of *Watersipora* in the field, and this interaction did not vary over time (*Density*Food*Flow:* χ^2= 3.8, p= 0.05, Fig. 1, for complete final model, see supplementary materials: table 1). Though the threeway interaction of food, flow and density did not vary over time, food and flow interacted with time, as did density and food, and density and flow (*Time*Food*Flow*: χ^2 = 6.0, p = 0.01, *Time*Density*Food:* χ^2 = 7.9, p = <0.001, *Time*Density*Flow*: χ^2 = 5.6, p = 0.02). For colonies in all environments, colony area increased with time. Exploring the three-way interaction of food, flow and density, we found that increased conspecific density reduced focal colony size in the low food, low flow environment, but the relationship between focal colony size and conspecific density tended to be positive in higher resource environments. In the environment where food availability was low but flow rate was high, colony area was unaffected by the neighbour density, though the range of densities was limited to four colonies per plate, rather than eight as was the maximal number of colonies per plate for all other treatments (due to haphazardly distributed plates with natural settlement to the treatments). In both environments where food availability was high, focal colony size was positively related to conspecific

density, regardless of flow regime.

For percentage of colony senescence from week 5 to week 11 in the field, we found an interaction of density and food, and this effect did vary with time (*Time*Density*Food*: $\chi^2 =$ 4.8, p = 0.03, Fig. 2, for final model, see supplimentary materials: Table 2). When food availability was low, the percentage of colony senescence did not vary with density. When the food availability was high, however, the senescence percentage of our colonies decreased with intraspecific density. For all colonies, the percentage of colony senescence increased with time.

Discussion

Overall, we found evidence that both facilitation and competition operate simultaneously in our system, and that resource availability alters the balance between the two. When resources were abundant, facilitation dominated, but when resources were scarce, competition dominated. In the environment where both food availability and flow rate were low, performance declined with density. In the environment where food was low but flow rate was high, density had no effect on performance. In both environments with added food however, performance increased with density, indicating that facilitation exceeded the effects of competition. It also appeared that colonies grew more in low flow compared to high flow environments. In addition, in environments with no competitors (density 1), colonies were smaller when food availability was high than when the availability of food was low, which may indicate specialisation towards low resource environments for this species. Whereas colony senescence did not change across densities in the low food environment, the percentage of colony senescence for colonies in the high food environment decreased with density. When no competitors were present, at density 1, the percentage of colony senescence was also marginally higher in the high food environment than in the low food environment. The lower growth and higher senescence rate in low densities under high food environments are puzzling. Our expectations were quite the reverse - namely,

that higher food availabilities would increase performance. One possible explanation may be a higher allocation for sexual reproduction, at the expense of colony size, for colonies in the high food environments. However, no colonies in our trials had reached sexual maturity in the space of 13 weeks when the trials concluded. Another explanation may be higher investment in calcification for defence at the expense of size.

The effects of resource availability on colony area and senescence with density varied through time, but there were some consistent signals. With the addition of resources, previously undetected facilitation effects were revealed. When stress is non-resource related, the SGH predicts that high stress environments promote facilitation between neighbours, whereas benign environments promote competition (Bertness and Callaway 1994). Maestre *et al.* (2009) introduced the exception of resource-related stress, where the interactions between neighbours may not follow the predictions of SGH. Our results suggest that altering resources in a population can mediate the competition-facilitation balance by suppressing the competition between neighbouring individuals, and thereby reveal facilitative interactions. In such scenarios, competition between neighbours dominates low resource environments (e.g. high resource-related stress) while in high resource environments (e.g. low resource-related stress), neighbours will no longer be affected by competition. If there are no benefits of neighbours, the interaction in a high resource environment would then be neutral. If there are benefits of neighbours, however, these positive interactions would dominate in high resource environments. Our results indicate the latter scenario, demonstrating a beneficial interaction between *Watersipora* individuals that becomes visible in the absence of competition.

However, the interactions in our study only describes those between *Watersipora* colonies in artificial monoculture communities. These conditions, where only *Watersipora* settlers are present in isolation from other species, may apply in the field in early stage communities if *Watersipora* settlers are the primary recruits before other species have had the opportunity to

colonise the habitat, and also before contact competition (space limitations) becomes

prominent. In other field scenarios, however, the presence of additional species is likely to alter

both the direction and magnitude of the interactions we observed. Svensson and Marshall

(2015) found that when they added the same food as used in our trials to a mixed species

community, most species benefitted and grew significantly larger than in their control

treatment. However, in the same study, the growth of *Watersipora* decreased with the addition

of food, likely because of the increased interspecies competition pressure.

The mechanism behind facilitation is unclear. Our results indicated that *Watersipora* generally

performs better in low-flow environments. It is possible that the slightly disruptive structures of

neighbouring conspecific colonies on the smooth settlement substrate decreased local flows and

enhanced performance (Sebens 1991), possibly by increasing filtration efficiency (Okamura

1985; Vogel 1996). In line with our results, other studies have shown that bryozoans facilitate

filtration by reducing the proportion of water that is refiltered (Pratt 2004), while faster flow

rates reduce feeding efficiency for some species (depending on colony size, Okamura 1985).

Encrusting species may burn more energy on feeding attempts in higher flow rates, while actual

feeding success and thus growth rate and performance may be greater in low flow rates,

somewhat explaining the preference for low flow in our species (Okamura 1988). It is also

possible that *Watersipora* colonies create a preferable microhabitat that enhances the conditions

for conspecific neighbours. These positive density dependent effects may play a crucial role for

the invasion success of *Watersipora* (Taylor & Hastings 2004).

Regardless of the reason behind the facilitation that we have observed, the results of our

study show that the abundance of resources alters the balanced relationship between

competition and facilitation at a population level. With this study, we have shown that

the balance between competition and facilitation at a population level can be shaped not

only by stressors, such as temperature or salinity, but also by resource availability as

discussed by Maestre et al. (2009). By directly manipulating resource availability, we

demonstrate its role in determining the balance of competition and facilitation in our

system. Our results add weight to earlier studies that found the role of intraspecific

facilitation to vary across environmental gradients (Fajardo and McIntire 2011;

Goldenheim et al. 2008; Leslie 2005a; McIntire and Fajardo 2011). With our results, we

present further evidence for the importance of acknowledging the impacts of both

competition and facilitation and how the balance between the two affects interactions

within and between populations.

Tables

Table 1. Final model and interactions removed from the model for the effects of time, density, food and flow on colony area for *Watersipora* colonies from week 1 to week 11 in the field. All significant effects are shown in bold.

	Colony area				
Final Model	Chi	p	Interactions removed	Chi	p
Panel	22.6	**0.00**	Time*Density*Food*Flow	1.6	0.21
Time*Density*Food	7.9	**0.00**			
Time*Density*Flow	5.6	**0.02**			
Time*Food*Flow	6	**0.01**			
Density*Food*Flow	3.8	**0.05**			
Time*Density	22.1	**0.00**			
Time*Food	10.4	**0.00**			
Time*Flow	22.0	**0.00**			
Density*Food	52.3	**0.00**			
Density*Flow	3.2	0.07			
Food*Flow	10.5	**0.00**			
Time	4175.7	**0.05**			
Density	4.0	**0.00**			
Food	21.7	**0.00**			
Flow	39.8	**0.00**			

Table 2. Final model and interactions removed from the model for the effects of time, density, food and flow on colony senescence percentage for *Watersipora* colonies from week 1 to week 11 in the field. All significant effects are shown in bold.

	Colony senescence %				
Final Model	Chi	p	Interactions removed	Chi	p
Panel	22.0	**0.00**	Time*Density*Food*Flow	3.3	0.07
Time*Density*Food	4.8	**0.03**	Density*Food*Flow	0.1	0.75
Time*Density*Flow	6.4	**0.01**			
Time*Food*Flow	5.7	**0.02**			
Time*Density	2.1	0.15			
Time*Food	4.3	**0.04**			
Time*Flow	0.4	0.53			
Density*Food	5.0	**0.03**			
Density*Flow	0.4	0.53			
Food*Flow	0.6	0.44			
Time	1611.5	0.44			
Density	0.6	1.00			
Food	0.0	0.37			
Flow	0.8	**0.00**			

Table 3. F-test for the parameters time, density, food and flow on the survival percentage of non-focal colonies per plate over time from week 1 to week 11 for *Watersipora* colonies in the field. All significant effects are presented in bold.

| Type 3 Tests of Fixed Effects, survival % of non-focal colonies per plate					
Effect	Numerator	Denominator	Chi	F	p
Density*Food*Flow*Time	3	559	0.53	0.18	0.91
Density*Food*Time	3	559	0.20	0.07	0.98
Density*Flow*Time	3	559	0.31	0.10	0.96
Food*Flow*Time	3	559	0.85	0.28	0.84
Density*Food*Flow	1	559	0.33	0.33	0.56
Density*Time	3	559	0.32	0.11	0.96
Food*Time	3	559	0.27	0.09	0.97
Flow*Time	3	559	0.16	0.05	0.98
Density*Food	1	559	0.51	0.51	0.48
Density*Flow	1	559	1.26	1.26	0.26
Food*Flow	1	559	0.64	0.64	0.43
Time	3	559	31.18	10.39	**<.0001**
Density	1	559	1.25	1.25	0.26
Food	1	559	2.07	2.07	0.15
Flow	1	559	1.16	1.16	0.28

Figures
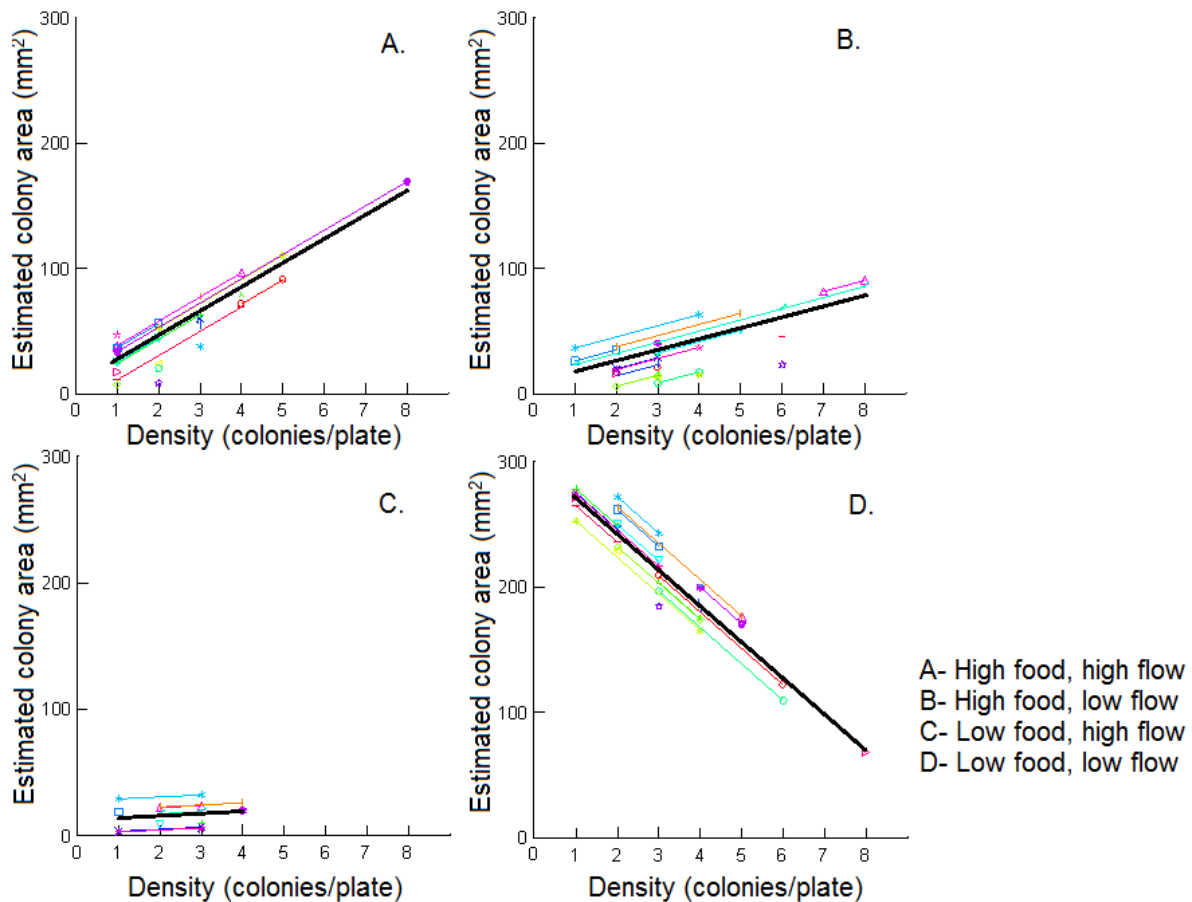
 Figure 1. Model estimates of the relationship between absolute colony area, food availability, flow rate and initial intraspecific density for *Watersipora* colonies at the latest stage of the experimental trial, at week 11 in the field, with variation due to un-correlated effects of the experimental panels the *Watersipora* colonies were deployed on. The estimates are presented for each of the four environments of food and flow combinations: high food (A and B), low food (C and D), high flow (A and C) and low flow (B and D). Lines of best fit for each environment is shown in black and bold, and coloured surrounding lines shows model variations due to the random effect of panel.
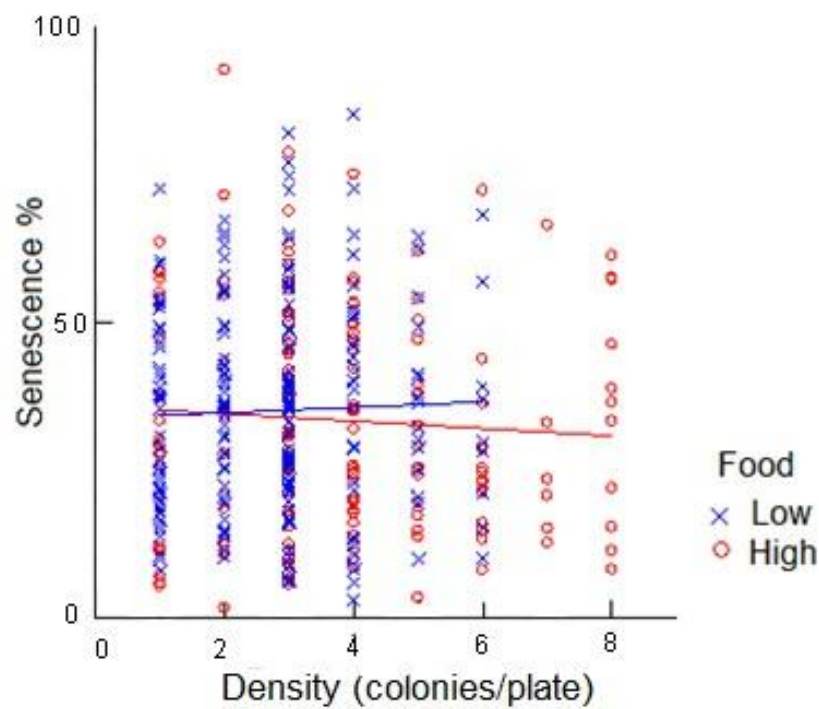
Figure 2. Relationship between colony senescence percentage, food availability (high or

low), and intraspecific density for *Watersipora* colonies from week 5 to week 11 in the field.

Colonies that experienced low food conditions are shown in blue and colonies that

experienced high food conditions are shown in red. Note that each point represents an

individual colony and lines represent the line of best fit for that treatment combination.

Chapter four

Environment mediates selection on module longevity in the field

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Key words: modularity, zooid lifetime, competition, partial mortality, resource availability, senescence, ageing, phenotypic selection, selection analysis

Abstract

The life histories of modular organisms are complicated, where selection and optimisation can occur at both organismal and modular levels. At a modular level, growth, reproduction, and death can occur in one part of the organism, independently of other parts. In plants, selection on module longevity and the components of modules (i.e., flowers and leaves) varies with environmental conditions, particularly resource regimes. Though the life histories modular animals are unmistakably similar to those of plants, there are no formal investigations of selection on module longevity in modular animals. We used two field experiments to test if selection acts on zooid (module) longevity, and how selection varies across successional gradients and resource regimes. We found that selection do act on zooid longevity, and that the strength of selection varies with environmental conditions. In environments where interspecific competition is high, selection favours colonies with longer zooid longevity if the ancestrula size of those colonies were large. In environments where food availability is high and flow rate is low, selection also favours colonies with longer zooid longevity. We find that the overall patterns in selection on zooid longevity across resource regimes suggest that modular optimisation theory conceived for plants may also apply to modular animals.

Introduction

Unlike solitary organisms, modular organisms have an indeterminate structure comprised of multiple sub-units, which can undergo independent development (Hamilton et al. 1987). This allows each module to experience its own life history, in some ways separated from, but at the same time within, the life history of the whole organism (Watkinson and White 1986). In principle, growth, reproduction and death can all occur autonomously in separate modules (Thomas 1994). Throughout the whole organism, resources are often shared between modules, and can be redistributed from one module to the next. This sharing and redistribution of resources throughout the colony allows modules to be allocated separate functions, such as feeding, growth or reproduction (Haggar and Ewel 1995; Palumbi and Jackson 1983; Watson 1984). Counterintuitively, this also allows deteriorating processes, like module mortality, to sometimes be beneficial for the organism — for example, when allocation of resources from older modules to newer, more vital ones maximises whole colony fitness.

In both theory and empiricism, module mortality has been best studied in plants. In plants the modular units are the totipotent shoots (Tuomi and Vuorisalo 1989), from which either flowers (reproductive units) or leaves (feeding units) can develop. The longevity of both flowers and leaves have been modelled. Since flowers do not produce energy themselves, they are reliant on the available energy of the plant. In perennial plants, it is predicted that the optimal longevity of flowers increase with increased resources and decrease with increased number of flowers, maintenance cost, and construction cost of a flower (Ashman and Schoen 1994). For leaves, it is predicted that the optimal leaf longevity is a result of whole-plant energy budgeting (Kikuzawa and Ackerly 1999; Wright et al. 2004). With time, the energy acquisition rate of a leaf will decline, either by shading from newer leaves ('self-

shading'), physical damage, or from the accumulation of metabolic waste products (Ackerly 1999; Gan and Amasino 1997; Guiboileau et al. 2010). Eventually, the energetic costs of maintaining the leaf will exceed what the leaf can produce. As a result, energy and nutrients (mainly nitrogen) are reabsorbed by the plant, and the old leaves will senesce and die (Smart 1994; Thomas and Stoddart 1980). This resource recycling strategy, based on nitrogen economics, is what allows plants to optimise resource allocation and maximise lifetime whole-plant fitness (Ackerly 1999; Kikuzawa 1991b; Kikuzawa 1995a; Kikuzawa and Ackerly 1999).

The timing of both leaf and flower senesce can be mediated by resource competition and environmental conditions (Ashman and Schoen 1994; Monk 1966). However, the strength and direction on selection for leaf and flower longevity in varying conditions is still debated. For example, it has been shown that leaf longevity is affected by the availability of local environmental resources. If sunlight is the limiting resource, then by partially shading a plant and hence limiting sunlight for some leaves, the available nitrogen will be redistributed from the shaded to the unshaded leaves. As leaf nitrogen content is positively correlated with leaf lifespan, the shaded leaves will therefore experience a shorter lifespan (Ackerly and Bazzaz 1995; Evans 1989; Hikosaka et al. 1994). Surprisingly, if the whole plant is shaded, leaf longevity will increase, and growth of the plant will decrease, due to energy retention within the plant (Hidema et al. 1991; Kikuzawa and Ackerly 1999; Terashima et al. 2005). For the same reasons, selection on plant traits, including leaf lifespan, is also affected by competition from neighbours. In line with studies on partial shading, neighbouring plants that compete for sunlight (and hence partially blocking the available sunlight for each other's canopies) have shorter leaf lifespans, as fast growth and fast leaf turnover is favoured (Grime and Hodgson 1987). If plants are competing for soil nutrients, however, the impact of competition is unclear, although evidence suggests that selection favours nutrient retention and thus prolonged leaf lifespan (for a full review, see Aerts 1999). These studies suggest that the interplay of

competition, resources and selection on module longevity is complex.

For modular animals, selection on module longevity is even less clear than for plants, and has been largely overlooked in the literature. The life histories of sessile marine organisms are almost identical to those of plants (Hughes 2005): what shoots are to plants, zooids are to colonial marine invertebrates (e.g. bryozoans, corals and ascidians). The zooids in these organisms contribute to a variety of functions, and many species experience zooid senescence separate to organism mortality. As for plants, zooids of some species have specialized functions, like feeding or reproduction, throughout their life cycles. In other species, the same zooid goes through different life stages with a variety of functions. Though there are studies indicating that senescence in some marine colonial species is a fixed intrinsic trait (Rinkevich et al. 1992), others suggest it is plastic (Harvell and Grosberg 1988). For example, laboratory trials show that zooids of the marine bryozoan *Electra pilosa* experience shorter lifespans in high food environments (Bayer et al. 1994), and that zooids of the marine bryozoan *Watersipora subtorquata* show different turnover rates at varying depths (Lange et al. 2016); however, manipulative studies examining selection on zooid longevity are exceedingly rare.

Typically, increases in available resources would increase the total energy, and thus increase organismal fitness. For maximal organismal fitness, the organism will experience trade-offs in life history traits, where for instance increased reproduction could decrease survival, due to whole organism energy budgeting (Stearns 1992). By restricting the amount of available energy, organismal aging will decrease and the lifespan of the organism will increase (Kirkwood 2005; Koubova and Guarente 2003). This is commonly known as 'calorie restriction', and is a typical example of life history trade-offs, where in low resource conditions, reproduction is inhibited due to increased investment in self-maintenance. For modular animals, conditions promoting calorie restriction should then extend the longevity of modules, by reducing both sexual and asexual reproduction where more energy would be allocated into

maintaining existing modules. To our knowledge, however, it has not been formally tested if this is the case for modules of modular organisms.

To examine how environments with varying resource conditions affect the selection on module longevity, we examined the strength and direction of selection on module longevity across a range of manipulated environments under field conditions. We used unanalysed data (on zooid longevity) from a field experiment conducted by Marshall and Monro (2013), and unanalysed data (also on the trait zooid longevity) from a field experiment conducted by Svanfeldt et al. (2017a). In the first experiment, interspecific competition (and thus variation in resource availability) was manipulated in three levels (Marshall and Monro, 2013). In the second experiment, food availability and flow rate were manipulated by altering the local abundances over a natural gradient of intraspecific density.

Methods

Study site and species

The first experiment was conducted at Redcliffe Marina, Brisbane, Australia in 2008, and the second at Royal Brighton Yacht Club (RBYC), Port Phillip Bay, Australia in 2013. These sites are both protected by manmade structures, and would be considered low exposure environments, with mainly directional flow from one effective inlet and one outlet. In RBYC, the local flow rate ranges from 0.1m/sec to 2.5cm/sec (Lagos et al. 2016) and flow rates are similar at Redcliffe Marina. In our trials, we used the colonial marine bryozoan *Watersipora subtorquata* (hereafter referred to as *Watersipora*), which is an invasive species, commonly found in coastal Australian waters (Hewitt et al. 2004). *Watersipora* colonies typically grow in sheltered areas and can easily be recognised by their characteristic bright red colour. New *Watersipora* colonies are formed by sexual reproduction and the subsequent release of free-

swimming larvae into the water column. The larvae are lecithotrophic and therefore completely

reliant on maternal investment in larval energy reserves until settlement (Svanfeldt et al. 2016).

After settlement, each larva goes through metamorphosis and forms the first feeding unit of the

colony - the ancestral zooid. The size of the ancestrula is highly correlated with larval size prior

to settlement, making it a good indicator of maternal provisioning (Marshall and Keough 2004).

Once metamorphosed, the ancestrula starts budding, where circular bands of feeding and

sexually-reproducing zooids starts forming the circular colony. Growth occurs in zooids on the

colony margin. With time, the zooids in the centre of the colony lose their colour and

irreversibly senesce. Though other bryozoan species can initiate senescence from other regions

of the colony than the centre (for some species to create excurrent chimneys), *Watersipora*

typically only senesces from the centre out. Zooid senescence is visible as the appearance of a

grey inner circle of older, dead zooids that expands as the colony grows (Hart and Keough

2009; Marshall and Monro 2013). Within the colony, energy acquired from food capture is

shared amongst zooids via porous cell walls (Banta 1969; Bone and Keough 2010).

Interspecific competition

At Redcliff Marina, interspecific competition was manipulated over a range of settler

(ancestrula) sizes. *Watersipora* colonies were collected at the field site, and spawned in the

laboratory. The released larvae were allowed to settle in water drops on acetate sheets, and the

sheets then glued onto 10x10cm PVC plates. To reflect the range of natural competition

conditions in the field in a way that affect performance for *Watersipora*, the environment was

manipulated in accordance with a previous study by Marshall and Keough (2009). Newly

settled ancestrulae were numbered and assigned randomly to one out of three field treatments:

low, intermediate and high competition. In the low competitor treatment, settlers were attached

to blank settlement plates that were cleaned weekly throughout the experiment to remove

natural recruitment from external organisms. In the intermediate competition treatment, settlers

were attached to blank settlement plates that were allowed to accumulate natural recruitment of

external organisms (including sponges, bryozoans, worms and ascidians) throughout the experimental period. In the high competition treatment, settlers were attached to settlement plates that had been exposed to natural recruitment of external organisms for three weeks in the field prior to attachment, and therefore already held young developing fouling communities. Treatments were applied independently on the scale of settlement plate. In total for this experiment, 60 replicates of each treatment were deployed. Plates with one of the three treatments were then haphazardly attached to PVC backing panels, such that all three treatments were represented on each panel, and the panels submerged at 1m depth in the field. Equal numbers of each treatment were attached to each backing panel and the position of the plates on the panels were rearranged weekly throughout the experiment, to prevent artefactual covariance between spatial arrangement and the life history traits of interest. In total, 30 backing panels were deployed, holding a total of 180 plates (60 per treatment). After deployment, the plates were photographed weekly for six weeks in the field, and the photos analysed in ImageJ software (available at http://imagej.net). For detailed description, see Marshall and Monro (2013). Colony traits were then analysed from the photos. In this study, we analysed ancestrula size, zooid longevity and the reproductive output of the colonies by counting ovicells per week as a measure of colony fitness.

Intraspecific density and resource availability

At RBYC, resource availability was manipulated over a natural gradient of intraspecific density of *Watersipora* colonies. *Watersipora* colonies are sessile filter-feeders that compete exploitatively for food particles, and depend on the rate at which the particles pass their feeding structures (Pratt 2008). We manipulated food availability by adding or not adding food blocks (see below for details), and flow rate by obstructing or not obstructing the natural flow. The two resource manipulations were then crossed over the range of *Watersipora* density in an orthogonal design, with the aim to create extremes in environmental conditions, overlapping with natural conditions experienced by *Watersipora*.

Resource availabilities and intraspecific density were manipulated according to following procedures. The range of intraspecific density was obtained by submerging 80 10x10cm acetate plates attached to backing panels at 1.5m depth at the field site. After a week in the field, the settlement plates were examined, and all non-*Watersipora* settlers were scraped off, resulting in 1-8 *Watersipora* settlers per plate. The plates were then haphazardly assigned to the resource manipulation treatments. To manipulate the availability of food, artificial slow releasing food blocks were made by mixing commercial filter feeder mixture and dental plaster (see Svensson & Marshall, 2015 for details). Control blocks were made with dental plaster and water. The food and control blocks were then attached with mesh cages at the centre of each settlement plate respective to treatment. Flow rate was manipulated by surrounding settlement plates with 10x10x5cm flow obstructing boxes. The boxes effectively obstructed the directional flow rates on the plates, without reducing access to open water. Controls for the flow obstruction treatment were open settlement plates. By crossing the food and flow treatments orthogonally, we created four environments with the combinations of high and low food and flow availabilities. In the field, 20 backing panels, each holding two replicates of each treatment (in total 8 plates per backing panel) were submerged over two runs (10 panels per run), with one week between the initiation of each run. In total, there was a replication of 40 plates per treatment. After deployment, the plates were monitored by weekly photographs from week 1 to week 13 in the field. The images were then analysed in ImageJ software. For detailed descriptions, see Svanfeldt et al. (2017a). For the analyses in this study, we measured zooid longevity and used asexual reproduction (colony size per week) as a fitness proxy. For colonial organisms, growth, just like sexual reproduction, increases reproductive output and is therefore integral to fitness (Pedersen and Tuomi 1995). Preferably, both sexual and asexual reproduction should be measured for total organismal fitness (Winkler and Fischer 1999), however, our colonies did not reach sexual maturity before deteriorating. For *Watersipora* and other colonial species, though, reproductive output is highly correlated to size (Hart and Keough 2009;

Hughes 1984) which further strengthens the appropriateness of our use of asexual rather than sexual reproduction as fitness measure.

Statistical analyses

To estimate the strength and direction of selection on the zooid traits in the two experiments (zooid longevity plus ancestrula size in the interspecific experiment, and zooid longevity in the intraspecific experiment), and to obtain standardised estimates of linear (β) and nonlinear (γ) gradients of selection, we used the classic multiple-regression approach of Lande and Arnold (1983). Prior to analysis, zooid traits were transformed to units of standard deviation within each environment, and relative fitness (asexual reproduction and reproductive output, respectively) was calculated by dividing each absolute value by the mean value within each environment, in accordance with standard methods for selection analyses (Kingsolver and Pfennig 2007). The strength of selection was tested by comparing models with maximum likelihood ratios.

To estimate selection on the zooid traits, we first analysed the effect of environment (competition for the interspecific experiment, and density, food and flow for the intraspecific experiment) on relative fitness. In our analyses, zooid traits were modelled as fixed effects and spatial arrangement (backing panel) was modelled as random. These mixed models for each environment thereafter served as our base models. Second, we tested for significant directional selection on the zooid traits (zooid longevity for the interspecific experiment, and zooid longevity and ancestrula size for the intraspecific experiment) on fitness, by adding only linear regression coefficients to our base model terms. Third, we tested for quadratic selection by adding quadratic terms to the linear model and again tested whether quadratic selection varied across environments. Since we had multiple traits for the interspecific experiment, we also added interactions between the zooid traits to our models to estimate correlational selection within environments and also if there was variation in

correlational selection on the traits across environments. All analyses were performed in

SAS, version 9.4 using ML estimation in PROC MIXED.

Results

For the interspecific competition experiment, we found a strong effect of environment

(competition level) on colony performance (ovicells per week; $\chi^2 = 106.4$, p<0.001), where

colonies performed best in the environment where competition was low, and worst in the

environment where competition was high. Further, we explored if there was selection on the

zooid traits (zooid longevity and ancestrula size), and if this selection differed across

environments. We found significant directional selection for the zooid traits that differed with

environment ($\chi^2 = 7.3$, p=0.03). However, when exploring selection for each trait, we found that

no significant directional selection with environment for either trait (zooid

longevity*competition: $\chi^2 = 3.7$, p=0.054, ancestrula size*competition: $\chi^2 = 3.7$, p=0.054, Table

1).We also found evidence for differences in correlational selection among environments ($\chi^2 =$

7.5, p<0.001); under high competition, individuals had highest fitness when ancestrula size was

larger and zooid longevity was longer, while individuals with lower values in both traits had

lower fitness Table 1, Figure 1). In low and intermediate competition environments, however,

there was no evidence for correlational selection. In addition, we found significant quadratic

selection on ancestrula size that differed with environment ($\chi^2 = 5.1$, p=0.02). In the

environments with low and high competition, quadratic selection on ancestrula size was non-

existent, whereas in the intermediate competition environment, there was strong stabilising

selection on ancestrula size ($\chi^2 = 5.1$, p=0.02). Quadratic selection on zooid longevity did not

differ among environments, ($\chi^2 = 0.00$, p=1.00), and there was no evidence for quadratic

selection on zooid longevity overall ($\chi^2 = 0.00$, p=1.00).

For the intraspecific density experiment, we found a strong main effect of overall environment (density, food and flow combinations) on colony performance (colony size per week; $\chi^2 = 37.5$, p= <0.001), where there was a significant interaction of density, food availability and flow rate ($\chi^2 = 3.8$, p= 0.05) on colony performance. Colonies in environments where food availability was high (regardless of flow), showed positive density dependence on fitness, as did colonies in the environment where food availability was low but flow rate was high. In the environment where both food availability and flow rate were low, however, colonies experienced negative density dependence (Svanfeldt et al. 2017a). Further, we explored if selection acted on zooid longevity and if this selection varied with environments. Directional selection did not vary with any of the interactions between zooid longevity, colony density and food ($\chi^2 = 0.0$, p=1.0) or zooid longevity, colony density and flow ($\chi^2 = 0.0$, p=1.0). However, the strength (and direction) of selection on zooid longevity varied with the interaction of zooid longevity, food and flow ($\chi^2 = 102.2$, p=0.01). In the environment where food availability was low and flow rate was high, selection favoured longer zooid longevity (Low food, high flow: $\chi^2 = 5.05$, p=0.02; Table 2, Figure 2). In all other environments, selection was weak or non-existent (High food, high flow: $\chi^2 = 2.55$, p=0.11; High food, low flow: $\chi^2 = 0.78$, p=0.38; Low food, low flow: $\chi^2 = 0.9$, p=0.34; Table 2, Figure 2). Quadratic selection on zooid longevity did not differ among environments, ($\chi^2 = 4.2$, p=0.65), and there was no evidence for quadratic selection on zooid longevity overall ($\chi^2 = 0.3$, p=0.58).

Discussion

We found that selection acts on module lifespan in a modular animal species, and that this selection is mediated by environmental conditions. In environments with low or intermediate interspecific competition, selection on zooid longevity was non-existent. In environments with

high interspecific competition, selection favoured colonies with longer zooid longevity if the ancestrula size of those colonies were larger. In this high competition environment, colonies with shorter zooid longevity were least favoured by selection, but only if the colonies were small as ancestrulae. Colonies in this environment that had higher values in one trait but lower in the other trait (smaller ancestrula and longer zooid longevity or larger ancestrula and shorter zooid longevity) had intermediate fitness. We also found quadratic selection for ancestrula size, as further discussed in Marshall and Monro (2013), where in environments with intermediate competition, there was strong stabilizing selection on ancestrula size. In our second experiment, selection favoured colonies with longer zooid longevity in environments with low food availability and high flow rate. In all other environments, colonies experienced no selection on zooid longevity. Consistent with terrestrial plant theory of module longevity (Ackerly 1999; Kikuzawa 1991b; Kikuzawa 1995a; Kikuzawa and Ackerly 1999), we have shown that module longevity in modular animals is under selection, and that selection varies with environmental conditions.

Previously, it has been shown that colonies growing in conditions with high food availability have shorter zooid longevity (Bayer et al. 1994), which aligns with module lifespan theories in plants. In plants, high nutrient availability promotes shorter leaf lifespans, encouraging plant growth overall. In high nutrient environments, the limiting factor for plant growth is sunlight. As new leaves grow, they will effectively shade the older leaves, inducing 'self-shading'. By adapting faster leaf turnover (and thereby shorter leaf longevity), the negative properties of the 'self-shading' effect are minimized (Grime and Hodgson 1987). Similarly, high nutrient environments will promote rapid growth of neighbours, which could further induce shading, and therefore enhance the benefits of shorter leaf lifespans (Grime and Hodgson 1987). In low nutrient availability, plants are shown to prolong leaf lifespans in order to efficiently retain nutrients (Escudero et al. 1992; Grime and Hodgson 1987). While it might be intuitively appealing to assume that low flow conditions for marine invertebrates are analogous to low

nutrient conditions in plants, the aspects of flow on food availability is somewhat complicated.

For colonial marine invertebrates, higher flow rates indicate that more food particles per unit time are passing the feeding apparatus (Cahalan et al. 1989). However, multiple studies by Okamura (1984; 1985; 1990) and also by Pratt (2008), have shown that feeding rates of sessile marine invertebrates, specifically bryozoans, are either unaffected by flow rate, or decrease with increased flow. The reason that increased flow rate does not increase feeding is explained in an additional study by Okamura (1988), where it was shown that for bryozoans, feeding attempts are higher under high flow rates, whereas the actual food particle capturing success is higher under lower flow rates. That colonies in the environment with low food availability in combination with higher flow rate experienced selection for longer zooid longevity could therefore be the result of nutrient retention, similar to the responses of plants in low nutrient environments (Escudero et al. 1992).

In both environments where selection favoured longer zooid longevity (low food and high flow, and high competition) colonies hence grew under conditions where the available food was restricted. Under calorie restriction, organisms typically allocate more energy to self-maintenance and less energy to reproduction (Koubova and Guarente 2003). In principle, this strategy is meant to optimise fitness for organisms in the restricted conditions. Therefore, it is possible that the energy acquired by the colonies in these conditions was allocated to maintain the existing zooids, which over the lifetime of the colony increased organismal fitness, even though less energy overall in these environments was allocated to reproduction. That we only found fitness benefits of longer zooid longevity in colonies that also were large as ancestrulae in our interspecies competition experiment, may be explained by that only the strongest, larger, ancestrulae in the nutrient poor (high competition) environments were able to benefit from the calorie restriction strategy, and in the end were the only colonies that had enough energy left for reproduction. Smaller, weaker ancestrulae on the other hand, probably struggled during these conditions where increased allocation to self-maintenance only allowed them to reproduce

poorly.

That selection favours the covariation of longer zooid longevity and larger offspring size (ancestrula) in environments with high interspecific competition, may also be a result of similar strategies to those acting on plant modules in competitive environments. In plants, it has been shown for several species that seed mass (offspring size) is positively correlated with leaf longevity (Seiwa and Kikuzawa 1991; Seiwa and Kikuzawa 1996). Classic offspring size theory predicts that there is a relationship between offspring size and fitness, where larger offspring have higher fitness than smaller offspring. It also predicts that this relationship is stronger in harsher rather than benign environments (Parker and Begon 1986). Therefore, our finding of a correlational selection between offspring size and zooid longevity for our marine invertebrate in the environment with high competition was not surprising, but rather confirms the patterns of classic theory of correlated evolution of these two traits.

Our results suggest that there are stabilising forces acting on module longevity, where the phenotypic diversity of this trait may persist due to variation in selection pressure across environments (Kassen 2002). The relationship between optimal module component longevity and maximal organismal gain over various conditions has been modelled and tested on plant spices in the field, where the longevity of leaves (the energy acquiring component of plant modules) is predicted (Kikuzawa 1991b). The model consists of three traits, two of which are likely to vary over environments: 1) increased time until module production reaches zero (environment dependent), which increases module longevity, 2) maximum daily assimilation (environment dependent), which decreases module longevity, and 3) fixed construction costs, which increase module longevity (Ackerly 1996; Kikuzawa 1995a; Kohei and Kikuzawa 2008). Whether this module optimality theory applies only to modular plants, or relates to all modular species across taxa, remains to be tested and requires empirical investigation. However, our results strengthens the suspicion that modular theory designed for plants may also apply to

other modular organisms.

In this study, we found that selection acts on zooid longevity in a modular species, and that this selection is mediated by environmental conditions. We also found that the direction of the selective forces on our study species, a marine invertebrate, act similar to those acting on terrestrial plants. These similarities allow us to relate our research to the already well established research on module theory developed for plants, though more empirical and theoretical studies are still required in order to draw general conclusions. Still, we believe that our study here has provided a starting point for understanding selection pressures in modular organisms, and the role of modules in the life history. By addressing the seemingly shared basic aspects of module longevity, we hope to improve our understanding of the highly diverse and numerous group of modular organisms.

Tables

Table 1. Standardized gradients of linear selection (β) and nonlinear selection (γ) on zooid longevity and ancestrula size with competition environments in *Watersipora subtorquata*. Errors are shown in parentheses next to each estimate and all significant effects ($p < 0.05$) are shown in bold.

			γ±SE	
		β±SE	Zooid longevity	Ancestrula size
Low competition	Zooid longevity	-0.07 (0.09)		
	Ancestrula size	0.06 (0.31)	0.01 (0.07)	0.03 (0.05)
Intermediate competition	Zooid longevity	-0.19 (0.11)		
	Ancestrula size	0.33 (0.41)	-0.06 (0.09)	**-0.17 (0.07)**
High competition	Zooid longevity	0.18 (0.31)		
	Ancestrula size	-1.05 (0.56)	**0.39 (0.13)**	-0.01 (0.10)

Table 2. Standardized gradients of linear selection (β) on zooid longevity with variation in resource (food and flow) environments in *Watersipora subtorquata*. Standard errors are shown in parentheses next to each estimate and all significant effects ($p < 0.05$) are shown in bold.

		β±SE
High food, high flow	Zooid longevity	-0.19 (0.12)
High food, low flow	Zooid longevity	0.16 (0.18)
Low food, high flow	Zooid longevity	**0.47** (0.20)
Low food, low flow	Zooid longevity	-0.22 (0.22)

Figures
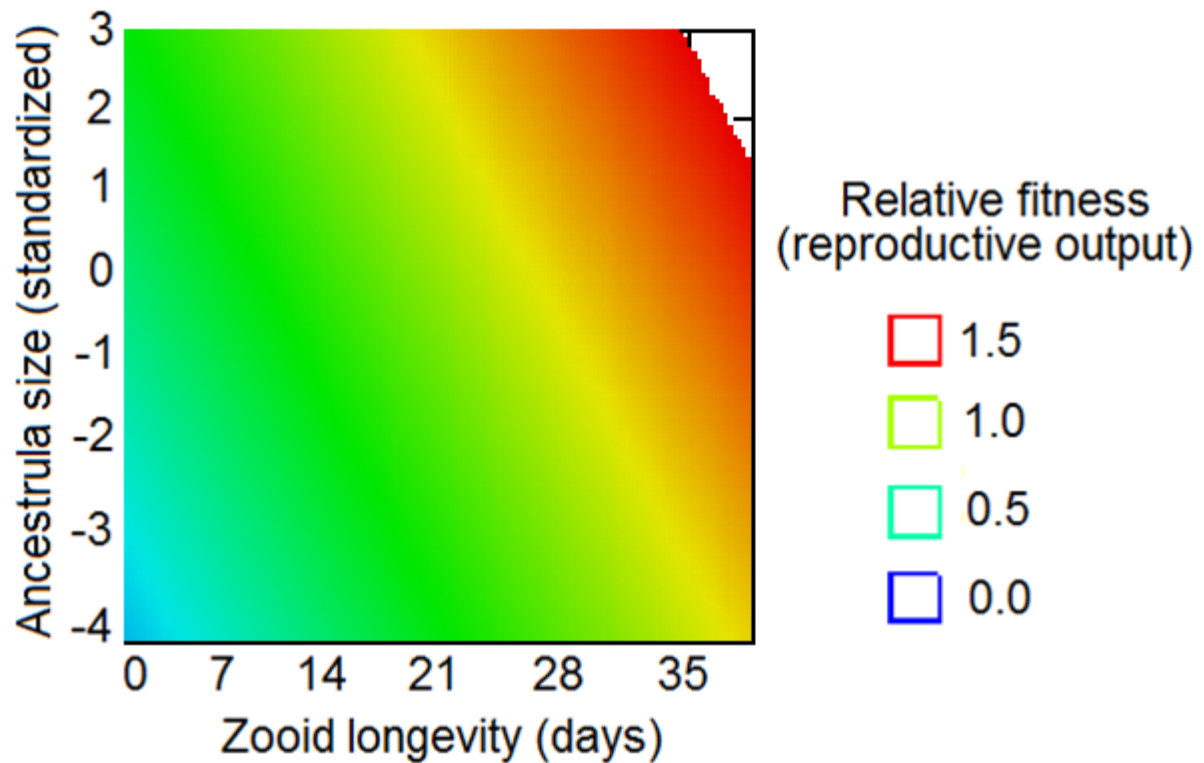
Figure 1. The predicted effect of zooid longevity and ancestrula size on fitness in high interspecific competitive environment for *Watersipora* colonies in the field. The x and y-axes show the standardized traits, and the heat plot shows relative fitness from low (blue) to high (red). In this environment (high competition) selection favours colonies originating from larger ancestrula with longer zooid longevity.
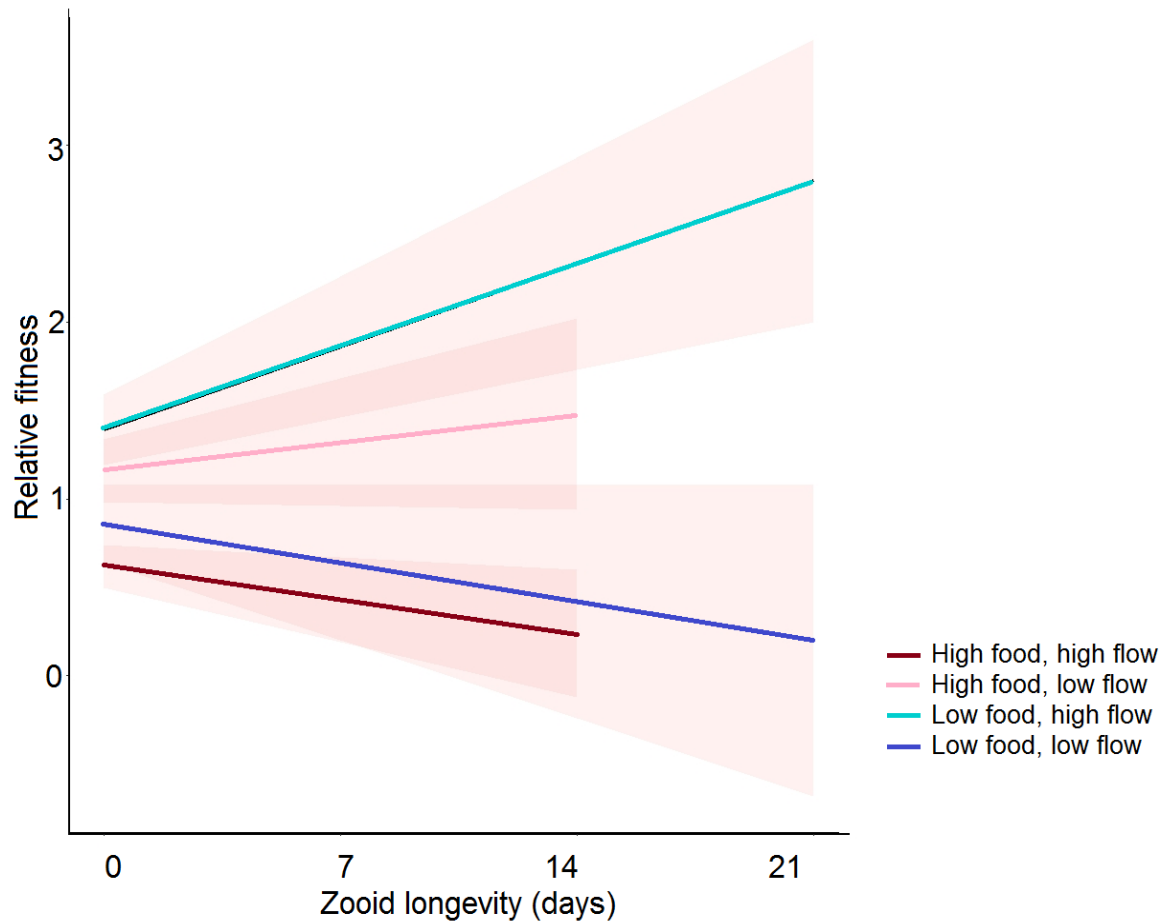
Figure 2. The predicted effects of zooid longevity on fitness in four environments with varying food and flow conditions on *Watersipora* colonies in the field. The x-axis shows zooid longevity and the y-axis shows relative fitness. The linear effects of zooid longevity on relative fitness is shown in maroon for the environment with high food and high flow, pink for the environment with high food and low flow, teal for the environment with low food and high flow, and indigo for the environment with low food and low flow (±SE as shades). When food availability is high and flow rate is low, selection favour colonies with longer zooid longevity. In all other combinations of food and flow environments, selection did not act on zooid longevity.

Chapter five

Do leaf optimality models successfully predict module longevity in animals?

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Abstract

The life histories of modular species are complex, and modules may die so as to maximize fitness of the whole organism. In plants, the optimal longevity of modules (leaves) is theoretically well resolved. Though modular animals share analogous life history characteristics to plants, no formal theories of module longevity have been developed, and discussions of module longevity in this group are largely conceptual. Here, we predict the longevity of modules (zooids) for a modular animal, the marine bryozoan *Watersipora subtorquata*, by parameterising a classic leaf optimality model. Our model predicted that optimal zooid longevity ranged between 14 and 25 days, and zooid longevity in the field has an average of 14 days. The successful application of this model raises novel testable hypotheses regarding the factors that drive module senescence in animals

Introduction

Relative to solitary organisms, aging in modular organisms is more complex. Modular

organisms have an indeterminate structure comprised by multiple subunits (modules, ramets,

polyps or zooids), which can experience (semi-)independent development. The independence of

these subunits allows life history to occur at different levels of biological organisation (Harvell

and Grosberg 1988). Senescence and regeneration can occur at both a modular level (i.e., partial

growth or partial mortality), and the level of the whole colony. This independence gives

modular organisms the advantage of using senescence as a developmental and adaptive resource

(Hamilton et al. 1987; Thomas 1994).

Senescence can benefit modular organisms, as module resources are able to be recycled and

redistributed from old modules to other, more vigorous, modules. This process is part of the

shared resource economy of the organism. Essentially, the redistribution of resources can

enhance growth and reproductive output at the organismal level, as ineffective modules are cut-

off from the remainder of the colony, and their resources reused. In removing these modules,

the timing of senescence can alter the entire metabolic economy of the organism (Gan and

Amasino 1997), and ultimately increase colony fitness (Lim et al. 2007). For many modular

species, the timing of senescence, and hence the lifetime of modules, is variable and responds to

selection, and these selective forces can vary with environmental conditions (Harvell and

Grosberg 1988; Sköld and Obst 2011; Wingler et al. 2006).

In plants, the relationship between environmental conditions and the timing of module

senescence is well established. Partial senescence in deciduous plants is a widespread

phenomenon seen in temperate regions of the world is reflected in the vast literature on this

topic (Ackerly 1999; Chabot and Hicks 1982; Kikuzawa 1995b; Williams 2001). Though leaves

are not considered modules from a semiautonomous sense, they are major features of the

totipotent shoots (Tuomi and Vuorisalo 1989). Fundamental theory underlying leaf senescence

models assumes that the decline in photosynthetic rate of leaves with age is a result of selection

to maximise net lifetime carbon gain lifetime (Ackerly 1999; Kikuzawa 1991a). Based on this

assumption, the lifetime of leaves in deciduous plants has been estimated using a simple

formula to obtain the maximal net carbon gain of the plant:

$$T_{opt} = (\frac{2 \times b \times C}{a})^{0.5}$$

The model includes three parameters and predicts that the optimal leaf longevity, T_{opt}, depends

on the maximal photosynthetic rate of a leaf (*a*), the time when photosynthetic rate reaches 0

(*b*), and the metabolic cost of producing a leaf (*C*). According to the model, the optimal leaf

lifetime increases with the active production duration of the leaf (the time during which the leaf

is photosynthetically active) and leaf construction cost, while it declines with the daily

photosynthetic rate of the leaf; (Kikuzawa 1991b). Also, depending on environmental

conditions, the value of the model parameters could change, and thus alter the optimal leaf

lifetime. If for example sunlight is the limiting resource, leaf turnover for a plant will accelerate

(and hence leaf longevity shorten) as a result of 'self-shading'- where leaves of the same plant

block the available sunlight for one another (Ackerly 1999).

Though there are still gaps in our understanding of the implications of modularity for plants,

our understanding of senescence in plants dwarfs our understanding of senescence in other

systems. In marine environments, hard substratum communities are dominated by sessile

colonial invertebrates, and these represent a major component of the world's biodiversity

(Jackson 1977). Like all modular organisms (including plants), colonial marine species are

formed by numerous individual but interconnected subunits, known in this group as zooids or

polyps. The tractable life histories of colonial organisms make them exceptional as model

species for investigating essential life-history traits (Bodnar 2009; Darling et al. 2013; Love

2009; Rinkevich and Matranga 2009). Nevertheless, the drivers of senescence in marine

colonial animals remain poorly understood with no formal theoretical explorations (Hughes

2005).

Both plants and colonial marine organisms share the life-history consequences of modularity and a sessile adult life phase. Just as for leaves of plants, zooids accumulate energy that is shared throughout the colony (Banta 1969; Bone and Keough 2010). As the colony grows, zooids also limit the accessibility of resources for one another, where younger zooids block older zooids from the directional flow, as in the case of 'self-shading' for plants (Ackerly 1999). If zooid longevity acts to optimise organismal fitness in marine invertebrates, it is likely that parameters that mediate leaf longevity may have analogues that mediate the longevity of zooids. Hence, it is likely that both groups show parallel responses to selective forces acting on similar life history traits (Hughes 2005). Evidence of convergent evolution of senescence and resource economics across plant taxa further supports this suspicion (Wright et al. 2004).

Here, we adapt and apply a classic leaf lifetime optimality model to colonial marine invertebrates in order to examine partial senescence for this group. We translated the key parameters from an optimal leaf longevity model (Kikuzawa 1991) to analogous zooid traits for animals, and then estimated these parameters in a series of laboratory trials on a marine invertebrate (Table 1). After parameterising the model, we made predictions of optimal module longevity. To test our predictions, we compared values to observations of zooid longevity in the field.

Methods

Study species

Watersipora subtorquata is an encrusting bryozoan found in most shallow temporal and subtropical waters, and is a common member of sessile marine communities of the Australian coast (Marshall and Keough 2009). Like all bryozoans, *Watersipora* forms colonies by asexual budding. New colonies start from a free-swimming sexually produce larva that attaches permanently to a surface (Marshall and Keough 2004). The colony's first feeding unit, the ancestrula zooid. Via budding, concentric bands of feeding and sexually reproducing zooids

will then grow outwards from the ancestrula. Growth occurs almost exclusively on the outer

ring of zooids (Hart and Keough 2009). With time, the centre of the colony, starting from the

ancestrula, will begin to senesce and form a grey inner circle of dead zooids. As the colony

grows, the circle of senesced zooids expands (Hart and Keough 2009; Marshall and Monro

2013).

Daily energy acquisition (a)

In the optimal leaf longevity model, parameter *a* estimates the maximum daily photosynthesis

of a leaf. For a perfect translation to zooids, we would ideally estimate the maximum daily food

acquisition of a zooid by measuring its daily energy consumption. However, estimating how

much a single zooid consumes is difficult due to logistical constraints. The food intake of

individual bryozoan zooids has been measured by feeding the colonies with indigestible

artificial beads that later could be dissected out of the digestive tract (Pratt 2008). However, the

calcified nature of Watersipora makes such dissections problematic. Instead, we calculated the

minimum energy intake that a zooid must consume by estimating several key values. First, we

estimated how much energy is spent on zooid maintenance by measuring the daily metabolic

rate (mR) of individual zooids. Second, to estimate how much a colony increases in size per

day, we measured the daily growth rate (gR) of a colony. Third, to approximate how much

captured food is retained in zooids during feeding, we used the assimilation efficiency

(conversion of food into energy; AE) of *Watersipora*. In this way, we achieved an estimate of

the minimum energy required for the self-maintenance of a growing zooid.

Below, we describe in more detail each step of our estimations of the components, (AE, gR and

mR) for the daily consumption of a zooid. Assimilation efficiencies of marine filter feeders vary

greatly, ranging from 20-80% (Jorgensen et al. 1984). Here, we used an intermediate value of

50% AE. For estimations of colony growth rate, we used the rates of colonies in natural

conditions in the field from Svanfeldt et al. (2017).   Metabolic rate was estimated from

laboratory trials from newly settled zooids to 13 days post settlement. Before each trial, the

zooids were starved overnight in sterilized filtered sea water. Since the metabolic rate of

organisms is strongly related to oxygen consumption (White et al. 2011), we performed oxygen

consumption (VO_2) trials. In these trials, newly spawned *Watersipora* larvae were settled on

individual acetate circles (5mm in diameter). Each acetate circle holding a larva was brushed

and triple dipped in sterilised filtered sea water (FSW). We measured the oxygen consumption

of the growing zooids using two PreSens sensor dish readers (Sensor Dish Reader SDR2,

PreSens), with 24-chamber 200ml glass micro vials with sensor spots (Loligo Systems Aps,

Tjele, Denmark), following standard protocols (as per SDR SensorDish® Reader manual,

PreSens Precision Sensing GmbH, Josef-Engert-Strasse 11, 93053 Regensburg, Germany). All

recordings were performed in complete darkness at a constant 17°C. The sensors were

calibrated with O_2 saturated sea water for 100% O_2-saturation, and seawater with 2% sodium

sulphite for 0% O_2-saturation. To estimate respiration, we placed 20 individual zooids (on

acetate) one by one in the sensor spot glass vials in FSW, and ran the sensor reader, taking

measurements every minute for two hours. Four vials per sensor plate were recorded with

empty acetate circles included as controls to account for microbial respiration. We then

calculated VO_2 (% h^{-1}) from the rate of change in O_2 saturation as $VO_2 = -1 (m_a - m_b/100) V\beta O_2$

(White et al. 2011), where m_b is the rate of O_2 saturation in the vials with empty acetate, βO_2 is

the capacitance of 17°C, O_2 saturated seawater and V is the water volume of the vials (0.002 l).

The procedure was repeated every second day from day 5 to day 13 post-settlement. The mR of

a single zooid was estimated from VO_2 at day 5 post-settlement, when the settled zooids had

opened their opercula but not yet started to bud. We then converted the respiration measures,

VO_2, to mR, (mJ h^{-1}) using the caloric conversion factor 20.08 J $ml^{-1}O_2$ (Crisp, 1971). In total,

we performed three runs of the trial (n=60 zooids).

Time until zooid consumption is 0 (b)

Zooids stop being productive when they stop acquiring food. *Watersipora* zooids accumulate

both food and oxygen by siphoning water from their close surroundings (Ferguson et al. 2013).

To estimate the time until zooid production is 0, we would ideally have followed single zooids

in the field and measured their food capturing activity over the entire zooid lifetime. Since this is not feasible, we used alternative methods. We used zooid position in the colony as a proxy of colony age, and resource availability at the zooid position in differing flow rates as at proxy for when zooid production is 0. Oxygen consumption is more easily estimated than food consumption, so we also used O_2 as a proxy for food availability across different zooid positions. Since oxygen molecules are smaller than food particles, they would be affected at a higher rate of the blocking effect from neighbours. Therefore, by estimating the effect using oxygen, we may at the most have slightly underestimated the blocking effect. Effectively, we measured the concentration of available O_2 for zooids with increasing distance from the colony edge, where an increased number of feeding 'mouths' blocked the available resources for the zooids of interest (similar to the case of 'self-shading' in plants), and then estimated the age of the zooids at that distance using colony growth rate from our field trials under varying flow conditions.

The zooid productivity trials were performed using the following procedure: Flat colonies were collected from the field and kept in aquaria at a constant 19˚C. To determine the location where oxygen was exhausted in a colony, we constructed a flume box (50x10cm) of 6mm acetate sheets, with a collimator creating a laminar flow throughout the box. The flume was set at controlled flow speeds (0 or 0.25 cm/s; representative speeds at the appropriate scale for our field site (Lagos et al. 2017) and one colony at a time was submerged in the centre of the flume. The available oxygen for zooids within a colony was measured in the direction of the flow at varying distances (1, 3, 5, 7, 9 and 11mm) from the colony edge. This was done by pointing a retractable needle Firesting™ O_2 micro sensor directly above the individual zooid, until a stable value was read for three minutes. The available oxygen measured for zooids (at each distance) was then recorded in Firesting Logger software (Pyro Science GmbH). In total, we measured 12 colonies per flow rate and 5 zooids per colony.

Zooid construction cost (C)

The construction cost of a zooid (C_{Tot}) was estimated as the energy required for maintenance of a zooid without buds (E_M), the ash-free dry weight of a zooid (E_w), added to the amount of energy used by a zooid during the budding process (E_B). The cost in energy of the production process was estimated by comparing the metabolic rate of singular zooids and zooids with a developing bud during the budding process: $C_{Tot} = E_B + E_M + E_W$. For this estimation, we used the same metabolic measures from the energy consumption trials (see above). The metabolic rate of a zooid without buds was estimated using the metabolic rate of zooids in the VO_2 trial at day 5 (mature zooids pre-budding). A bud took eight days to form (from day 5 to day 13, from ancestral settlement) before becoming self-sufficient, and for comparison, the metabolic rate of a zooid at day five was multiplied by eight (for an approximation metabolic rate over eight days with no budding).

Ash free dry weight was measured according to standard procedures for marine invertebrates (Norrbin and Båmstedt 1984). We compared the weight of dried (60˚C for 12hrs) colony fragments with known number of zooids with their burned weight (550 ˚C for 4hrs). Six colonies with three fragments per colony were measured and each fragment was photographed and analysed with ImageJ software (available at http:// imagej.net) to determine fragment area and number of zooids per fragment.

Zooid longevity in the field

To examine zooid longevity in the field, we collated published data on zooid longevity from a recent field study on *Watersipora* at our study site (Svanfeldt et al. 2017a). Specifically, we estimated zooid longevity from unmanipulated controls.

Results

Daily energy acquisition (a)

Estimates from our oxygen consumption trial showed that the average metabolic rate of a fully

grown zooid (at day five after settlement) was 0.013 (±0.0001) J/day. The average area of a

grown zooid was 0.274mm^2. We used the growth rate of field grown colonies in natural

conditions from Svanfeldt et al. (2017). The average growth rate of natural zooids was 0.180

(±0.024) mm/day. Based on these estimations, the minimum daily food consumption was 0.031

(±0.003) J/day.

Time until zooid production capacity is 0 (b)

The critical limit for flat marine colonial organisms is 14% O_2 saturation (Ferguson et al.,

2013), and zooids that experience lower oxygen concentrations are not able to function.

Therefore, the distance from the colony edge at which O_2 drops below 14% is our estimate for a

production capacity of 0. In our trials, for flow rates at 0.25cm/sec and in still water (0cm/sec),

the O_2 concentration reached 14% at 7.5mm and 2.5mm respectively from the colony edge (Fig.

1) – we calculated *b* using those two measures, as field trials from our research sites indicate

they are representative of local flow conditions (Lagos et al. 2017). Once again, we used growth

estimates of field grown colonies in natural conditions by Svanfeldt et al. (2017b). Based on

these growth rates, our calculations indicated that it took 41.7 days for colonies to grow 7.5mm

and 13.9 days for colonies to grow 2.5mm. Thus, our estimation of parameter *b* was estimated

to be between 41.7 and 13.9 days, depending on the flow rate.

Zooid construction cost (C)

Our estimation for the metabolism of a zooid at day five was 0.013 (±0.0001)J per day,

meaning that the total sum of the costs of maintaining a zooid for eight days (the equivalent

time of producing a bud) was 0.104 J. During budding, the increase in metabolic cost was

estimated daily. In total of the eight days during budding, a zooid producing one single bud had

a metabolic cost of 0.152J. Subtracting the cost of metabolic maintenance of the zooid itself, the

metabolic cost of budding was 0.049 (±<0.0001) J -an increase in metabolic cost by 46.7% per

zooid. Our estimation of the ash-free dry weight of *Watersipora* showed this species has

12.23% organic tissue and 87.77% inorganic calcified compounds. With the estimated cost of

dry mass tissue, 8.4mg^{-1} (Hou et al., 2008), and inorganic calcification, 1.5Jmg^{-1} (Palmer,

1992), the total energetic dry weight cost of *Watersipora* was 2.344Jmg^{-1}. Dry weight per zooid

was 78.44µg and the total energy content per zooid 0.184 ($\pm$0.001) J. The total cost to produce a

zooid, cost of the production process (0.049J), added to cost of the final product (0.184J) was

0.232 ($\pm$0.0012) J.

Assembled parameters

After estimating values for our model parameters, we used the model to predict the optimal

zooid longevity. We estimated a single value for parameter *a, and C*, respectively, representing

natural conditions at our field site. The variation in the effect of 'self-shading' and growth rates

in different flow rates, yielded two varying values for parameter *b*, time until production is 0.

According to the optimal leaf longevity model, the estimates of our model parameters predicted

an optimal zooid longevity of 25.0 ($\pm$0.06) days in environments with a flow of 0.25cm/sec and

13.9 ($\pm$0.06) days in environments with no directional flow.

Zooid longevity in the field

The data on zooid longevity we collected from field data was in range of our model

estimations (Fig. 2). The average zooid longevity under field conditions was 13.93 ($\pm$0.46)

days, whereas our model predictions estimated that zooid longevity should range from 13.9-

25.0 ($\pm$0.06) days, depending on the local flow rate.

Discussion

In this study, we successfully applied an optimal leaf longevity model to predict the longevity of zooids of a sessile marine invertebrate under field conditions. We suggest that future studies of senescence in other modular organisms (e.g. corals) utilize the theoretical framework developed for plants, as such models are likely to translate well.

Given how successfully the model used in our study predicts module longevity in a colonial marine invertebrate, some novel hypotheses arise from its application. For example for sessile marine invertebrates, high food availability or favourable flow conditions would increase maximum feeding capacity and thereby increase the value of parameter *a,* daily food consumption. If *a* increase, zooid longevity would decrease. However, food availability would also affect the colony growth rate, which in turn affects parameter *b*, the time until zooid consumption is 0. An increase in food availability would either increase the 'self-shading effect', and thereby decrease *b*, or increase the local food abundancy which would increase *b*. An increase in *b*, would increase zooid longevity, whereas a decrease in *b* would do the opposite. All else being equal, the model would therefore predict that an increase in food availability would either increase or decrease zooid longevity, depending on which force, parameter *a* or either of the two components of parameter *b*, that has the highest impact. These effects are likely visible in seasonal changes, as both the abundance of food and the growth rates of colonies in the field typically increase in summer and decrease in winter (Underwood and Anderson 1994).

Given offspring size often affects colony growth in colonial marine invertebrates (including *Watersipora*), the model would predict that faster growing colonies from larger offspring would have either shorter or longer zooid longevity, also depending on the influence of parameter *a* and parameter *b*. Interestingly, Marshall and Monro (2013) found that for *Watersipora* colonies

in harsh environments, selection favoured individuals with lower senescence rates (and hence longer zooid longevity) if the individuals were large as offspring. This finding is in accordance with our model and suggest that such correlational selection is likely to be widespread in colonial organisms.

While there have been numerous studies of estimating leaf construction cost for plants (Baruch and Goldstein 1999a; Griffin 1994), we know of no previous studies that have estimated the construction cost of zooids. Here we calculated not only of the energy content the new zooid, but also, the metabolic costs associated with the construction of that zooid. Through our trials, we found that in producing a zooid, about 20% of the total cost was energy lost during the production process and the remaining 80% went into the final product of the new zooid. This additional cost of increased maintenance during the construction period is not considered when estimating leaf construction costs in plants, where energy content of the final leaf is the only consideration (Ackerly 1999; Baruch and Goldstein 1999b; Navas et al. 2003).If our results apply more generally, considering only the energy content of the module may underestimate its total construction cost.

Modularity evokes links between both plant and colonial marine species, and the possible links in theoretical assumptions have been considered for decades (Hughes 1984; McKinney and Jackson 1991; Rosen and Larwood 1979). Through the trials in this study, we conclude that there are enough similarities between plant traits and traits of colonial marine invertebrates for further investigations between the links between these fields. The timing of module senescence for both plants and colonial marine species respond to selective forces (Harvell and Grosberg 1988; Sköld and Obst 2011; Wingler et al. 2006), and our results indicate that the vast theories designed for plants can be translated to fit other modular organisms. We argue that the generalised conditions of the leaf optimality model that apply to leaves of plants, can be applied colonial marine invertebrates and possibly other modular organisms.

Tables

Table 1. Plant parameters for estimating optimal leaf longevity translated to parameters suitable for estimating optimal zooid longevity in marine invertebrates.

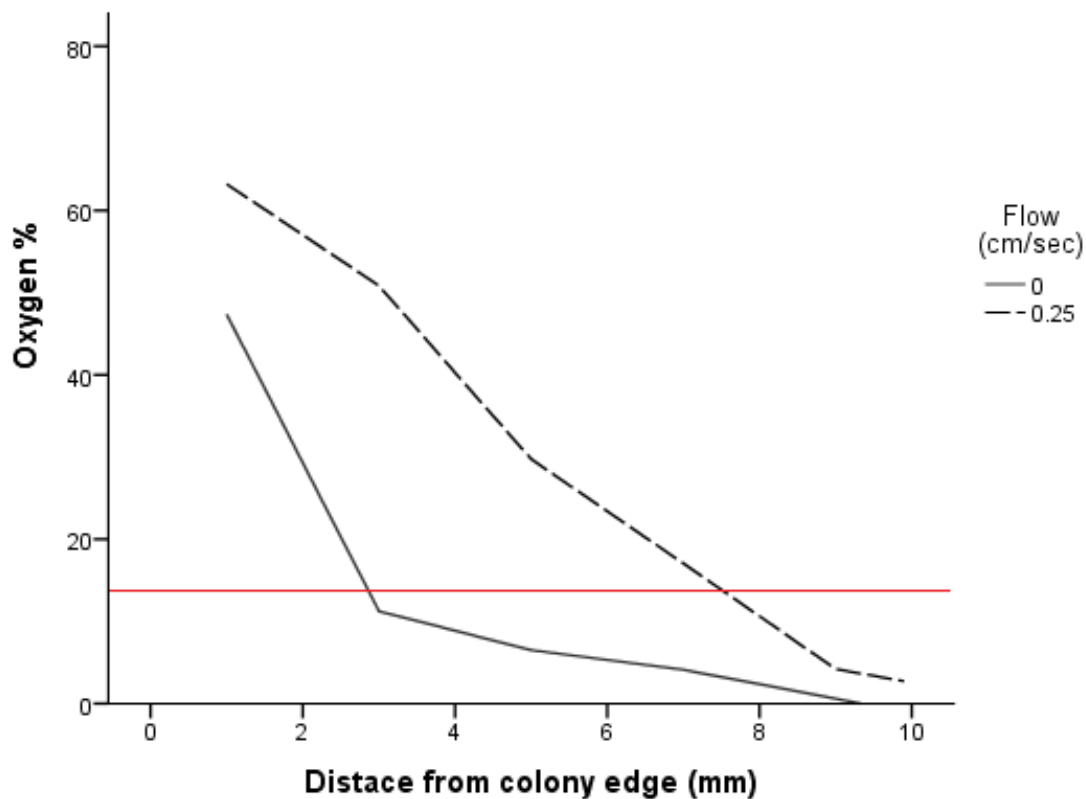|	Deciduous plants	Colonial invertebrates
a	Daily photosynthesis of a leaf	Daily food acquisition of a zooid
b	Leaf age when photosynthetic capacity $=0$	Zooid age when food assimilation $=0$
c	Construction cost of a leaf	Construction cost of a zooid

Figures

Figure 1. O_2 availability at increased distances (mm) from the colony edge to colony centre of *Watersipora* in the flow direction, at flow rates 0cm/sec (full line) and 0.25cm/sec (dashed line). In red, we show the critical O_2 limit (14% O_2) for marine sessile species with flat growth form (including *Watersipora*). The critical O_2 limit was reached 3mm from the edge in 0cm/sec flow, and 8mm from the edge at 0.25cm7sec flow. The O_2 saturation of the experimental basin was 100%.
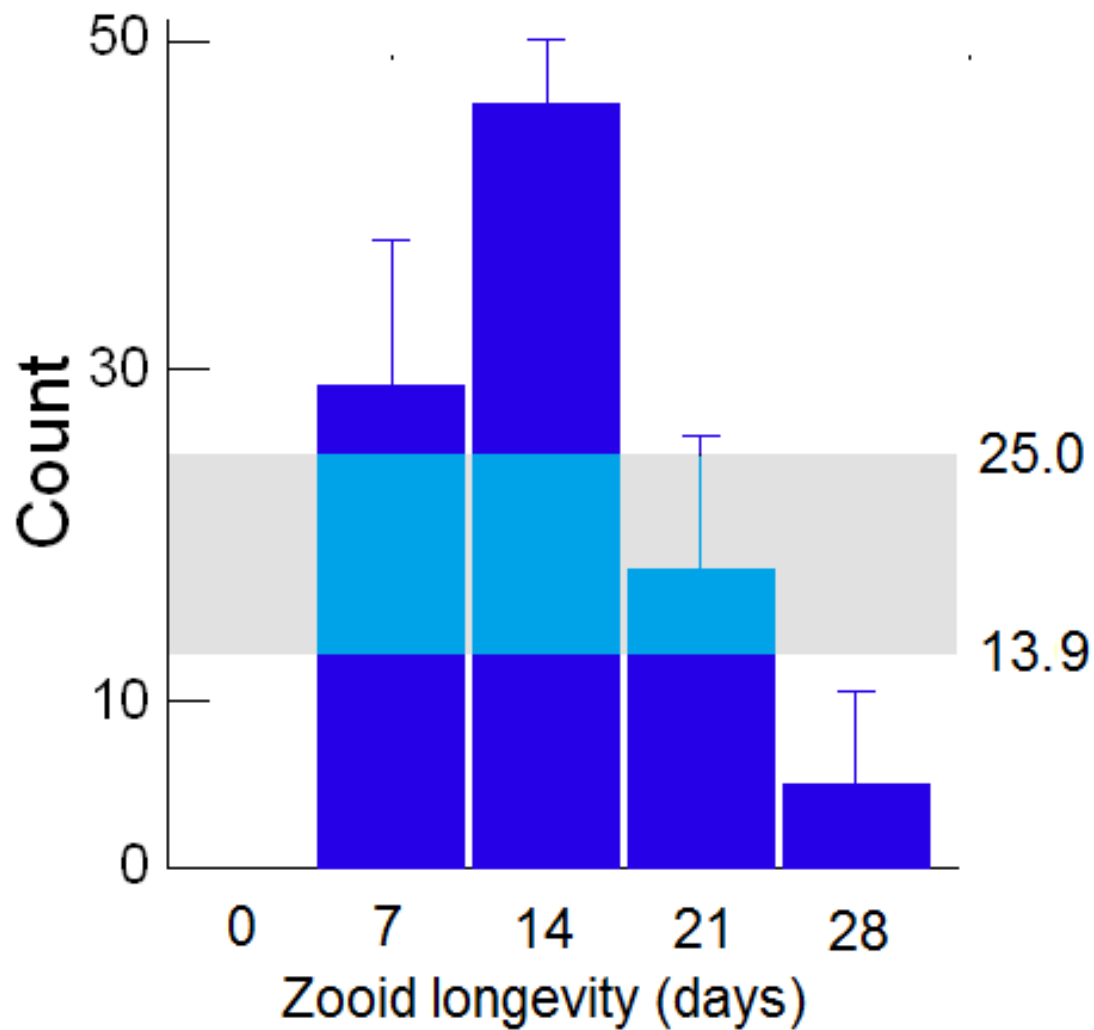
Figure 2. The distribution of observed longevity of zooids from *Watersipora* colonies in the field, collected from data by Svanfeldt et al. (2017). On average, zooid longevity of the field grown *Watersipora* colonies were 13.93 (±0.46) days. The grey box represent the range (13.9-25.0 days) of predicted zooid longevity, based on the optimal zooid longevity model.

Chapter six

Discussion

The life history of modular organisms is complicated, since it occurs simultaneously both at the level of modules and at the level of genets (Harvell and Grosberg 1988). Whereas plant biology offers abundant theory and empirical investigations to the field of modularity (Kikuzawa 1991b; Kitajima et al. 1997; Ackerly 1999; Kikuzawa and Ackerly 1999), the consequences of a modular life history, particularly on the level of modules, is much less understood in colonial animals (Hughes 2005). By the redistribution of resources, modular organisms possess the ability to maximize organismal fitness by altering module longevity, which is established to be the case for plants. Whether this strategy is widespread across modular organismal groups, is still not well resolved. However, I have through my chapters shown indications of that my study species, *Watersipora*, adapts strategies similar to those of modular plants in regards to energy distribution between modules to increase organismal fitness. *Watersipora* experiences variation in life history strategies, and in my previous chapters I have shown that some of this variation is due to the resource acquisition and allocation experienced by *Watersipora* colonies. Through my research, I have found that selection acts on life history traits of *Watersipora* and throughout the life history, variation in resource acquisition alters the strength and direction of selection on life history traits.

In Chapter 2, I found that the fitness consequences of maternal resource allocation per offspring depend on offspring experience. Whereas the current offspring size theory predicts that larger offspring with more resource reserves perform better than smaller offspring with lesser resource reserves (Smith and Fretwell 1974; McGinley et al. 1987), I

found that depending on offspring experience, the opposite may be true. If offspring experienced conditions with short dispersal periods and minimal resource depletions prior to settlement, larger offspring performed better as adults than smaller offspring. In conditions with extended dispersal periods and high energy depletions, though, smaller offspring performed better as adults than larger offspring. However, in these conditions, smaller offspring also experienced marginally higher offspring mortality rates. Regardless of underlying mechanisms, I showed that selection for maternal resource allocation per offspring (offspring size) is mediated by offspring experience.

 If larger larvae are preferred when dispersal distances are short, it is likely that mothers in preferable habitats with high resource availability produce large larvae, with intrinsic fast early growth (multiple initial buds). Just like modular plants in high resource environments, fast growth rates in these conditions would be beneficial to diminish the effects of 'self-shading' and to outcompete neighbours (Ackerly 1999). For plants, neighbours then would compete for sunlight (Grime and Hodgson 1987), whereas colonial marine invertebrates would experience similar competition pressure, but for the resource, space (Connell 1972; Jackson 1977; Benayahu and Loya 1981; Paine 1984). In poor resource conditions, mothers would instead benefit from producing many, small, offspring that once dispersed and settled likely will exhibit slower growth rates, but potentially in habitats with less competition pressure. Habitat quality, and spacing (Burgess et al. 2013), likely both affect the offspring size and number trade-off.

By adding resources to the *Watersipora* system (Chapter 3), competition effects were reduced and the positive effects of facilitation were revealed. I found that in low resource environments, density affected colony performance negatively. However, in environments where resource availabilities were high, I found mainly positive density-dependent effects on colony performance. These results suggest that resource availability mediates the relative strength of competition and facilitation in this system. Though density-dependent interactions

are most studied in plants, there are a number of studies examining the relationship of

conspecific density and performance in marine invertebrates (Bertness 1989; Leslie 2005b).

However, little is known of how resource-related stressors affect density-dependent

interactions (Maestre et al. 2009).

My results in Chapter 3 indicate that intraspecific facilitation may be more common than

currently appreciated, and that environmental variation in resource availability may mediate

the balance between negative and positive density-dependence. In my trials, I found that

Watersipora thrived in environments with low flow rate. According to previous studies, it is

apparent that though higher flow rates would have more food particles passing the feeding

apparatus, the food capturing success of bryozoans are higher in lower flow rates (Okamura

1988). In environments where flow was high, it is likely that the structure of neighbours

obstructed the direct flow, and thereby increased each other's capturing success, explaining

the positive density dependent interactions I observed.

In Chapter 4, I found that selection acts on zooid longevity, and that this selection is mediated

by resource availability and competition. Further, the strength and direction of selection on

zooid longevity in this chapter strengthens the suspicion that modular theory, designed for

plants, may also apply to sessile marine invertebrates. Though previous studies have shown

how longevity of zooids change in varying resource conditions (Bayer et al. 1994; Lange et

al. 2016), my trials in Chapter 4 are the first formal selection analyses examining the effects

of zooid longevity on colony fitness.

Though numerous studies are continuously performed to evaluate fitness consequences of

colonial marine organisms across environments, there is little focus on the modular-specific

strategies underlying the responses in fitness cues. In several plant species, seed mass is

positively correlated to leaf longevity (Seiwa and Kikuzawa 1991, 1996), and as described

earlier, in plants, leaf longevity is long in low nutrient environments, competition because

of nutrient retention (Grime and Hodgson 1987). Though my observations of zooid

longevity in Chapter 4 are not perfectly comparable to responses in plants, they still

indicate that similar forces of selection may act on both plants and colonial marine

invertebrates.

To further investigate the similarities of modular plants and colonial marine invertebrates, I

formally tested the applicability of plant theory on *Watersipora* in Chapter 5. By adapting a

model designed for leaves (Kikuzawa, 1991), I was able to make predictions on zooid

longevity. By estimating zooid parameters, analogous to those in the leaf optimality model, I

found that, by adapting the optimal leaf longevity model, I was able to estimate the lifetime

of Watersipora zooids, and that my estimations mirrored the zooid longevity of colonies in

the field. My results therefore support the hypothesis that plant theory could be applied to

other modular organisms, like colonial animals. Though my predictions in this chapter are

not perfect translations, and need complimentary refinements, they still provide a starting

point in connecting the various fields of modularity.

Modularity evokes links between the disciplines of both plant and colonial animal, and the

possible links in theoretical assumptions have been considered for decades (Rosen and

Larwood 1979; Hughes 1984; McKinney and Jackson 1991). Whereas numerous

characteristics unite modular organisms, it is still common with variation in module

morphology between species. Modules of some species, like the leaves of many perennial trees and zooids of encrusting bryozoans (including *Watersipora*), are highly isomorphic in their module composition. Other species, however, like thorny desert shrubs and erect bryozoans, have polymorphic modules, comprising of specialised modules for feeding, defense and reproduction (Harvell 1991; Halpern et al. 2007). This variations complicates the applicability of general theory. However, the complications are equal for both plants and colonial animals, and I argue that generalizations should therefore still be applied across modular organisms as a united group. However, though the life histories of plants and colonial animals are similar, there are differences between the systems, and there are limitations in applying models, such as the leaf longevity model, to marine invertebrate zooids. One such difference is the role of sexual reproduction in modules.

Whereas sexual reproduction in plants almost always occurs in modules of specialised function (flowers), it is common (though not exclusive) for sessile marine species that even if modules are isomorphic, their function may vary over time, where the same zooid have different roles throughout the zooid life time (Hart and Keough 2009). For *Watersipora,* colonies in the field show distinct bands of zooids with varying functions, starting from the oldest, senescing zooids in the colony centre, followed by bands of feeding and sexually reproducing zooids and finally a band of growing zooids at the colony edge (Lange et al. 2016). In contrast, a leaf will never experience sexual reproduction (though other plant modules, like shoots, will), and the fitness value of a leaf for the plant is solely dependent on energy acquisition (photosynthesis). Separately from leaf longevity, the longevity of flowers is estimated as a function of available resources allocated to sexual reproduction, the number of flowers produced, the maintenance cost of a flower, and construction cost of a flower (Ashman and Schoen 1994). According to the flower longevity model, the (Ashman and Schoen 1994). However, as opposed to flowers, isomorphic zooids of sessile marine species

like *Watersipora* are still autonomous and accumulate resources. Therefore, it would be inappropriate to estimate declines in zooid longevity with increased number of zooids. It is also worth noting that fitness for a sessile marine invertebrate include asexual as well as sexual reproduction. Whereas sexual reproduction is one strategy to increase fitness, clonal organisms in stable environments may also rely on asexual reproduction (Pedersen and Tuomi 1995). In turn, since every zooid (for most species) is able to sexually reproduce, asexual reproduction promotes increase sexual reproduction. Regardless, sexual reproduction does occur in sessile marine invertebrates, and should not be ignored.

There are unmistakably large gaps in our understanding of modularity and the implications of the resource economy underlying a modular life history. Though our current insights in the evolution and function of plants offers a great starting point, we may be missing crucial evidence by excluding other modular organisms, like colonial marine invertebrates, when investigating the evolution and functionality of modular species. It has been previously suggested that in medical science, colonial marine invertebrates are excellent model systems for ageing research, due to their clonal traits where somatic cells are seemingly totipotent and exhibit substantially superior regenerative abilities than their non-clonal counterparts (Bodnar 2009; Rinkevich and Matranga 2009; Sköld and Obst 2011). If possible, further advances in plant theory could be applied to the field of colonial marine invertebrates and other groups of modular organisms, which in turn could contribute to our understanding of the evolutionary ecology of ageing in both modular and solitary organisms.

Conclusions

Modularity is a common trait among both plant and colonial invertebrate species, yet the evolutionary ecology of clonal life history is hardly understood. For modular organisms, general theory and empirical investigations are mostly found on plants (Kikuzawa 1991b; Kitajima et al. 1997; Ackerly 1999; Kikuzawa and Ackerly 1999). In my four data chapters, I examined different aspects of the life history of a colonial marine invertebrate, with the aim to understand the implications of the colony, the zooids within the colony, and the external consequences of how resources affect life history traits at both levels. My studies imply that convergent evolutionary forces, similar to those of higher plants, act on my study species (and possibly other colonial marine invertebrates), and that evolutionary theory should cautiously be applied across modular organisms as a united group.

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