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JAMES COOK UNIVERSITY AUSTRALIA

The energetics of scleractinian coral larvae and implications for dispersal

Thesis submitted by

Erin Marie GRAHAM

BSc (Hons), James Cook University

in August 2012

For the degree of Doctor of Philosophy

in the School of Marine and Tropical Biology

James Cook University

Statement on the contribution of others

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I dedicate this thesis to my father, Ian Keith Graham.

Abstract

Dispersal is a key process in the ecology and evolution of species. For sessile marine invertebrates like corals, the larval stage is the only means of dispersal, making this stage fundamentally important. Factors governing the dispersal potential of scleractinian corals include oceanographic conditions and the length of time coral larvae spend in the plankton. For lecithotrophic, broadcast-spawned coral larvae, the pelagic larval duration (PLD) will largely depend on larval energetics; that is, the amount of maternally-derived energy reserves available and the rate at which these reserves are used. The overall aim of this thesis was to investigate the energetics of scleractinian coral larvae to enhance understanding of the dispersal potential of corals, knowledge that is necessary for the design of marine protected areas and other management strategies needed to protect coral reefs. I first established the temporal dynamics of larval energy use by quantifying temporal changes in lipid content and respiration rates throughout the larval phase for a range of species. Building on these findings, I quantified how the dynamics of larval energetics changed under different experimental temperatures, to improve understanding of how climate change may influence larval dispersal and coral population connectivity. Finally, I considered how larval energetics affected post-settlement survival and growth to gain insights into whether time spent in the plankton might reduce a coral's ability to contribute to post-settlement demography (i.e., "realized" dispersal).

Lecithotrophic marine invertebrate larvae generally have shorter PLDs than planktotrophic larvae. However, non-feeding coral larvae have larval durations far exceeding predictions based on their energetics, raising questions about how they achieve such longevity. In this thesis, I measured temporal changes in metabolic rates

and lipid content of larvae of four species of reef corals (*Goniastea aspera*, *Acropora tenuis*, *A. nasuta*, and *A. spathulata*) to determine whether changes in energy use through time contribute to their extended PLDs (Chapter 2). The temporal dynamics of both metabolic rates and lipid content were highly consistent among species. Metabolic rates pre-fertilization were low, and then increased rapidly during development to peak 1-2 days after spawning, when larvae began swimming. Rates then declined by up to two orders of magnitude over the following week, and remained low thereafter. Consistent with patterns in metabolic rates, lipid depletion was rapid during development, before slowing dramatically from about ten days onwards. Throughout this extended period of low metabolism, larvae continued to swim, complete metamorphosis, and showed no increase in mortality rates. The capacity of non-feeding coral larvae to enter a low-metabolism state soon after becoming competent significantly extends their dispersal potential, thereby accruing connectivity advantages typically associated with planktotrophy.

Temperature is an important environmental variable affecting the metabolism of ectothermic organisms. Predicted increases in sea-surface temperatures due to climate change are likely to alter the energy use of coral larvae and thus influence the dispersal potential of corals. Using a regression-based approach, I quantified the effect of five temperatures on the survival and energy use of *A. tenuis* larvae (Chapter 3). Temperature had a significant effect on larval survival, with increasing temperature leading to a monotonic increase in mortality rates. Contrary to my expectation that metabolic rates would increase with temperature, however, temperature had a parabolic effect on peak respiration rates and lipid use during development: rates declined as temperatures either increased above or decreased below ambient. Moreover, temperature did not appear to affect larvae during the extended period of low

metabolism. My results suggest that even small differences in temperature from ambient affect coral larval dispersal potential. In particular, increased metabolism associated with warming temperatures leads to faster development, which increases the potential for self-recruitment, while higher mortality decreases the proportion of a larval cohort that survives for longer dispersal distances. Thus, connectivity among coral populations, which critically underpins reef resilience, is likely to decline in the near future.

Demographic connectivity requires both the dispersal of individuals between sub-populations, and their subsequent contribution to population dynamics. For non-feeding marine larvae, the capacity to delay settlement enables greater dispersal distances, but the energetic cost of delayed settlement can adversely impact post-settlement fitness. Accordingly, I assessed whether delayed settlement influences either mortality rates or growth rates for the first six weeks following settlement of *A. tenuis* larvae (Chapter 4). Larvae that were settled at two, four, and six weeks after spawning, and then deployed in the field, showed negligible effects of delayed settlement on post-settlement survival and time to initial budding for colony formation. Between-cohort differences in budding rate were best explained by temporal variation in the post-settlement acquisition of zooxanthellae. The potential for coral larvae to remain in the pelagic zone for increased periods of time, with little or no effect on post-settlement survival and growth, suggests that the costs of delayed settlement are largely confined to those accrued during the larval phase itself. This indicates that larvae that successfully settle after extended periods in the plankton are likely to make meaningful demographic contributions to benthic dynamics. Thus the predicted trade-off between delayed settlement and post-settlement fitness appears to be less applicable to reef-building scleractinian corals than other taxa with non-feeding larvae.

In conclusion, my research has identified a potentially novel physiological attribute of coral larvae that offers an explanation for their exceptionally long larval durations compared to other non-feeding marine invertebrate larvae. Specifically, coral larvae enter a low-metabolism state soon after competence is acquired, and they are able to maintain this state for many weeks, even at temperatures several degrees above ambient. Consistent with this, coral larvae that successfully settle after spending more time in the plankton survive and grow at rates similar to those of corals spending less time in the plankton. Nevertheless, in a warmer world, coral connectivity will likely decline due to temperature-dependent increases in larval mortality and development. The broader implications of these findings for the long-term persistence of coral metapopulations under climate change will depend on the relative importance of local population maintenance, versus replenishment from other sub-populations.

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1 General Introduction

Dispersal is an important process in the ecology and evolution of species. Larval dispersal affects the distribution and abundance of organisms, enabling replenishment of populations, colonization of new habitats and the expansion of geographic ranges (Underwood and Fairweather 1989, Hanski 1999, Clobert et al. 2001, Gaston 2003). Patterns of dispersal also have important consequences for the genetic structure of populations, affecting gene flow and rates of speciation and extinction (Bohonak 1999, Weersing and Toonen 2009). Species with limited means for dispersal generally have greater genetic structure than species with high dispersal potential (Nishikawa et al. 2003). Therefore, understanding dispersal is essential to understanding a species' ecology and evolution. Just as importantly, understanding dispersal is essential for developing conservation and management strategies, particularly for the design of protected areas (Shanks et al. 2003) and for evaluating whether and how species can alter their geographical distributions in response to predicted changes in climate (Watkinson and Gill 2002). Despite its importance, there is considerable uncertainty about the extent to which local marine populations are self-seeded, compared to being maintained by recruits from nearby or distant habitats (Cowen et al. 2000). On coral reefs, this is normally expressed as a distinction between the extent to which recruitment consists of individuals recruiting back to the natal reef, compared to arriving from nearby or distant reefs.

Like most marine invertebrates, corals have a complex life cycle consisting of a benthic adult phase and a pelagic larval phase. Most corals are broadcast spawners, releasing their gametes into the water column where fertilization and embryogenesis occurs externally (Baird et al. 2009). Initially, coral larvae are in a pre-competent stage lasting between two and five days (Nozawa and Harrison 2005), in which rapid

morphological and physiological changes occur before they can become competent, or capable of settlement. This is followed by a competent stage, in which the larvae are capable of metamorphosing into juvenile corals to begin their sessile phase. Thus the dispersal potential of corals will depend on the onset and duration of competence, in addition to survival during the dispersal stage. The majority of a cohort will typically acquire competence and settle within 10 days after spawning in the presence of a suitable settlement surface (Connolly and Baird 2010). In the absence of the right cue, however, settlement can be delayed many months (Table 1.1).

The poor swimming capacity of coral larvae and their dependence on oceanographic processes for dispersal (Chia et al. 1984, Willis and Oliver 1990) highlight their need for adequate energy reserves to survive unpredictable planktonic durations and remain competent to settle. Accordingly, for broadcast-spawned corals, one of the most important biological characteristics affecting how long this competence period lasts is that larvae are lecithotrophic, or non-feeding. While the larvae of a few species of corals inherit symbiotic photosynthesizing dinoflagellates, or zooxanthellae, from their parents and are capable of supplementing their energy reserves, most larvae derive all of their energetic requirements from the yolk, which is inherently limited (Baird et al. 2009). Both survival and the duration of the competent period will be constrained by the amount of energy available in the egg and the rate at which this energy is expended, which makes understanding the energetics of competence and survival essential for understanding dispersal. Despite being lecithotrophic, coral larvae have remarkably long larval durations (Table 1.1). Larvae of some coral species are competent to settle for at least 100 days and several others are capable of surviving for at least 200 days (Graham et al. 2008, Connolly and Baird 2010). Moreover, because very few studies maintain larvae and conduct settlement assays for extended periods,

the total number of coral species capable of long larval lifespans may be considerable. Solving the puzzle of how coral larvae with limited energy reserves can have such long larval durations is therefore necessary to be able to make accurate predictions of dispersal potential.

To date, there has been only one attempt to understand the length of the competent period based on an energetic approach. In the late 1980's, Richmond (1987) measured the energy content and respiration rates of one species of brooding coral, *Pocillopora damicornis*, and used a simple model to predict the duration of the competent period:

$$C_p = \frac{2}{3} E_c (O_c \times T_e)^{-1}$$

where C_p = potential competency in days, E_c = energy content of the larva, O_c = net oxygen consumption rate in mg oxygen d^{-1}, T_e = energetic equivalency in joules used mg^{-1} oxygen consumed, and 2/3 is a constant for the amount of body mass Richmond believed was required for successful settlement. The main assumptions of Richmond's model were 1) that *P. damicornis* planulae consisted of 17% protein, 70% lipid and 13% carbohydrate by weight; 2) these components were utilized at same rate in proportion to their occurrence within the larvae; and 3) larvae were competent until they reached 1/3 of their original size. Richmond (1988) later measured the respiration rate of *Acropora tenuis* larvae and used his model to predict the dispersal potential of this broadcast-spawned species. Assuming *A. tenuis* had the same tissue energy content as *P. damicornis*, Richmond's model predicted a 20 d competent period for *A. tenuis*. By removing the size restriction for successful settlement, an upper bound estimate of 30 d can be made for larval longevity – this is the length of time predicted by the equation above for all of a coral larva's initial energy content to be consumed. The

model estimate is substantially less than empirical observations (69 d, *A. tenuis*, Nishikawa and Sakai 2003) and suggests a need to revisit our understanding of larval energetics. In particular, there is a need to assess a number of larval physiological parameters that may contribute to this discrepancy, including temporal variation in biochemical composition of larvae; rates at which biochemical components are used; the capacity to supplement their energetic reserves; the capacity to regulate their energy expenditure; or some combination of these parameters. Empirically evaluating which of these alternatives contribute to coral larval energetics will improve our ability to make more accurate estimates of dispersal potential.

Once competent, the length of time a larva can maintain this state will depend on its energy reserves. This is particularly true for lecithotrophic larvae, which are unable to supplement their endogenous reserves during the dispersal phase by feeding. Energy must be available for development, dispersal, and metamorphosis. A newly settled juvenile will then rely on its endogenous reserves until it develops into a self-sufficient coral with tentacles for heterotrophy and zooxanthellae for autotrophy. Long periods of time spent in the plankton should mean less energy remains for these other needs associated with metamorphosis and settlement and could have flow-on effects on the success of coral larvae post-settlement (e.g., Wendt 1998, Maldonado and Young 1999, Onituska et al. 2010). The potential for decreased growth and increased mortality after settlement should therefore increase for larvae with longer pelagic durations compared to larvae that settle immediately. This implies there may be an energetic threshold associated with the loss of competence, the onset of larval senescence, or successful settlement, which places an upper bound on dispersal potential.

Ultimately, a better understanding of the energetics of coral larvae is essential for determining how reefs might cope with climate change. Temperature is one of the

most important environmental determinants of metabolic rate (Gillooly et al. 2001), particularly for those organisms that are unable to regulate their own body temperatures. Sessile marine invertebrates are exposed to continual fluxes in temperature that they are unable to avoid, and temperature is known to affect the physiology of marine species by increasing development and mortality rates and influencing rates of settlement (e.g., Negri et al. 2007, Nozawa and Harrison 2007, Randall and Szmant 2009a). Thus, temperature will likely be particularly important in causing variation in energy use patterns, competence dynamics, and survival in nature. Moreover, understanding how temperature affects physiological factors governing dispersal potential is relevant for understanding effects of climate change on population dynamics (O'Connor et al. 2007). For example, increased temperatures may substantially increase or decrease the number of larvae settling locally, as well as decrease the number of successful long distance dispersers. If increased temperatures lead to increased energy use, then larval durations are likely to decrease. Given the increases in sea-surface temperature predicted to occur within the next few decades, temperature will play an important role in determining which species are able to cope with climate change (IPCC 2007).

The overall aim of my thesis is to investigate patterns of energy use of coral larvae to increase our understanding of the dispersal potential of scleractinian corals and the ecological implications of dispersal. In Chapter 2, I quantify temporal changes in larval lipid content and metabolic rates in order to reconcile the differences between the (short) pelagic larval durations estimated with Richmond's competence model and the (much greater) empirical observations made in recent studies of scleractinian coral larvae. I show that energy use of coral larvae is highest during early development and remains high until larvae become competent to settle, after which larvae enter a state of

reduced metabolic rates which minimizes energy use and is maintained for as long as two months. These findings indicate that estimates of oxygen consumption taken early in the larval duration will lead to over-estimates of energy utilization over the entire lifespan. This temporal change in energy use offers a potential explanation for the discrepancy between Richmond's calculations and long larval lifespans documented empirically. Chapter 2 combines data from studies of different species in two years that were originally planned as two separate chapters. However, to minimize repetition in introduction and methods sections, I have combined them into a single chapter.

In Chapter 3, I examine the effect of temperature on the energetics of *Acropora tenuis* larvae in order to quantify the effect of temperature on survival, respiration rates, and lipid utilization. Most research in this area is designed to determine whether there are differences in survival or energy use among a small set of fixed temperatures; very few studies have actually tried to quantify the functional response of these variables to temperature. My results indicate that there are two types of responses to temperature elicited in coral larvae: 1) a monotonic response in the survival of larvae, where increased temperatures lead to progressively increasing mortality; and 2) a parabolic response in energy use, where respiration rates and lipid utilization appear to be maximized at ambient temperature at the time of spawning and performance decreases as temperatures move away from ambient.

In Chapter 4, I test whether *A. tenuis* larvae that delay settlement for up to six weeks suffer post-settlement costs that would reduce "realized dispersal" – the extent to which larvae dispersing long distances actually contribute to post-settlement demography –compared to predictions of "potential dispersal" based on survival and competence dynamics. Although Chapter 2 indicates that coral larvae have enormous potential for dispersal given their extended competence periods and longevities, energy

expended during dispersal means less energy is available to complete metamorphosis, survive, and grow. I find that larvae that delay settlement for up to six weeks after spawning (five weeks after competence is acquired) do not suffer higher mortality, nor experience any decrease in growth, compared to larvae settling just two weeks after spawning. In fact, my data suggest that the acquisition of zooxanthellae is more important to the growth of coral juveniles than the length of the larval phase. This is consistent with the low energy expenditure and limited lipid depletion revealed by my study of the temporal dynamics of larval energy use, as documented in Chapter 2. My results indicate that coral larvae are equipped with ample energy reserves to survive for extended periods in the plankton, and suggest that the realized dispersal of coral larvae may be higher than predicted based on studies of other non-feeding marine invertebrates.

In Chapter 5, I summarise the outcomes of the research described in the thesis, assess its implications for understanding the ecology, evolution, conservation and management of corals and coral reefs, and suggest some productive directions for future research. In particular, my thesis shows that high dispersal potential of scleractinian coral larvae is due to the reduction in energy use that occurs during an extended period of reduced metabolism soon after larvae acquire competence. Significantly, this reduction in energy use allows coral larvae to delay settlement for up to six weeks after spawning without suffering heightened mortality or reduced post-settlement growth. Although increased temperatures lead to monotonically increasing mortality in coral larvae, respiration rates and lipid use decrease at temperatures on either side of ambient. These findings highlight the extraordinary dispersal potential of coral larvae and help to explain the large geographic range sizes of most broadcast-spawning species. They indicate that, although coral larvae have the means (energy reserves) to colonize areas outside of their

present day boundaries in response to increases in sea surface temperatures, they will suffer increased mortality. Further work is needed, however, to determine the mechanism of temperature-dependent mortality in coral larvae because energy stores are not limited and therefore it is unlikely that larvae are dying of starvation. Further work is necessary to determine if coral larvae utilize other sources of energy, such as protein reserves and/or dissolved organic matter absorbed directly from seawater. Quantifying the minimum amount of energy required for a coral larva to be able to metamorphose, and how much energy is used during metamorphosis, would also help place an upper limit on dispersal potential.

Table 1.1 Summary of the maximum length of the competent stage and longevity for a range of scleractinian broadcast spawning species (updated from Graham et al. 2008).

Species	Maximum Competence (d)	Maximum Longevity (d)	Reference
Goniastrea aspera	123	215	Graham 2007, Graham et al 2008
Platygyra daedalea	105	124	Nozawa and Harrison 2000
Acropora valida	90	130	Baird 1998, 2001
Acanthastrea lordhowensis	78	78	Wilson and Harrison 1998
Acropora tenuis	69	69	Nishikawa and Sakai 2003
Montastrea magnistellata	67	244	Graham 2007, Graham et al 2008
Favites chinensis	63	63	Nozawa and Harrison 2005
Acropora millepora	60	110	Baird 1998, 2001
Goniastrea australiensis	56	56	Wilson and Harrison 1998
Acropora digitifera	54	45	Nishikawa and Sakai 2005
Acropora latistella	47	209	Graham 2007, Graham et al 2008
Pectinia paeonia		209	Graham et al 2008
Favia pallida		195	Graham et al 2008
Acropora gemmifera		90	Baird 2001
Goniastrea retiformis		90	Baird 2001

2 Rapid declines in metabolism explain extended coral larval longevity

2.1 Introduction

Marine invertebrate larvae can be broadly classified into two categories, lecithotrophs or planktotrophs, depending on their source of nutrition during development. Planktotrophic larvae require external food sources to complete development, whereas lecithotrophic larvae are capable of completing development based solely on maternal provisions (Thorson 1950). The potential to feed should enable planktotrophic larvae to survive longer in the plankton (Scheltema 1986), and there are numerous examples of planktotrophic larvae that spend more time in the plankton than closely related species with lecithotrophic development (e.g. Emlet et al. 1987, Kempf and Todd 1989, Shanks et al. 2003). The longer PLDs of planktotrophic larvae are thought to confer greater dispersal potential for species with such larvae, enabling higher levels of gene flow over larger areas and potentially larger geographic ranges compared to species with non-feeding larvae (Jablonski and Lutz 1983, Pechenik 1999). Relationships between PLD, genetic population structure and range size have been documented for echinoids (Hunt 1993, Emlet 1995), gastropods (Hoskin 1997, Collin 2003, Paulay and Meyer 2006), and various other invertebrates (Foggo et al. 2007, Selkoe and Toonen 2011). However, the role of PLDs in driving such relationships is not always clear (Weersing and Toonen 2009), because population connectivity depends on many factors, including post-settlement processes (Marshall et al. 2010), that may obscure the role of larval duration alone.

In reef-building scleractinian corals, larval development mode is generally a good predictor of patterns of dispersal and connectivity. Populations of brooding

species, whose larvae are ready to settle on release, typically have higher genetic structure than broadcast spawning species, whose larvae have an obligate planktonic period of 2-4 days (e.g., Hellberg 1996, Nishikawa et al. 2003). Corals, along with some high-latitude echinoderm taxa, are the only groups with non-feeding larvae for which extremely long PLDs have been documented (Birkeland et al. 1971, Hartnoll 1975, Sebens 1983, Bosch and Pearse 1990, Bryan 2004, Graham et al. 2008, Connolly and Baird 2010). For example, coral larvae can survive up to 200 days (Graham et al. 2008), and can complete metamorphosis up to at least 100 days after spawning (Connolly and Baird 2010). These examples indicate that species with lecithotrophic larvae, including many coral species, have developed strategies to extend larval duration, and thus accrue the advantages of dispersal traditionally associated with planktotrophy.

If lecithotrophic larvae are to survive long periods in the plankton, they must possess a large supply of stored energy, have low metabolic rates, or be able to supplement their endogenous reserves. Large initial energy stores, slow development rates, and low rates of metabolism in Antarctic echinoderms imply that these larvae can persist for up to five years (Shilling and Manahan 1994). For scleractinian corals, a few species equip their propagules with photosynthetic symbionts (zooxanthellae), which may provide energy to larvae during dispersal and support PLDs over 100 days (Richmond 1987, Harii et al. 2010). However, most (>75%) coral species have larvae that lack such symbionts (Baird et al. 2009), yet even larvae of these species have exceedingly long PLDs. In *Acropora tenuis* larvae, initial energy content and metabolic rates observed during the first few weeks after fertilization imply larval longevities of only ~30 days (Richmond 1987, Graham et al. 2008). This estimate is less than half the 69 days observed for this species (Nishikawa et al. 2003), and many months less than

larval longevities reported for other *Acropora* species (Graham et al. 2008, Connolly and Baird 2010). Similar discrepancies between energy reserves, metabolic rates, and observed larval durations have been found in echinoderms (Bryan 2004). This suggests that corals and at least some other lecithotrophic larvae must either reduce their metabolic rates substantially as they age, take up additional energy (e.g., by absorption of dissolved organic matter (DOM)), or combine elements of both of these strategies. Although there is some evidence for uptake of DOM in soft corals (Ben-David-Zaslow and Y. Benayahu 2000), it has not, to date, been documented in scleractinian corals. Similarly, knowledge of metabolic rates for coral larvae is limited. Temporal changes in lipids over the first month after fertilization in one species suggest a slowing in the depletion of lipids after the first week, although lipids were still declining relatively rapidly towards the end of the study (e.g., by about 25% in the final week) (Harii et al. 2007). Consistent with this, Okubo and others (Okubo et al. 2008) found respiration rates of *Acropora intermedia* larvae declined to about one-third of peak values by seven days after spawning. These studies indicate that metabolic rates can decrease as coral larvae age, although these changes do not appear to be of sufficient magnitude to account for the extended PLDs documented in coral larvae.

Determining whether long PLDs are widespread among coral species with lecithotrophic larvae, and understanding how these PLDs are attained, is critical to estimating the dispersal potential of this dominant group of reef builders. Such knowledge has important implications for understanding the ecology, evolution, and biogeography of corals and for anticipating how dispersal potential and population connectivity may be impacted by changing ocean conditions that have implications for metabolic rate, particularly increased seawater temperature. Therefore, in this chapter, I investigated physiological mechanisms underpinning extended PLDs in scleractinian

corals with non-zooxanthellate, lecithotrophic larvae, by quantifying respiration rates and energy use over larval lifespans. I found strong evidence in both respiration rates and lipid levels for a rapid decline in rates of energy use within approximately one week of spawning, even though larvae are still capable of settlement. I conclude that decreased metabolic rates (i.e, hypometabolism) allow non-feeding coral larvae to extend larval life by minimising depletion of their energy reserves.

2.2 Materials and methods

2.2.1 Study site and larval cultures

The study took place at Orpheus Island, Australia, in December 2008, and November and December 2009. Gametes from a total of four broadcast spawning scleractinian species whose larvae lack zooxanthellae (*Goniastrea aspera*, *Acropora tenuis*, *A. nasuta*, and *A. spathulata)*, were collected and cultured using established methods (Willis et al. 1997). Four to six adult colonies of each species were collected immediately prior to anticipated spawning dates and brought onshore. For each species, gametes were collected within an hour of release and combined. Once fertilized, developing embryos were transferred to 500 L fibreglass aquaria with flow through 0.2 µm filtered seawater (FSW) and continuous aeration, one tank per species. The aquaria were maintained in temperature controlled rooms at near-ambient temperature (27 +/- 1°C) and a 12 h light:dark cycle.

2.2.2 Sampling design

At regular sampling intervals, subsamples of eggs, embryos, or larvae (hereafter "propagules") were randomly selected, for each species, and used for respiration measurements and lipid analysis. The first sample was taken from newly released

gametes, prior to fertilization. For respiration measurements, five replicates of 50 propagules were used. For lipid analysis of each *Acropora* species, the same 50 propagules used to measure respiration rates were subsequently frozen and used for lipid analysis. However, for lipid analysis of *G. aspera* propagules, due to their smaller size, three replicates of 600 propagules were used. Sampling took place every 12 h for the first 36-48 h to capture larval development through embryogenesis to a swimming larva, followed by daily sampling until the majority of larvae were competent to settle at 5 DAS. From 5 DAS, sampling was further reduced to every three days, unless low numbers of surviving larvae forced a further reduction to weekly sampling. Survival experiments and settlement assays, described in detail below, were conducted at each sampling point after larvae began swimming to determine whether patterns in energy use affected larval mortality rates or the larvae's capability to metamorphose.

2.2.3 Respirometry

To measure egg, embryo, and larval respiration rates, a temperature compensated, fiber-optic oxygen meter called the Fibox was used (PreSens GmbH). Respiration chambers were custom made, with each chamber consisting of a 1.5 ml glass vial integrated with a 5 mm diameter oxygen sensitive sensor foil spot on the inside of the chamber. Prior to each experiment, the respirometer was calibrated using sodium dithionite ($Na_2S_2O_4$) and air-saturated FSW for a two-point (0, 100%) calibration following the manufacturer's instructions. A sterilized miniature magnet was placed inside each chamber and magnetically stirred to ensure adequate mixing. For each of the five replicates of each species, 50 propagules were counted into the chamber and topped with fresh 0.2 µm FSW. The change in oxygen concentration in the chamber was measured for 5 min. Following each replicate, the chamber was flushed and refilled with fresh FSW, and oxygen measurements were taken of the individual-

free seawater for an additional 5 min to serve as a control. Oxygen consumption was calculated as the slope of oxygen concentration over the 5 min measuring period, and then converted into nmol O_2 $larva^{-1}$ $hour^{-1}$.

There is no *a priori* theory that predicts a particular functional form to describe how oxygen consumption should change with DAS. Moreover, initial plots of oxygen consumption rate as a function of DAS suggested a complex nonlinear relationship between the two variables. A log transformation of the observed values improved the homogeneity of variances, but the underlying relationship remained highly nonlinear. Therefore, to analyse the change in oxygen consumption rate, a nonparametric generalized additive model (GAM) was fitted to the log-transformed data. GAM uses a locally-weighted smoothing function to characterize arbitrary nonlinear relationships between the response (respiration rate) and predictor (DAS) variables (Zuur et al. 2009). GAM was implemented using the mgcv package in R (Wood 1994).

2.2.4 Lipid analysis

To measure the total amount of lipid in each sample, a TLC-FID detection system was used (Iatroscan MK-5). Lipids were extracted using a modified Bligh-Dyer chloroform:methanol method, with an internal standard added to provide an estimate of lipid recovery (Sewell 2005). Two developments were used to separate the lipid sample into the following classes: aliphatic hydrocarbons, wax esters (WE), triacylglycerides (TG), free fatty acids, free aliphatic alcohols, cholesterols (ST), and phospholipids (PL) (Parrish 1999). The lipid classes were then divided into two groups -- energetic lipids (WE and TG) and structural lipids (ST and PL) -- and analysed separately. Since energetic lipids are most likely to be available for maintenance of metabolism during the larval phase, I present the energetic lipids in the results. However, I also assess the extent to which qualitative trends in total lipids reflect those of energetic lipids.

Like the respiration data, there is no *a priori* reason for favouring a particular mathematical function to describe lipid depletion over time. Moreover, visual inspection of lipid data revealed a complex nonlinear relationship with time. Therefore, after applying a square-root transformation to the lipid data (to homogenize variances), a GAM was used to characterize the nonlinearity in the change in lipid levels over time. My approach was similar to that described above for respiration. However, because coral larvae are non-feeding and lack zooxanthellae, I also applied a monotonicity constraint to the GAM (i.e., I constrained fitted lipid levels to decrease over time, as per Wood 1994). This helped to avoid over-fitting of the model, and yielded narrower confidence intervals than an unconstrained fit. I obtained 95% confidence intervals for this constrained GAM fit by bootstrapping residuals (Efron and Tibshirani 1993).

2.2.5 Survival

To determine if larval survival was affected by energy use, once swimming larvae had developed, I set up five replicate 70 ml specimen jars containing 0.2 µm FSW and 100 larvae each. At each sampling time, the number of surviving larvae was recorded and larvae were transferred into new specimen jars. Because coral larvae typically lyse within 24 hours of death, there was no need to distinguish between live and dead larvae, i.e., the larvae remaining at each interval were assumed alive (Baird et al. 2006). A Kaplan-Meier product-limit analysis was used to obtain nonparametric estimates of the median survival time and 95% confidence intervals around this estimate for each species.

2.2.6 Settlement assays

To determine the onset of competence and whether this capability was maintained throughout larval duration, at each sampling point following the onset of swimming, a subsample of 120 larvae was placed into a 6-well plate. Twenty larvae

were introduced into each well containing 0.2 µm FSW and a small piece of crustose coralline algae, a known settlement inducer for *Acropora* species (Morse et al. 1996). After 24 h, I recorded whether or not successful metamorphosis had occurred.

2.3 Results

2.3.1 Respirometry

Qualitative patterns in respiration rates through time were highly consistent among all four study species (Figure 2.1). Oxygen consumption increased from very low levels in unfertilized eggs to a peak 1-2 days after spawning (DAS). Peak oxygen consumption coincided with the onset of larval motility (Figure 2.1, vertical dashed lines). After a week, oxygen consumption had fallen substantially, reaching levels similar to those of unfertilized eggs, and remained low until the conclusion of the experiments (up to 60 days later). Of the four study species, *Acropora tenuis* exhibited the most pronounced peak in oxygen consumption; respiration rates fell by approximately two orders of magnitude over the week following peak respiration (Figure 2.1B). In contrast, *A. spathulata* exhibited the smallest change, with an approximately two-fold decline from peak levels over approximately two weeks (Figure 2.1A, D). Unique among the four species, there was a delay until approximately 12 h after fertilization before larval respiration rates of *A. nasuta* began to increase, but otherwise its overall pattern of oxygen consumption was very similar to those of the other species (Figure 2.1C).

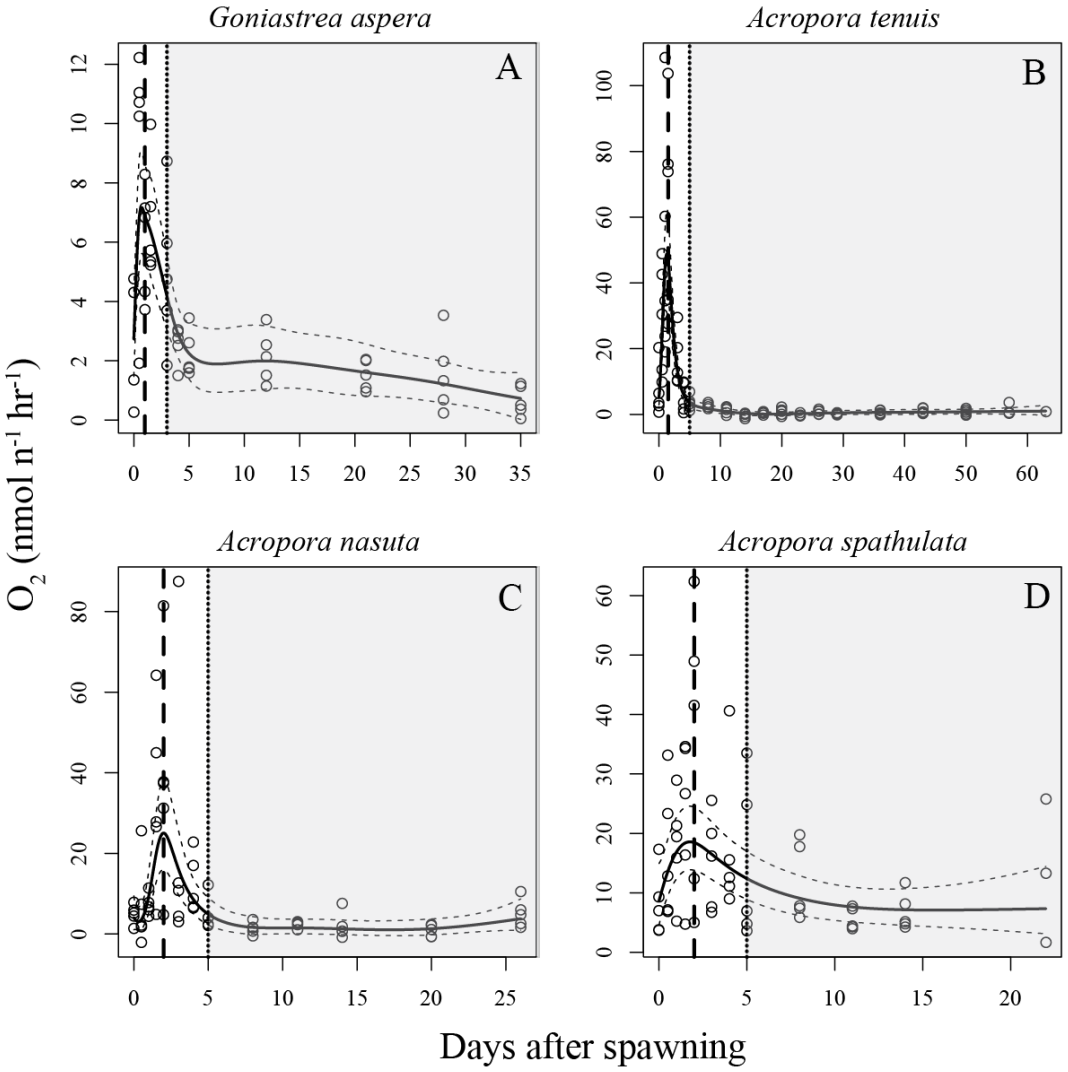

Figure 2.1 Rates of oxygen consumption through time in four scleractinian coral species. Each open circle represents one replicate measurement. Solid lines represent fitted mean respiration rates from the GAM. Dashed lines show upper and lower 95% confidence intervals on the fitted GAM values. Fitted values and confidence intervals were obtained on the log-scale (on which the analysis was conducted), and have been back-transformed to the arithmetic scale for plotting. Two vertical lines show developmental stage; a dashed line for time to swim and a dotted line for the time larvae first become competent to settle. Shaded areas indicate sampling times when settlement was observed (i.e, larvae were competent).

2.3.2 Lipid

Total lipids consisted overwhelmingly of energy lipids, and these two quantities exhibited quantitatively very similar temporal dynamics (Figure A.1 in Appendix A), so energetic lipids only were used for the analysis. Consistent with the trends found for respiration rates, larvae of all four species exhibited qualitatively similar patterns of energy lipid depletion (Figure 2.2). Initial lipid levels declined rapidly through embryogenesis and development approximately until larvae became competent (i.e, capable of metamorphosis) (Figure 2.2, vertical dotted line). Subsequently, energy lipid levels declined very slowly throughout the remainder of the experiment. In contrast to the three other species, lipid levels in *A. nasuta* larvae remained high for the first 12-24 h after fertilization, before declining rapidly (Figure 2.2C), consistent with the delayed increase in metabolic rates observed for this species (Figure 2.1C). Of the acroporids, *Acropora tenuis* larvae had the greatest initial decline in energy lipid levels, with an approximately three-fold reduction occurring in the first week (Figure 2.2B), consistent with its greater decline in metabolic rates after swimming commenced (Figure 2.1B). In contrast, *A. spathulata* exhibited the smallest decline in lipid levels, decreasing by only two-fold in the first week (Figure 2.2D), consistent with its smaller decline in metabolic rates (Figure 2.1D). Once competence was acquired, larvae from all species remained capable of settlement at every sampling point over the remainder of the experiment (Figure 2.1, Figure 2.2, shaded areas).

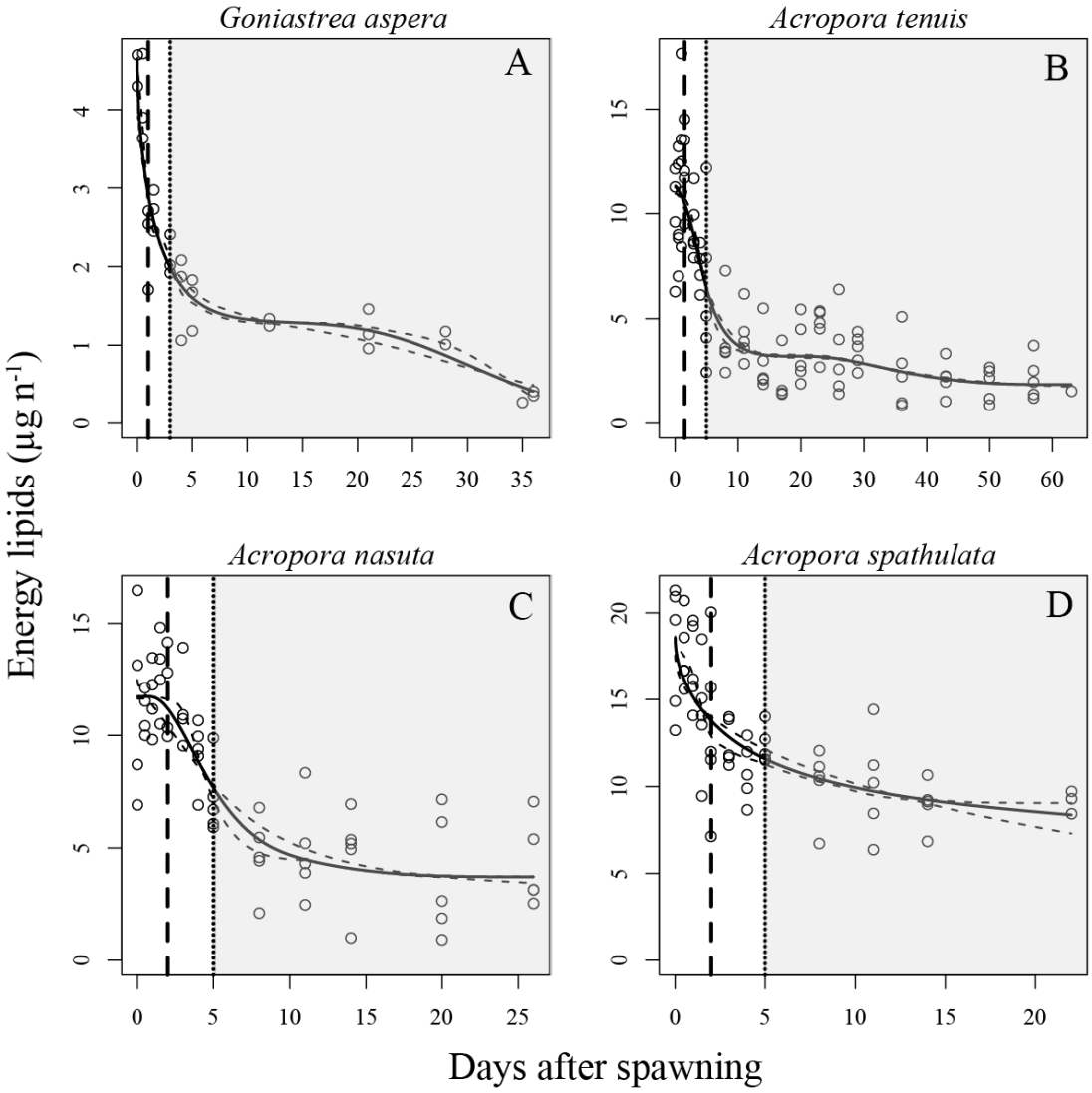

Figure 2.2 Depletion of energy lipids through time in four scleractinian coral species. Each open circle represents one replicate measurement. Solid lines represent mean respiration rates, and dashed lines show upper and lower 95% confidence intervals. Means and confidence intervals were obtained from the GAM fits by back-transforming from the log-scale (on which fits were made) to the arithmetic scale (for plotting). Two vertical lines show developmental stage: a dashed line for time to swim, and a dotted line for the time larvae first become competent to settle. Shaded areas indicate sampling times when settlement was observed (i.e, larvae were competent).

2.3.3 Survival

Survival times varied among species, with estimated median lifetimes ranging from 4 d for *A. spathulata* to 57 d for *A. tenuis* (Figure 2.3). A median lifetime for *G.aspera* was not estimable, due to the very high survival in this species (>98% after 35 d; Figure 2.3A). Mortality rates also varied, but in most cases, increased mortality did not occur until after larvae were competent to settle (slope of the lines in Figure 2.3, shaded areas). The exception was *A. spathulata*, whose survival decreased the most between the onset of swimming and the acquisition of competence (vertical dashed and dotted lines, Figure 2.3D). For each species, surviving larvae remained at the conclusion of the experiment.

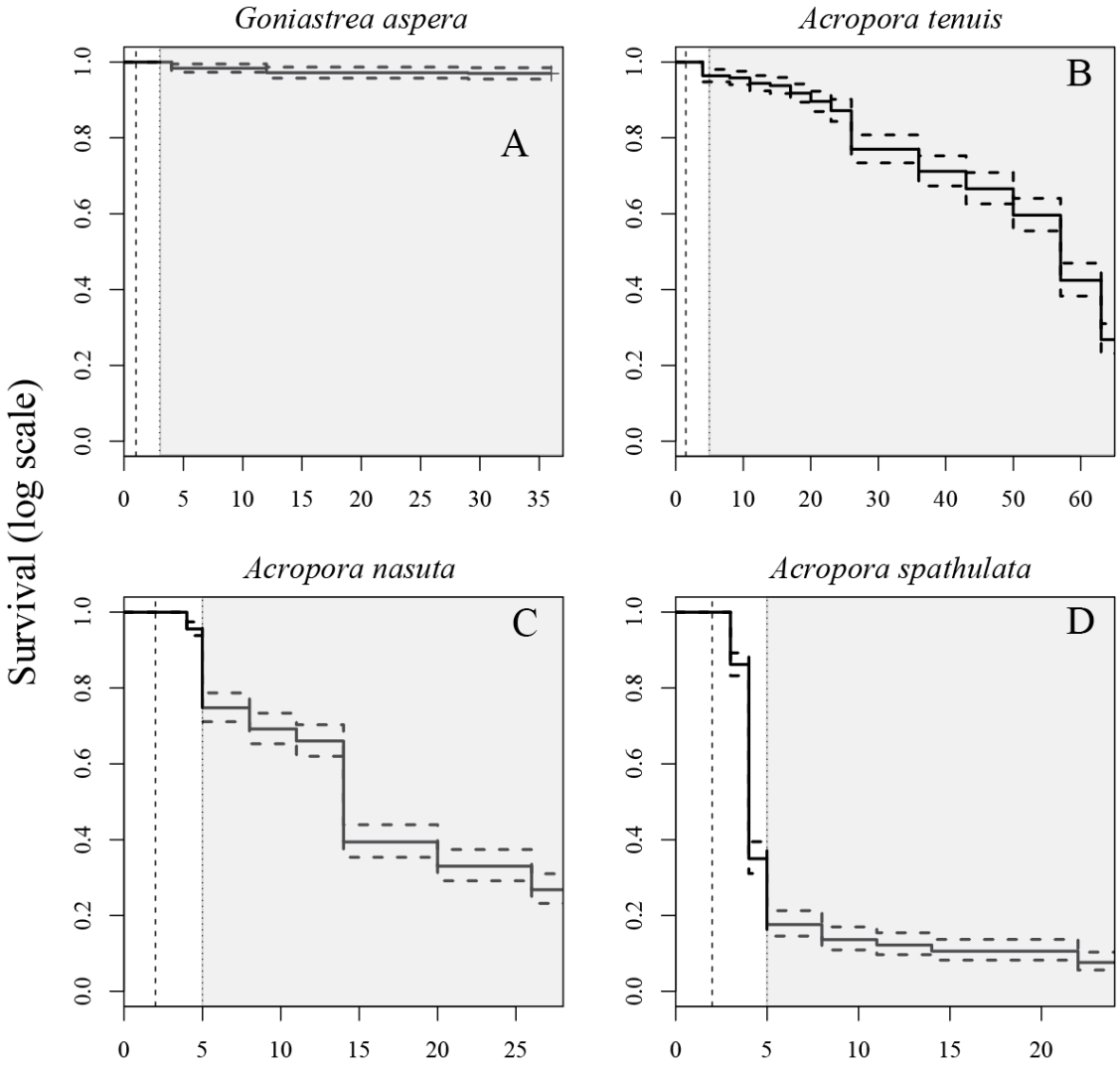

Figure 2.3 Kaplan-Meier survival estimates for four scleractinian coral species. Solid lines represent median estimates; dashed lines show upper and lower 95% confidence intervals. Vertical dashed lines indicate when larvae began swimming; vertical dotted lines when larvae acquired competence to settle, and gray shaded areas indicate when settlement was observed. Note the different scales on the y-axes.

2.3.4 Settlement

Once competence was acquired, larvae from all species maintained competence to settle throughout the duration of the study (Figure 2.4). The proportion of competent larvae was highest for *A. nasuta* and *A. spathulata*, with the majority of the larvae of these two species acquiring competence 8-14 days after spawning (Figure 2.4C, D). On the other hand, the proportion of *G. aspera* and *A. tenuis* larvae that had acquired competence never reached more than 50%, but a relatively large proportion of larvae was capable of settlement at the conclusion of the experiment (Figure 2.4A, B).

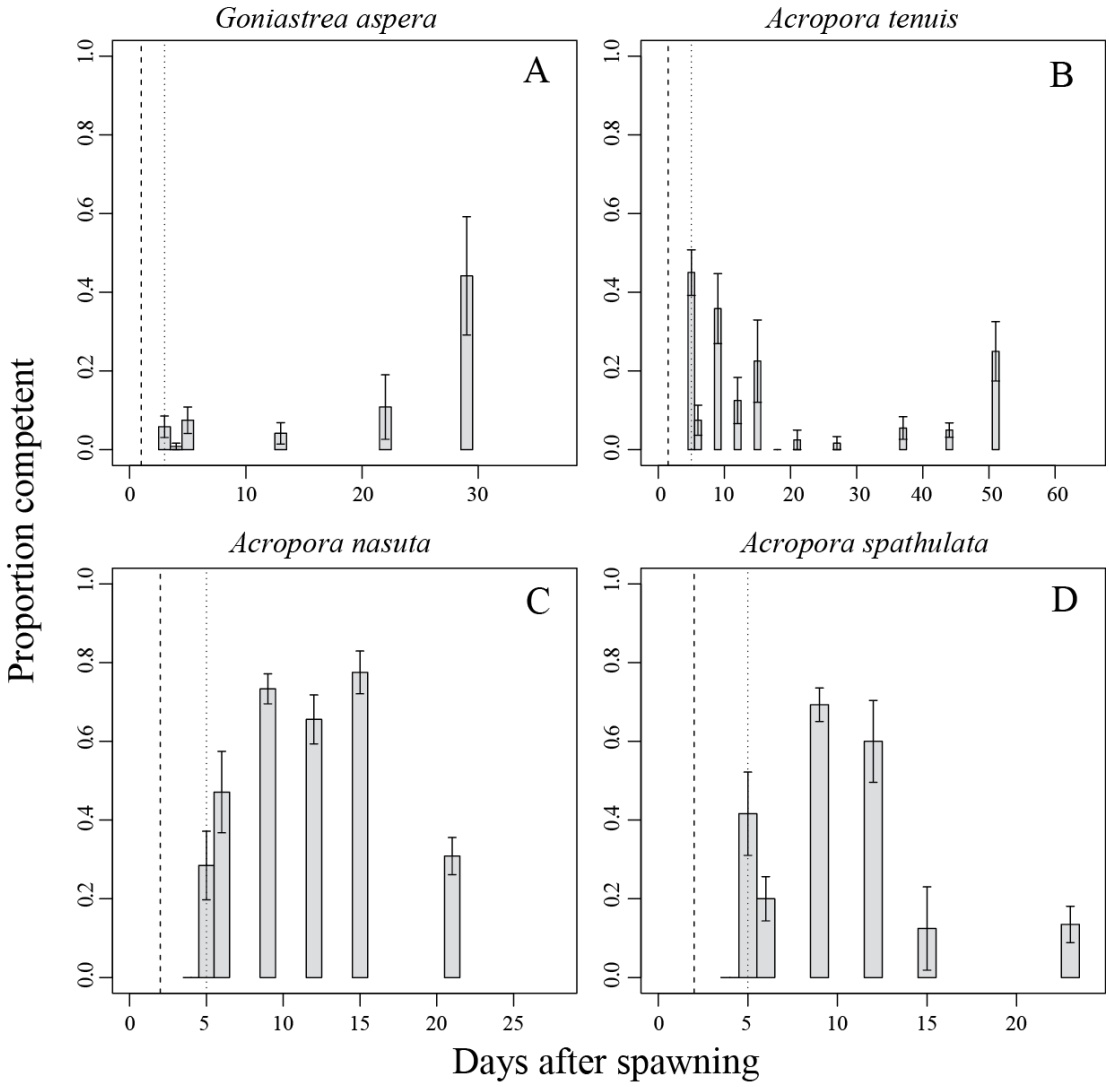

Figure 2.4 Proportion of larvae that are competent to settle for four species of scleractinian corals. Each open circle represents a settlement assay. Error bars represent one standard error.

2.4 Discussion

Temporal patterns in the metabolic rates and lipid levels of lecithotrophic, non-zooxanthellate larvae for four broadcast spawning species of corals were strikingly similar in the first three weeks after spawning. Although the specific values of oxygen consumption varied among species, in all cases, respiration rates were relatively low for eggs, followed by a rapid increase through embryogenesis to a peak, the timing of which varied among species from 12-48 h after fertilization. This spike in respiration rates is short-lived, and rates quickly fall back to initial levels within 3-6 days, where they remain for up to eight weeks. Consistent with these observations, lipids are depleted rapidly during development, until a few days after larvae become competent to metamorphose, at which time the rate of lipid utilization slows dramatically. The high concordance between these two measures of energy use strongly support the hypothesis that an extended period of reduced larval metabolism explains, at least partly, the long PLDs observed in many coral species with non-feeding larvae. Moreover, the capacity of larvae to maintain settlement competence throughout this extended period of reduced energy use implies the capacity to settle over a broad range of dispersal distances.

Although embryogenesis and larval development are the most energetically demanding periods in the larval life of many marine invertebrates (e.g., Shilling and Manahan 1994, Anger 1996, Hoegh-Guldberg and Emlet 1997, Bryan 2004), the magnitude of the declines observed in some species suggests that lecithotrophic larvae have the capacity to achieve much lower levels of energy use than have previously been documented for corals. High rates of respiration correspond firstly with high rates of cell division during embryogenesis (approximately 12-36 h post-fertilization) and secondly with the development of specialised cells, such as spirocysts, that are associated with attachment and metamorphosis (36-96 h post fertilization) (Hayashibara

et al. 2000, Okubo and Motokawa 2007). Once these energetically demanding processes are complete, larvae enter a state of substantially reduced metabolism. The magnitude of decrease in respiration varied from ~2.5-fold in *A. spathulata*, to about 100-fold in *A. tenuis*. Temporal changes in respiration have been examined previously in only one species, *Acropora intermedia* (Okubo et al. 2008), and were found to decrease by ~3-fold, which is within, but towards the low end of the range of declines observed in this chapter.

The pattern of rapid decline in lipid content during the first week of embryogenesis and larval development, followed by a period when further lipid depletion was minimal, is consistent with the trends I observed in respiration rate. Overall, larvae lost between half (*A. spathulata*) to ~75% (*A. tenuis*) of their initial lipids during the first week. This is similar to, but larger than, the ~30-40% depletion of lipids observed over a similar period in the one previous study that reports comparable data (Harii et al. 2007). For at least three of my four study species, lipid levels were stable after this period. The exception was *G. aspera*, which exhibited a secondary decline over the final (fifth) week. However, this decline must be treated with some caution, because it was driven entirely by samples on the final sampling date, and lipid levels were very stable over the preceding three weeks. For the three *Acropora* species, the rates of lipid depletion apparent at the end of the study are consistent with very long PLDs observed in other *Acropora* coral species, such as *A. valida*, which has been observed to successfully metamorphose at ~130 days (Connolly and Baird 2010). On the final sampling date (63, 26, and 22 DAS for *A. tenuis*, *A. nasuta*, and *A. spathulata*, respectively), the observed rates of lipid depletion in larvae of these species (the slopes of the fitted lipid line in Figure 2.2) varied from ~0.11 μg day^{-1} to <<0.01 μg day^{-1} (Table 2.1). At these rates, after a further 100 days in the plankton, *A. tenuis* and *A.*

nasuta larvae would have used less than approximately 6% and 16% of their remaining energetic lipids, respectively (Figure 2.2B,C), suggesting that energy reserves are consistent with the very long (100+ days) PLDs that have been documented for corals (Connolly and Baird 2010). *Acropora spathulata* had enough remaining lipid for an additional 77 d at their rate of consumption at the end of the study, with a total estimated PLD for this species of 99 d (Table 2.1).

Table 2.1 Estimates of lipid remaining after an additional 100 days in the plankton for three *Acropora* species, assuming maintenance of the lipid utilization rate observed at the end of the study (i.e., change over the last two sampling points).

	Acropora tenuis	*Acropora nasuta*	*Acropora spathulata*
Age (d)			
Second to last sampling point	57	20	14
End of study	63	26	22
Lipid remaining (µg)			
Second to last sampling point	1.860	3.753	9.221
End of study	1.853	3.717	8.361
Estimated rate of lipid used (day^{-1})	0.001	0.006	0.108
Next 100 days			
Lipid used (µg)	0.113	0.597	10.752
Percent of remaining lipid used (%)	6	16	129

In contrast to estimates based on rates of energy lipid decline, estimates of PLDs based on respiration rates prevailing at the end of the experiment still fall short of empirically observed PLDs in the literature (Table 2.2). One possible explanation for this apparent discrepancy between metabolic rates and rates of energy lipid depletion is that the larvae are supplementing their endogenous reserves by absorbing dissolved organic matter (Ben-David-Zaslow and Benayahu 2000), which would have been present both in the filtered seawater being supplied to them, and as a consequence of the lysing of dead coral larvae. Alternatively, the handling necessary to place larvae in respirometry vials for measurement of oxygen consumption may have stimulated a temporary elevation in metabolic rates for these sampled larvae, causing respirometry measures to be biased upwards, relative to average levels prevailing for larvae remaining in the tanks.

Table 2.2 Estimates of pelagic larval duration (PLD) for four study species using lipid content and oxygen consumption, converted to their energetic equivalents (39.5 kJ / g lipid; 441 kJ / mol O2; (Gnaiger 1983)).

	Goniastrea aspera	*Acropora tenuis*	*Acropora nasuta*	*Acropora spathulata*
Age (d)	35	63	26	22
O2 consumption (nmol/n/hr)	0.70	0.90	3.45	6.79
O2 consumption (mJ/n/hr)	0.31	0.40	1.52	3.00
Lipid remaining (µg)	0.41	1.85	3.72	8.36
Energy remaining (mJ)	16.18	73.19	146.83	330.25
Esimated remaining (d)	2	8	4	5
Age + Est. remaining (d)	37	71	30	27
Maximum observed PLD (d)	215[a]	69[b]	NA	NA

a - Graham et al. 2008; b - Nishikawa et al. 2003

Even though metabolic rates measured in vials may be high relative to rates for larvae in tanks, the rates are still substantially lower than those predicted from metabolic scaling theory. This strongly suggests that metabolic rates are indeed unusually low after competence is achieved. The metabolic theory of ecology (MTE) predicts whole organism metabolic rate scales with body mass raised to ¾ power (Gillooly et al. 2001). In particular, for temperatures between 8-27°C, mass-normalized resting metabolic rates of multicellular invertebrates are predicted to lie between 0.002 and 0.135 W $g^{-3/4}$, where W is metabolic rate in Watts (joules per second) (Gillooly et

al. 2001). Using egg dry weights that I measured for *G. aspera* (0.000012 g) and *A. nasuta* (0.000031 g) during an earlier study (Honours thesis; Graham 2007), and assuming *A. tenuis* and *A. spathulata* have comparable egg dry weights as similar-sized eggs of *A. digitifiera* (0.000043 g) and *A. divaricata* (0.000027 g) respectively, I calculated mass-normalized metabolic rates for larvae in this study (at 27°C). My estimates are much lower than expected (Figure 2.5), even though the larvae were actively swimming (i.e., not resting).

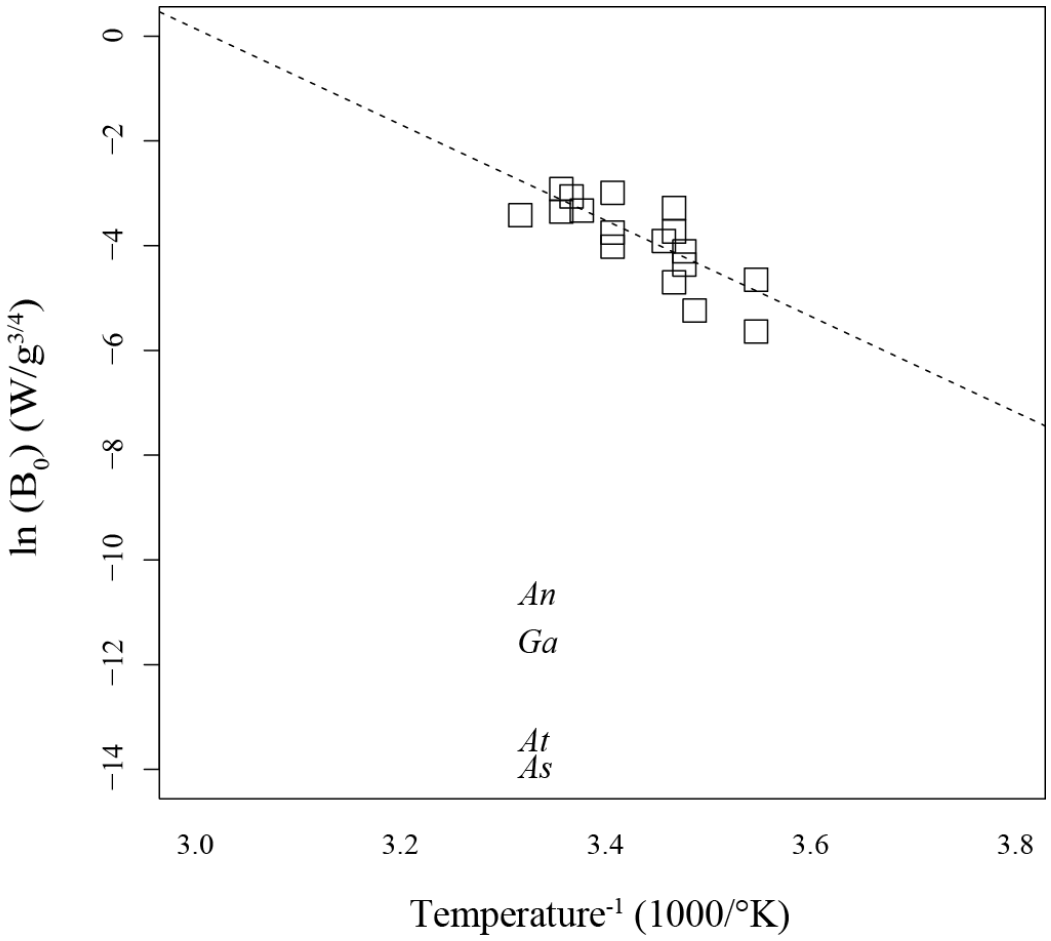

Figure 2.5 Mass-normalized resting respiration rates for multicellular invertebrates (recreated from Gillooly et al. 2001), in comparison to mass-normalized respiration rates of the four scleractinian corals maintained at 27°C in this study. *An = Acropora nasuta*; *Ga = Goniastrea aspera*; *At = Acropora tenuis*; *As = Acropora spathulata*.

The consistency in the overall patterns of reduced oxygen consumption and lipid depletion that I observed for all four study species is striking, and implies that the energetic cost of delaying metamorphosis may be much smaller than is commonly assumed for lecithotrophic larvae. These findings help to explain large discrepancies that have been reported between energetic estimates of larval duration based on metabolic rates measured early in larval life (Richmond 1987) and the much greater

durations measured empirically (Graham et al. 2008, Connolly and Baird 2010). This suggests that very low basal metabolic rates underpin the extended competence periods and larval durations of scleractinian corals, which are on par with or greater than those of most planktotrophs (Shanks et al. 2003, Shanks 2009). While there are undoubtedly costs associated with increased time in the plankton, my results suggest that some of the hypothesized post-settlement costs, such as increased mortality and decreased growth (Pechenik 2006), may be less severe for scleractinian coral larvae than might be expected based on the metabolic rates prevailing early in larval life, or typical of similar-sized invertebrates. Few studies of the temporal dynamics of metabolism in other lecithotrophic larvae have lasted more than two weeks (e.g., Okubo et al. 2008, Hoegh-Guldberg and Emlet 1997, Moran and Manahan 2003). This raises the possibility that other invertebrate lecithotrophs may extend their larval durations in a similar fashion. If reduced larval metabolism leading to extended larval duration is a common early life history trait underpinning current coral population structures, then ocean warming, which should elevate these basal metabolic rates, is likely to have significant consequences for dispersal potential and hence population connectivity, not only for the majority of reef-building corals but for other marine invertebrates as well.

3 Temperature effects on the energetics of scleractinian coral larvae and their implications for dispersal

3.1 Introduction

Temperature has a major effect on the physiology of organisms, most notably by affecting the rates of biochemical reactions that collectively govern organism functions. Typically, biochemical reactions catalysed by enzymes exhibit a parabolic response to temperature, such that reaction rates increase with temperature until an upper tolerance threshold is reached, after which rates start to decline (Sibly et al. 2012). Biological performance follows a similar trajectory, rising gradually with temperature to an optimum before dropping (Huey and Stevenson 1979). Metabolic rates, development rates and lifespans are all similarly affected by temperature (Gillooly et al. 2001, 2002). While some organisms are capable of regulating their internal body temperature to maintain optimum enzyme activity, internal temperatures of others, including most invertebrates, are dependent on their environments. Given the increases in global temperatures over the last century and the predicted increases to come in the near future (IPCC 2007), temperature will likely play a major role in determining the future distribution and abundance of animal populations.

In the marine environment, most invertebrate species have a complex life-cycle consisting of a benthic, mostly sedentary, adult stage and a dispersing larval stage. Many coral species appear to live close to the upper limit of their thermal tolerances, and even small increases in temperature can lead to the loss of their symbiotic photosynthesizing dinoflagellates from the genus *Symbiodinium* (zooxanthellae), a condition known as bleaching (Jokiel and Coles 1990, Berkelmans and Willis 1999). If temperatures remain above this threshold for extended periods of time, death of the

colony can occur (Glynn 1984). Increased temperatures also affect corals during the larval stage, with increases of 3-4°C causing rates of development to increase by 10-25%, the number of larvae developing abnormally to increase 20-40% (Bassim et al. 2002, Negri et al. 2007, Randall and Szmant 2009b), and mortality to increase by 20-80% (Edmunds et al. 2001, Bassim and Sammarco 2003, Brooke and Young 2005, Randall and Szmant 2009a, 2009b). Short term exposure to increases in temperature of 6-8°C can increase overall settlement by 30-50% (Coles 1985, Nozawa and Harrison 2007), while long term exposure to temperatures only 3-4°C above ambient may decrease settlement during the exposure period (Randall and Szmant 2009a, 2009b). Although it is generally assumed that increased metabolic rates are the cause of such changes, very few researchers have measured the respiration rates of coral larvae at different temperatures, and no studies to date have quantified the effect of temperature on the energy content of larvae.

Respiration rates of larvae from several species of brooding corals exhibit a parabolic response to temperature, with peaks occurring near ambient seawater temperatures at the time of larval release (*Porites astreoides*, Edmunds et al. 2001; *Pocillopora damicornis, Seriatopora hystrix and Stylophora pistillata*, Edmunds et al. 2011). However, brooded larvae develop internally and, considering that *Symbiodinium* symbionts are vertically transmitted from parents to offspring in all brooding hermatypic scleractinians except species of the genus *Isopora* (Baird et al. 2009), they are physiologically different from larvae of broadcast-spawned corals with horizontal *Symbiodinium* transmission, which make up the majority (> 80%) of coral species (Baird et al. 2009). To date, only one study has looked at the effect of temperature on respiration of broadcast-spawned larvae lacking symbionts. Rodriquez-Lanetty et al. (2009) found increased temperature increased rates of larval respiration in *Acropora*

millepora monotonically, but the larvae in this study were 10 d old and exposed for 10 hours at most. More research is needed to determine if a monotonic response of metabolic rates to temperature is typical of other broadcast spawning species, and if differences in respiration rate are correlated with increasing rates of development and mortality rates.

In this chapter, I aim to quantify the effects of temperature on the survival, metabolic activity, and energy expenditure of scleractinian coral larvae over the entire larval duration. The results from Chapter 2 demonstrate that levels of oxygen consumption of coral larvae peak with the onset of larval swimming and then decline to low levels characteristic of unfertilized eggs between 7-10 days after spawning, after which respiration rates remain constant for up to two months. If temperature increases rates of early development in coral larvae, the peak in oxygen consumption should be higher and occur sooner for larvae maintained at higher temperatures. Moreover, larvae at higher temperatures should have higher rates of respiration during the extended period of low metabolism. Similarly, if respiration is increased at higher temperatures, the rate of lipid depletion, the putative energy source for coral larval metabolism (Arai et al. 1993), should also increase at higher temperatures. Higher rates of energy use should then lead to higher mortality rates. Here, I test these hypotheses using laboratory experiments on *Acropora tenuis* larvae, from newly fertilized gametes, through larval development, for up to two months.

3.2 Materials and methods

The study was conducted at Orpheus Island Research Station (OIRS), part of the Palm Island Group in the Central Section of the Great Barrier Reef (18.61 S, 146.48 E),

during the austral summer in 2009-2010. *Acropora tenuis* was selected as the target species because it is a locally abundant, broadcast spawning species, whose eggs lack zooxanthellae at release. Three days prior to the full moon in November, six adult colonies of *A. tenuis* were collected, three each from Orpheus and Pelorus Islands, and brought to OIRS where they were maintained in flow-through aquaria at ambient temperature at the time of spawning (27°C). On November 7, spawned gametes from all colonies were collected, combined, and fertilized at ambient temperature. Once cleavage had started in the majority of *A. tenuis* eggs, ~50,000 developing embryos were equally and randomly distributed into five 10 L aquaria containing 0.2 µm filtered seawater (FSW) maintained at different target temperatures: 25.0°C, 27.0°C (ambient), 28.5°C, 30.0°C, and 31.5°C. Five temperatures were chosen in order to analyse the data using a regression-based approach, explained below. These temperatures are within the range of temperatures larvae typically experience around Orpheus Island in the summer months.

Sampling commenced immediately after transfer of larvae into the treatment tanks and was then repeated: every 12 h until all larvae were actively swimming (36 h), daily until larvae in all treatments were competent to settle (5 DAS), every 3 d in the remainder of the first month (29 DAS), and every 7 d in the second month (up to 63 DAS in treatments with larvae remaining). At each sampling time, respirometry measurements were made on a subset of larvae from each treatment, after which the same larvae were immediately frozen in liquid nitrogen for subsequent lipid analysis. A survival experiment, described below, was set up when larvae in all treatments were swimming (4 DAS).

3.2.1 Modelling approach

To determine how temperature affected the mortality rate of *A. tenuis* larvae, as well as their rates of lipid depletion and oxygen consumption, a regression-based approach was used, where temperature was treated as a continuous predictor variable because it enabled estimates of the size of the effect of temperature. The standard approach to analysing temperature effects is to treat temperature as a categorical variable and use analysis of variance (ANOVA). This type of analysis can determine if temperature effects differed among treatments, but it cannot calibrate how effect size varies as a continuous function of temperature. Temperature effects were predicted to have one of two response types, monotonic or parabolic (Figure 3.1). For the monotonic response, response rates (mortality, lipid depletion and respiration respectively) were modelled as increasing linearly with temperature (Figure 3.1, dotted line). Alternatively, if a parabolic response to increasing temperature occurred, response rates should increase (or decrease) to an optimal value before decreasing (or increasing) (e.g., Figure 3.1, solid line). If this was the case, the optimal temperature would be expected to be at or near ambient temperature (Portner 2002). As experimental temperatures move away from ambient, effects on response rates should become increasingly large. Thus, the squared deviation of absolute temperature from ambient was used instead of absolute temperature as the predictor variable to test for a parabolic response in metabolic rates. Both types of response were tested for in analyses of the survival, lipid, and respiration data. All analyses were done using R (R Development Core Team 2011).

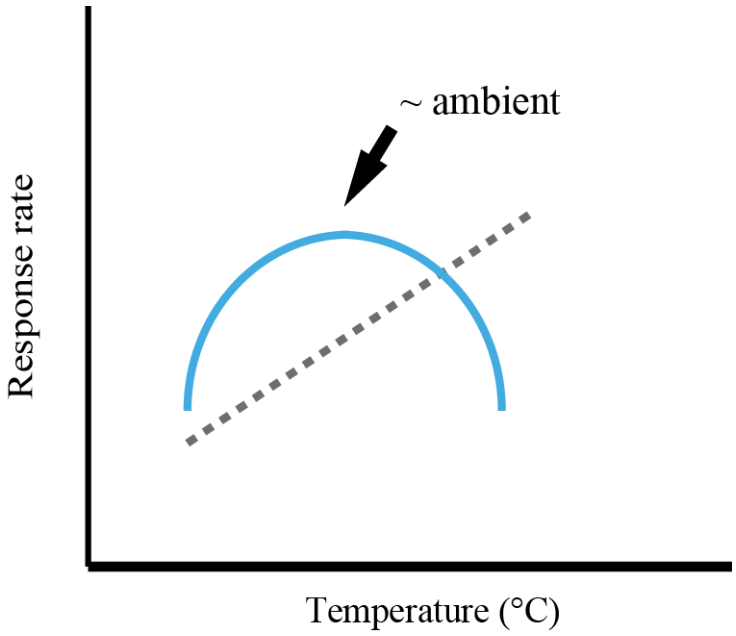

Figure 3.1 Illustration of two types of expected responses of metabolic rates due to increases in temperature. The dotted line represents a monotonic response and the solid line a parabolic response. Note that the "parabolic" response analysis also allowed for ambient temperature to be a minimum value, rather than a maximum, as shown.

3.2.2 Survival

At 4 DAS, when larvae in all treatments were swimming, 500 randomly selected larvae were removed from each temperature treatment, transferred into one of five replicate 70 ml specimen jars filled with heated FSW (100 larvae each), and placed into water baths maintained at the same temperatures as the treatment aquaria. At each sampling time, the number of surviving larvae in each jar was recorded and the larvae transferred into a clean specimen jar with heated FSW.

To determine if temperature had an effect on the survival of *A. tenuis* larvae and how median lifetimes varied with temperature, a nonparametric Kaplan-Meier analysis was first used to obtain estimates of median lifetimes for each treatment. Then, to

directly compare larval survival between treatments and obtain a more quantitative estimate of the effect of temperature on survival, a parametric regression was used to fit a Weibull distribution to the survival times. The Weibull distribution is one of the most commonly used distributions for survival analysis. It is a two-parameter model with the initial parameter (λ) denoting the overall level of hazard and the shape parameter (γ) that allows mortality rates to increase (γ > 1) or decrease (0 < γ < 1) monotonically, or to remain constant (γ =1), over time (*t*). The survival function for the Weibull distribution is:

$$S(t) = e^{-\lambda t^{\gamma}};$$

Incorporating temperature dependence into the survival function means when testing for a monotonic response (linear model):

$$\lambda = a + b * T_{\circ C};$$

where *a* and *b* are the coefficients of the linear predictors produced by the model fit and $T_{\circ C}$ is the actual temperature in °C. When modelled for a parabolic response:

$$\lambda = a + b * (T_{\circ C} - T_A)^2;$$

where T_A is ambient temperature. Using the function "survreg" in the "survival" package in R, the model used consisted of survival as a function of temperature, incorporated as a continuous fixed effect. Random effects of replicates were included in the model using the "frailty" function (Therneau et al. 2003).

3.2.3 Lipid

The amounts of lipid in larvae from the different temperature treatments were measured for the same five replicate samples, each comprising ~50 larvae, which were used for the respirometry measurements. Lipids were extracted using a modified Bligh-

Dyer chloroform:methanol procedure, adding ketone as a standard for lipid recovery (Sewell 2005). A thin layer chromatography-flame ionization device (Iatroscan, SES GmbH) was used to measure lipids, which enabled the individual lipid classes to be quantified separately from the total amount of lipid. The lipid classes measured were the wax esters (WE), triacylglycerides (TG), sterols (ST), and phospholipids (PL). The WE and TG lipid classes were combined for analyses, as these have been identified as the primary energy storage lipids in marine invertebrates (Villinski et al. 2002, Kattner et al. 2007).

To analyse the energetic lipid data in this chapter, a linear regression model was used instead of a GAM (as in Chapter 2) because I wanted a model whose shape could be modelled as a continuous function of temperature. Also, a log-log transformation of the data was used in this Chapter, rather than the square-root transformation used in Chapter 2, because a near-linear relationship between days after spawning and respiration rates was observed after the initial pre-swimming period had ended (Figure B.1 in Appendix B). Due to this relationship, and because this Chapter aimed to discern differences among treatments, and not just a quantitative description of the overall pattern of lipid depletion, the starting point for the analysis of lipid samples was selected as the time when larvae commenced active swimming. This time corresponded to 36 h for the lowest three temperatures and 24 h for the highest two temperatures. Using time to motility as the starting point for the analysis, multiple linear regression was used to model lipid depletion, with time and temperature as the explanatory variables. The following possible outcomes were tested for: i) an interaction between time and temperature (different intercepts, different slopes); ii) no interaction (same slope, different intercepts); iii) the case where the intercept was the same but the slopes were different; and iv) the slopes and intercepts were the same for all treatments (Figure

3.2). Biologically, these models represent: i) differing amounts of lipid remaining following development through to the swimming stage among larvae in the five temperature treatments, and differing rates of lipid depletion thereafter; ii) differing amounts of lipid remaining after development, but equal rates of lipid depletion thereafter; iii) equal amounts of lipid remaining after development, but differing rates of depletion thereafter; and iv) no differences in initial lipid levels or rates of depletion. Akaike's Information Criterion (AIC) was then used to select the model that provided the best fit to the data. In each case, the relevant parameter (slope and/or intercept of lipid levels as a function of time) was modelled as a linear function of either temperature, or the squared deviation of temperature from ambient, as described in *Modelling Approach*, above.

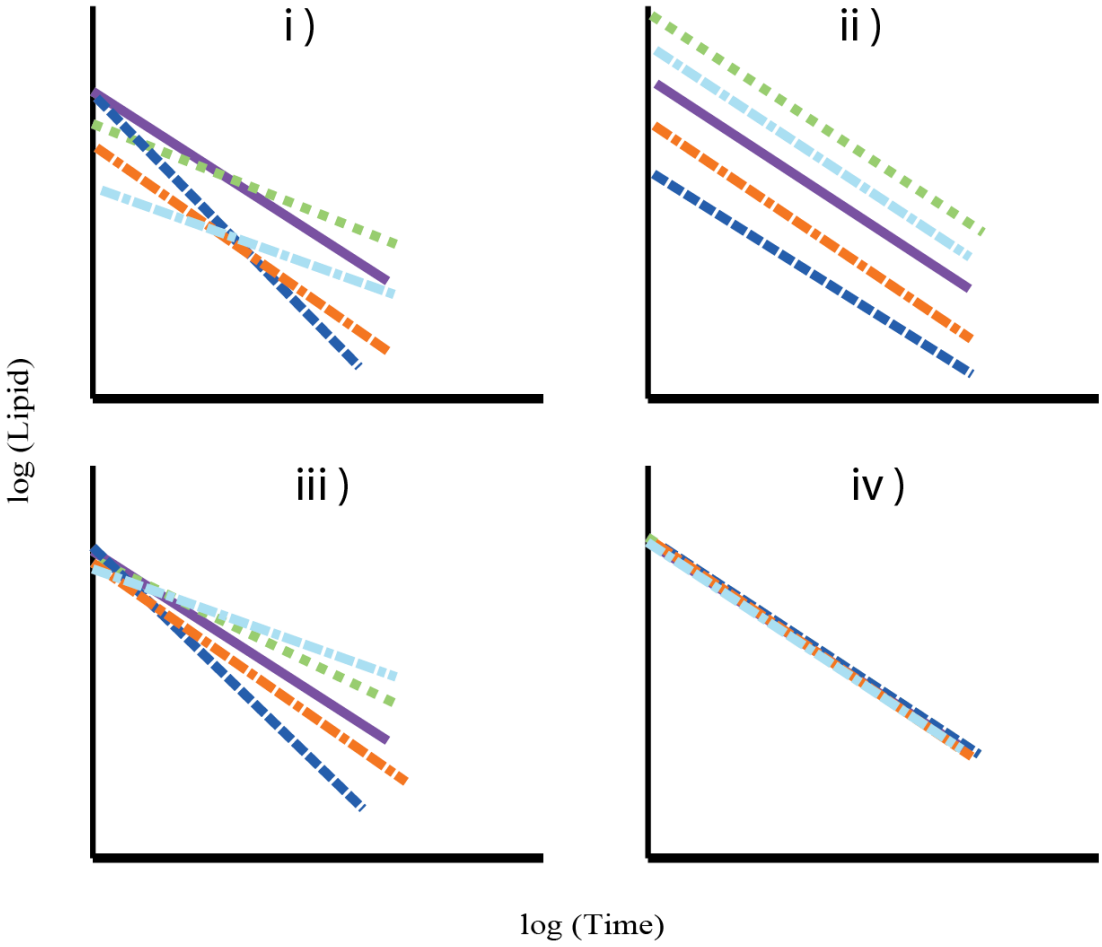

Figure 3.2 Illustration of possible outcomes tested for in the analysis of energetic lipid depletion in *Acropora tenuis* larvae maintained at five temperatures. Biologically, the outcomes shown represent i) differing amounts of initial lipid and differing rates of lipid depletion; ii) differing amounts of initial lipid but equal rates of lipid depletion; iii) equal amounts of initial lipid but differing rates of depletion, and iv) no differences in initial lipid levels or rates of depletion.

3.2.4 Respirometry

Oxygen consumption was measured at each sampling time using a temperature compensated fiber-optic microrespirometry system (Fibox, PreSens). Respirometry

chambers were custom made using 1.5 ml glass vials with 5 mm oxygen sensor spots attached to the inside of the vial. The system was first calibrated following manufacturer instructions using air-saturated (100% O_2) water and oxygen-free water (a solution containing Na_2SO_3). At each sampling time, five replicate samples, each comprised of 50 randomly selected larvae, were measured. For each measurement, larvae were transferred from treatment aquaria into the chamber, the chamber topped up with heated FSW, and the chamber placed into a temperature bath maintained at the desired temperature. Measurements were taken for 5 min before larvae were removed from the chamber, placed into a 1.5 ml cryovial and put directly into liquid nitrogen. Following each measurement of respiration, the respirometry chamber was rinsed and refilled with heated FSW only, and another 5 min measurement was taken as a control. Oxygen consumption was calculated as the slope for the 5 min measurement and converted into nmol O_2 $larvae^{-1}$ $hour^{-1}$.

To determine if there were differences in larval oxygen consumption among temperature treatments, a generalized additive model (GAM) was fit to the data for each temperature. The linear approach used for the lipid analysis was deemed inappropriate because of the non-linear response found for oxygen consumption which could not be eliminated via data transformation. A GAM is a non-parametric smoother that is used to describe relationships between variables that exhibit complicated, non-linear, non-monotonic shapes (Guisan et al. 2002). Although GAMs do not assume an explicit functional form for the relationship between variables, they do assume normality and homoscedasticity of residuals. Therefore, values for the variables days after spawning (DAS) and respiration were log-transformed to comply with assumptions, and then a GAM was fit to the transformed data for each treatment individually. The “gam” function in the “mgcv” package in R was implemented with the formula: gam (

respiration rate ~ s (DAS) (Wood 1994). The function “s” in the formula means the predictor variable, DAS, is the smooth term.

Patterns in respirometry measurements for controls (i.e. chambers without larvae) clearly reflected the relative difference in temperature from ambient for each of the five temperature treatments (Figure B.2 in Appendix B). At ambient temperature, the control measurements were close to zero as expected, i.e. there was no change in oxygen concentration when no larvae were present (Figure B.2B in Appendix B). However, below ambient, the amount of oxygen in the control vials decreased (Figure B.2A in Appendix B), while above ambient, the amount of oxygen increased proportionally with the temperature difference from ambient (Figure B.2C-E in Appendix B). At all temperatures, the magnitude of the change in oxygen, and the difference between replicates, was greatest during the first week. I believe the cause of this pattern in the control measurements was a combination of handling and temperature effects. The process of removing the respirometry chamber from the temperature bath, removing the larvae from the previous measurement, rinsing and then refilling the chamber with FSW and returning the chamber to the bath for the next measurement meant that the temperature of the FSW in the vial was changing. Although the Fibox compensates for temperature, the temperature sensor should ideally be placed inside the respirometry chamber itself. Because respirometry chambers were closed, the temperature sensor was placed into the same temperature bath as used for larval measurements. I suspect that, as the temperature inside the chamber changed to match the bath, the partial pressure of the oxygen inside the vial also changed. Since the Fibox is actually measuring the partial pressure of oxygen inside the chamber, and then performing a conversion to mol O_2 based on a number of variables, including atmospheric pressure and temperature, systematic changes in temperature in the vial

could have produced spurious changes in oxygen content. Given this pattern in the control measurements, it is likely that the larval measurements would have been affected similarly. Several approaches were used to account for this apparent bias in the controls: 1) pairing the larval measurement together with the subsequent control measurement, and then using the difference between the two as a measure of respiration rate; 2) calculating the mean control measurement on the sampling day and adding it to each larval measurement from that day; and 3) adding the median control measurement from each day to the larval measurements on that day. These corrections are based on the assumption that the biases in the control were most closely related to the previous measurement for that day in particular. The resulting oxygen consumption calculated from the GAM fits changed quantitatively based on the different method used, but the qualitative differences between temperatures remained. Accordingly, larval measurements corrected with the mean control measurement on the sampling day are used in analyses. To compare respiration among temperature treatments in a way that minimized the impact of bias in the controls, the back-transformed GAM fits were used to compare the height and the timing of the peak in respiration. Residuals from the GAM fits were used in a resampling bootstrap procedure to obtain 95% confidence intervals on these estimates.

3.3 Results

3.3.1 Survival

Temperature had a substantial effect on the survival of *Acropora tenuis* larvae. Mortality rates increased monotonically with increasing temperature, even though there was variation among replicates (Table 3.1, Figure 3.3). Estimated survival times for

each temperature indicated a 10-fold decrease in median lifetime between the lowest (57 d) and highest temperatures (5 d) (Figure 3.4). At the end of the experiment at 63 d, the three tanks with the lowest temperatures all had surviving larvae.

Table 3.1 Parameter estimates for the parabolic survival models and Akaike's Information Criterion (AIC) used for model comparison. Estimated parameters are: a, the coefficient of the linear predictor used to calculate λ (the survival rate parameter; and γ, the shape parameter of the Weibull distribution.

Model	a	γ	AIC
NULL	3.48	1.43	12728
RELATIVE_TEMP	3.89	1.57	12191
RELATIVE_TEMP + frailty(REPLICATE)	3.89	2.40	**10469**

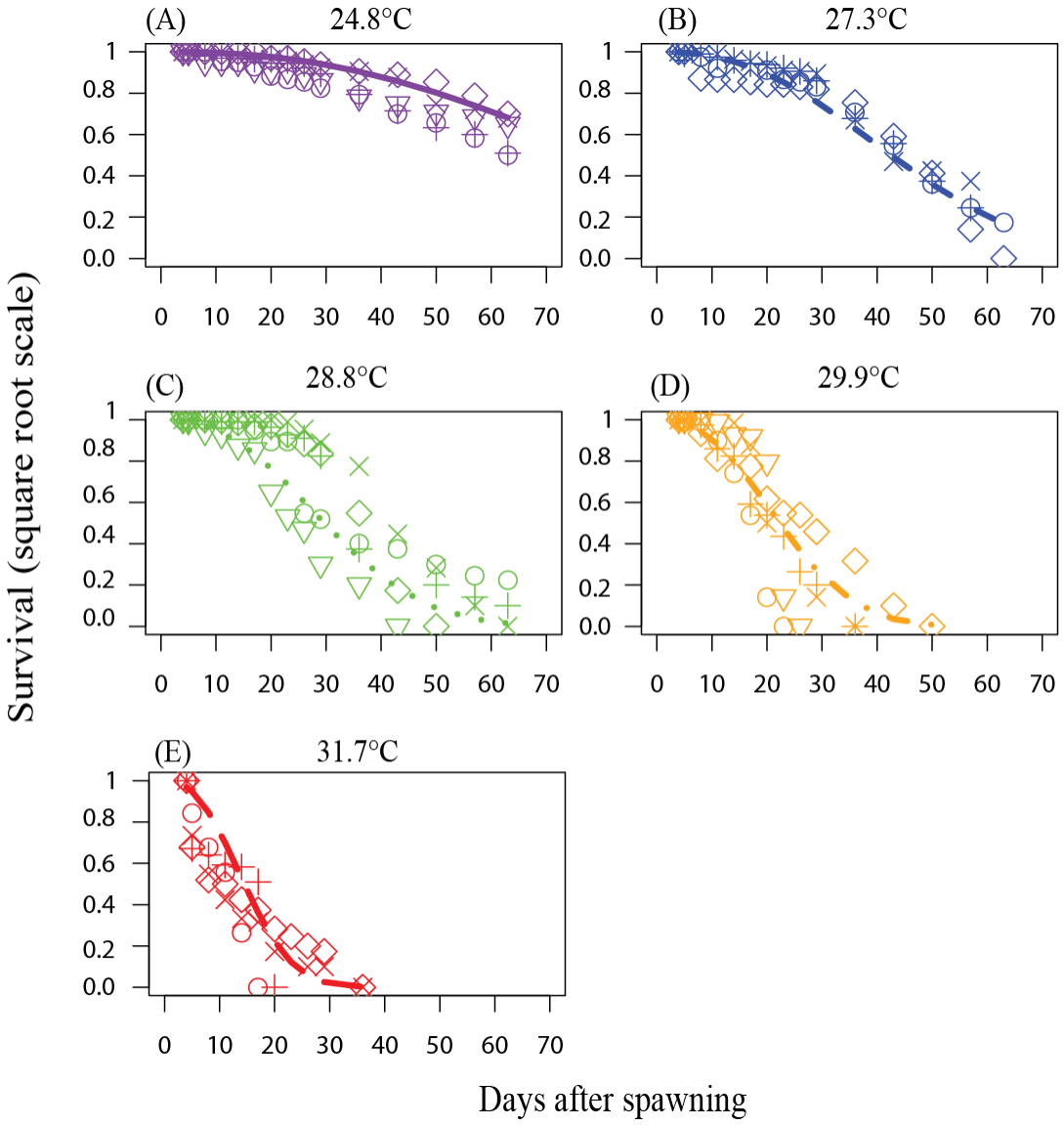

Figure 3.3 Estimated parametric survival function for larvae of *Acropora tenuis* fitted to the empirical survival data with the Weibull distribution (lines). Each symbol represents a replicate vial containing 50 initial larvae (these vials were the random effects in the analysis), and the shape of each symbol (◊,x,+,Δ,○) represents which of the five replicates the observation came from.

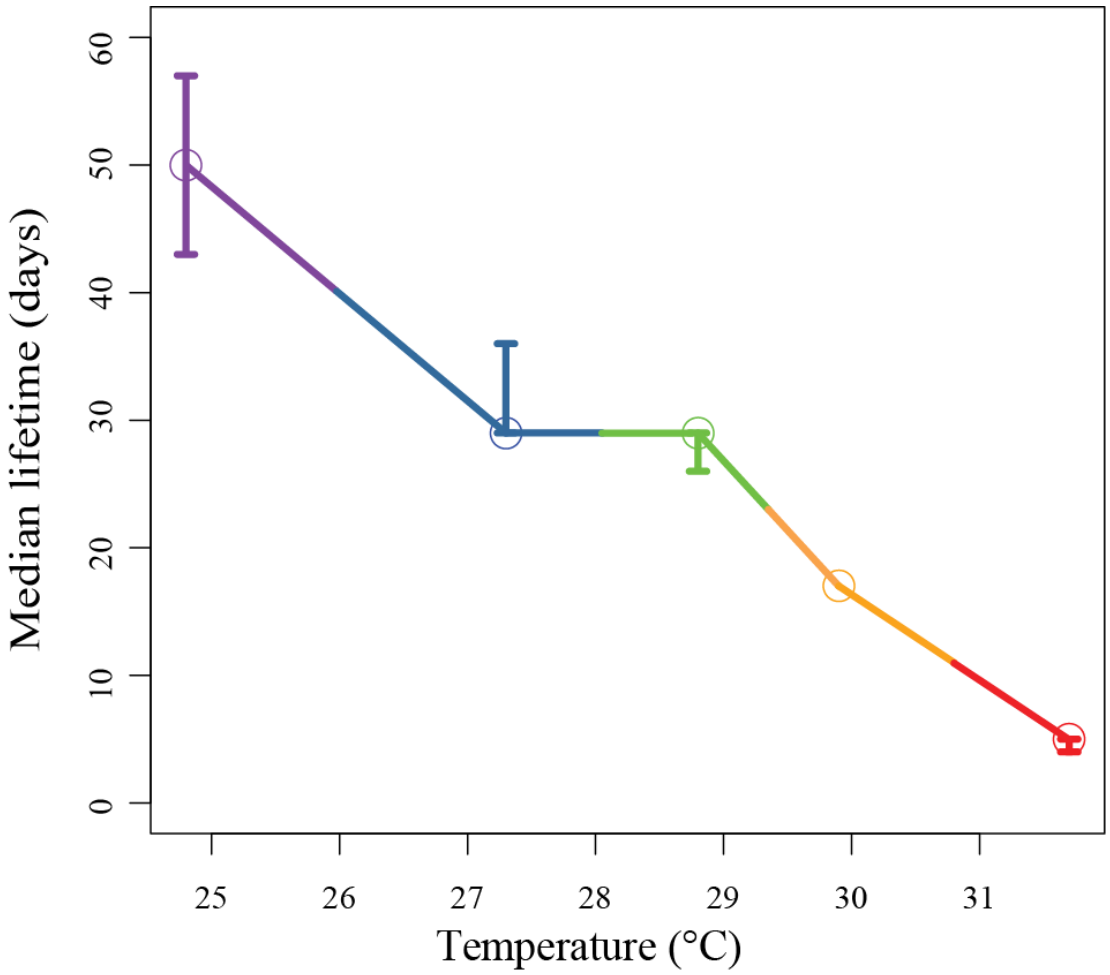

Figure 3.4 Nonparametric Kaplan-Meier estimated median survival times for larvae of *Acropora tenuis* for each temperature treatment. Error bars indicate upper and lower confidence intervals.

3.3.2 Lipid

The best model for the lipid data was the one that fixed the intercept (same amount of lipid remaining after development to a swimming larvae) to be the same for all temperatures but allowed for different slopes (differing rates of lipid depletion) (Table 3.2, Figure 3.5). There was a parabolic response of lipid depletion rate to temperature: maximum depletion rates of energy lipids occurred in the ambient

temperature, with larvae in the low and the higher temperature treatments consuming fewer lipids (Figure 3.6).

Table 3.2 Model selection results for the effect of temperature on lipid depletion of *Acropora tenuis* larvae. Models were: (i) differing intercepts and slopes, (ii) differing intercepts, same slopes, (iii) same intercepts, differing slopes, and (iv) same intercepts and slopes, where the intercept is the amount of lipid remaining after development to a swimming larvae, and the slope is the rate of lipid depletion. Aikaike's Information Criterion (AIC) as calculated for each model as -2*-Loglikelihood+2*degrees of freedom. The lowest AIC indicates the model with the best-fit to the data.

	Model							
	i) time * temperature		ii) time + temperature		iii) time + time:temperature		iv) time	
Parameter	Estimate	p	Estimate	p	Estimate	p	Estimate	p
(Intercept)	2.50	< 0.01	2.36	< 0.01	2.48	< 0.01	2.51	< 0.01
log(TIME)	-0.45	< 0.01	-0.40	< 0.01	-0.45	< 0.01	-0.41	< 0.01
RELATIVE_TEMP	0.00	0.82	0.02	< 0.01	--	--	--	--
log(TIME): RELATIVE_TEMP	0.01	< 0.01	--	--	0.01	< 0.01	--	--
AIC	446.93		452.98		**444.98**		476.41	

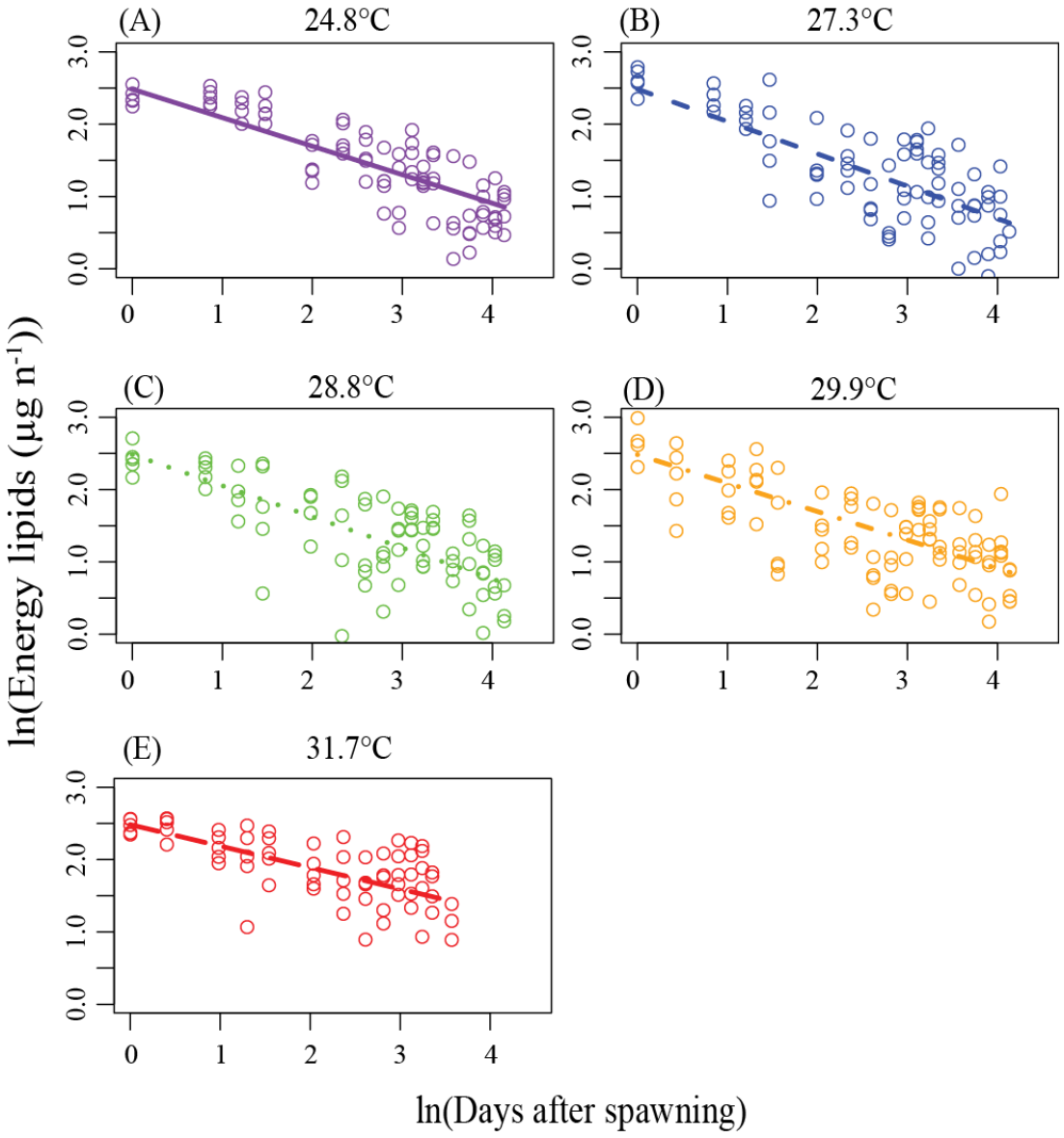

Figure 3.5 Best-model fits for the depletion of energy lipids through time by *Acropora tenuis* larvae maintained at five different temperatures. Each circle represents a replicate measurement, with lines for each fit. Model selection results found that the model with the same amount of lipid remaining after development but different rates of lipid depletion provided the best-fit for the data.

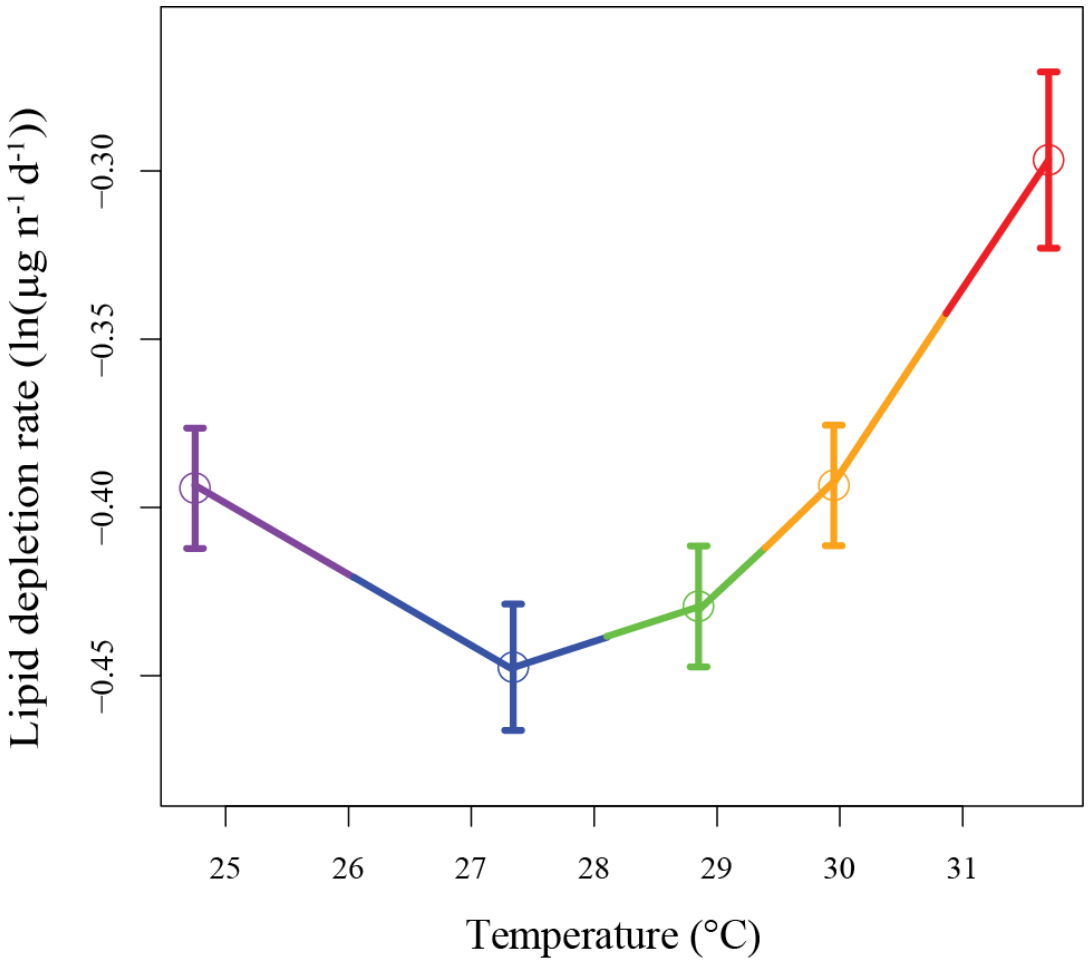

Figure 3.6 Estimated lipid depletion rates for *Acropora tenuis* larvae maintained at different temperatures, from the best-fit lipid depletion model shown in Table 3.2 and Figure 3.5. Error bars indicate standard errors calculated using the variance-covariance matrix of the best-model fit.

3.3.3 Respirometry

In two of the three temperature treatments, oxygen consumption rates rapidly increased to a peak within the first 48 h after spawning before declining to the level of newly fertilized eggs, where they remained for 63 d (Figure 3.7). For the highest temperature, the initial peak in respiration rates occurred prior to the first sampling time at ~12 h after spawning (Figure 3.7E). For the 28.8°C larvae (Figure 3.7C), the pattern

of an initial decrease in respiration rates followed by an increase before decreasing again (essentially two initial peaks) was inconsistent with the other treatment data, as well as with oxygen consumption patterns found in other species (Chapter 2). Therefore, data from this temperature treatment was considered anomalous and excluded from further analyses.

The height of the peak in respiration for the low temperature was the lowest of all treatments, and occurred approximately 12 h later than the peak at ambient temperature and 24 h later than the peak at high temperature (Figure 3.8). The highest peak occurred at ambient temperature, although all of the higher temperatures had peaks that occurred sooner than the low temperature. Peak oxygen consumption in the higher temperatures was more than double that of the low temperature. After 10 d, respiration rates of larvae were nearly the same across all temperatures, except for larvae in the second highest temperature, which respired at a slightly higher rate. Consistent with the lipid data, peak respiration rates had a parabolic response to temperature, with the maximum at ambient temperature (Figure 3.9). However, the timing of the peak was more consistent with a monotonic response; as temperature increased, the height of the peak occurred sooner (Figure 3.10).

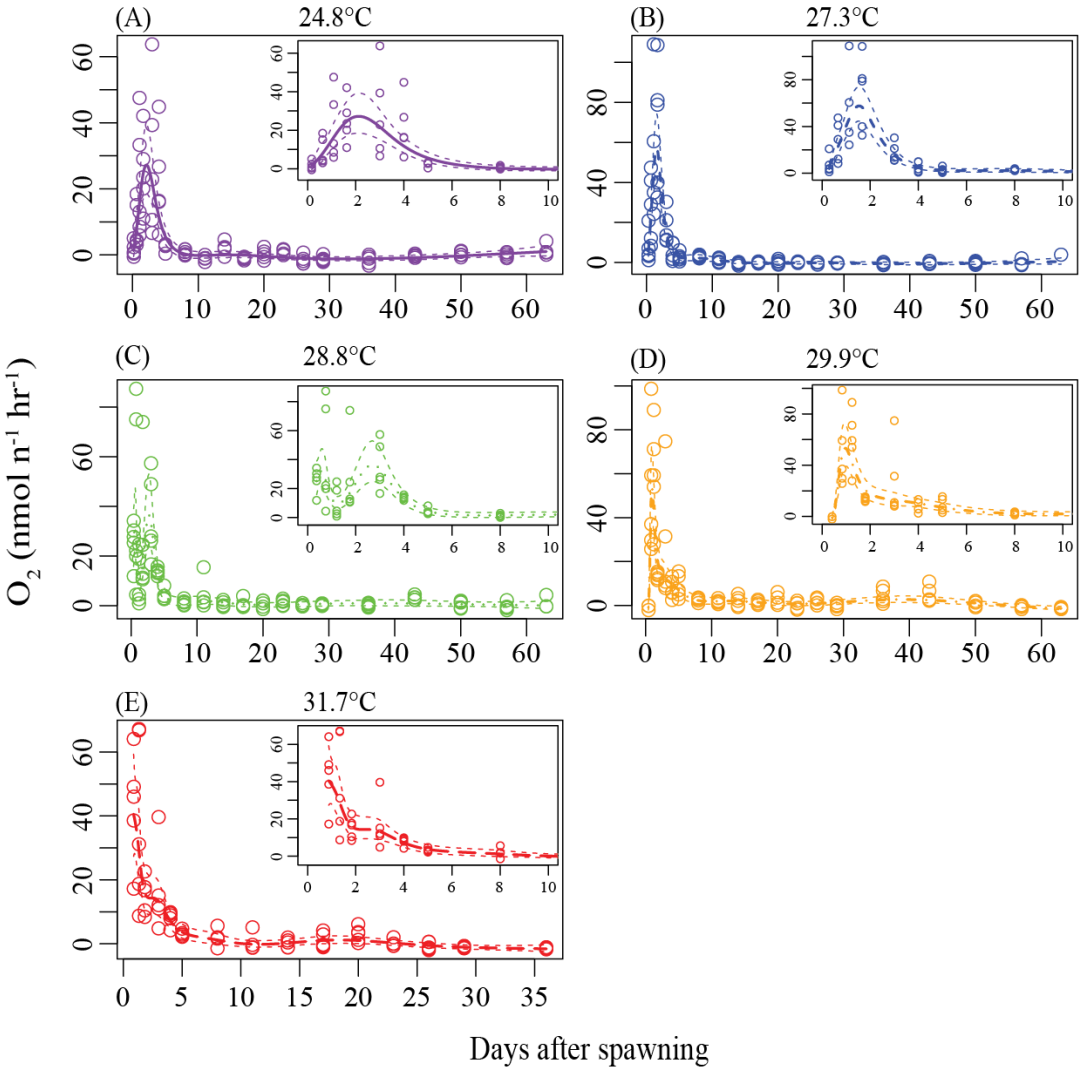

Figure 3.7 Rates of oxygen consumption through time for *Acropora tenuis* larvae maintained at five different temperatures. Each circle represents a measurement. Solid lines represent (back-transformed) GAM fitted mean respiration rates. Dashed lines show upper and lower 95% confidence intervals on the (back-transformed) fitted GAM values. Inset shows the first 10 days to more clearly show the initial changes in respiration.

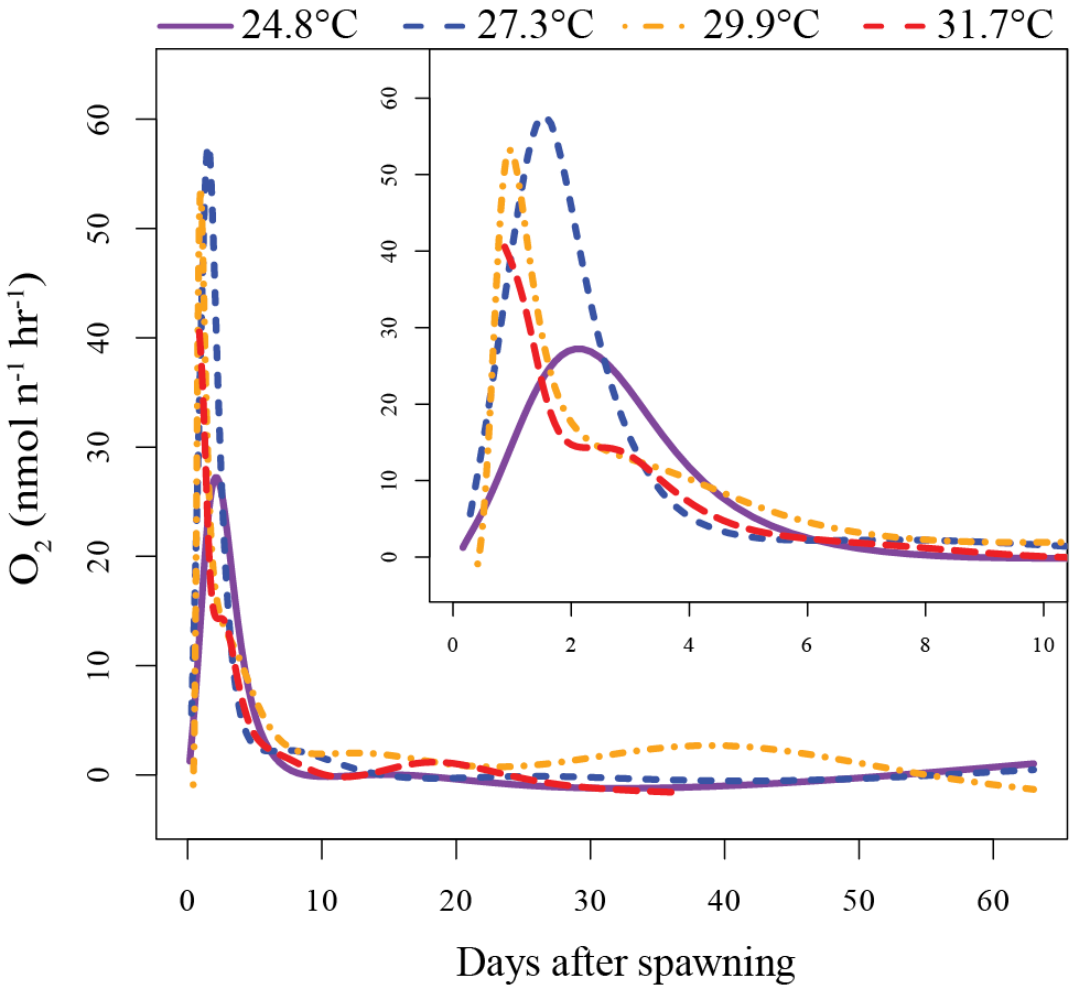

Figure 3.8 Rates of oxygen consumption through time for *Acropora tenuis* larvae maintained at four different temperatures. Lines represent (back-transformed) GAM fitted mean respiration rates. Inset shows the first 10 days to more clearly show the initial changes in respiration.

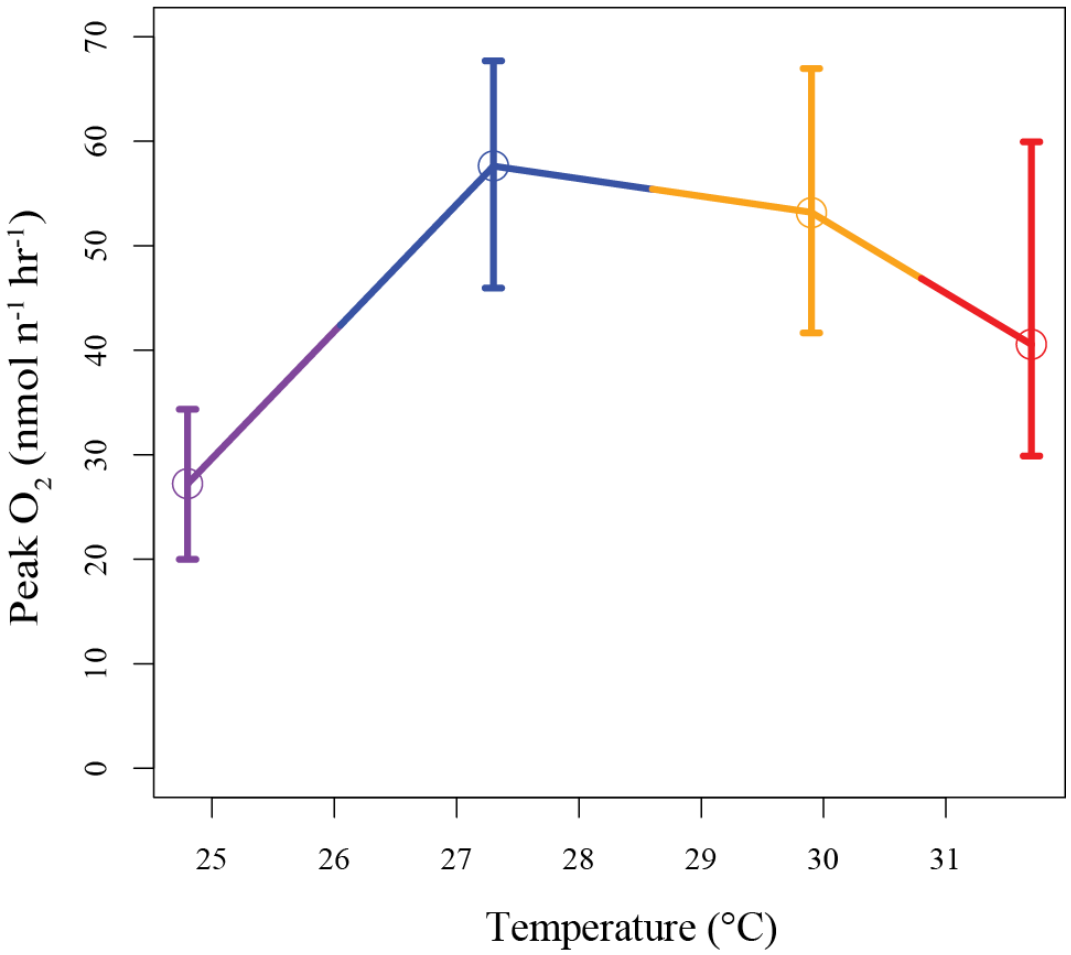

Figure 3.9 Peak rate of oxygen consumption for *Acropora tenuis* larvae maintained at four different temperatures. Error bars represent the 95% confidence intervals of the estimate calculated using a resampling residual bootstrap approach.

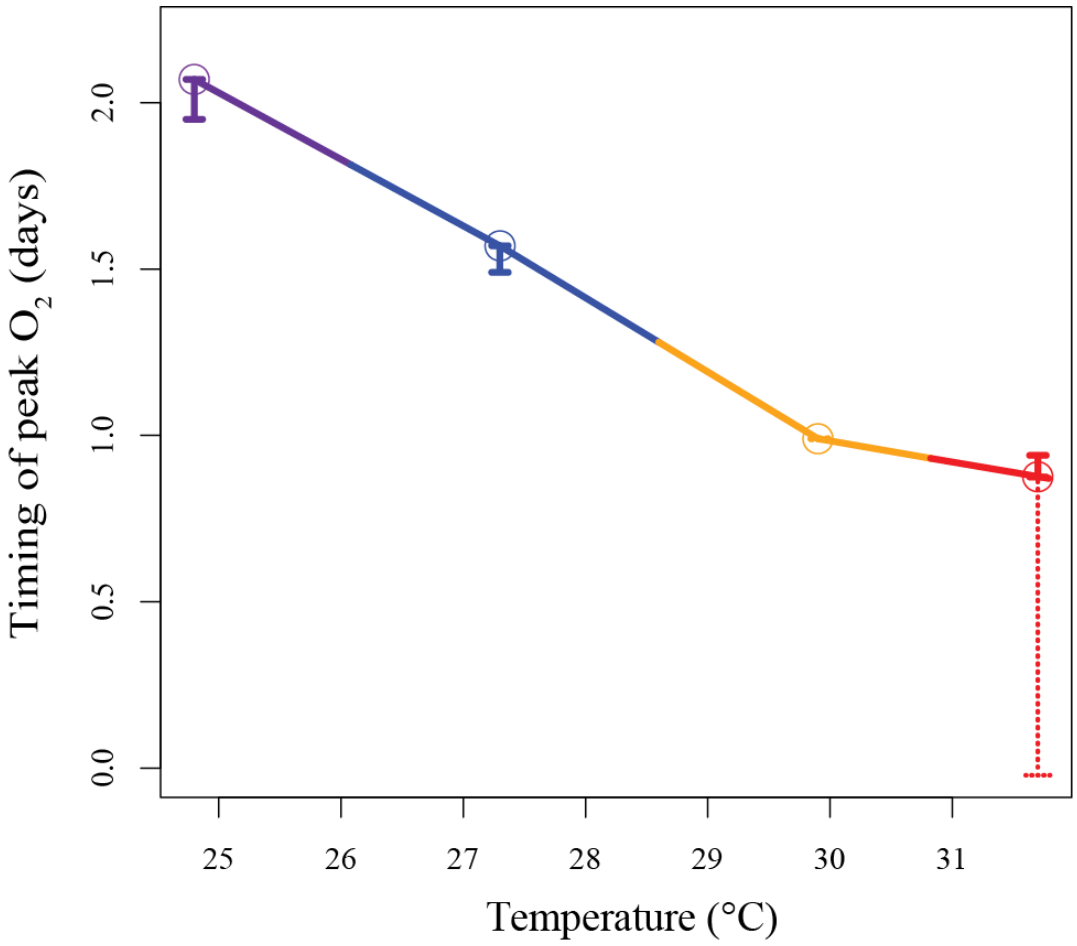

Figure 3.10 Timing of peak rate of oxygen consumption for *Acropora tenuis* larvae maintained at four different temperatures. Error bars represent the 95% confidence intervals of the estimate calculated using a resampling residual bootstrap approach. Peak respiration occurred prior to the first sampling time for larvae maintained at 31.7°C.

3.4 Discussion

Temperature has a strong effect on the survival and energy use of larvae of the broadcast spawning coral *Acropora tenuis*. Of the physiological and ecological processes quantified, rising temperatures had the greatest impact on larval survival, with a 10-fold decrease in median survival times occurring between the lowest and the highest temperature treatments. Respiration rates were higher and peaked sooner for

larvae at higher temperatures, verifying higher metabolic rates as the mechanism for faster development times at high temperatures reported in previous work (e.g. Negri et al. 2007). However, the parabolic responses found for both lipid depletion and respiration, with the greatest rates occurring at ambient temperature and declining on either side of ambient, suggest an optimum temperature threshold for coral larvae at or near ambient temperature at the time of spawning.

This study is the first to examine the effect of temperature on the energy content of lecithotrophic larvae for a marine invertebrate. The parabolic responses to temperature found for rates of both lipid consumption and peak respiration by larvae of the broadcast spawning coral *A. tenuis* are in agreement with results found previously for respiration rates of larvae of brooding species (Edmunds et al. 2001, Edmunds et al. 2011). Over time, organisms become optimized to local environmental conditions in order to maximize enzyme activity and associated metabolic functions (Somero 1978). On either side of optimum temperature, biological functions necessarily decrease. However, there is an upper temperature threshold, which, when exceeded, can lead beyond suboptimal functioning to damaging, potentially terminal, effects (Willmer et al. 2000). The increased mortality of larvae maintained in my higher temperature treatments may reflect such negative impacts of temperature on biological processes rather than lack of energy reserves, as slower depletion of energy reserves at high than at ambient temperatures indicates that reserves were not limiting.

There are several different methods for measuring the effect of temperature on biological processes. One of the most widely used is the Q_{10} coefficient, which is a measure of the change in the rate of a reaction due to a change in temperature of 10°C. But, because Q_{10} is itself dependent on temperature and applies only to processes that increase exponentially, Gillooly and co-authors (2001) have proposed an alternative

Universal Temperature Dependence (UTD) model, which is part of the Metabolic Theory of Ecology (MTE) (Brown et al. 2004). The UTD predicts a monotonic response of biological rates to increased temperature, in contrast to the hump-shaped response observed in this experiment, suggesting that nonlinear temperature dependence models may be more applicable (i.e., Schoolfield et al. 1981). However, the MTE can also be applied to "biological times", such as development times and lifespans, by considering the log of the biological time of interest as a function of 1000/T (Gillooly et al. 2001). Using the timing of the height of the peak in respiration as a proxy for the end of development, the log-transformed development time was plotted as a function of 1000/Temperature (in Kelvin), and the slope calculated using least-squares linear regression (Figure 3.11A). The slope of the regression line through log-transformed development time as a function of 1000/Temperature was 11.96 ± 1.16, which is almost twice the slope predicted by the MTE for aquatic invertebrates (6.50; Gillooly et al. 2001). Following the same methodology, but analyzing median lifetimes, the slope of the regression is 27.70 ± 7.15, which is also much greater than is predicted by the MTE (Figure 3.11B). These differences between the slopes expected by the MTE and the observed slopes suggests that other factors in addition to temperature, such as genotype, are affecting the development times and lifetimes of coral larvae in this study. It is possible the differences actually represent the contribution of mal-adaptation to those high and low temperatures.

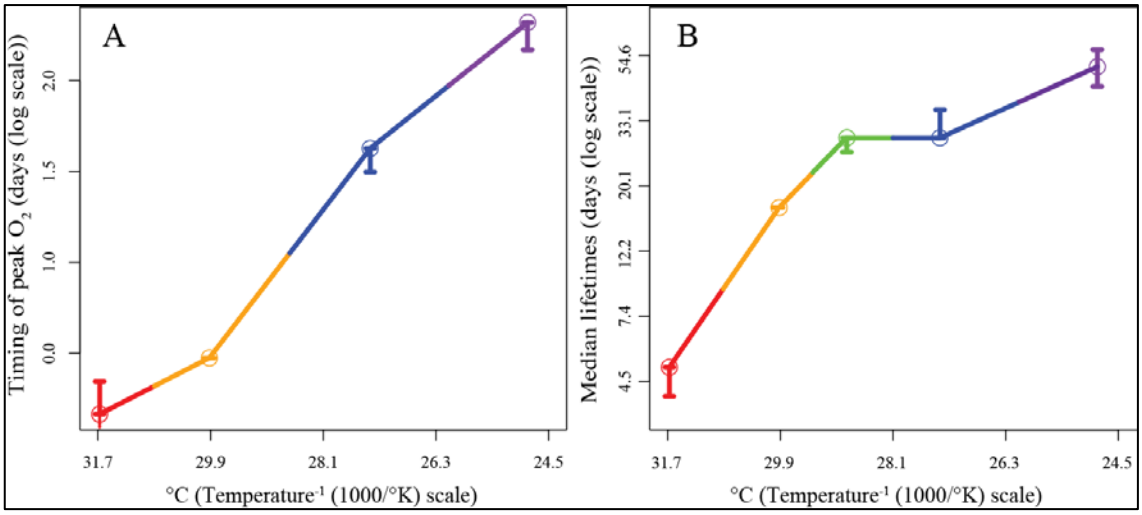

Figure 3.11 Patterns in the timing of A) peak oxygen consumption, and B) median larval lifespan larvae of *Acropora tenuis* maintained at four different temperatures. Note the transformation of the x-axis means the temperature decreases from left to right. (This was done to be consistent with the figures in Gillooly et al. 2001). Error bars represent the 95% confidence intervals (CI) of the estimate calculated using a resampling residual bootstrap approach. At the highest temperature, the CI extends to time 0 h, as no measurement was taken until 12 h.

Once development was complete and larvae were competent to settle, respiration rates dropped to the low levels seen in newly fertilized eggs (as in Chapter 2). While the parabolic effect of temperature on respiration rates was clear during development, the rank order of oxygen consumption among temperature treatments did not persist through time after larvae began swimming. For most of the remainder of the study, respiration rates were quite similar between the low and ambient temperatures. This suggests that even at the highest temperature, once larvae enter their state of reduced metabolism soon after competence is achieved, they are relatively robust to changes in temperature, at least in terms of their metabolism.

In contrast, survival of *Acropora tenuis* larvae was significantly decreased by increases in temperature. This is the first study to calibrate how mortality rate varies as a function of temperature in contrast to studies that simply determine that survival rates differ among temperature treatments. Interestingly, Edmunds et al. (2001) found a parabolic effect of temperature on mortality of *Porites asteroides*, with increased mortality at temperatures both above and below ambient, whereas my study and others (Bassim and Sammarco 2003, Brooke and Young 2005, Randall and Szmant 2009a, 2009b) document a linear effect. The parabolic response found for *P. asteroides* may have something to do with their algal symbionts, which are transmitted from adults to larvae, i.e., photosynthesis may be more sensitive to reduced temperatures than animal metabolic processes.

An energetic cause is often assumed as the cause of larval mortality, with increased temperatures raising metabolic rates and depleting limited energy stores causing starvation (e.g., Edmunds et al. 2001). In contrast, results presented in this Chapter indicate that energy stores are not depleted at higher rates; in fact, the opposite occurs. Other potential causes of mortality include carry-over effects from developmental abnormalities (e.g., Negri et al. 2007, Randall and Szmant 2009b), disruption of the balance between reactions of differing thermal sensitivity (Willmer et al. 2000), and/or problems with membrane structures so that transport systems into and between cells become unbalanced or inoperative (Hofmann and Todgham 2010). Determining which of these mechanisms is causing larval mortality in this study will require further work. Mortality is typically exceptionally high during the larval phase (Thorson 1950); increased mortality due to warming oceans will further affect the dispersal potential of larvae and most likely lead to reduced connectivity among populations.

The highest temperature used in this study, 31.7°C, is towards the high end of the present-day range (23.9°C to 33.2°C) recorded for inshore shallow reef flats at Orpheus Island in the austral summer months of November to March (mean temperatures between 2002 and 2010 were 28.3°C at 1.9 m and 28.6°C at 6 m; AIMS 2011). Nevertheless, this temperature has been experienced on 11 days between 2002 and 2010, for between 1-3.5 hours. It is important to note that larvae in this study were taken as fertilized embryos from 27°C and placed directly into temperature tanks at various temperatures, and continuously exposed to those temperatures. Realistically, the duration of exposure to the higher temperatures is likely to be much shorter in the current ocean. However, even conservative predictions of increases in global sea surface temperatures will push sea temperatures above the highest temperatures tested here by 2100 (IPCC 2007), thus larvae are likely to become exposed to these high temperatures more often, if not continually, in the relatively near future. Together with strong effects on mortality, and other work suggesting an effect on fertilization (e.g., Krupp et al. 2006, Negri et al. 2007), development (e.g., Bassim et al. 2002, Randall and Szmant 2009b), and settlement (e.g., Edmunds et al. 2001, Nozawa and Harrison 2007), the dispersal potential of corals is likely to change fundamentally in the near future, given the expected increases in temperature due to anthropogenic climate change.

4 Effects of delayed settlement on post-settlement growth and survival of scleractinian coral larvae

4.1 Introduction

Dispersal affects many aspects of a species ecology and life history evolution, including metapopulation dynamics, biogeography, and the genetic structure of populations. Benefits of dispersal include escape from density-dependent competition and predation, colonization of new habitats, and recolonization of previously-occupied habitats (Bowler and Benton 2005). Increased gene flow resulting from dispersal can reduce inbreeding depression, increase genetic variability, and reduce extinction rates (Clobert et al. 2001). However, to be effective, dispersal requires more than just movement of propagules; propagules must also successfully establish themselves in their new environment long enough to contribute to the new population ("effective" or "realized" dispersal: Kinlan and Gaines 2003). A trade-off exists between the benefits of an extended dispersal period and increased population connectivity, and the potential for post-settlement fitness costs, including reduced survival and reproductive success (Bonte et al. 2012). Understanding the consequences of delays in settlement (or an extended period in the plankton) is therefore essential to our understanding of a species' ecology and evolution (Marshall and Morgan 2011). Moreover, improved understanding of realized dispersal is required to assess the evolutionary benefits of dispersal and regulation of populations (Travis et al. in press).

In the marine environment, most invertebrate species have a complex life cycle with a benthic, relatively sedentary or sessile, adult phase and a dispersive larval stage. Dispersal times can range from hours to months, but generally there is a minimum amount of time that must elapse before a marine larva is competent to settle. Some

larvae can delay settlement beyond this obligate pre-competent stage, which increases the likelihood of encountering suitable habitat and can enhance population connectivity (Pechenik 1999). However, increased time in the plankton also has costs. Energy expended during dispersal is unavailable for use post-settlement, so in addition to the risk of starvation during dispersal, propagules that delay settlement are likely to have lower energy reserves at settlement than those that settle immediately after competence is acquired. For non-feeding species, the effects of delayed dispersal on larval energetics are expected to be more severe than for planktotrophic species (Miller 1993). Most studies on non-feeding larvae have examined species with short larval durations lasting only hours to days, such as bryozans, ascidians, and sponges. In most cases, delayed settlement caused a decrease in post-settlement survival and growth (Wendt 1998, Maldonado and Young 1999, Marshall et al. 2003; but see Hunter et al. 1999, Marshall et al. 2003 for exceptions). Much less is known about the effect of delaying settlement on non-feeding larvae with longer larval durations. A delay of two to three weeks resulted in a 25-50% decrease in survival and a 20-30% decrease in post-settlement growth for abalone (Roberts and Lapworth 2001, Takami et al. 2002, Onitsuka et al. 2010), which suggests that the energetic costs of dispersal may be larger and more demographically significant for taxa with longer-lived, non-feeding larvae.

Scleractinian corals are the primary architects of coral reefs, which are among the most productive ecosystems in the world. Most reef-building coral species are broadcast spawners, releasing their gametes into the water column where fertilization and embryogenesis occur externally (Baird et al. 2009). Initially, coral larvae pass through an obligate pre-competent period, during which larvae undergo rapid morphological and physiological changes. This is followed by a competent period, in which the larvae are capable of metamorphosis into juvenile corals. Competence can be

acquired as soon as 2-3 days after spawning for some species (Nozawa and Harrison 2005), but peak competence, when the majority of a cohort is capable of settling, does not generally occur until 4-13 days after spawning (Connolly and Baird 2010). Coral larvae have unusually long competence periods, with some species capable of delaying settlement for over 100 days (Connolly and Baird 2010). Most broadcast-spawned larvae are non-feeding and derive all of their energetic requirements during dispersal from the yolk (Baird et al. 2009).

The extended periods of competency found for coral larvae suggest the potential for considerable variation in age at settlement. However, we do not presently know the costs of delayed settlement for non-feeding coral larvae, such as the effects of depletion of energy reserves during dispersal on post-settlement growth and survival. In particular, the acquisition of zooxanthellae is an important event in the life cycle of corals because the majority of a coral's energy needs post-settlement are met by the translocation of carbon from the photosynthesizing zooxanthellae (Muscatine et al. 1981). Although some coral larvae inherit zooxanthellae maternally and can supplement their energy reserves (Richmond 1987), the majority of species must acquire zooxanthellae from the environment. Thus the rate at which zooxanthellae are acquired after propagules arrive at the settlement site also has important implications for post-settlement survival and growth.

Here, I examine the effects of delayed settlement on the post-settlement success of the common, broadcast-spawning reef coral, *Acropora tenuis*. My aim was to determine if coral larvae that delay settlement have higher post-settlement mortality than larvae that settle without a delay, and whether or not the process of colony growth, which occurs in corals through replication of module polyps by a process known as budding, is hindered by a delay. I also quantify the timing of zooxanthellae acquisition,

to assess the extent to which it may provide an alternative explanation for patterns in survival or growth. Demographic costs of delayed settlement, if significant, could mean that the apparently high dispersal potential of corals, which has been identified in studies of coral larvae, is unlikely to translate into realized dispersal that provides meaningful demographic connectivity over large distances.

4.2 Materials and methods

4.2.1 Larval cultures

The study took place at Orpheus Island Research Station (OIRS), on Orpheus Island in the central Great Barrier Reef (18° 61'S 146° 48'E) from November 2009 to January 2010. *Acropora tenuis* is a locally abundant, broadcast spawning species with non-feeding larvae that lack zooxanthellae when released. Larvae become competent to settle after ~4 days but have been observed to settle as much as 69 days after spawning (Nishikawa et al. 2003).

Larvae were cultured from *A. tenuis* colonies collected from Pioneer and Cattle Bays as follows. Six adult, gravid colonies were collected 2-3 days prior to the full moon and maintained in outdoor aquaria. On 26 November 2009, spawned gametes from all colonies were collected, combined, and left for two hours to fertilize. After fertilization, the developing embryos were transferred to 500 L aquaria containing 0.2 µm filtered seawater (FSW), where swimming larvae developed between 36-48 h later. The aquaria were continuously supplied with fresh FSW, at a flow rate of approximately 1.5 L/min, and air stones were provided to increase oxygenation. The aquaria were maintained for 40 days in a temperature controlled room at 29°C with a 12:12 h light:dark cycle.

4.2.2 Sampling design

At 12 days after spawning (DAS), when most larvae were competent to settle, ~ 2,000 larvae were transferred into 70 L settlement tanks containing 150mm x 15mm Petri dishes that had been drilled with a hole through the centre, roughened with sandpaper and soaked in FSW for 24 h. The dishes were also sprinkled with crushed crustose coralline algae, a known settlement inducer for *Acropora* species (Morse et al. 1996). To maximize the number of settlers, larvae were left in the settlement tanks for 48 h. Water was changed after 24 hours and ~2,000 more larvae then introduced. After 48 h, a census of the number of successfully attached, solitary juveniles on the settlement surfaces was completed. Within 24 h of the first census, the 40 dishes with the greatest number of settled juveniles were randomly distributed onto four racks (10 dishes each) and transported to Cattle Bay. The racks were suspended from star pickets at 3 m depth in habitat containing adult colonies of *A. tenuis*. Censuses were then made weekly, with the dishes collected from Cattle Bay, taken to OIRS for the census, and then returned to the field within 24-36 h. Additional batches of larvae were settled after both a two-week (26 DAS) and four-week delay (40 DAS), deployed to Cattle Bay, and censused weekly in the same manner as described above. At each census, the number of living juveniles was recorded for survival analysis and a digital photograph of each juvenile was taken for the determination of size at settlement, number of secondary polyps, and onset of zooxanthellae infection. Size was measured using ImageJ software (Abramoff et al. 2004) and calculated as the average of the longest diameter through the mouth of the settler and the diameter perpendicular to the first measurement. Zooxanthellae infection was defined as the first time zooxanthellae were observed inside the tissue of the settler using a stereo dissecting microscope. Censusing continued until 55 DAS. The study was terminated by Cyclone Yasi in February 2011,

which destroyed most of the racks and dishes. The three cohorts of juveniles will be referred to as 2 wk, 4 wk, and 6 wk settlers in the following sections.

4.2.3 Data analysis

I used mixed effects models to determine if delayed settlement had an effect on the size at settlement, survival, time to bud secondary polyps, or time to acquire zooxanthellae. The mixed-effects approach allowed me to explicitly account for random variation associated with settlement dish, or (where appropriate) with the deployment rack to which the settlement dish was affixed in the field. All analyses were done using R 2.13.0 (R Development Core Team 2011). Specifically, to determine if delayed settlement had an effect on size at settlement, I fit a linear mixed effects (LME) model with age at settlement, measured as DAS, as a fixed effect, and settlement dish as a random effect using the function "lme" in package "nlme" (Pinheiro et al. 2011). To determine whether delayed settlement had an effect on post-settlement survival, time to bud, or acquisition of zooxanthellae after deployment in the field, I used a mixed effects Cox Proportional Hazards (CPH) model ("coxme" package; Therneau 2011). The CPH model is a time-to-event analysis, which is designed for data that record when a defined event occurs (Muenchow 1986). CPH models allow for the hazard rate (the stochastic rate at which an event occurs) to vary over time in arbitrary fashion, but make a "proportional hazards assumption" that the relative effect of any treatment (i.e., the ratio of hazards between two treatments) is consistent over time. For each CPH analysis, I first tested the proportional hazards assumption using the "cox.zph" function in the "survival" package in R (Therneau and Lumley 2011), which fits a least-squares regression to Schoenfeld's partial residuals to diagnose nonproportionality (Grambsch and Therneau 1994). Provided the assumption was met, I then fit a series of models with and without the fixed effect of age at settlement, and all possible combinations of

the random effects of deployment rack and settlement dish (rack effect only, dish effect only, dish effect nested within rack). To select the best model for the data, I used Akaike's Information Criterion (AIC). I calculated Akaike weights for each model, which are estimates of the relative likelihood of a model, given the data, compared to the other models being considered (Burnham and Anderson 2002). I also used Akaike weights to calculate model-averaged parameter estimates of the fixed effect for each analysis. This is essentially an average effect size across all models, weighted by the models' relative likelihood, given the data. This approach yields more robust estimates of effect size than inference based solely on the best-fitting model (Burnham and Anderson 2002).

4.3 Results

Delayed settlement had a negative effect on the initial size of settlers. The estimated mean size of larvae that settled at 2 weeks (1139 ±10.97 µm) was significantly greater than that of 4 wk settlers (944 ± 14.56 µm), which, in turn, was greater than that of 6 wk settlers (895 ± 14.58 µm) (LME model, effect of DAS: $F_{2, 102}$=151.41, p < 0.001).

4.3.1 Survival

In contrast to the initial size of settlers, delayed settlement had no detectable effect on survival of *A. tenuis* juveniles (Figure 4.1A; Table 4.1). An initial analysis using all three cohorts found highly significant violation of the proportional hazards assumption of the Cox model (X^2 = 29.68; P<0.001). Inspection of the data suggested that this was due to the 6 wk cohort, which had disproportionately high survival during the first week post-settlement, but not the second week (Figure 4.1A). Therefore, I

excluded this third cohort and compared only the 2 wk and 4 wk cohorts. For this subset of the data, the proportional hazards assumption was met (X^2 = 0.685; P=0.408). The best-fit model included a random effect of dish nested within rack, but no fixed effect of delayed settlement (Table 4.1). Indeed, survival was virtually indistinguishable between 2 wk and 4 wk cohorts, a result that is apparent in their highly coincident survivorship curves (Figure 4.1A), and the fact that confidence intervals on the model-averaged hazard ratio are narrowly centered around one (i.e., no difference: Table 4.2)

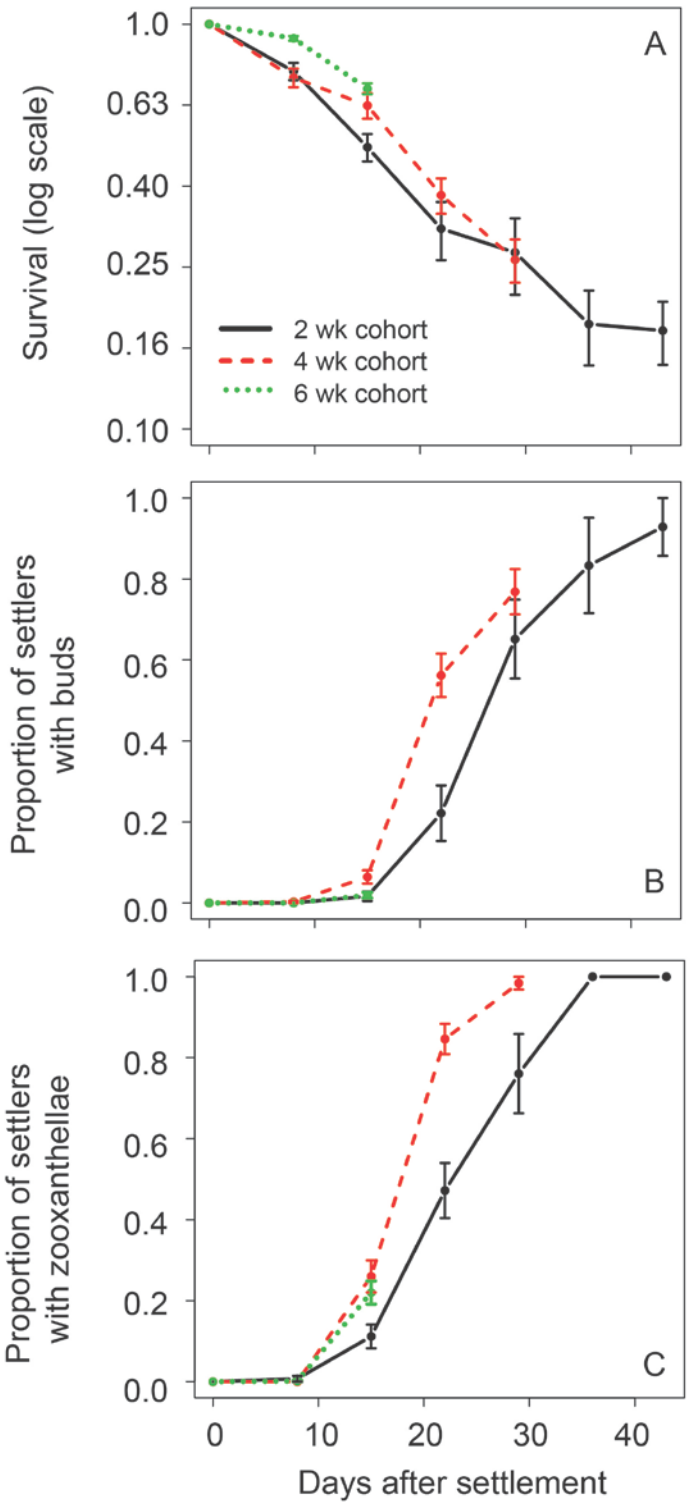

Figure 4.1 Effect of delayed settlement on the survival, time to bud, and acquisition of zooxanthellae of three cohorts of *Acropora tenuis* settlers. The points represent the mean proportion of settlers on each dish that A) survived, B) budded additional polyps, or C) acquired zooxanthellae. Error bars represent one standard error.

Table 4.1 Model selection results for time-to-event analyses of three cohorts of *Acropora tenuis* juveniles. "NULL" is the simplest model with no fixed or random effects. Random effects are noted in parentheses, with the forward slash (/) indicating nesting. "Cohort" refers to a fixed effect associated with when settlement occurred (2 wk, 4 wk, or 6 wk). ΔAIC is the difference between the AIC of the indicated model and the best-fitting model, which is indicated in bold type. w_i indicates the Akaike weight (the estimated probability that the model is the best in the model set).

	Survival		Budding		Zooxanthellae Acquisition	
Model	**ΔAIC**	**w_i**	**ΔAIC**	**w_i**	**ΔAIC**	**w_i**
1) NULL	213.68	0.00	20.50	0.00	76.49	0.00
2) (rack)	122.28	0.00	12.12	0.00	49.07	0.00
3) (dish)	7.63	0.02	14.11	0.00	16.14	0.00
4) (rack/dish)	**0.00**	**0.71**	9.62	0.01	7.14	0.02
5) cohort	214.07	0.00	4.30	0.08	48.85	0.00
6) cohort + (rack)	124.27	0.00	5.96	0.03	42.12	0.00
7) cohort + (dish)	9.59	0.01	**0.00**	**0.65**	**0.00**	**0.57**
8) cohort + (rack/dish)	1.99	0.26	2.01	0.24	0.65	0.41

Table 4.2 Model-averaged estimates of the hazard ratio (with 95% confidence intervals) for the effect of delayed settlement on survival, time to bud, and acquisition of zooxanthellae of three cohorts of *Acropora tenuis* juveniles. "Hazard Ratio" is the rate at which the relevant event occurs (mortality, zooxanthellae acquisition, or budding) for the indicated cohort, relative to the 2 wk cohort (e.g., a hazard ratio of 2.5 for the 4 wk cohort indicates that the event occurs at approximately 2.5 times the rate at which it occurs in the 2 wk cohort). Random effects are noted in parentheses in the "Best Model" column, with the forward slash (/) indicating nesting. Note that there is no parameter estimate for the 6 wk cohort in the survival analysis because the proportional hazards assumption was violated.

			Hazard Ratio Parameter Estimate		
Analysis	**Best Model**	**Fixed Effect**	**Best Model**	**P**	**Model-averaged**
Survival	(rack/dish)	--	--	--	0.99 (0.93, 1.04)
Budding	cohort + (dish)	4 wk	2.10 (1.30, 3.40)	0.00	2.06 (1.28, 3.33)
		6 wk	0.64 (0.29, 1.42)	0.27	0.64 (0.29, 1.40)
Zooxanthellae Acquisition	cohort + (dish)	4 wk	2.58 (1.68, 3.96)	<0.001	2.59 (1.59, 4.24)
		6 wk	2.37 (1.48, 3.78)	<0.001	2.39 (1.41, 4.05)

4.3.2 Growth

For budding and zooxanthellae acquisition, there was strong evidence for between-cohort differences, but they were contrary to my expectations (Figure 4.1B, C). In both analyses, the proportional hazards assumption between groups was met for the analysis with all three cohorts (budding: 4 wk X^2=2.53, P=0.11; 6 wk X^2=0.50, P=0.48; zooxanthellae acquisition: 4 wk X^2=1.71, P=0.19; 6 wk X^2=0.95, P=0.33). Also for both analyses, the best-fitting model included a fixed effect of settlement cohort and a random effect of dish (Table 4.1). Budding commenced approximately 2.1 times sooner after settlement in the 4 wk cohort, compared to the 2 wk cohort (Table 4.2). In contrast, time to commence budding in the 2 wk and 6 wk cohorts were not significantly different (i.e., confidence intervals on the model-averaged hazard ratio encompassed 1: Table 4.2). Zooxanthellae were acquired sooner in both the 4 wk and 6 wk cohorts, compared to the 2 wk cohort (Figure 4.1C, Table 4.2).

4.4 Discussion

Despite the fact that newly settled lecithotrophic larvae of a broadcast spawning coral were smaller when settlement was delayed, I found no evidence that the delay deleteriously affected the post-settlement processes measured in this study. Larval energetics are often invoked to explain the costs of delayed settlement for non-feeding invertebrate larvae (e.g., Jaeckle 1994, Marshall et al. 2003). For example, it is generally assumed that long larval durations cause larvae to expend more energy during the planktonic phase, leaving fewer reserves for post-settlement survival and growth. However, my results suggest that coral larvae have ample energy reserves to sustain at least a four-week delay in settlement with no appreciable adverse effects on three

critical post-settlement processes: survival, budding, and the acquisition of zooxanthellae.

Contrary to my expectation, 4 wk settlers began budding approximately twice as quickly after settlement than 2 wk settlers, although 6 wk settlers did not. Presently, little is known about the initial period of growth after settlement for corals, as they are difficult to track and usually not studied in the field until they are large enough to be seen with the naked eye (e.g., Babcock 1985, Harriott & Fisk 1985, Fitzhardinge 1988). Although growth of marine invertebrates (including colonial organisms) generally increases with warmer water temperatures and increased food availability (Hunter and Hughes 1993, Lambert 2005, Saunders and Metaxas 2009), difference in field seawater temperatures among the grow-out periods of the three larval cohorts is an unlikely explanation for the more rapid growth of the 4 wk settlers. Average seawater temperatures during the second week after settlement, when differences in budding rate first became apparent, differed by $<0.1^{0}$C between the 2 wk (28.76^{0}C) and 4 wk (28.73^{0}C) settlers, and was actually highest for the 6 wk settlers (29.74^{0}C) (AIMS 2011).

One possible explanation for the lack of a negative effect of delayed settlement on polyp budding is that this process might be more strongly dependent on the uptake of zooxanthellae than on energy reserves at settlement and zooxanthellae uptake might not be affected by delayed settlement. Corals derive the majority of their carbon from their endosymbionts (Muscatine et al. 1981), so the acquisition of zooxanthellae is a key event in the life cycle of a coral. Budding may well be sufficiently energetically expensive for a newly-settled coral that it is strongly dependent on the successful establishment of symbiosis. Consistent with this, the proportion of juveniles with zooxanthellae increased slightly faster than the proportion with buds (Figure 4.1B, C),

and, of the 190 settlers that produced secondary polyps during the study, only one of these lacked zooxanthellae.

To examine the possibility that the acquisition of *Symbiodinium* endosymbionts precedes the initiation of polyp budding more quantitatively, I used a generalized linear mixed model with a binomial response (success or failure to bud), fit only to the subset of settlers that had acquired zooxanthellae, on each day separately ("glmer" function, "lme4" package; Bates et al. 2011). This analysis suggests that, once the presence of zooxanthellae is accounted for, budding responds nearly identically in all three cohorts (Figure 4.2). Consequently, the variation in time to budding between cohorts appears likely to be explained, at least in part, by the timing of zooxanthellae acquisition.

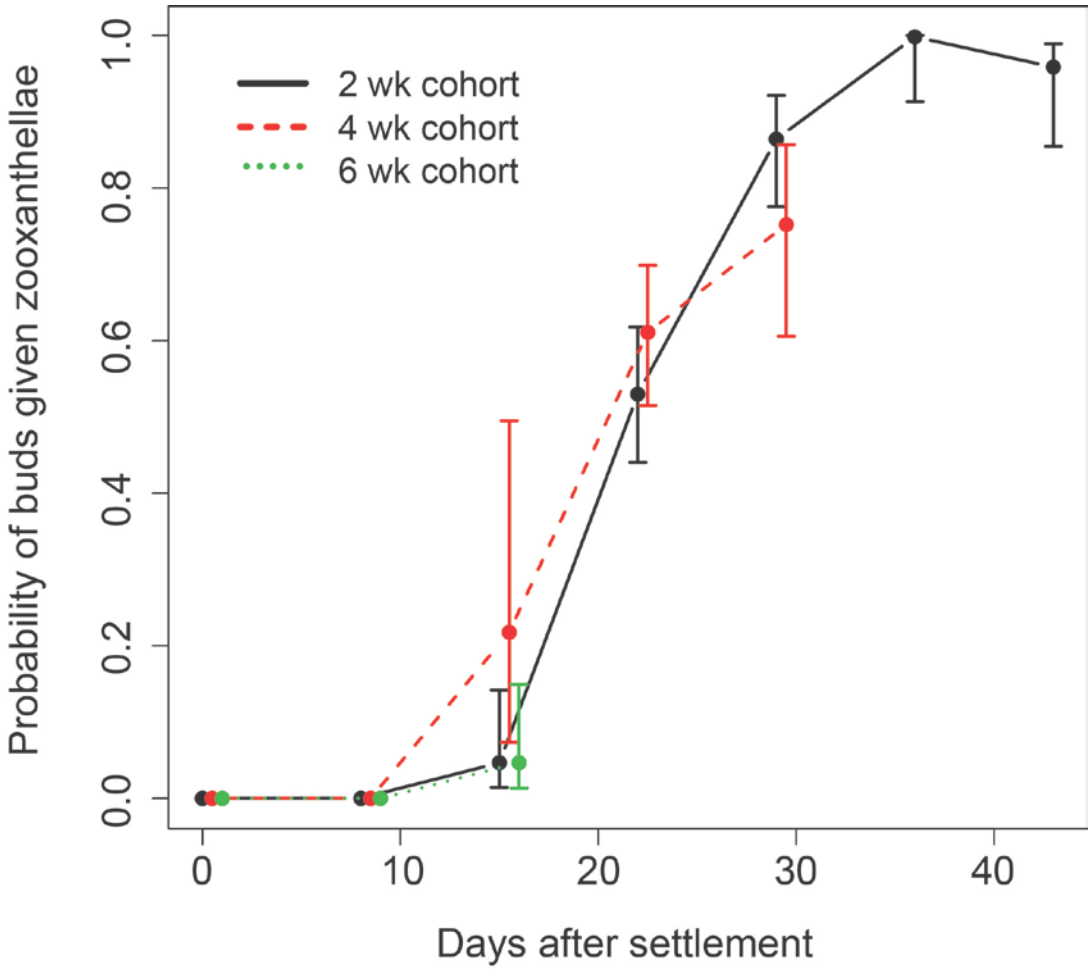

Figure 4.2 Proportion of juveniles with zooxanthellae that also have buds for the three cohorts of *Acropora tenuis* settlers. Error bars represent the standard error of the estimated proportion, obtained from a generalized linear mixed effects model fit to each day, with a random effect of dish and binomial error. Note that standard errors are asymmetric because they have been back-transformed from an inverse logistic scale.

There are two possible explanations for differences in the temporal pattern of zooxanthellae acquisition among larval cohorts. The higher rate of acquisition in the 4 wk cohort could, in principle, be due to developmental constraints on how soon after fertilization corals can acquire zooxanthellae, i.e., the age of the individual rather than the time since settlement. However, this seems unlikely, given that several *Acropora* species have been shown to acquire zooxanthellae as early as 5 DAS, with the proportion infected approaching 100% by 7 DAS, when zooxanthellae were supplied in an aquarium study (Harii et al. 2009). This suggests that temporal variation in prevailing environmental conditions that influence zooxanthellae availability or uptake is a more likely explanation. Unfortunately, although the abundance of *Symbiodinium* cells has been found to be greater in reef sediments than in the water column near a northern reef in the Great Barrier Reef (Littman et al. 2008), virtually nothing is known about the temporal variation in the distribution and abundance free-living zooxanthellae in reef habitats. Regardless of the mechanism driving the observed variation in time to acquire zooxanthellae, however, the onset of symbiosis appears to provide a much better explanation for the among-cohort variation in budding rate than the length of delay prior to settlement.

The ability to delay settlement for a period of time after competence is achieved, without suffering major deleterious effects, supports the hypothesis that the long pelagic larval durations and extended competence periods of corals enhance their potential for realized dispersal. This is not to say that delayed settlement has no deleterious effects. For instance, the proportion of a cohort that survives and is capable of successfully metamorphosing declines by 3-100-fold between two and six weeks for three species in the coral genus *Acropora* (Connolly and Baird 2010). Studies of a range of marine invertebrates have shown that species with non-feeding larvae are more likely to suffer

physiological costs of dispersal after settlement, compared to species with planktotrophic larvae, and thus costs of delayed settlement may constitute a greater barrier to population connectivity in non-feeding larvae (Pechenik 2006). In contrast, my results suggest that the trade-off between extended dispersal periods and post-settlement fitness is less severe than expected, and therefore that the costs of dispersal in broadcast spawning corals are principally those accrued during the extended larval phase, rather than after settlement has occurred.

5 General Discussion

Research presented in this thesis significantly advances understanding of the energetics of lecithotrophic larvae of scleractinian corals, filling key knowledge gaps that have limited our ability to estimate the dispersal potential of corals and fully understand the importance of the early (larval) life history stage in the ecology and evolution of corals. Results presented in Chapter 2 move us closer to solving the discrepancy between early energetically-based calculations of larval longevity and the longer pelagic larval durations found empirically in more recent larval studies, revealing that a significant decrease in larval respiration and lipid utilization rates, occurring soon after larvae become competent to settle, and lasting for up to two months, accounts for the extended larval longevities found for several coral species. In Chapter 3, I demonstrate that temperature can alter this general pattern of larval energetics, but that rates of fundamental biological processes, specifically of oxygen consumption and lipid use, are parabolically, rather than monotonically, related to temperature in larvae of *Acropora tenuis*. In contrast, larval mortality rates do increase monotonically with temperature, adding to the litany of impacts that warming oceans are likely to have on coral populations. In Chapter 4, I show that extending larval durations for up to four weeks after the majority of a cohort acquires competence does not lead to additional, deleterious effects on post-settlement mortality or budding rates of coral juveniles. These findings provide further corroborative evidence that the period of reduced metabolism that coral larvae enter soon after competence is achieved has important implications for realized dispersal of coral larvae and the connectivity of coral populations.

In the remaining sections of this final chapter, I provide an integrated assessment of the outcomes of the research described in each chapter, including how

outcomes match the objectives outlined in my introductory chapter, and the broader significance of these findings for our current understanding of the effects of dispersal on coral populations in particular, and organisms with lecithotrophic larvae in general. The results from all three chapters are integrated with previous work on larval dispersal. Finally, where appropriate, I identify productive directions for future research.

5.1 Larval energetics

My thesis research identifies a mechanism underpinning the ability of non-feeding (lecithotrophic) larvae of marine invertebrates to survive for months in the plankton, thereby providing important opportunities for enhanced inter-reef connectivity. My results indicate that scleractinian coral larvae are exceptional among lecithotrophic marine invertebrates because of their capacity to successfully metamorphose after lengthy larval durations that are on par with, or even exceed, larval durations of many planktotrophic larvae (e.g., Bradbury et al. 2008, Shanks et al. 2003). Changes in respiration rates and lipid content of larvae from four scleractinian coral species demonstrate that coral larvae enter a state of reduced metabolism within a week of spawning, substantially reducing rates at which they deplete endogenous energy reserves. Despite low metabolic rates, coral larvae continued to survive, swim, and successfully complete metamorphosis. To my knowledge, this is the first study to examine changes in the metabolic rates of lecithotrophic larvae at a fine temporal scale over such a long period of larval life. Because few studies of non-feeding larval energetics extend past two weeks (see Okubo et al. 2008, Hoegh-Guldberg and Emlet 1997, Moran and Manahan 2003 for exceptions), my results raise the possibility that other taxa with lecithotrophic larvae may adopt similar strategies to extend larval

longevity and increase energy reserves available for metamorphosis and growth after long-distance dispersal.

It has been known for many years that scleractinian coral larvae have the potential to spend several weeks to months in the plankton prior to settlement (e.g., Richmond 1987, 1988; Baird 1998, Nozawa and Harrison 2000, Graham et al. 2008, Connolly and Baird 2010), yet there has been only one attempt to explain the larval duration of corals using an energetic approach (Richmond 1987, 1988). Richmond's estimate of larval duration is substantially shorter than recent observed lifespans for broadcast-spawned, non-feeding coral larvae. The rapid and marked declines in rates of oxygen consumption and lipid use found in Chapter 2, once larvae commence swimming, suggest that estimates of energy use made relatively early in larval life are likely to overestimate the amount of energy being consumed and therefore underestimate the length of time a larva can survive with those energy stores. Providing a physiological explanation for the extended larval durations observed empirically for broadcast spawning corals is an important step forward in the development of accurate models for the dispersal of coral larvae.

The small discrepancy that still exists between model estimates and observed larval duration for some of the species studied here, when energy use is converted to energetic equivalents and directly compared (Table 2.2), indicates that there are further questions related to larval energetics that need to be addressed. Although it is possible that the estimates of energy reserves made in Chapter 2 are biased towards the low end of the range, a number of other factors could also contribute to explaining the discrepancy. Based on previous work on coral (Arai et al. 1993, Harii et al. 2007) and echinoderm larvae (Sewell 2005, Prowse et al. 2008), I chose two classes of lipid, wax esters and triacylglycerides, as the sources of energy in coral larvae. Theoretically,

other lipid classes, protein, or carbohydrates, which I did not quantify, could make major contributions to the energy budgets of coral larvae. At present, not much is known about the protein or carbohydrate content of coral eggs but some data are available for coral larvae. Planulae of the brooding coral *Pocillopora damicornis* are composed of 17% protein and 13% carbohydrate (Richmond 1987), while planulae of the soft coral *Heteroxenia fuscescens* consist of 33.6% protein and 1.3% carbohydrate (Ben-David-Zaslow and Benayahu 2000). Although the latter study tracked temporal changes in biochemical content of *H. fuscescens* and found that the carbohydrate content declined, neither protein (24.0 kJ g^{-1}) nor carbohydrate (17.5 kJ g^{-1}) provide as much energy as lipid (39.5 kJ g^{-1}; Gnaiger 1983), and neither are present in sufficient quantities to make up for the demand calculated using oxygen consumption of the coral larvae in this study (Chapter 2). Therefore, contributions from alternative energy sources are unlikely to fully explain the discrepancy.

The apparent shortfall in energetic reserves calculated from respirometry data, even after potential carbohydrate and protein sources are considered, suggests another possible explanation for the longevity of lecithotrophic coral larvae. It is possible that coral larvae supplement their endogenous reserves by assimilating dissolved organic material (DOM) from their environment to increase their overall energy budget. Many marine invertebrate larvae have been shown to take up DOM, including echinoderms (De Burgh and Burke 1982, Hoegh-Guldberg 1994), molluscs (Manahan and Crisp 1982, Jaeckle and Manahan 1989), and bryozoans (Jaeckle 1994, Johnson and Wendt 2007). The contribution of DOM to the energy budgets of these invertebrate larvae ranged from 0.6-72% in these studies. In corals, only larvae of the soft coral *H. fuscescens* have been shown to uptake DOM, with a potential contribution of 11% to the metabolic demand of planulae (Ben David Zaslow and Benayahu 2000). This

suggests that even if the larvae in this study were capable of acquiring energy from their environment, they would need to do so at a much higher rate than observed previously in corals.

As discussed in Chapter 2, I consider the possibility that my oxygen consumption measurements overestimated metabolism to be the most plausible explanation for the remaining discrepancy between calculated and observed larval durations, because the handling involved in transferring larvae from aquaria to the respirometry chamber is likely to have stimulated a metabolic response in the larvae. One way to assess the possibility of bias in the estimates of metabolic rate is to develop a method of measuring the respiration of coral larvae without agitating the larvae just prior to measurements, for example, by using an open respirometry system in which the larvae can remain in chambers for longer periods of time without risking anoxia. Determining which, if any, of the possibilities outlined above is responsible for the discrepancy between model estimates and empirical observations should be a research priority, given the importance of larval dispersal for the long term persistence of coral populations in the face of climate change and other anthropogenic impacts on reefs.

Despite the discrepancy between energetic measurements and empirical evidence, the exceedingly long larval durations observed for scleractinian coral larvae and the overall consistency between larval patterns in lipid use and oxygen consumption documented in this thesis point to a reduction in energy use with larva age as a major contributing factor. Further evidence of the likelihood that reduced energy during larval stages promote inter-reef connectivity is provided by the survival and competence dynamics of larvae during this period of low metabolism; both of which are necessary for realized dispersal. There were no marked declines in mortality rates for any of the four species that coincided with the period of reduced metabolism. Survival

of *G. aspera* larvae remained high throughout the study, while mortality rates in *A. tenuis* larvae increased smoothly over two months. *Acropora spathulata* mortality rates were actually lower during the period of reduced metabolism compared to development. And while mortality rates did increase for *A. nasuta* after competence was acquired, it was only over a period of three days. Overall, these data provide further evidence that low larval metabolic rates and energy use maintain mortality rates at low levels as larvae age, with increasing mortality rates only occurring much later in their larval lifetimes. Consistent with this interpretation, previous work indicated that mortality rates in aquaria did not increase until ~100 days after spawning (Graham et al. 2008). On the other hand, the temporal dynamics of settlement documented here and elsewhere (Connolly and Baird 2010) reveal dramatic declines in competence over the period of low metabolism. Given results presented in this thesis, it is unlikely that such declines occur because larvae no longer have the energy to metamorphose, but further work should quantify how much energy is actually required for metamorphosis. Because metamorphosis in many marine invertebrates is in response to an external cue, one hypothesis for declining competence is that larvae lose their ability to respond to metamorphic inducers due to the degeneration of receptors or neural connections (Pechenik 1980, but see Swanson et al. 2006). There is still much to learn about the induction of metamorphosis in coral larvae (Hadfield 2011), and determining the relationship between declining energy reserves and declining responsiveness to inducers required for metamorphosis would help to resolve outstanding questions.

5.2 Temperature effects on larval energetics

Temperature is an important environmental variable known to affect the metabolism of organisms, particularly those unable to control their internal body temperatures, such as marine invertebrates. Increased temperatures lead to increased mortality, decreased fertilization rates, and increased development rates in coral larvae (e.g., Bassim and Sammarco 2003, Edmunds et al. 2001, Krupp et al. 2006, Negri et al. 2007, Nozawa and Harrison 2007). The effects of temperature on settlement rates are less clear, but appear to be related to both the duration of exposure and the magnitude of the temperature increase, with short-term exposures (10 min to 24 h) to higher temperatures increasing settlement (Coles 1985, Edmunds et al. 2001, Nozawa and Harrison 2007), but long-term exposures (8-9 d) decreasing settlement (Bassim and Sammarco 2003; Randall and Szmant 2009a, 2009b; but see Nozawa and Harrison 2007). However, very few studies have actually measured the respiration rates of coral larvae at different temperatures (but see Edmunds et al. 2001, Rodriquez-Lanetty et al. 2009, Edmunds et al. 2011) and none have measured larval energy content. This makes it difficult to directly link metabolism and declining energy reserves to mortality or settlement. Moreover, most of these studies were primarily interested in whether there were statistically-detectable differences in physiological responses at temperatures above ambient; none have calibrated how physiological rates vary as a continuous function of temperature, which is important for estimating likely responses at temperatures other than the specific values used in experimental treatments.

In Chapter 3, the parabolic response to temperature that I quantified for metabolic rates of coral larvae, with optima at ambient temperatures, has important implications for understanding the potential for adaptation of larval physiological processes to warming oceans. Temperature typically has a two-phase effect on

biological response rates, with an increase in rates up to a particular temperature optimum, after which rates decrease. Optima typically vary among populations that have different environmental histories, depending on the level of genetic variation among populations, and local adaptation in indicated when populations shift their metabolic properties to maximize performance under local temperature conditions (Levinton 1983). Edmunds and co-authors (2011) were the first to note this effect of temperature on respiration rates in a range of scleractinian corals. A similar parabolic response to temperature can be seen in the oxygen consumption of adults of the starfish *Acanthaster planci* (Yamaguchi 1974) and even in larval settlement patterns of the brooding coral *Pocillopora damicornis* (Jokiel and Guinther 1978). What my results mean for the dispersal potential of scleractinian corals will depend on the rate at which their metabolic optima can adapt to changing temperature. Overall, metabolic rates influence dispersal potential, so increasing temperatures are likely to increase metabolism of coral larvae up to a point and, when combined with increased mortality, decrease dispersal potential of coral larvae.

Assuming that the parabolic responses documented here are indicative of local adaptation, there is the potential for peak metabolic rates to evolve towards higher temperatures as global sea temperatures increase; however, the key question is how fast such adaptation can occur. One way to test this would be to empirically derive a temperature performance curve (Huey and Stevenson 1979) at various latitudes across a species' range and compare temperature thresholds and tolerance ranges for respiration rates among populations. Evidence for adaptation would be reflected in a tendency for the temperature optimum of peak respiration to shift according to local environmental conditions. Also, one might predict populations from higher latitudes to have wider performance or tolerance breadths (Huey and Kingsolver 1989). Conversely, the fact

that larval mortality responses are monotonic suggests that the capacity for adaptation to ameliorate the effects of temperature on larval metabolic rates would nevertheless have limited impact on larval survival. Although Edmunds et al. (2001) found a parabolic effect of temperature on larval mortality of *Porites astreoides*, most studies, including this one, have found a monotonic effect, with survival decreasing by 50-90% over a range of 4-5°C (Bassim and Sammarco 2003, Randall and Szmant 2009a, 2009b). The 80% decrease in median survival times found in this thesis, while at the high end over a similar temperature range, is comparable and indicates a significant barrier to adaptation of coral larvae to warming oceans.

5.3 Effects of delayed settlement

The cost of dispersal is one of the most poorly understood aspects of metapopulation dynamics. In the marine environment, dispersive larvae utilize energy during dispersal, and there is a widely-held view that, as a consequence, longer times in the plankton imply reduced post-dispersal survival and growth. This reduced "realized dispersal" is believed to be particularly pronounced in organisms with non-feeding larvae. My thesis tests this hypothesis for non-feeding larvae of a broadcast-spawned scleractinian coral that are known to be able to successfully metamorphose after long periods in the plankton. In Chapter 4, the negligible effects of 2-6 weeks delayed settlement on juvenile survival and growth for *A. tenuis* overturn this paradigm. This is the first study to examine the effects of delayed settlement on scleractinian corals, the primary architects of coral reefs. The results are consistent with the pattern of energy use found in Chapter 2, which shows that larvae of *A. tenuis* lost less than 1% of their original lipid between 2 and 4 wks, and only 7% between 4 and 6 wks. Similar patterns

of lipid use for the three other coral species studied here suggest that larvae of broadcast spawning corals are likely to have comparable post-settlement success.

The capacity of coral larvae to delay settlement for weeks to months is likely to translate into realized dispersal that has important demographic implications for coral populations. In the broadest sense, my results challenge the view that the energetic costs of time spent in the plankton can generally be expected to decrease demographically significant population connectivity. However, because growth of juvenile corals appears to be dependent on zooxanthellae acquisition following settlement, the potential for delayed settlement to enhance realized dispersal in other marine invertebrates that do not have similar phototrophic sources of nutrition may be limited. Moreover, although survival of juvenile corals does not appear to be affected by delayed settlement, larval mortality during dispersal, though variable, is generally thought to be quite high (Rumrill 1990), and, combined with declining competence after peaking 1-2 weeks after spawning, at least in some species (Connolly and Baird 2010), may significantly reduce levels of realized dispersal. Considering all costs of dispersal, including larval mortality, competence, and post-settlement effects, the positive effects of delayed settlement on coral population connectivity are likely to be overshadowed by processes that occur during the larval phase, i.e., reduced larval survival and loss of competence). Thus larval processes are likely to play a bigger role in inter-reef connectivity of coral populations than processes occurring after metamorphosis.

5.4 Dispersal potential of scleractinian corals

To estimate the dispersal potential of marine organisms, an understanding of both hydrodynamics and biological traits are required (Cowen et al. 2007). Great strides

have been made in our understanding of both the oceanographic conditions affecting planktonic larvae and the biological parameters influencing dispersal, including larval durations, mortality rates, and larval behaviour, that improve our ability to model connectivity among reefs (e.g., Cowen et al. 2006, Treml et al. 2008, Kool et al. 2011). At large scales, ocean currents enable the long distance transport of larvae, while at local scales, topographically complex coral reefs interact with currents, tides, and winds to create water flows that may retain larvae (Willis and Oliver 1990, Baums et al. 2006, Cetina-Heredia and Connolly 2011, Andutta et al. 2012). Because they are poor swimmers (Chia et al. 1984), coral larvae are generally considered passive particles, although changes in buoyancy during larval life may allow them limited vertical movement (Willis and Oliver 1988, Carlon and Olson 1993, Stake and Sammarco 2003, Szmant and Meadows 2006). Recently-documented temporal patterns in larval survival (Graham et al. 2008) and in the acquisition and loss of competence (Connolly and Baird 2010) have filled some knowledge gaps necessary to make better predictions about dispersal in corals. However, few biophysical models of dispersal dynamics incorporate considerations of the impacts of environmental variables on larval physiology (reviewed in Lett et al. 2010). Global sea surface temperatures have increased over the past century and will continue to do so into the near future, at accelerating rates (IPCC 2007). Therefore, future models will require quantifiable effects of temperature on larval physiology, such as the ones documented in this thesis, to make more accurate predictions of patterns of population connectivity under different climate change scenarios. Given my results that coral larvae are likely to acquire competence faster and die at a higher rate in a warmer ocean, it is likely that an increasing number of larvae will settle locally while the potential for long distance dispersal will be decreased, thereby reducing connectivity between reefs. Decreased connectivity between reefs will

significantly reduce the capacity of coral communities to recover following disturbances, which are also predicted to increase with climate change (Wilkinson 2008).

5.5 Conclusion

Marine invertebrate larvae can be broadly classified as being either planktotrophic or lecithtrophic, depending on their source of nutrition during the dispersal phase. Conventional wisdom holds that planktotrophic larvae capable of feeding will necessarily have longer pelagic larval durations than species whose nutrition is derived solely from the egg and for the most part, this is true. Scleractinian coral larvae, however, are unique among marine lecithotrophs in that their PLD's are on par with, and often exceed, those of planktotrophs. For most non-feeding marine larvae, delaying settlement by even a few hours is enough to increase mortality and decrease growth post-settlement. However, as I demonstrate in Chapter 2, coral larvae enter a state of reduced metabolism soon after competence is acquired, suggesting that coral larvae are equipped with more than enough energy to disperse long distances. In Chapter 4, I corroborate these findings and show that increasing the larval duration from two to six weeks does not have a negative effect on coral larvae post-settlement. Thus the predicted trade-off between delayed settlement and post-settlement fitness appears to be less applicable to reef-building scleractinian corals than other taxa with non-feeding larvae. Although these findings increase the likelihood that the dispersal potential of larvae will be realized for corals, an increased amount of time in the plankton does expose coral larvae to higher risks of predation and potentially to suboptimal temperatures. Increases in mortality due to anticipated increases in sea

temperatures will likely decrease inter-reef connectivity, with major implications for the capacity of coral communities to recover from ever-increasing disturbances.

References

Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics International 11:36-42

AIMS (Australian Institute of Marine Science) (2011) Table generated 12th December 2011 using Reef Weather, Orpheus Island. Long Term Monitoring and Data Centre, AIMS. http://data.aims.gov.au/aimsrtds/station_data.xhtml?station=9

Andutta FP, Kingsford MJ, Wolanski E (2012) 'Sticky water' enables the retention of larvae in a reef mosaic. Estuar Coast Shelf Sci 101:54-63

Anger K (1996) Physiological and biochemical changes during lecithotrophic larval development and early juvenile growth in the northern stone crab, *Lithodes maja* (Decapoda: Anomura). Mar Biol 126:283-296.

Arai T, Kato M, Heyward A, Ikeda Y, Iizuka T, Maruyama T (1993) Lipid composition of positively buoyant eggs of reef building corals. Coral Reefs 12:71-75

Babcock RC (1985) Growth and mortality in juvenile corals (*Goniastrea*, *Platygyra* and *Acropora*): the first year. Proc Fifth Int Coral Reef Congress 4:355-360

Baird AH (1998) The length of the larval phase in corals: new insights into patterns of reef connectivity, Australian Coral Reef Society Newsletter pp 6-8

Baird AH (2001) The ecology of coral larvae: settlement patterns, habitat selection and the length of the larval phase. Ph.D. thesis, James Cook University, p181

Baird AH, Gilmour JP, Kamiki TM, Nonaka M, Pratchett MS, Yamamoto HH, Yamasaki H (2006) Temperature tolerance of symbiotic and non-symbiotic coral larvae. Proc Tenth Int Coral Reef Symp 4:38-42

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551-571

Bassim KM, Sammarco PW (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). Mar Biol 142:241-252

Bassim KM, Sammarco PW, Snell TL (2002) Effects of temperature on success of (self and non-self) fertilization and embryogenesis in *Diploria strigosa* (Cnidaria, Scleractinia). Mar Biol 140:479-488

Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-41

Baums IB, Paris CB, Cherubin LM (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. Limnol Oceanogr 51:1969-1981

Ben-David-Zaslow R, Benayahu Y (2000) Biochemical composition, metabolism, and amino acid transport in planula-larvae of the soft coral *Heteroxenia fuscescens*. J Exp Zool 287:401-412

Berkelmans R, Willis BL (1999) Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs 18:219-228

Birkeland C, Chia FS, Strathmann RR (1971) Development, substratum selection, delay of metamorphosis and growth in seastar, *Mediaster aequalis* Stimpson. Biol Bull 141:99-108

Bohonak AJ (1999) Dispersal, gene flow, and population structure. Q Rev Biol 74:21-45

Bonte D, Van Dyck H, Bullock JM, Coulon A, Delgado M, Gibbs M, Lehouck V, Matthysen E, Mustin K, Saastamoinen M, Schtickzelle N, Stevens VM, Vandewoestijne S, Baguette M, Barton K, Benton TG, Chaput-Bardy A, Clobert J, Dytham C, Hovestadt T, Meier CM, Palmer SCF, Turlure C, Travis JMJ (2012) Costs of dispersal. Biol Rev 87:290-312

Bosch I, Pearse JS (1990) Developmental types of shallow-water asteroids of Mcmurdo Sound, Antarctica. Mar Biol 104:41-46

Bowler DE, Benton TG (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biol Rev 80:205-225

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE (2008) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc R Soc Biol Sci Ser B 275:1803-1809

Brooke S, Young CM (2005) Embryogenesis and larval biology of the ahermatypic scleractinian *Oculina varicosa*. Mar Biol 146:665-675

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789

Bryan PJ (2004) Energetic cost of development through metamorphosis for the seastar *Mediaster aequalis* (Stimpson). Mar Biol 145:293-302

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. 2nd edition. Springer, New York

Carlon DB, Olson RR (1993) Larval dispersal distance as an explanation for adult spatial pattern in 2 Caribbean reef corals. J Exp Mar Biol Ecol 173:247-263

Cetina-Heredia P, Connolly SR (2011) A simple approximation for larval retention around reefs. Coral Reefs 30:593-605

Chia FS, Bucklandnicks J, Young CM (1984) Locomotion of marine invertebrate larvae - a review. Can J Zool 62:1205-1222

Clobert J, Danchin E, Dhondt AA, Nichols JD (eds) (2001) Dispersal. Oxford University Press, New York

Coles SL (1985) The effects of elevated temperature on reef coral planula settlement as related to power station entrainment. Proc Fifth Int Coral Reef Congress 4:171-176

Collin R (2003) Worldwide patterns in mode of development in calyptraeid gastropods. Mar Ecol Prog Ser 247:103-122

Connolly SR, Baird AH (2010) Estimating dispersal potential for marine larvae: dynamic models applied to scleractinian corals. Ecology 91:3572-3583

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: Open or closed? Science 287:857-859

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522-527

Cowen RK, Gawarkiewic G, Pineda J, Thorrold SR, Werner FE (2007) Population connectivity in marine systems: An overview. Oceanography 20:14-21

De Burgh ME, Burke RD (1982) Uptake of dissolved amino acids by embryos and larvae of *Dendraster excentricus* (Eschscholtz) (Echinodermata: Echinoidea). Can J Zool 61:349-354

Efron B, Tibshirani RJ (1993) An Introduction to the Bootstrap. Boca Raton: Chapman & Hall/CRC

Edmunds PJ, Gates RD, Gleason DF (2001) The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances. Mar Biol 139:981-989

Edmunds PJ, Cumbo V, Fan TY (2011) Effects of temperature on the respiration of brooded larvae from tropical reef corals. J Exp Biol 214:2783-2790

Emlet RB (1995) Developmental mode and species geographic range in regular sea-urchins (Echinodermata, Echinoidea). Evolution 49:476-489

Emlet RB, McEdward LR, Strathmann RR (1987) Echinoderm larval ecology viewed from the egg. In: Jangoux M, Lawrence JM (eds) Echinoderm Studies, Vol 2. Rotterdam, Balkema, pp 55-136

Fitzhardinge RC (1988) Coral recruitment: the importance of the interspecific differences in juvenile growth and mortality. Proc Sixth Int Coral Reef Symp, Australia 2:673-678

Foggo A, Bilton DT, Rundle SD (2007) Do developmental mode and dispersal shape abundance-occupancy relationships in marine macroinvertebrates? J Anim Ecol 76:695-702

Gaston KJ (2003) The Structure and Dynamics of Geographic Ranges. Oxford University Press, Oxford

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251

Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH (2002) Effects of size and temperature on developmental time. Nature 417:70-73

Glynn PW (1984) Widespread coral mortality and the 1982-83 El Nino warming event. Environ Conserv 11:133-146

Gnaiger E (1983) Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger E, Forstner H (eds) Polarographic Oxygen Sensors: Aquatic and Physiological Applications. Springer-Verlag, Berlin, pp 337-345

Graham EM (2007) Settlement competence and survival in azooxanthellate scleractinian coral larvae. Ph.D. thesis, James Cook University, p77

Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. Coral Reefs 27:529-539

Grambsch PM, Therneau TM (1994) Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 81:515-526

Guisan A, Edwards Jr TC, Hastie T (2002) Generalized linear and generalized additive models in studies of species distributions: setting the scene. Ecol Model 157:89-100

Hadfield MG (2011) Biofilms and marine invertebrate larvae: What bacteria produce that larvae use to choose settlement sites. Annu Rev Mar Sci 3:453-470

Hanski I (1999) Metapopulation Ecology. Oxford University Press, New York

Harii S, Nadaoka K, Yamamoto M, Iwao K (2007) Temporal changes in settlement, lipid content and lipid composition of larvae of the spawning hermatypic coral *Acropora tenuis*. Mar Ecol Prog Ser 346:89-96

Harii S, Yasuda N, Rodriguez-Lanetty M, Irie T, Hidaka M (2009) Onset of symbiosis and distribution patterns of symbiotic dinoflagellates in the larvae of scleractinian corals. Mar Biol 156:1203-1212

Harii S, Yamamoto M, Hoegh-Guldberg O (2010) The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. Mar Biol 157:1215-1224

Harriott VJ, Fisk DA (1987) A comparison of settlement plate types for experiments on the recruitment of scleractinian corals. Mar Ecol Progr Ser 37:201-208

Hartnoll RG (1975) The annual cycle of *Alcyonium digitatum*. Estuar Coast Mar Sci 3:71-78

Hayashibara T, Kimura T, Hatta M (2000) Changes of cnida composition during planula development of a reef-building coral *Acropora nasuta*. Galaxea 2:39-42

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167-1175

Hoegh-Guldberg O (1994) Uptake of dissolved organic-matter by larval stage of the crown-of-thorns starfish *Acanthaster planci*. Mar Biol 120:55-63

Hoegh-Guldberg O, Emlet RB (1997) Energy use during the development of a lecithotrophic and a planktotrophic echinoid. Biol Bull 192:27-40

Hofmann GE, Todgham AE (2010) Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72:127-145

Hoskin MG (1997) Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. Mar Biol 127:647-656

Huey RB, Stevenson RD (1979) Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Am Zool 19:357-366

Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. Trends Ecol Evol 4:131-135

Hunt A (1993) Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of 2 intertidal starfish, *Patiriella calcar* and *P. exigua*. Mar Ecol Prog Ser 92:179-186

Hunter E, Hughes RN (1993) Effects of diet on life-history parameters of the marine bryozoan, *Celleporella hyalina* (L). J Exp Mar Biol Ecol 167:163-177

Hunter E, Shimizu K, Fusetani N (1999) Role of protein in larval swimming and metamorphosis of *Bugula neritina* (Bryozoa: Cheilostomatida). Mar Biol 133:701-707

IPCC (International Panel on Climate Change) (2007) Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Geneva, Switzerland: International Panel on Climate Change

Jablonski D, Lutz RA (1983) Larval ecology of marine benthic invertebrates - paleobiological implications. Biol Rev Camb Philos Soc 58:21-89

Jaeckle WB (1994) Rates of energy-consumption and acquisition by lecithotrophic larvae of *Bugula neritina* (Bryozoa, Cheilostomata). Mar Biol 119:517-523

Jaeckle WB, Manahan DT (1989) Feeding by a nonfeeding larva - uptake of dissolved amino-acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. Mar Biol 103:87-94

Johnson CH, Wendt DE (2007) Availability of dissolved organic matter offsets metabolic costs of a protracted larval period for *Bugula neritina* (Bryozoa). Mar Biol 151:301-311

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155-162

Jokiel PL, Guinther EB (1978) Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*. Bull Mar Sci 28:786-789

Kattner G, Hagen W, Lee RF, Campbell R, Deibel D, Falk-Petersen S, Graeve M, Hansen BW, Hirche HJ, Jonasdottir SH, Madsen ML, Mayzaud P, Muller-Navarra D, Nichols PD, Paffenhofer GA, Pond D, Saito H, Stubing D, Virtue P (2007) Perspectives on marine zooplankton lipids. Can J Fish Aquat Sci 64:1628-1639

Kempf SC, Todd CD (1989) Feeding potential in the lecithotrophic larvae of *Adalaria proxima* and *Tritonia hombergi* - an evolutionary perspective. J Mar Biol Assoc UK 69:659-682

Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: A community perspective. Ecology 84:2007-2020

Kool JT, Paris CB, Barber PH, Cowen RK (2011) Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Glob Ecol Biogeogr 20:695-706

Krupp DA, Hollingsworth LL, Peterka J (2006) Elevated temperature sensitivity of fertilization and early development in the mushroom coral *Fungia scutaria* Lamarck 1801. Proc Tenth Int Coral Reef Symp pp 71-77

Lambert G (2005) Ecology and natural history of the protochordates. Can J Zool 83:34-50

Lett C, Ayata S-D, Huret M, Irisson J-O (2010) Biophysical modelling to investigate the effects of climate change on marine population dispersal and connectivity. Prog Oceanogr 87:106-113

Levinton JS (1983) The latitudinal compensation hypothesis: Growth data and a model of latitudinal growth-differentiation based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). Biol Bull 165:686-698

Littman RA, van Oppen MJH, Willis BL (2008) Methods for sampling free-living *Symbiodinium* (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef). J Exp Mar Biol Ecol 364:48-53

Maldonado M, Young CM (1999) Effects of the duration of larval life on postlarval stages of the demosponge *Sigmadocia caerulea*. J Exp Mar Biol Ecol 232:9-21

Manahan DT, Crisp DJ (1982) The role of dissolved organic material in the nutrition of pelagic larvae - amino-acid-uptake by bivalve veligers. Am Zool 22:635-646

Marshall DJ, Morgan SG (2011) Ecological and evolutionary consequences of linked life-history stages in the sea. Curr Biol 21:R718-R725

Marshall DJ, Pechenik JA, Keough MJ (2003) Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial, ascidian *Diplosoma listerianum*. Mar Ecol Prog Ser 246:153-162

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea. Ecol Lett 13:128-140

Miller SE (1993) Larval period and its influence on postlarval life-history - Comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae*. Mar Biol 117:635-645

Moran AL, Manahan DT (2003) Energy metabolism during larval development of green and white abalone, *Haliotis fulgens* and *H. sorenseni*. Biol Bull 204:270-277

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M (1996) An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149-154

Muenchow G (1986) Ecological use of failure time analysis. Ecology 67:246-250

Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26:601-611

Negri AP, Marshall PA, Heyward AJ (2007) Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs 26:759-763

Nishikawa A, Sakai K (2003) Genetic variation and gene flow of broadcast spawning and planula brooding coral, *Goniastrea aspera* (Scleractinia) in the Ryukyu Archipelago, southern Japan. Zool Sci 20:1031-1038

Nishikawa A, Katoh M, Sakai K (2003) Larval settlement rates and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87-97

Nozawa Y, Harrison PL (2000) Larval settlement patterns, dispersal potential, and the effect of temperature on settlement of larvae of the reef coral, *Platygyra daedalea*, from the Great Barrier Reef. Proc Ninth Int Coral Reef Symp 1:409-415

Nozawa Y, Harrison PL (2005) Temporal settlement patterns of larvae of the broadcast spawning reef coral *Favites chinensis* and the broadcast spawning and brooding reef coral *Goniastrea aspera* from Okinawa, Japan. Coral Reefs 24:274-282

Nozawa Y, Harrison PL (2007) Effects of elevated temperature on larval settlement and post-settlement survival in scleractinian corals, *Acropora solitaryensis* and *Favites chinensis*. Mar Biol 152:1181-1185

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proc Natl Acad Sci USA 104:1266-1271

Okubo N, Motokawa T (2007) Embryogenesis in the reef-building coral *Acropora spp*. Zool Sci 24:1169-1177

Okubo N, Yamamoto HH, Nakaya F, Okaji K (2008) Oxygen consumption of a single embryo/planula in the reef-building coral *Acropora intermedia*. Mar Ecol Prog Ser 366:305-309

Onitsuka T, Kawamura T, Ohashi S, Iwanaga S, Horii T, Watanabe Y (2010) Effects of delayed metamorphosis and delayed post-settlement feeding on post-larval survival and growth of the abalone *Haliotis diversicolor*. Aquaculture 298:239-244

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer-Verlag, New York, pp 4-20

Paulay G, Meyer C (2006) Dispersal and divergence across the greatest ocean region: Do larvae matter? Integr Comp Biol 46:269-281

Pechenik JA (1980) Growth and energy-balance during the larval lives of three prosobranch gastropods. J Exp Mar Biol Ecol 44:1-28

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269-297

Pechenik JA (2006) Larval experience and latent effects - metamorphosis is not a new beginning. Integr Comp Biol 46:323-333

Pinheiro JC, Bates MD, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: Linear and nonlinear mixed effects models. R package version 3.1-100

Pörtner HO (2002) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol Part A Mol Integr Physiol 132:739-761

Prowse TAA, Sewell MA, Byrne M (2008) Fuels for development: evolution of maternal provisioning in asterinid sea stars. Mar Biol 153:337-349

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Randall CJ, Szmant AM (2009a) Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, *Favia fragum* (Esper). Coral Reefs 28:537-545

Randall CJ, Szmant AM (2009b) Elevated temperature affects development, survivorship, and settlement of the elkhorn coral, *Acropora palmata* (Lamarck 1816). Biol Bull 217:269-282

Richmond RH (1987) Energetics, competence, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. Mar Biol 93:527-533

Richmond RH (1988) Competency and dispersal potential of planula larvae of a spawning versus a brooding coral. Proc Sixth Int Coral Reef Symp 2:827-831

Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. Mol Ecol 18:5101-5114

Roberts RD, Lapworth C (2001) Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. J Exp Mar Biol Ecol 258:1-13

Rumrill SS (1990) Natural mortality of marine invertebrate larvae. Ophelia 32:163-198

Saunders MI, Metaxas A (2009) Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. Mar Biol 156:2267-2276

Scheltema RS (1986) On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull Mar Sci 39:290-322

Schoolfield RM, Sharpe PJH, Magnuson CE (1981) Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J Theor Biol 88:719-731

Sebens KP (1983) Settlement and metamorphosis of a temperate soft-coral larva (Alcyonium siderium Verrill): Induction by crustose algae. Biol Bull 165:286-304

Selkoe KA, Toonen RJ (2011) Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. Mar Ecol Prog Ser 436:291-305

Sewell MA (2005) Utilization of lipids during early development of the sea urchin *Evechinus chloroticus*. Mar Ecol Prog Ser 304:133-142

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. Ecol Appl 13:S159-S169

Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373-385

Shilling FM, Manahan DT (1994) Energy-metabolism and amino-acid-transport during early development of Antarctic and temperate echinoderms. Biol Bull 187:398-407

Sibly RM, Brown JH, Kodric-Brown A (eds) (2012) Metabolic Ecology: A Scaling Approach. John Wiley & Sons, Ltd, Oxford

Somero GN (1978) Temperature adaptation of enzymes: biological optimation through structure-function compromises. Annu Rev Ecol Syst 9:1-29

Stake JL, Sammarco PW (2003) Effects of pressure on swimming behavior in planula larvae of the coral *Porites astreoides* (Cnidaria, Scleractinia). J Exp Mar Biol Ecol 288:181-201

Swanson RL, de Nys R, Huggett MJ, Green JK, Steinberg PD (2006) In situ quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens*. Mar Ecol Prog Ser 314:1-14

Szmant AM, Meadows MG (2006) Developmental changes in coral larval buoyancy and vertical swimming behavior: implications for dispersal and connectivity. Proc Tenth Int Coral Reef Symp pp 431-437

Takami H, Kawamura T, Yamashita Y (2002) Effects of delayed metamorphosis on larval competence, and postlarval survival and growth of abalone *Haliotis discus hannai*. Aquaculture 213:311-322

Therneau T (2011) coxme: Mixed Effects Cox Models. R package version 2.2-1

Therneau T, Lumley T (2011) survival: Survival analysis, including penalised likelihood. R package version 2.36-5

Therneau TM, Grambsch PM, Pankratz VS (2003) Penalized survival models and frailty. Journal of Computational and Graphical Statistics 12:156-175

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol Rev Camb Philos Soc 25:1-45

Travis JMJ, Mustin K, Bartoń KA, Benton TG, Clobert J, Delgado MM, Dytham C, Hovestadt T, Palmer SCF, Van Dyck H, Bonte D (in press) Modelling dispersal: an eco-evolutionary framework incorporating emigration, movement, settlement behaviour and the multiple costs involved. Methods Ecol Evol

Treml EA, Halpin PN, Urban DL, Pratson LF (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landsc Ecol 23:19-36

Underwood AJ, Fairweather PG (1989) Supply-side ecology and benthic marine assemblages. Trends Ecol Evol 4:16-20

Villinski JT, Villinski JC, Byrne M, Raff RA (2002) Convergent maternal provisioning and life-history evolution in echinoderms. Evolution 56:1764-1775

Yamaguchi M (1974) Effect of elevated temperature on the metabolic activity of the coral reef asteroid *Acanthaster planci* (L). Pac Sci 28:139-146

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds) Dispersal Ecology. Blackwell Science, Oxford pp 410-428

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1-12

Wendt DE (1998) Effect of larval swimming duration on growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. Biol Bull 195:126-135

Wilkinson C (2008) Status of coral reefs of the world: 2008. Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Australia 296 p.

Willis BL, Oliver JK (1988) Inter-reef dispersal of coral larvae following the annual mass spawning on the Great Barrier Reef. Proc Sixth Int Coral Reef Symp 2:853-859

Willis BL, Oliver JK (1990) Direct tracking of coral larvae - Implications for dispersal studies of planktonic larvae in topographically complex environments. Ophelia 32:145-162

Willis BL, Babcock RC, Harrison PL, Wallace CC (1997) Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. Coral Reefs 16:S53-S65

Willmer P, Stone G, Johnston IA (2000) Environmental physiology of animals. Blackwell Publishing, New York

Wilson JR, Harrison PL (1998) Settlement-competency periods of larvae of three species of scleractinian corals. Mar Biol 131:339-345

Wood SN (1994) Monotonic smoothing splines fitted by cross-validation. Siam Journal on Scientific Computing 15:1126-1133

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. New York: Springer

Appendix A Supplementary analysis of lipid depletion

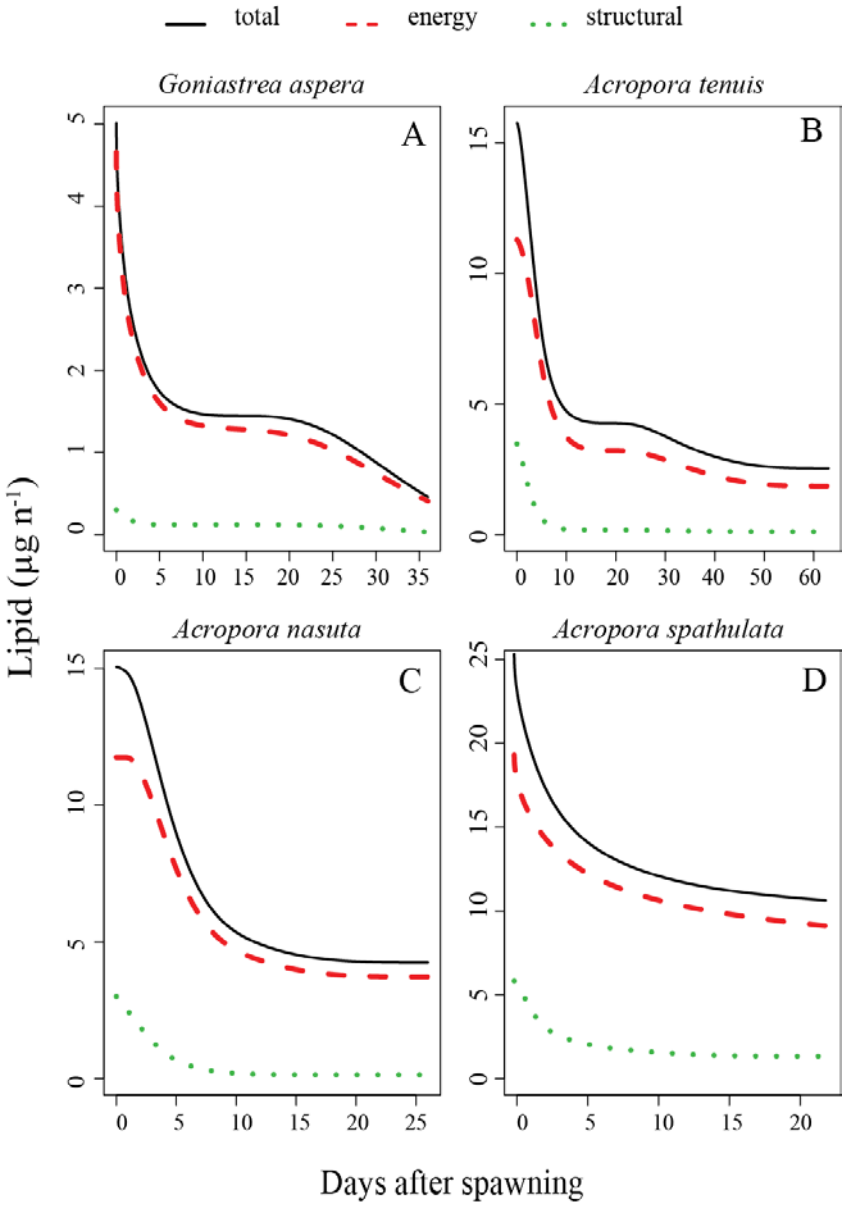

Figure A.1 Depletion of different lipid classes through time in four scleractinian coral species. Solid lines represent the total lipid (i.e., all lipid classes combined), dashed lines represent energetic lipids (WE and TG), and dotted lines represents structural lipids (ST and PL). The lines are fitted values from the GAM fits to measured lipid data, and have been back-transformed from the log-scale (on which fitting was done) to the arithmetic scale.

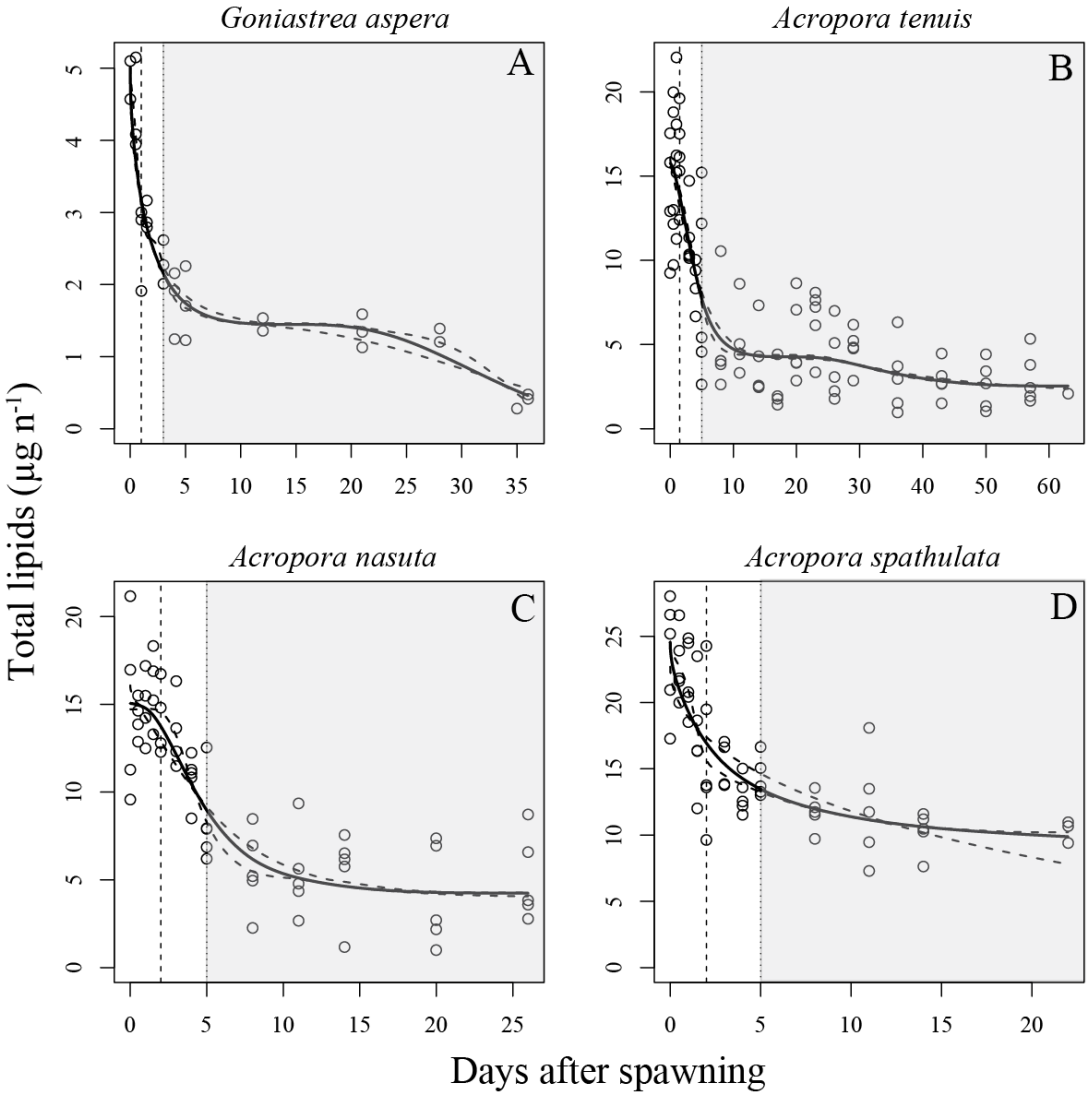

Figure A.2 Depletion of total lipids through time in four scleractinian coral species. Each open circle represents one replicate measurement. Solid lines represent mean respiration rates, and dashed lines show upper and lower 95% confidence intervals. Means and confidence intervals were obtained from the GAM fits by back-transforming from the log-scale (on which fits were made) to the arithmetic scale (for plotting). Two vertical lines show developmental stage: a dashed line for time to swim, and a dotted line for the time larvae first become competent to settle. Shaded areas indicate sampling times when settlement was observed (i.e, larvae were competent).

Appendix B Supplementary lipid and oxygen consumption analyses

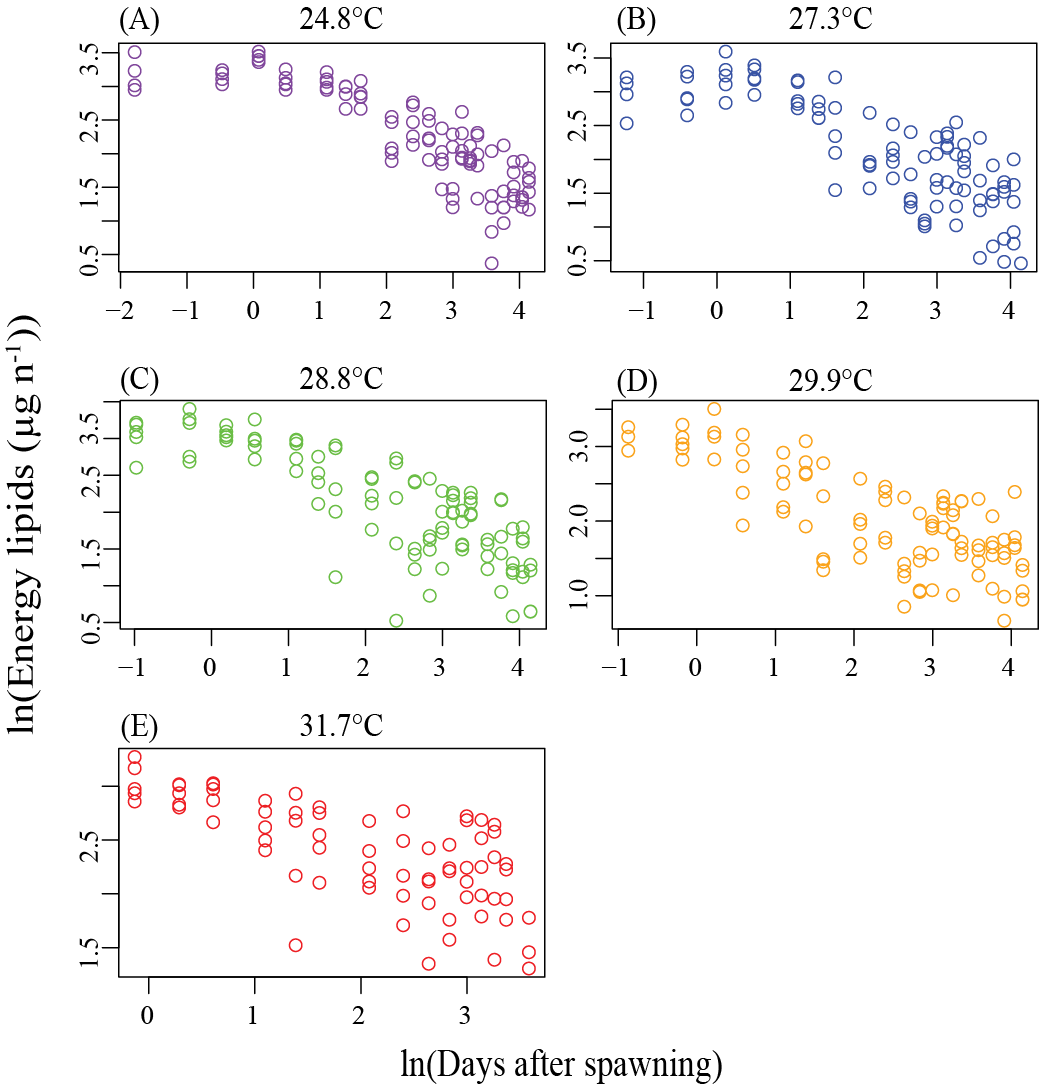

Figure B.1 Log transformation of energetic lipid content for *Acropora tenuis* larvae maintained in five temperature treatments. A near-linear relationship can be seen in lipid depletion after an initial period of no decline.

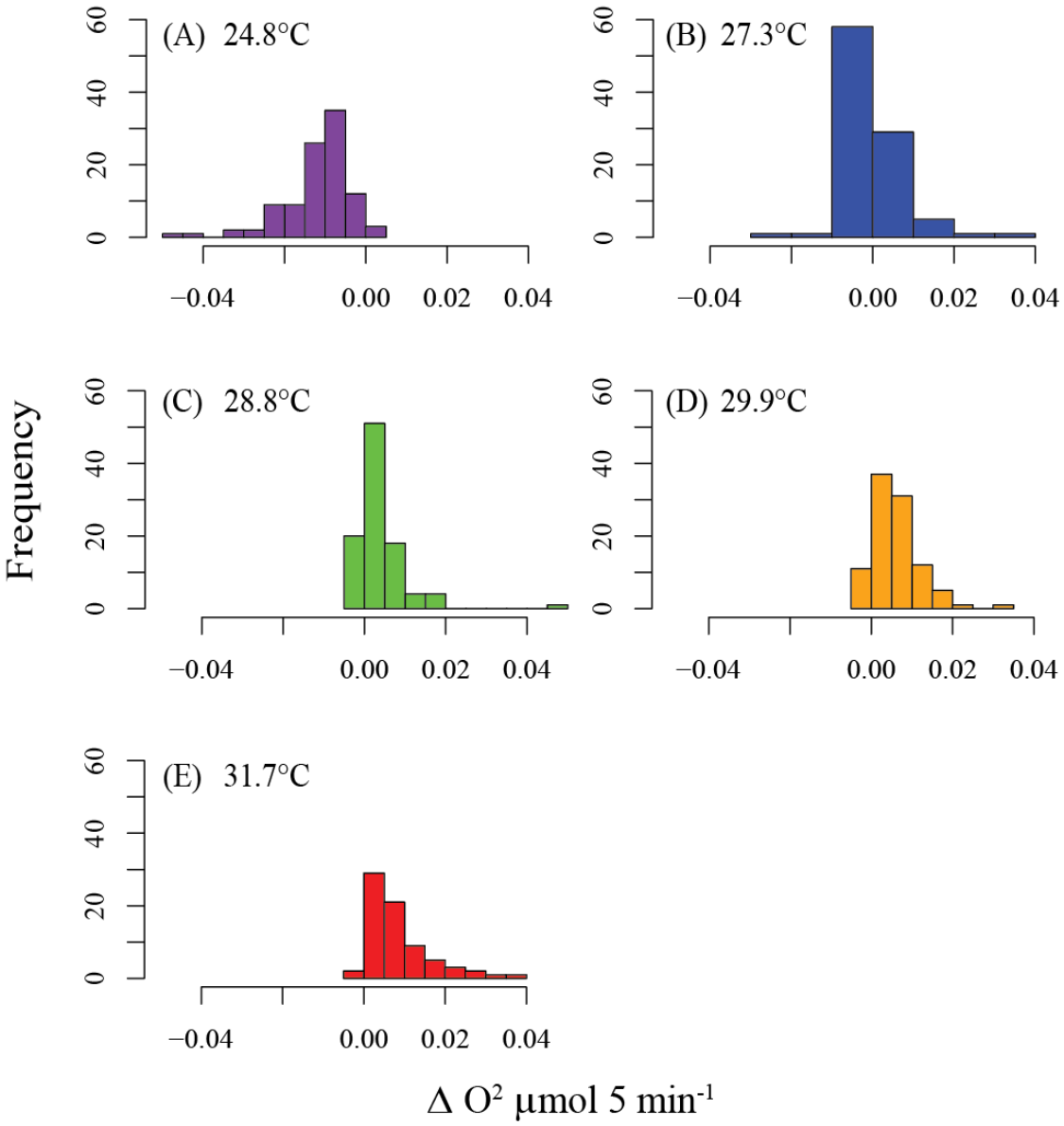

Figure B.2 Frequency distributions of control measurements for different temperatures. The slope was calculated as the change in oxygen over five minutes, and, as the respiration chambers were empty, the expected slope was zero.

Table B.1 Mean change in oxygen consumption in control respiration chambers (filtered seawater only) for different temperatures.

Temperature (°C)	Mean Δ O2 µmol 5 min-1 (95% Confidence Intervals)
24.8	-0.0115 (-0.0132, -0.0098)
27.3	-0.0004 (-0.0018, 0.0011)
28.8	0.0038 (-0.0025, 0.0051)
29.9	0.0061 (0.0049, 0.0073)
31.7	0.0084 (0.0067, 0.0102)