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Climate-driven changes in the recruitment success of marine invertebrates: the role of food supply and temperature

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Climate-driven changes in the recruitment success of marine invertebrates:

The role of food supply and temperature

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By

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CONTENTS

SUMMARY

Current trends indicate that key events in the life cycle of marine organisms (e.g. spawning, reproduction, larval release) are taking place earlier in the season in response to global warming associated with climate change. Specific changes in the phenology (i.e. the timing of these life cycle events) of planktonic organisms suggest that responses to climate change are not consistent for all functional groups in the pelagic community. As a result, the tight synchrony that exists between pulses of production of larvae and their food sources could be disrupted; this may cause a decline in survival and affect recruitment levels into a population. Marine invertebrates with complex life cycles from temperate-cold regions may be particularly sensitive to changes in food availability and temperature as, during the larval phase, many of these species are strongly dependent on seasonal peaks of pelagic food sources. The degree to which mismatches between the timing of larval development and seasonal peaks of planktonic production may influence recruitment success of a species is unknown, and may vary depending on species life-history. Intertidal barnacles are widely distributed and ecologically important species which serve as a good model species to investigate the impact of potential shifts in food and temperature associated with climate change. *Semibalanus balanoides* is boreal species which synchronously releases its larvae once a year to coincide with the spring phytoplankton bloom. In contrast, *Austrominius modestus* is a lusitanean species which releases multiple broods over a much longer period of time, with larval production at its peak during the summer months when food supply is lower. These two contrasting species are ideal models to use in a comparative study aimed at understanding the effects of food limitation and temperature on the recruitment success of marine invertebrates.

In this study, I focused on the combined effects of food limitation and temperature on larval performance of *S. balanoides* and *A. modestus.* Firstly, I examined whether a reduction in available food at different temperature conditions would have an impact on the recruitment success of either species. This approach was to assess if there were any differences between species responses to potential low food

environments and also explored whether changes in temperature could exacerbate any larval responses to low food. I also evaluated the responses of both species to temporal mismatches, simulating food availability as consequence of larval release occurring before or after peaks in food abundance, to identify whether the timing of food availability during larval development was important for recruitment success. Following this, I concentrated on identifying the mechanisms by which these two species may differ in their responses to food limitation and temperature. Here, the tolerance of both species to starvation at different temperatures was examined and the elemental composition of the energy reserves they have upon hatching was assessed to identify any differences that may exist between the species. Finally, I focused on the ingestion rates of both species to see whether they exhibit any differences in their feeding rates in response to a range of food concentrations at different temperatures. A partial energy budget for larvae was also estimated to evaluate if species differed in their capabilities to use food for growth, development and maintenance in different food/temperature environments.

Results showed that the timing and abundance of food sources were critical for the recruitment success of *S. balanoides* and less important for *A. modestus*. A temperature of 15^{o}C was sub-optimal for *S. balanoides* and tended to increase the negative effects of food limitation. Both food limitation and temperature had strong effects on naupliar mortality and cyprid settlement success rates for *S. balanoides* and, thus, were important in determining the supply and quality of settlers to a population. When compared to *A. modestus*, the poorer performance of *S. balanoides* could not be explained by differences in initial energy reserves or starvation tolerance immediately after hatching as these were comparable between the two species. Considerable differences were observed in the ingestion rates of both species during the early stages and suggested that differences in mass specific feeding rates may be linked to the better performance of *A. modestus* in food limited environments.

In summary, results suggest that recruitment success of *S. balanoides* would be particularly vulnerable to mismatches of larval pulses with their food sources,

particularly at higher temperatures, due to the strong effects of these factors on larval supply and larval quality. In contrast, even under food limited conditions, moderate increases in temperature should not result in strong negative consequences for *A. modestus*. Work presented here highlights that species-specific responses to climate-driven changes in the environment may profoundly affect the abundance and distribution of marine invertebrate species with complex life cycles, through direct and interactive effects of these changes on larval mortality and settlement success.

CHAPTER 1

GENERAL INTRODUCTION

1.0. General Introduction

Global changes in the climate, associated with rises in greenhouse gas emissions, have led to a rapid warming of the atmosphere over recent years (IPCC, 2007). Over the past 30 years the global average temperature has increased by about 0.4 and 0.8^{o}C and is expected to continue to rise at a rapid rate in the current century (Houghton, 2001; IPCC, 2007). Global warming has a number of important consequences for marine ecosystems in particular (Hoegh-Guldberg and Bruno, 2010). Oceans act a heat sink, and the added heat energy in the atmosphere has caused an increase in ocean heat content, with the average temperature of the upper layers of the ocean rising by 0.6^{o}C over the past 100 years (Levitus et al. 2009). Increases in heat content of the ocean can drive a number of other changes that may have great repercussions for marine ecosystems. Whilst changes in temperature can have direct effects upon species through its influence on biological processes (Hochachka and Somero, 2002), temperature can also influence water column stability, nutrient enrichment and the degree of primary production (see review Hoegh-Guldberg and Bruno, 2010).

There is now ample evidence that these climatic changes have had a number of biological impacts upon a broad range of marine organisms (Beaugrand et al. 2002; 2003; Root et al. 2003; Hays et al. 2005; Hoegh-Guldberg and Bruno, 2010); these changes include geographical shifts in range boundaries of species (Mieszkowska et al. 2006; Hawkins et al. 2008), and mass mortality events (Harley, 2008; Wethey et al. 2011) that are related to climate change. Some of the best documented changes have been seen on rocky shore communities in Europe (see review Hawkins et al. 2008) where cold-water adapted species are decreasing in abundance and retreating poleward whereas warm-water species are increasing in abundance and advancing poleward. Other important impacts of climate on biology are related to temporal changes in the timing of events occurring during the life cycle of organisms (phenology), and on seasonal variations in the environment (Parmesan and Yohe, 2003; Edwards and Richardson, 2004. These impacts are currently under intensive research and are explained in the next section.

1.1. Changes in phenology

Phenology is the study of periodic natural life-cycle events, such as flowering or migration, which are influenced by the environment. Current trends indicate that a number of species have advanced the timing of their life cycle events in response to the warming conditions associated with climatic changes (Hughes, 2000; Parmesan and Yohe, 2003; Moller et al. 2008). Therefore, long-term phenological data sets can provide a sensitive indicator for tracking changes to the ecology of species in response to climate change (Walther et al. 2002; Edwards and Richardson, 2004; Durant et al. 2007). Increases in temperature have been shown to have major effects on bird populations and the timing of breeding and migration now occurs much earlier than it did 50 years ago (Moller et al. 2008). Parmesan and Yohe (2003) reviewed data on shifts in spring phenologies for 667 species over a period of 50 years and found a general trend of earlier spring events by 2.3 days per decade for a wide range of taxa (e.g. trees, birds and amphibians).

Using long-term data from 1958 to 2002 of 66 plankton taxa in the North Sea, Edwards and Richardson (2004) studied changes in phenology across three trophic levels using five functional groups. Crucially, their evidence suggested that a shift to earlier occurrences in phenology in response to increased water temperature is not consistent for all functional groups in the pelagic community. Interestingly, diatom blooms in spring have remained relatively fixed in time since they are dependent on day length or light intensity, which is invariant to global warming (Sommer et al. 1986). Conversely, organisms that are dependent on temperature to stimulate physiological developments and larval release have significantly moved forward in their seasonal cycle in response to the warming temperature. Over the past 45 years, dinoflagellates in the North Sea are peaking earlier by 23 days, copepods by 10 days, and other holozooplankton by 10 days (Fig 1.1). The timing of the seasonal cycles of meroplankton (including the larvae of cirripedes, decapods, echinoderms and fish) seems particularly sensitive to climate change. As a group they have shown the largest shifts forward in seasonality (27 days) compared to the holoplankton (10 days). Some of the meroplankton groups have reacted more strongly than others.

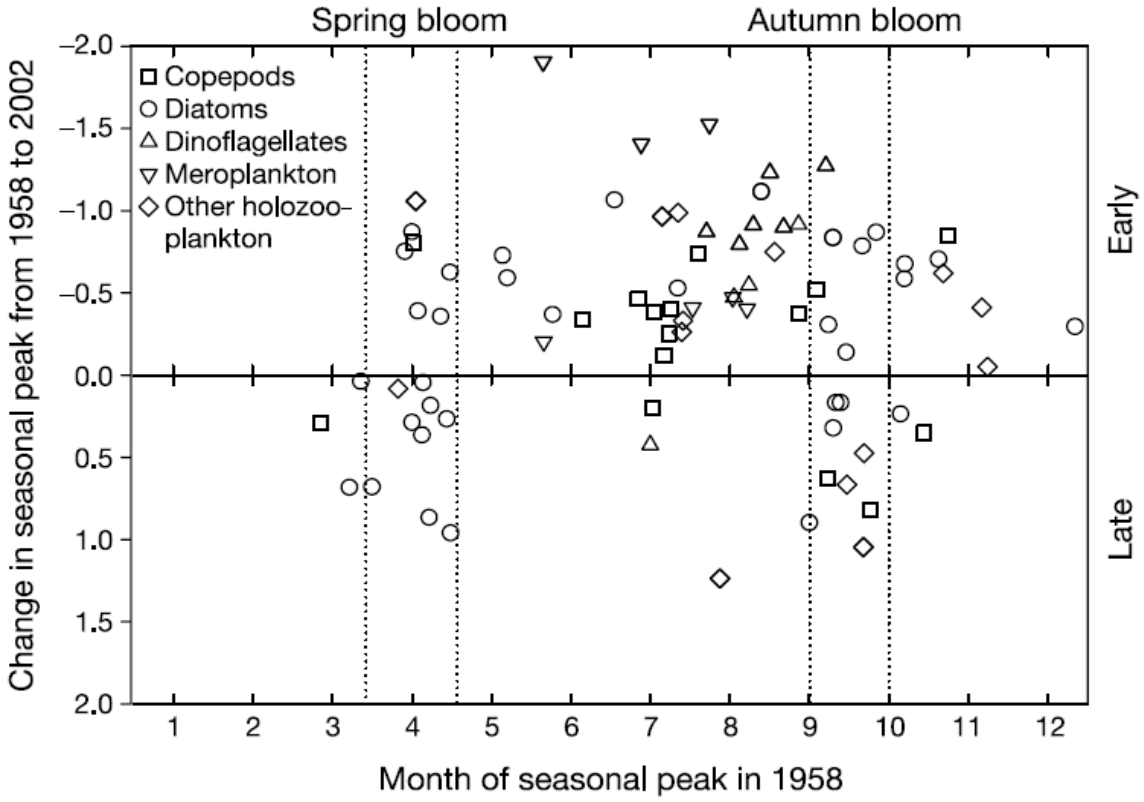

Fig 1.1 Changes in phenology in the central North Sea from 1958 to 2002. The change in timing of the seasonal peaks (in months) for 66 taxa over a 45 year period (1958-2002), plotted against the timing of their seasonal peak in 1958. A negative difference between 1958 and 2002 indicates seasonal cycles are occurring earlier. From Edwards and Richardson (2004).

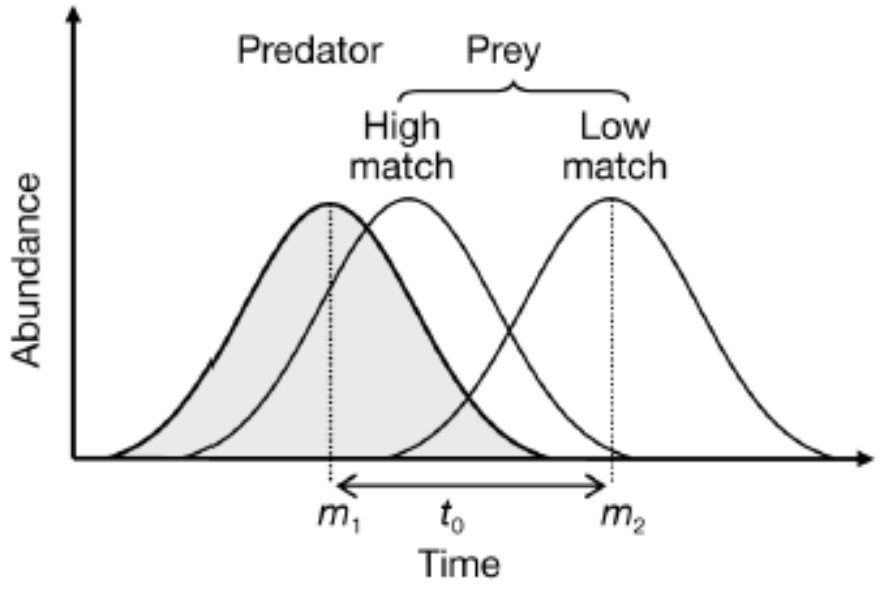

Fig. 1.2 The match/mismatch hypothesis (MMH). Interaction between 2 trophic levels explained by the MMH. A high match is represented by a temporal overlap of the predator and its prey. An increase in time-lag (t_0) between 2 population peaks (m_1, m_2; mean peak time for Population 1 and 2, respectively) leads to a low match. From Durant et al. (2007)

For example, larvae of benthic echinoderms in the North Sea are now appearing in the plankton 47 days earlier than they did 50 years ago. These large phenological shifts have occurred with an increase in SST of 0.9^{o}C during the study period (1958-2002). It appears that although the marine pelagic community is responding to changes in the climate the intensity of the response varies considerably throughout the community and the seasonal cycle.

1.2. Phenological decoupling – Match-mismatch hypothesis (MMH)

A large number of terrestrial and marine studies have shown that although a number of species have shown a marked shift towards earlier spring phenological events, the magnitude of this shift can vary among species (e.g. Both and Visser, 2001; Parmesan et al. 2003; Edwards and Richardson, 2004; Moller et al. 2008). As a result, the response of a species may differ from responses of organisms at lower levels of the food chain, disrupting the tight synchrony that exists between the timing of reproduction and peaks of main food supply, causing a decline in offspring survival (Both and Visser, 2001). The idea of how a mismatch between a species and its food source can influence recruitment to a population has been developed by Cushing (match-mismatch hypothesis: 1969; 1990).

The “match-mismatch hypothesis” (MMH) proposes that recruitment variation in a population is determined by the relationship between that of a predator and its prey species at the immediate lower level (see Fig 1.2). A scenario where the peak abundance of a species coincides with the peak of its food source is described as a “match” and results in high survival and recruitment (Cushing, 1990). A “mismatch” situation occurs when there is a disruption in the temporal synchrony between the peak abundances of the predator species and its food source which can result in increased mortality and low recruitment. For example, recent warm springs have resulted in the winter moth *Operophtera brumata* hatching up to 3 weeks before their food source (the oak bud burst of *Quercus robur*) (Visser & Holleman 2001). Because newly hatched moths can only survive a few days without food, this has led to higher mortality and lower reproductive success.

A number of studies have provided support for the MMH and demonstrated that climate-driven decoupling of phenological relationships can affect recruitment levels into populations (see Visser & Holleman 2001; Both and Visser, 2001; Cushing, 1990; Beaugrand et al. 2003; Winder and Schindler, 2004; Moller et al. 2008). The timing of a mismatch seems to be particularly important for population dynamics and ecosystem functioning, as species populations that are most mistimed have declined the most in number (e.g. Moller et al. 2008).

1.3. Potential vulnerability of marine invertebrate larvae to mismatches

Within the marine pelagic community, the disruption of synchronicity may be important for the recruitment success of marine invertebrate species. Many marine invertebrate species have complex life cycles, in which one or more free-living developmental stages eventually metamorphoses to a morphologically and often ecologically and physiologically distinct juvenile stage (Pechenik et al. 1998). These complex life cycles are widely distributed among such diverse animals as sponges; gastropod and bivalved molluscs; polychaete worms; crustaceans; bryozoans; and echinoderms (Thorson, 1950). Larvae may feed on phytoplankton and other particulates or rely entirely on yolk or other nutrients provided by the mother. They may spend as little as a few minutes or as long as several to many months in the plankton before metamorphosing to adult form and habitat (Pechenik, 1990; 1998).

In marine species with complex life cycles, adult population size depends to a large extent on the transport of larvae into and away from adult populations (Thorson, 1950; Jackson and Strathmann, 1981; Bailey and Houde, 1989; Hill, 1991; Shanks, 1995; Alexander and Roughgarden, 1996), the number of larvae that survive to metamorphose (Thorson, 1950; Istock, 1967; Bailey and Houde, 1989; Berven, 1990; Kerrigan, 1996), and the extent of post-metamorphic mortality (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Larval processes can have a strong effect upon recruitment success and potentially determine adult population distribution and abundance (Olson and Olson, 1989). The most obvious factor to determine

recruitment success is larval mortality which can be determined by a number of environmental factors, such as food availability and temperature (Pechenik, 1987).

Many marine invertebrate larvae develop from small eggs with low energy content (Vance, 1973) that are highly dependent on exogenous food sources, such as phytoplankton, for nourishment (Lang and Marcy, 1982; Anger, 1987; Starr et al. 1990). Resource patchiness is often implicated in larval mortality as it may cause periods of nutritional stress throughout pelagic development (Holland, 1978; Anger et al. 1981b). For example, low concentrations of phytoplankton at certain key times of the year can lead to mass mortality of larvae (Barnes, 1956, 1957; Lucas, 1982, Starr et al. 1990). As a result, to reduce the effect of stochastic variations in the environment, marine invertebrates have evolved mechanisms that couple larval production with phytoplankton blooms (Starr et al. 1990; 1991; Cushing, 1990; Kirby, 2007).

However, not all marine invertebrate species synchronise their larval release to periods of phytoplankton abundance, suggesting potential differences in the physiological tolerance of low food levels or the use of alternative food sources. For example, even after 33 days without food (phytoplankton) the larvae of the Pacific oyster (*Crassostrea gigas*) were still swimming and did not lose their ability to capture and digest algal cells when provided with food. It was believed that this was due to their capacity to survive on alternative sources of energy, such as dissolved organic matter (Moran and Manahan, 2004). Therefore, the importance of food limitation can vary taxonomically and geographically (Huntley and Boyd, 1984; see review by Olson and Olson, 1989). Evidence suggests that fluctuations in food availability in the field are of low importance for survival of polychaetes, molluscs and echinoderms but are a critical factor in the development of larval crustaceans (Olson and Olson 1989; Fenaux et al. 1994). Given the strong dependency of these larvae upon external food sources, and the large shifts forward in seasonality shown by meroplankton (Edwards and Richardson, 2004), it is likely that survival and recruitment of larval crustaceans are vulnerable to the food limited situation linked with mismatches.

Increases in temperature linked with climate change have the potential to increase the effects of food limitation for marine invertebrate larvae. Temperature has a fundamental effect on biological processes simply by its influence on molecular kinetic energy, which determines the rate of fundamental processes such as enzyme reactions, diffusion and membrane transport (Hochachka and Somero, 2002). Moderate increases in temperature have the potential to affect many life stages of marine invertebrates (Rumrill, 1990); increases in temperature increase metabolism and thus influences larval growth, development and survival as well as the rate of energy reserve utilisation (Anger et al. 1981b; Olson, 1985; Pechenik, 1990). The increased metabolic demands of larvae at a higher temperature will lead to faster degradation of consumed energy reserves, under food limited conditions this could lead to a decrease in larval performance or even mortality.

1.4. Objectives

Global warming over the last few decades has clearly already had an effect on marine ecosystems; a number of marine invertebrates have shifted the timing of their larval production causing a mismatch in timing between larvae and their sources of food. This may result in extensive mortality and decreased recruitment success of a species as predicted by the match-mismatch hypothesis. The degree to which such mismatches could influence the recruitment success of a population is unknown, but previous research has shown that larvae developing at low food concentrations have lower growth rates, longer development times and reduced survivorship compared to those in a high food environment.

The intertidal barnacle *Semibalanus balanoides* is a boreal species that releases larvae in spring, during a brief period of usually 3-4 weeks, around the time of the spring peak of chlorophyll. A co-occurring species, *Austrominius modestus,* releases larvae during a longer period of time, from late spring to late summer (Crisp and Davies, 1955). Field studies on *S. balanoides* have suggested that recruitment success depends on a match between the timing of larval release and the spring phytoplankton bloom (Barnes, 1956; 1957; 1962). *S. balanoides* and *A. modestus* are therefore ideal models to use in a comparative study aimed to understand potential

effects of mismatch on recruitment and physiological underpinnings for a potential recruitment failure.

The specific objectives of this thesis were to examine the extent to which recruitment success of marine invertebrate larvae may be affected by climate-driven changes in food availability and temperature. By studying the performance of these two model species with different life strategies in manipulative laboratory experiments, the combined role of varying food availability and temperature on the development, survival and recruitment success of marine invertebrates can be investigated. In the following section the complex life-cycle of the barnacle is described and their suitability as model species for recruitment based experiments is justified.

1.5. Barnacle life cycle and the model species

Barnacles have a global distribution and are a dominant group on many rocky shores (Hayward et al. 1995). As such, they are a major component of many intertidal communities and have been used extensively as a model species of benthic invertebrates for a number of reasons, namely they are common, sessile and very accessible. Upon liberation of the nauplii from the adult, the larval development of both species is tightly constrained through six naupliar stages (West and Costlow, 1988). The larval stages of *S. balanoides* was first described by Bassindale (1936) who introduced the setation formulae which are now widely used, these descriptions have since been updated by Norris & Crisp (1953) and Crisp (1962). The first morphological description of *A. modestus* was given by Knight-Jones and Waugh (1949). Planktotrophic development proceeds over several weeks during which moulting and growth occur until the sixth stage. Following the final nauplius stage, the larvae metamorphose into the non-feeding cypris larvae which have a bivalve carapace and do not feed. The cyprid is specially adapted to locate suitable substrata for permanent fixation. Following fixation a second metamorphosis into a sessile juvenile occurs which then grown and develop into adults (Fig 1.3).

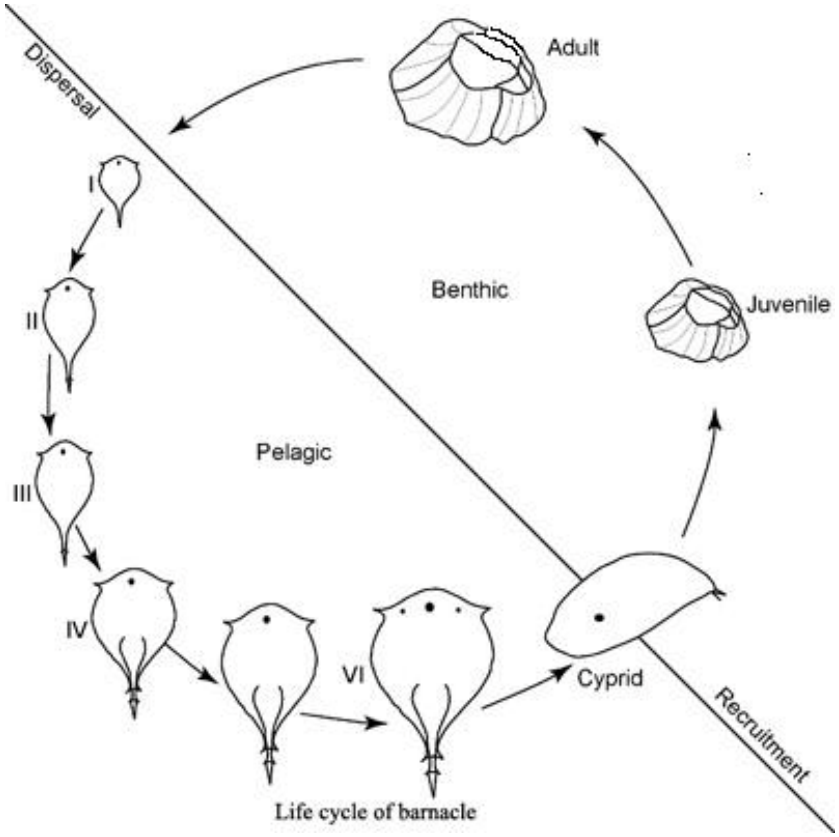

Fig. 1.3 The life cycle of a barnacle
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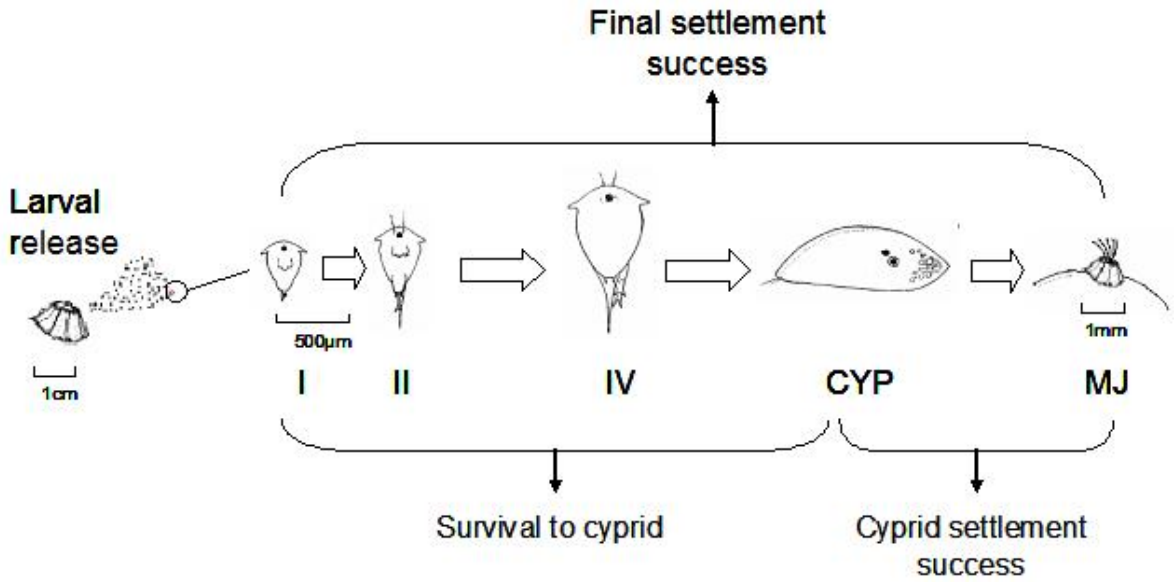

Fig. 1.4 Response variables measured during the experiments to determine recruitment success. Abbreviations: CYP - Cyprid; MJ - metamorphosed juvenile

There is a wealth of literature available on how to rear barnacle larvae successfully in the laboratory and larvae can be seen with the naked eye (e.g. Crisp, 1956; Lucas, 1980; Harms, 1984). Cyprids will also settle on most surfaces allowing easy quantification of settlement. With regular and easy access to adult populations that occur in only two dimensions, barnacles have been studied extensively in the field as they are an ideal group with which to investigate propagule input to populations. In this study the two model species studied were the intertidal acorn barnacles *S. balanoides* and *A. modestus* which are described in the following sections.

1.5.1. *Semibalanus balanoides*

The barnacle *Semibalanus balanoides* is an ecologically important intertidal species known to synchronously release a single brood of pelagic larvae during the spring. The boreoarctic *S. balanoides* is the most widespread barnacle in the UK, being distributed around the coast in the eulittoral zone, except on southerly portions of the Irish and Cornish coasts (Rainbow, 1984). Following fertilization in November/December, the embryos develop within the mantle cavity of the adult and are often retained for a considerable period after development is complete until synchronous larval release is triggered (Barnes 1962; Crisp 1956). The major period of larval release is usually restricted to a few weeks, sometimes with the peak release taking place over an even shorter period (Barnes, 1957). Previous work has suggested that *S. balanoides* has evolved a mechanism to match its larval release to a food source; field and laboratory evidence has suggested that larval release in *S. balanoides* may occur in response to the spring phytoplankton bloom (Barnes, 1956, 1957, 1962; Hawkins and Hartnoll, 1982; Starr et al. 1991). In contrast, recent work in North America by Gyory et al. (2011) has suggested that larval release may be associated with turbulence and storm events rather than being directly linked to the abundance of phytoplankton. Although the cue which triggers larval release remains ambiguous, ultimately, it appears that synchronising larval release with an abundant food source is fundamental for the recruitment success of this species. For example, Barnes (1956) observed that years when there was failure of the larval population of

S. balanoides were, to a large degree, coincident with the failure or irregularities of the diatom population.

1.5.2. *Austrominius modestus*

Austrominius modestus is Australasian in origin and was first recorded in the UK in 1945 (Crisp, 1958). The species was introduced by shipping activities and the first colonisation was thought to be Chichester Harbour, from where it spread rapidly around the coast through shipping and natural drift of larvae (Crisp, 1958). *A. modestus* is now a common inhabitant of the low and mid shore around the UK. This warm water species does not show a restricted period of larval production and usually produces several broods per individual per year (Crisp and Davies, 1955). *A. modestus* grows rapidly, matures early and can begin to reproduce within 12 weeks of settlement. In *A. modestus* the individual cycles or broods of larvae are non-seasonal, although the rate of breeding does vary within a season, and is fundamentally different to that of *S. balanoides. A. modestus* is able to breed over wide temperature limits, the whole breeding cycle being capable of completion at any temperature from 6 to 20^{o}C and is entirely dependent on food supply and temperature (Crisp and Davies, 1955). Larvae of *A. modestus* may therefore be found in the plankton at all times of the year but are most abundant during the summer months. In spring and summer the supply of food is sufficient to allow a steady output of broods of nauplii as *A. modestus* is able to remove food from suspension and transform it into large numbers of dispersive larvae in a very short time (Rainbow, 1984).

1.6. Thesis structure

In this thesis, the potential effects of climate-driven changes in food availability and temperature upon marine invertebrates was considered by exploring the larval performance of *S. balanoides* and *A. modestus* in a range of different food manipulation and temperature experiments. Given the different larval strategies of both species, and the apparent dependence of *S. balanoides* upon an abundant food

source, it is predicted that *S. balanoides* will exhibit a stronger negative response to food limitation than *A. modestus* throughout the experiments.

To examine the effects of the treatments and account for genetic variation among adults, larvae from different adults were reared separately under experimental treatments. Several aspects of larval performance were evaluated throughout the experiments (Fig 1.4). The responses of duration of development, survival rates to cyprid and cyprid size of larvae hatched from different parents were investigated in a fully crossed design of food, temperature and parent. The responses for cyprid settlement success and final settlement success were investigated in a fully crossed design of food and temperature. Recruitment success was measured as final recruitment success (total number of nauplii surviving from hatching though to metamorphosed juvenile). However, recruitment failure can occur due to low larval supply (number of hatching nauplii surviving to cyprid) or poor larval quality (number of cyprids that can complete settlement and metamorphosis). Therefore, in these experiments, survival rates to cyprid and the subsequent settlement of these cyprids were both observed to identify the life stage at which larval mortality occurred (as shown in Fig 1.4)

In the first part of this study (Chapter 2) focus was placed on investigating the larval performance of *S. balanoides* and *A. modestus* under periods of constant food limitation at different temperatures. Chapter 3 explored whether the pattern of mismatch was important for recruitment success by investigating the effects of temporal food availability and temperature on larval performance. Chapter 4 examined the starvation tolerance of both model species and quantified their initial larval reserves upon hatching to explore whether this was related to their responses to food limitation. The same experimental approach was undertaken to examine the effects of food limitation and temperature on larval performance through Chapters 2 – 4. Chapter 5 examined the feeding rates of both species across a range of different food concentrations at different temperatures to identify any differences between the two species. Chapter 6 summarises the major outcomes of this study and discusses its limitations and ecological relevance.

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CHAPTER 2

Effects of food limitation and temperature on the recruitment success of *Semibalanus balanoides* and *Austrominius modestus*

2.1. Introduction

Phenology is the study of annually recurring life-cycle events (e.g. migration, flowering, egg-laying, larval production) that are driven by environmental factors such as temperature, photo-period, food abundance or precipitation. The phenology of a species has evolved to match the environmental conditions that maximise its fitness (Futuyma, 1998). In recent years, increases in temperature and changes in weather associated with climate change have affected the environmental cues that animals use for timing their life-cycle events (Parmesan and Yohe, 2003; Sydeman and Bograd, 2009). This is causing seasonal events, such as insect emergence and the arrival of migratory birds, to occur earlier than they did in the past (Parmesan and Yohe, 2003; Root et al. 2003). In general, while many species have shown advances in their phenology, some species have shown no detectable change (Moller et al. 2008). For example, it is well documented that plants and insects have advanced their phenology in conjunction with the increases in spring temperatures (Parmesan and Yole, 2003; Walther et al. 2002; Root et al. 2003), but the bird species which consume these plants and insects have responded to a lesser degree or not at all (Visser et al. 1998; Both and Visser, 2001; Moller et al. 2008). This has resulted in a mistiming in reproduction relative to peaks of available food. Because the reproductive success and survival of a species depends on its ability to encounter and consume a sufficient quantity of food, these differential changes in phenology of consumers and their prey have generated variability in survival rates of species (Visser & Holleman 2001; Both and Visser, 2001; Phillipart et al. 2003; Durant et al. 2007). The mechanisms by which phenological relationships may influence recruitment success are proposed by the match/mismatch hypothesis (MMH) (Cushing, 1969; 1990).

The MMH states that recruitment variation in a population is caused by the relationship between the phenology of a predator/consumer and that of its prey/food species at the immediate lower level (Durant et al. 2007). A scenario where the peak abundance of a species coincides with the peak of its food source is described as a “match” and results in high survival and recruitment (Cushing, 1990).

A “mismatch” situation occurs when there is a disruption in the temporal synchrony between the peak abundances of the predator species and its food source. Such mismatches result in varying degrees of food limitation for the predator species and, under such scenarios, the MMH would predict increased mortality and low recruitment (Cushing, 1990; Durant et al. 2007). Changes in absolute peak food abundance can also disrupt or amplify the phenomenon described by the MMH, for example, leading to situations where there is a match in time and space but the predator is unsuccessful owing to lack of food (Durant et al. 2005). Various terrestrial and marine studies have examined the temporal and spatial overlap between predators and their food sources. These studies provide support for the MMH and, moreover, establish that climate-driven decoupling of phenological relationships has occurred between species (see Visser & Holleman 2001; Both and Visser, 2001; Cushing, 1990; Beaugrand et al. 2003; Winder and Schindler, 2004; Moller et al. 2008).

Within the marine pelagic community, climate-driven shifts in phenology have been shown to differ in magnitude among a range of species and functional groups, with the potential consequence of mismatches between trophic levels (Edwards and Richardson, 2004). The disruption of synchronicity may be important for the recruitment success of marine invertebrate species, particularly those which have a complex life cycle involving a pelagic larval phase. This is because these larvae develop from small eggs with low energy content (Vance, 1973) that are highly dependent on exogenous food sources (Lang and Marcy, 1982; Anger, 1987; Starr et al. 1990). As a result, many marine invertebrates synchronise their larval production with periods of peak food abundance to ensure conditions are optimal for larval survival, (Starr et al. 1990; 1991; Cushing, 1990; Kirby et al. 2007). For example, Starr et al. (1990) demonstrated a direct coupling of spawning in green sea urchins and blue mussels with the spring phytoplankton bloom.

The potential for marine invertebrates to experience food limitation varies taxonomically and geographically (Huntley and Boyd, 1984; see review by Olson and Olson, 1989, Moran and Manahan, 2004; Pechenik, 2006). It has been proposed that

food limitation for invertebrate larvae is more likely to occur in taxonomic groups that feed at higher trophic levels (e.g. fish and crustaceans) than at lower trophic levels (e.g. molluscs and echinoderms) (review in Olson and Olson, 1989). For example, experimental evidence has shown that fluctuations in food availability are a critical factor in the development of larval crustaceans and fish, with extensive mortality occurring if suitable food is unavailable (Lang and Marcy, 1982; Anger, 1987; Fenaux et al. 1994; Gotceitas et al. 1996). Given this dependency of larvae upon external food sources for nourishment, food limitation is likely to influence yearly larval recruitment success as predicted by the MMH.

The implications of food limitation for marine invertebrates have also been shown to depend upon physical variables (Anger and Dawris, 1981). In particular, temperature should be considered in the context of food limitation, due to its control upon the metabolism of ectothermic organisms (Desai and Anil, 2000). It is well documented that temperature exerts an influence upon larval growth, development and survival, as well as the rate of reserve utilisation (Anger et al. 1981a; Olson, 1985; Pechenik, 1990). As feeding rates increase with temperature, this allows for higher growth, reproduction and fitness (Scheltema and Williams, 1982; Harms, 1986; Monaco and Helmuth, 2011). However, exposure to sub-optimal environmental conditions may induce physiological changes in an organism that increase its ‘‘cost of living’’ (Somero, 2002). For example, higher temperatures lead to faster degradation of energy reserves and an exponential increase in metabolic demands; above a maximum temperature, growth decreases as increased metabolic demands are greater than larval feeding capabilities (Hodgson and Bourne, 1988). If food conditions are limited, this response to increasing temperatures is likely to lead to a decrease in fitness. Given that different species exhibit different thermal tolerances and optima based on their natural environment (Portner, 2001; Monaco and Helmuth, 2011), the magnitude of species responses to increases in temperature would be expected to vary, particularly under food limited conditions.

In temperate marine ecosystems, interactions between phytoplankton and zooplankton form the basis for energy flux to higher trophic levels (Platt et al. 2003;

Edwards and Richardson, 2004). Consequently, a reduction in the recruitment success of marine invertebrates, due to phenological mismatches, may have a large impact on the structure of marine food webs (Edwards and Richardson, 2004). The barnacle *Semibalanus balanoides* is an ecologically important intertidal species known to synchronously release a single brood of pelagic larvae during the spring (Barnes, 1956, 1957, 1962). It appears that synchronising larval release with an abundant food source is fundamental for the recruitment success of this species. For example, Barnes (1956) observed that years when there was failure of the larval population of *S. balanoides* were, to a large degree, coincident with the failure or irregularities of the diatom population. However, not all marine invertebrate species synchronise their larval release to periods of phytoplankton abundance, suggesting potential differences in the physiological tolerance of low food levels or the use of alternative food sources. For instance, the barnacle *Austrominius modestus*, an exotic species to the European coast, does not show a synchronous release of its larvae (Crisp and Davies, 1955). This warm water species does not show a restricted period of larval production and usually produces several broods per individual per year.

The planktotrophic development of *S. balanoides* and *A. modestus* culminates in a non-feeding cyprid stage, which attach and settle upon the shore. This transition from pelagic to benthic form is a critical period for survival as mortality rates for settled cyprids are reportedly high within the first 24 hours (see review Gosselin and Qian, 1997). Research has demonstrated that food conditions experienced during larval development can determine the physiological condition of cyprids, and thus, have a profound effect upon their capacity to settle and metamorphose successfully (Jarrett and Pechenik, 1997; Hentschel and Emlet, 2000; Tremblay et al. 2007). The magnitudes of this effect are expected to vary with species-specific tolerance to food limitation.

This study proposes to assess the influence of food abundance upon the settlement success of two contrasting barnacle species; *S. balanoides* and *A. modestus.* To evaluate this, the influence of different food levels upon the rate of development,

survival of larvae, cyprid size and the subsequent metamorphosis rates of both model species were examined in manipulative laboratory experiments. As the model species exhibit different strategies in reproduction and larval release, it is proposed they will differ in their response to low food environments. It is predicted that *S. balanoides*, with larval release synchronised to phytoplankton blooms, may show a reduced tolerance to low food levels than *A. modestus*, which does not synchronise its larval release to an environmental cue. The first food concentration experiments in this study were conducted at constant temperatures of 15^{o}C as previous work has shown that larvae of both species can be successfully reared at this temperature (Harms, 1984). The second experiments aimed to examine whether temperature modifies the larval response to food density. In order to do this, the first experiments were repeated for each species with the addition of another temperature level which was also representative of a temperature that larvae would experience during their time in the plankton. For *S. balanoides* a lower temperature of 9^{o}C was selected as this is an environmental temperature the larvae are likely to experience during their peak abundance in the plankton during the spring in North Wales. In contrast, *A. modestus* prefers warmer waters (Harms, 1984) so an elevated environmental temperature of 18^{o}C was chosen. Furthermore, this temperature is in accordance with a temperature that *A. modestus* larvae could experience in the plankton during a particularly hot summer in North Wales. Given the relationship between temperature and metabolism, it is predicted that a higher temperature would exacerbate any impacts seen at reduced food levels. Therefore, it may be expected that a mismatch of larvae with optimal food and temperature conditions will have a stronger influence upon larval survival and settlement of *S. balanoides* than it would upon *A. modestus*.

2.2. Materials and Methods

2.2.1. Larval collection and culturing procedures

Adult barnacles of *S. balanoides* and *A. modestus* were collected from intertidal locations along the Menai Strait, Anglesey, UK, by scraping intact individuals from

the substratum. Upon return to the laboratory, adults that contained well-developed larval broods were identified and placed individually in separate 100ml plastic containers containing 0.45 µm filtered seawater (FSW). These were left for up to 2 hours to allow hatching of stage I nauplii (Crisp, 1956). Only adults where most of the larval brood had readily hatched were deemed appropriate for experimental use and, from these, four adults were randomly selected for the experiment and larvae transferred at appropriate densities to experimental culture vessels (10 larvae per x 100ml plastic vessel). All replicates were reared in automated incubators with a 12:12h light: dark photoperiod at the designated experimental temperature.

Larvae were fed the diatom *Skeletonema costatum* (strain: CCAP 1077/5) at appropriate cell densities every 2 days. Larval feeding also co-incided with a full water change.

Prior to each feeding, *S costatum* were collected from cultures (grown in Conway medium with added silicates and FSW) and culture cell density was determined with a haemocytometer. Algal cultures were reared in 4 or 10 litre Pyrex borosilicate flasks at 18-19^{o}C with a 24 hr light cycle. Larval culture water was changed by pouring the contents of a replicate jar into a small glass bowl and, using a Pasteur pipette, larvae were moved to a watch glass for microscopic examination. After examination, larvae were returned to a clean jar already containing *S. costatum* and FSW at the appropriate experimental temperature and food density. During microscopic examination, larvae were checked for mortality and for average larval development stage. Larvae within each replicate were observed every 2 days until the first appearance of cyprids, after which time, larval cultures were checked daily (with water change and feeding still taking place every 2 days). All cultures ran until larvae had either died or moulted to the final larval cyprid stage. When the cyprid stage was reached, cyprids were removed from cultures and subsequently used to determine settlement rates.

2.2.2. Experiment 1

In this experiment environmental food levels were manipulated in order to determine their effect upon the performance of larvae from both study species. Larval performance was measured in terms of duration of larval development to the cyprid stage, percentage survival to the cyprid stage and subsequent settlement success. Larvae were hatched from adults collected from two shores to examine for any differences in performance between larvae which had developed in different locations.

Adult barnacles of *S. balanoides* and *A. modestus* were collected from 2 intertidal locations along the Menai Strait, Anglesey, UK (Shore 1: 53°13'16N, 04°09'47W; Shore 2: 53°13'27N, 04°09'38W); *S. balanoides* were collected in April 2010 and *A. modestus* in June-July 2010. Using the method described in the previous section, freshly hatched larvae from a total of 8 adults (4 from each location) were harvested to be reared under 3 different food levels of *S. costatum,* as illustrated in Fig 2.1A. The food treatments selected for the experiments were based upon those known to affect levels of *S. balanoides* larval release in the laboratory (Starr et al. 1991). From this information, food levels were selected that correspond to those known to elicit 80%, 50% and 25% larval release in gravid adults. The three food density treatments were high food (HF): 4×10^5; mid food (MF): 2×10^5 and low food (LF): 4×10^4 cells ml^{-1}; all three of these are high enough for both species to complete metamorphosis (*pers obs*). The larvae were reared at a temperature of 15°C under these three food treatments as described in the larval culturing section.

When larvae had developed to the cyprid stage, cyprids were harvested to determine the effects of the naupliar food treatments upon the settlement rates. At this point, cyprids from different replicates and different adults were pooled together by food treatment into a jar on a daily basis. Cyprids were maintained without food in the incubators in jars of FSW at a temperature of 15°C. A rock (approx 40mm x 40mm) collected from shore 2, which previously had barnacles living upon it, was introduced into each jar as settlement surface at the same time as

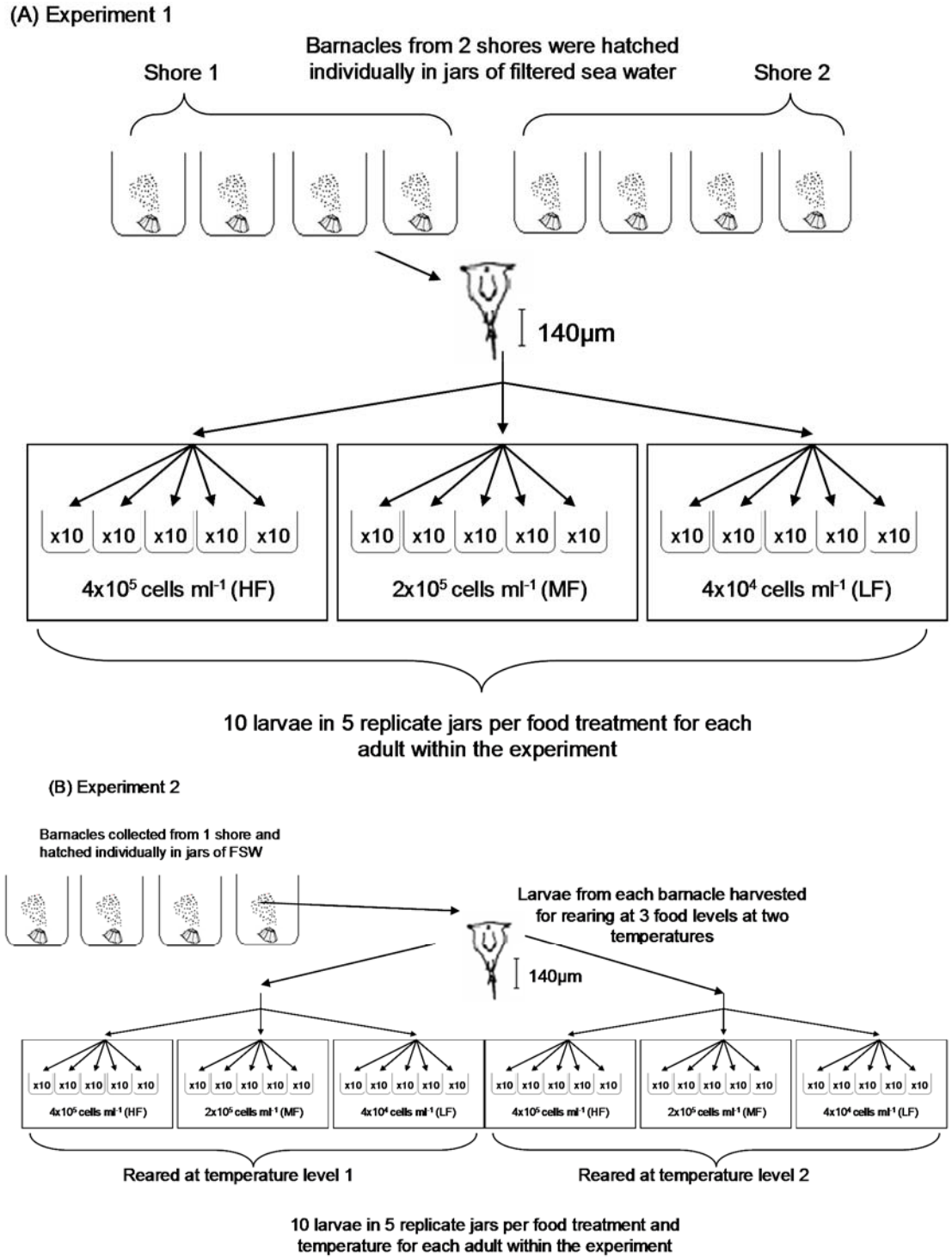

Fig. 2.1 ***Semibalanus balanoides*** **and** ***Austrominius modestus*****. Procedure for collection of larvae and experimental design of (A) experiment 1; effects of food density on larval performance and (B) experiment 2; effects of food density and temperature on larval performance. Larvae of each species were reared in separate experiments.**

the first cyprids. The barnacles (living and dead) upon the rock had been picked off carefully with a knife and it was presumed a settlement cue and biofilm would remain to attract settlement of the cyprids (Knight-Jones, 1953).

2.2.3. Experiment 2

This experiment evaluated whether temperature modified the larval response to food density for both species. Larval performance in these experiments was measured in terms of duration of larval development to the cyprid stage, percentage survival to the cyprid stage, cyprid size and subsequent settlement success.

Experiments were carried out in February 2012 for *S. balanoides* and in October 2012 for *A. modestus*. For each experiment, adults were collected from one location on the Menai Strait (53°13'16N, 04°09'47W) and left to hatch as described in the larval collection section. For each experiment, 4 adults with ripe embryos were selected. Using the larval culturing procedure, larvae from each adult were reared under 2 different temperatures for each of the 3 experimental food levels. *S. balanoides* were reared at 9°C and 15°C; *A. modestus* were reared at 15°C and 18°C, as illustrated in Fig 2.1B.

Experiment 1 showed no significant effects of shore upon larval performance, but there was important variability in performance among larvae from different adults within shores (see results). This second experiment aimed to explore potential sources of adult derived variability in larval performance and, in particular, whether performance would be related to larval body size and/or adult body size. Therefore, additional larvae from each adult were collected and preserved in ethanol to eventually determine nauplius body size at hatching.

When larvae had developed to the cyprid stage, the cyprids from each adult reared under each food treatment and temperature condition were pooled together in 1 jar. Cyprids were kept in the incubator in FSW at the same temperature they were

reared under. 10 cyprids from each adult at each treatment were selected at random and photographed using a microscope camera GX-CAM 5. Photographs were subsequently analysed using Image J to calculate the length, width and cyprid area. Cyprids were then returned to the cultures for settlement rates analysis. For this, a settlement surface was introduced to the vessel containing the cyprids and the resulting settlement rates were observed. As described in experiment 1, a scraped clean rock was provided for the *S. balanoides* cyprids. For *A. modestus* a piece of slate with the adults removed was offered as a settlement surface. On day 2, 4, 6 and 8 (after the rock had been added) the cyprids cultures were examined and the water was changed. During examination, numbers of metamorphosed cyprids upon the rocks were counted and any dead cyprids in the jar were removed.

2.2.4. Larval Mortality

The instantaneous mortality rates (M) of larvae during naupliar development (hatching to cyprid) and cyprid settlement were calculated using the equation

$$M = \ln (N_0/N_t)/-t$$

where t = specific time interval; N_t = number of larvae after a specific time interval (t); and N_o = initial number of larvae. Using this method, the average M for each food and temperature treatment were calculated for both the period of hatching to cyprid and the subsequent cyprid to settlement period for each species.

2.2.5. Statistical analyses

For the first experiment, the response variables were the duration of development from hatching to the cyprid stage per replicate, percentage survival to cyprid per replicate, and percentage settlement. The duration of development and percentage survival variables were analysed in GMAV5 (Underwood, 2002) using mixed model ANOVAs with shore, parent, (nested within shore) as random factors and food treatment as a fixed factor (orthogonal to shore and parent). Chi-square analysis was used to examine differences in percentage settlement among food treatments. For the second experiment the response variables were duration of development from hatching to the cyprid stage per replicate, percentage survival to cyprid per replicate,

percentage settlement and mean cyprid size. These variables were also analysed with mixed model ANOVAs with parent as a random factor and temperature and food as fixed factors. Cochran's test was used to test for homogeneity of variance and Student Newman Keul's (SNK) was used for *post-hoc* multiple comparisons. Where data transformation did not remove heterogeneity, non-transformed data were analysed using the ANOVA described, owing to a lack of alternative approaches. Thus, for data with heterogeneous variances, the critical value was adjusted to a more conservative level of $p < 0.01$. Here significant results should be interpreted with caution owing to an increased probability of type I error (Underwood, 1997).

Estimates of variance components were used to evaluate patterns of variability in the response variables among random factors (shore, parent and replicate) for each experimental treatment. The relative variance components were estimated for each source of variation by using the observed mean squares to estimate terms identified in the expected mean squares (Searle et al. 1992; Underwood, 1997; Graham and Edwards, 2001). Sometimes one or more estimates from the ANOVA method were negative; then, these estimates were set to zero, removed from the model and the estimates for the remaining factor re-calculated according to Fletcher & Underwood (2002). Untransformed data were used throughout to provide variance components comparable across all data (Fraschetti et al. 2005).

2.3. Results

2.3.1. Experiment 1

For both species, survival to the cyprid stage was high (60-90%) for all food treatments (Fig 2.2 A-B). In *S. balanoides*, the percentage survival to the cyprid stage under the low food treatment was significantly lower (65%) when compared to the high and mid food levels (Fig 2.2A); for *A. modestus* it was not significantly different (81%) (Fig 2.2B; Table 2.1). There was also significant variability in percentage

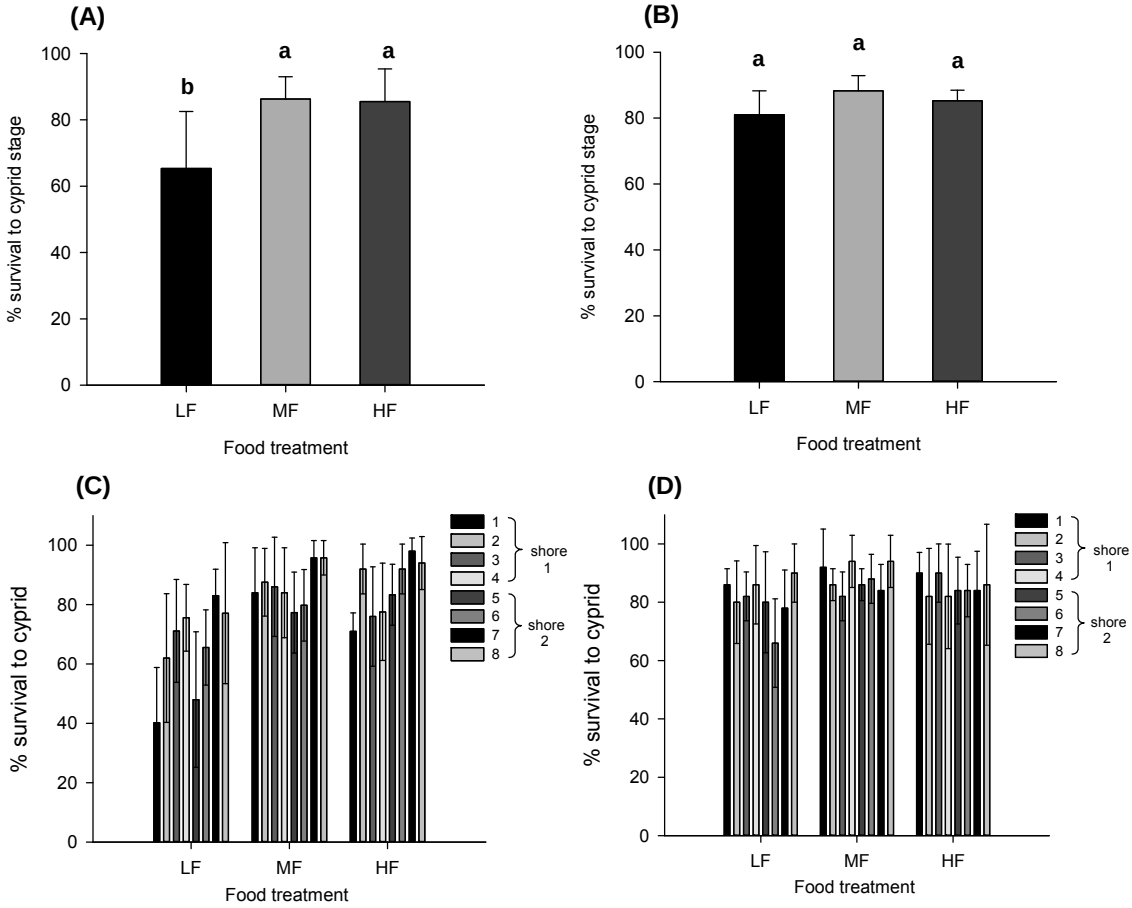

Fig. 2.2 Effects of food density on survival to the cyprid stage for *Semibalanus balanoides* and *Austrominius modestus*. (A) *S. balanoides* and (B) *A. modestus;* average percentage survival to the cyprid stage at each food level. (C) *S. balanoides* and (D) *A. modestus*; average percentage survival to cyprid for larvae from each parent at different food treatments. Error bars: (A) and (B): SD based on replicate parents. (C) and (D). Error bars: SD. Different lower case letters show significant differences (p=0.05) between food treatments for each species

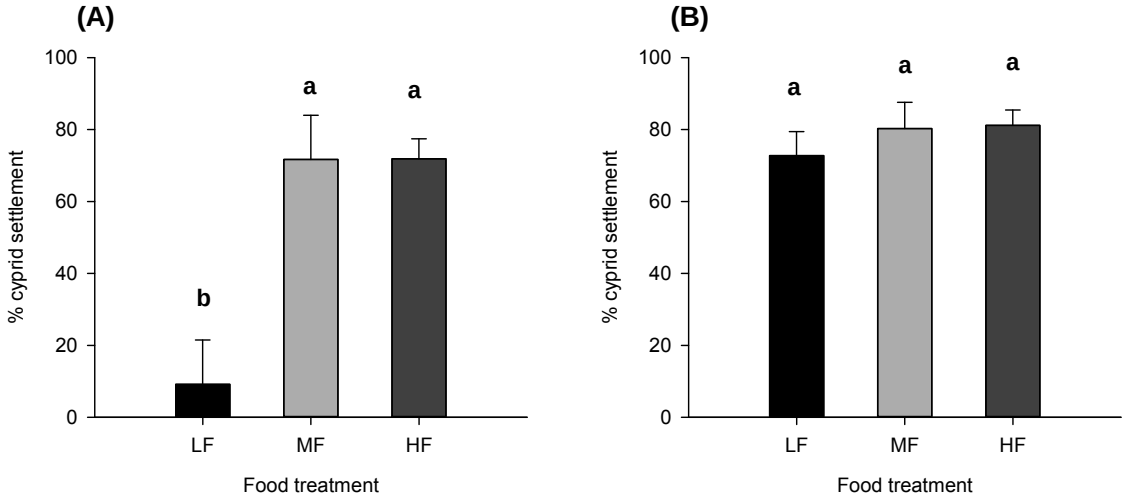

Fig. 2.3 Effects of food density upon settlement rates of cyprids of *S. balanoides* (A) and *A. modestus* (B): LF; low food MF; Mid food, and HF; high food. Different lower case letters show significant differences (p<0.05) among food treatments for each species.

Table 2.1 Mixed model ANOVA on percentage survival to the cyprid stage for larvae from 2 shores fed different food treatment. Parents were nested within shore and both these factors were considered random. Food was a fixed factor

Factor	SS	DF	MS	F	P
Experiment 1 – *S. balanoides*					
Shore	1425.6	1	1425.61	1.22	0.3118
parent(shore)	7014.6	6	1169.1	5.89	≤0.0001
Food	11280.0	2	5640.01	18.71	0.0500
shoreXfood	602.9	2	301.45	1.00	0.3951
foodXparent(shore)	3601.8	12	300.15	1.51	0.1332
Experiment 1 – *A.modestus*					
Shore	163.3	1	163.33	0.75	0.4209
parent(shore)	1313.3	6	218.88	1.52	0.1789
Food	1061.7	2	530.83	9.51	0.0952
shoreXfood	111.7	2	55.83	0.50	0.6200
foodXparent(shore)	1346.7	12	112.22	0.78	0.6688

Table 2.2 *Semibalanus balanoides:* The cross-tabulation for settlement at each food treatment in Experiment 1 which gave a significant association using Pearson Chi-squared ($\chi^2 = 220.55$, $df = 2$, $p < 0.001$)

		settled		
Food		yes	no	Total
HF	Count	167	73	240
	Expected Count	126.8	113.2	240
	% within food	69.60%	30.40%	100.00%
	% within settled	44.90%	22.00%	34.10%
	% of total	23.70%	10.40%	34.10%
	Std. Residual	3.6	-3.8	
MF	Count	188	76	264
	Expected Count	139.5	124.5	264
	% within food	71.20%	28.80%	100.00%
	% within settled	50.50%	22.90%	37.50%
	% of total	26.70%	10.80%	37.50%
	Std. Residual	4.1	-4.3	
LF	Count	17	183	200
	Expected Count	105.7	94.3	200
	% within food	8.50%	91.50%	100.00%
	% within settled	4.60%	55.10%	28.40%
	% of total	2.40%	26.00%	28.40%
	Std. Residual	-8.6	9.1	
Total	Count	372	332	704
	Expected Count	372	332	704
	% within food	52.80%	47.20%	100.00%
	% within settled	100.00%	100.00%	100.00%
	% of total	52.80%	47.20%	100.00%

survival to cyprid among larvae hatched from individual *S. balanoides* parents (Table 2.1, Fig 2.2C) but not for *A. modestus* (Table 2.1; Fig 2.2D). SNK analysis identified that these important parental influences were only present at the low food level for *S. balanoides*.

Rates of cyprid metamorphosis responded strongly to the experimental food treatments for *S. balanoides*. At the low food level, rates of metamorphosis were considerably reduced (8.5%) compared to those at mid and high food levels (70%) (Fig 2.3A; Table 2.2). There was a significant association between the larval food treatment and whether or not the cyprids were able to settle ($\chi^2 = 220.55$, $df = 2$, $p < 0.001$). Based on the odds ratio, cyprids of *S. balanoides* reared under the low food treatment were 25 times less likely to complete settlement than when they were reared at the high and mid food treatment (Table 2.2). By contrast, rates of metamorphosis in *A. modestus* were high at all food treatments (Fig 2.3B) and statistical tests revealed that none of the experimental factors affected settlement rates (shore, $F_{1,18} = 0.36$, $p > 0.5$; food treatment, $F_{2,18} = 5.96$, $p > 0.1$).

Food level had a significant effect on development time for both species with larvae developing quicker at the high and middle than at the low food levels (Fig 2.4A-B). The low food treatment increased this development time by 58% for *S. balanoides* and by 10% for *A. modestus*. These results were consistent across shores (no significant shore effect: Table 2.3). There were significant interactions between parent and food treatment in both species (Table 2.3), indicating that the effect of food on larval duration of development varied among larvae from different adults (Fig 2.4C-D). *Post hoc* SNK identified that the effect of parents was stronger at low food than at other food conditions, but the response varied among species. In *S. balanoides* there was always a significant effect of low food level on duration of development irrespective of the parent; in *A. modestus* larvae from some particular adults did not show an increase in duration of development in response to food.

Variance components analysis identified that at the high and mid food levels most of the variation in development time occurred within the replicates for *S. balanoides*,

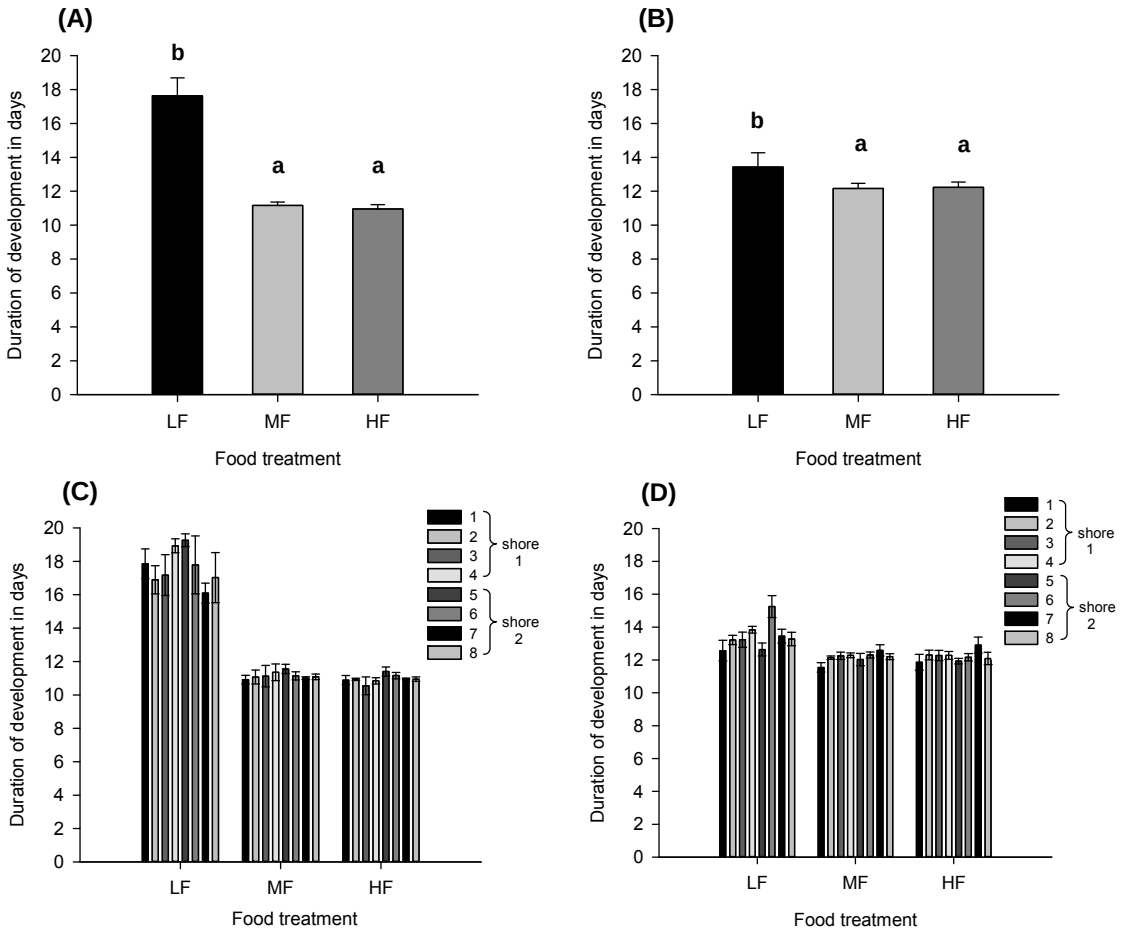

Fig. 2.4 Effects of food density on duration of development of *Semibalanus balanoides* and *Austrominius modestus*. (A) *S. balanoides* and (B) *A. modestus*; average duration of development to the cyprid stage at each food level. (C) and (D) average duration of development for larvae from each parent at each food level(C) *S. balanoides* and (D) *A. modestus*; Error bars: (A) and (B): SD based on replicate parents. (C) and (D). Error bars: SD. Different lower case letters show significant differences ($p<0.01$) between food treatments for each species

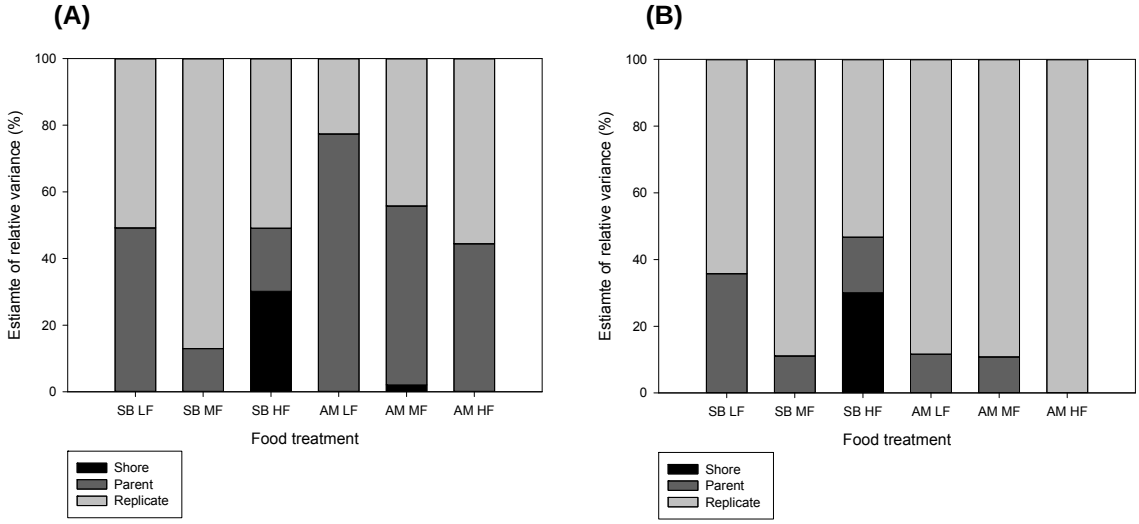

Fig. 2.5 Estimates of variance components for (A) duration of development and (B) survival to cyprid under each experimental food treatment at shore, parent and replicate scale. Abbreviations; SB, *S. balanoides*; AM, *A. modestus*; LF, Low Food; MF, Mid food; HF High food.

Table 2.3 Mixed model ANOVA on duration of development to the cyprid stage for larvae from 2 shores fed different food treatment. Parents were nested within shore and both these factors were considered random. Food was a fixed factor. For *S. balanoides* significance level was judged at p < 0.01 as variances were heterogeneous (Cochran's *C*-test, p < 0.05)

Factor	SS	DF	MS	F	P
Experiment 1 – *S. balanoides*					
Shore	0.16	1	0.16	0.05	0.8378
parent(shore)	21.47	6	3.58	8.15	**≤0.0001**
Food	1151.40	2	575.72	993.78	**0.0010**
shoreXfood	1.16	2	0.58	0.34	0.7160
foodXparent(shore)	20.23	12	1.69	3.84	***0.0001**
Experiment1 – *A.modestus*					
Shore	1.88	1	1.88	0.75	0.4200
parent(shore)	15.06	6	2.51	19.41	**≤0.0001**
Food	40.87	2	20.43	67.84	**0.0145**
shoreXfood	0.60	2	0.30	0.26	0.7741
foodXparent(shore)	13.82	12	1.15	8.90	**≤0.0001**

Table 2.4 Mixed effects ANOVA on percentage survival to the cyprid stage for larvae reared under different food and temperature treatments. Parents were considered a random factor. Food and temperature were fixed factors.

Factor	SS	DF	MS	F	P
Experiment 2 – *S. balanoides*					
Parent	749.16	3	249.72	3.92	0.0110
temperature	1020.83	1	1020.83	2.60	0.2050
Food	1140.00	2	570.00	2.69	0.1469
parentXtemp	1175.83	3	391.94	6.15	**0.0007**
parentXfood	1273.33	6	212.22	3.33	**0.0051**
tempXfood	126.67	2	63.33	1.16	0.3742
parentXtempXfood	326.67	6	54.44	0.85	0.5317
Experiment 2 – *A.modestus*					
Parent	229.17	3	76.39	1.93	0.1299
temperature	240.83	1	240.83	3.25	0.1693
Food	351.67	2	175.83	4.62	0.0610
parentXtemp	222.5	3	74.17	1.87	0.1392
parentXfood	228.33	6	38.06	0.96	0.4556
tempXfood	71.67	2	35.83	0.47	0.6448
parentXtempXfood	455.00	6	75.83	1.92	0.0859

as indicated by the large estimates of relative variance (Fig 2.5A). In contrast, at the low food level most variability occurred among parents. Most of the variability for *A. modestus* was found among parents at all the food levels, with the largest proportion again being at the low food level (Fig 2.5A). Most of the variation in survival occurred within the replicates for both species at >50%, but parent had a greater degree of variation at the low food level than at the high and mid food levels for *S. balanoides* (Fig 2.5B).

2.3.2. Experiment 2

2.3.2.1. Naupliar survival and settlement rates

Both species exhibited high average survival rates (>80%) to the cyprid stage under all of the experimental treatments (Fig 2.6A,B). For larvae of *S. balanoides*, the effect of food and temperature on survival rates varied strongly among larvae from different parents (significant (food x parent) and (temperature x parent) interactions: Table 2.4). An increase in temperature from 9 to 15^{o}C generally decreased the survival of larvae across the food treatments by 7% (Fig 2.6A), but this effect depended on the parent (Table 2.4). For example, SNK analysis identified that larvae from parent 1 had significantly decreased survival at 15^{o}C compared to the other parents (Fig 2.6C). Parental effects also interacted with the food factor, with parent 1 demonstrating significantly decreased survival at the low food conditions compared to larvae reared from other parents. Variance components suggested that the most important variation among parents of *S. balanoides* occurred at the high temperature, especially at low food levels (Fig 2.7A). Survival rates of *A. modestus* larvae were high >90% under all experimental conditions (Fig 2.6B). Survival of this species was not significantly affected by any of the experimental factors (Table 2.4) and there were no detectable differences between the responses of the parents (Fig 2.6D). Variance components indicated low contribution of parents to the total variability for this species (Fig 2.7B).

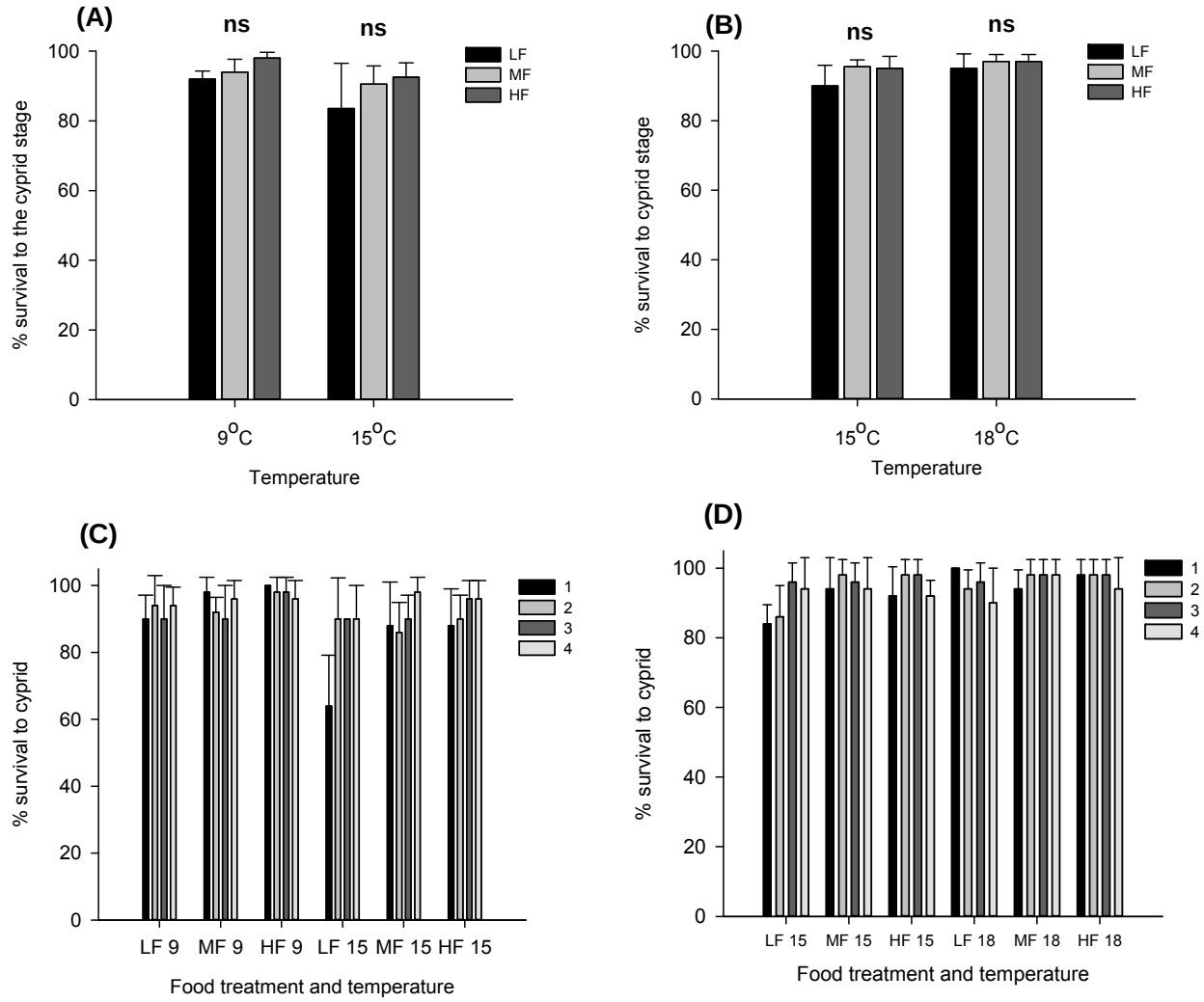

Fig. 2.6 Percentage survival to the cyprid stage for (A) *S. balanoides* and (B) *A. modestus;* and percentage survival to the cyprid stage for each parent(C) *S. balanoides* and (D) *A. modestus* at different food treatments and temperatures. Error bars: (A) and (B) SD based on replicate parents. (C) and (D):SD. Letters "ns" indicate non-significant differences among treatments.

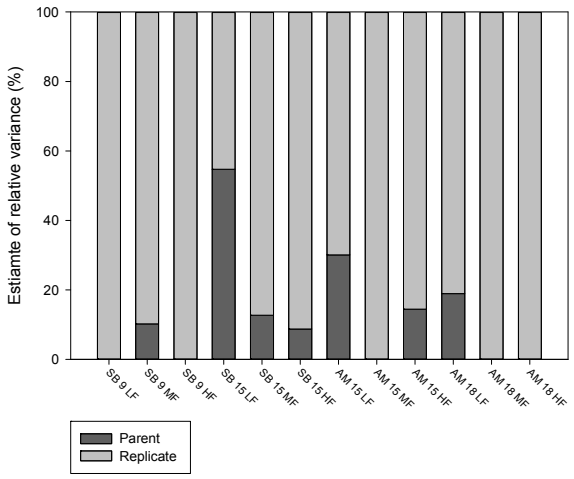

Fig. 2.7 Estimates of variance components for percentage survival for *S. balanoides* (SB) and *A. modestus* (AM) under each experimental food and temperature treatment at the parent and replicate scale

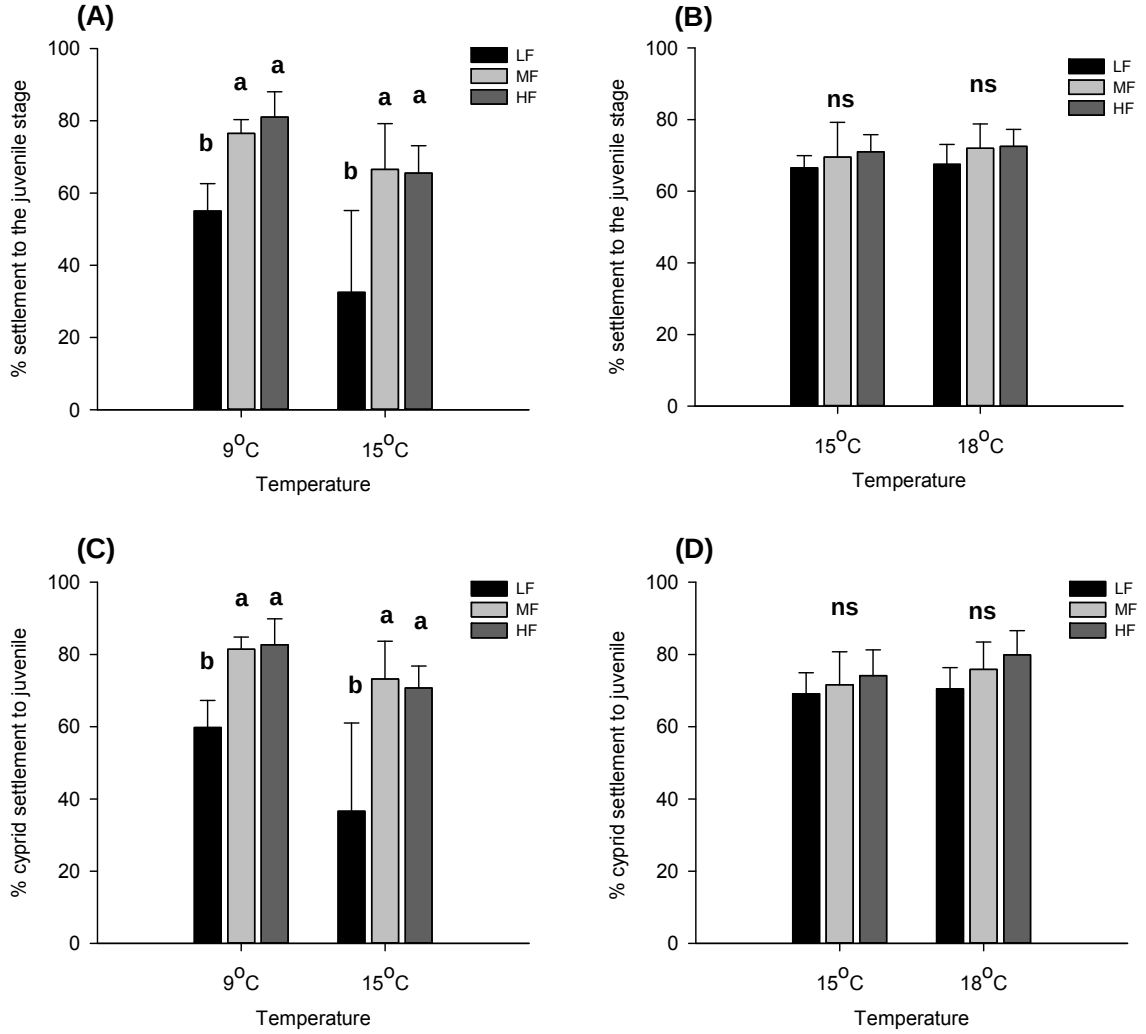

Fig. 2.8 Effects of food density and temperature treatments upon *S. balanoides* and *A. modestus*: Final-settlement rates as a percentage of initial number of larvae for (A) *S. balanoides* and (B) *A. modestus*; and rates of cyprid settlement as a percentage of developed cyprids for (C) *S. balanoides* and (D) *A. modestus*.Error bars; SD. Lower case letters indicate significant differences ($p < 0.005$) among food treatments; ns indicates non-significant differences among treatments.

Table 2.5 ANOVA on settlement success as a percentage of larvae reared under different food and temperature treatments. Food and temperature were fixed factors(*) denotes that for *S. balanoides* significance level was judged at $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$)

Factor	SS	DF	MS	F	P
Experiment 2 – *S. balanoides*					
Temperature	1536.00	1	1536.00	10.82	***0.0041**
Food	4382.33	2	2191.16	15.43	***0.0001**
Tempxfood	157.00	2	78.50	0.55	0.5848
Experiment 2 – *A.modestus*					
Temperature	16.67	1	16.67	0.44	0.5150
Food	100.33	2	50.17	1.33	0.2898
Tempxfood	2.33	2	1.17	0.03	0.9696

The two species demonstrated considerable differences in their performance of rates of final settlement rates (Fig 2.8A,B). For *S. balanoides* final settlement was reduced at the higher temperature (15^{o}C) and at the lowest food level (Fig 2.8A). Temperature and food both significantly influenced final settlement success, but the two factors did not interact (Table 2.5). An increase in temperature led to a significant decrease of 15% in settlement success across the food treatments (Fig 2.8A; Table 2.5). SNK identified that final settlement success was significantly lower at the low food treatments (Fig 2.8A). Average survival rates at this food level were 48% compared to success rates of 77% at mid and high food (with no significant differences between high and mid food). By contrast, metamorphic rate was high (>70%) for *A. modestus* under all experimental conditions with no significant differences between any of the treatments (Fig 2.8B; Table 2.5).

Cyprid settlement rates of *S. balanoides* (as a proportion of developed cyprids and not initial nauplii), were also dependent upon food and temperature treatment, but these factors did not interact (Table 2.6); settlement was reduced at higher temperatures or at low food (Fig 2.8C). For *A. modestus* cyprid settlement was similar for all food treatments and did not differ at a higher temperature (Fig 2.8D; Table 2.6).

2.3.2.2. Mortality at different larval developmental stages

For both species, cyprid mortality during settlement was higher than naupliar mortality (Fig 2.9A,B; Table 2.7). The food environment that *S. balanoides* nauplii experienced seemed particularly important during the cyprid settlement; on average, mortality in the low food treatments was increased by 22% in comparison to the high and mid food treatments (Fig 2.9A). An increase in temperature also reduced cyprid settlement success for all food treatments with average survival being decreased by 10% (Fig 2.9A). Compared to naupliar mortality, cyprid mortality was high for *A. modestus* but was consistent among food and temperature treatments. Estimated instantaneous mortality rates (M) supported these findings

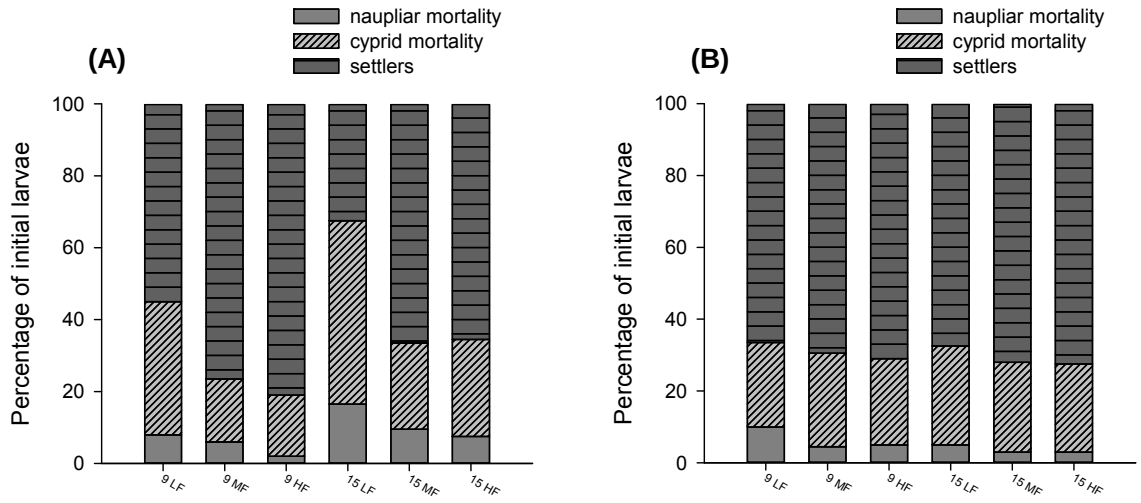

Fig. 2.9 Performance of larvae under different food densities and temperature treatments for (A) *S. balanoides* and (B) *A. modestus*

Table 2.6 ANOVA on settlement success rates of cyprids produced under different food and temperature treatments. Food and temperature were fixed factors (*) denotes that for *S. balanoides* significance level was judged at p < 0.01 as variances were heterogeneous (Cochran's *C*-test, p < 0.05)

Factor	SS	DF	MS	F	P
Experiment 2 – *S. balanoides*					
temperature	620.17	1	620.17	9.28	***0.0069**
food	2736.33	2	1368.17	20.47	***≤0.0001**
tempxfood	56.33	2	28.17	0.42	0.6624
Experiment 2 – *A.modestus*					
temperature	8.17	1	8.17	0.13	0.7200
food	8.33	2	4.17	0.07	0.9348
tempxfood	26.33	2	13.17	0.21	0.8096

Table 2.7 Estimated instantaneous mortality rates of larvae (d^{-1}) from hatching to cyprid and cyprid to post-settlement.

species	food treatment	Temperature in ^{o}C	mortality rates	
			From hatching to cyprid	From cyprid to settlement
S. balanoides	LF	9	0.00465	0.08574
	MF	9	0.00406	0.03433
	HF	9	0.00131	0.03175
	LF	15	0.01334	0.15727
	MF	15	0.00972	0.05136
	HF	15	0.00767	0.05753
A. modestus	LF	15	0.00826	0.02832
	MF	15	0.00426	0.03000
	HF	15	0.00476	0.01712
	LF	18	0.00526	0.02735
	MF	18	0.00363	0.03981
	HF	18	0.00364	0.04510

and indicated that mortality was higher during the cyprid settlement stage in comparison to the naupliar stages (Table 2.7). During the cyprid settlement phase for *S. balanoides*, estimated mortality rates were at their highest in the low food treatments (at 9°C M=0.085 d^{-1}; at 15°C M=0.157 d^{-1}) when compared to the high and mid food treatments where M<0.058 d^{-1} (Table 2.7). In contrast, for *A. modestus* settlement mortality remained low (0.017< M <0.045) and did not vary substantially among food and temperature treatments.

2.3.2.3. Duration of development

As in experiment 1, both species had a shorter development time when the larvae experienced the high/mid food rations and/or the higher temperature condition (Fig 2.10A,B). In *S. balanoides*, the effect of food limitation on duration of development was exacerbated by a higher temperature. Increasing the temperature had a disproportionate impact upon duration of larval development under food limited conditions; at the lower temperature, development took 18% longer at the low food conditions compared to rates observed at high and mid food levels, but duration of development was increased by 32% at the higher temperature (Table 2.8 and Fig 2.10A). The effect of temperature and food on duration of development varied significantly among larvae from different parents (significant interactions of parent x food and parent x temperature: Table 2.8). However, for all parents, larvae took a longer time to develop at the low food level and lower temperature (Fig 2.10C).

For *A. modestus*, both low food and low temperature resulted in longer duration of development (Fig 2.10B), but there was a significant 3 way interaction between parent, temperature and food (Table 2.8), indicating that the magnitude of the food and temperature effect on development time was different across larvae from different parents. SNK analysis identified that, across the food treatments and temperatures, larvae from certain parents consistently performed significantly better than others, as can be seen in Fig 2.10D.

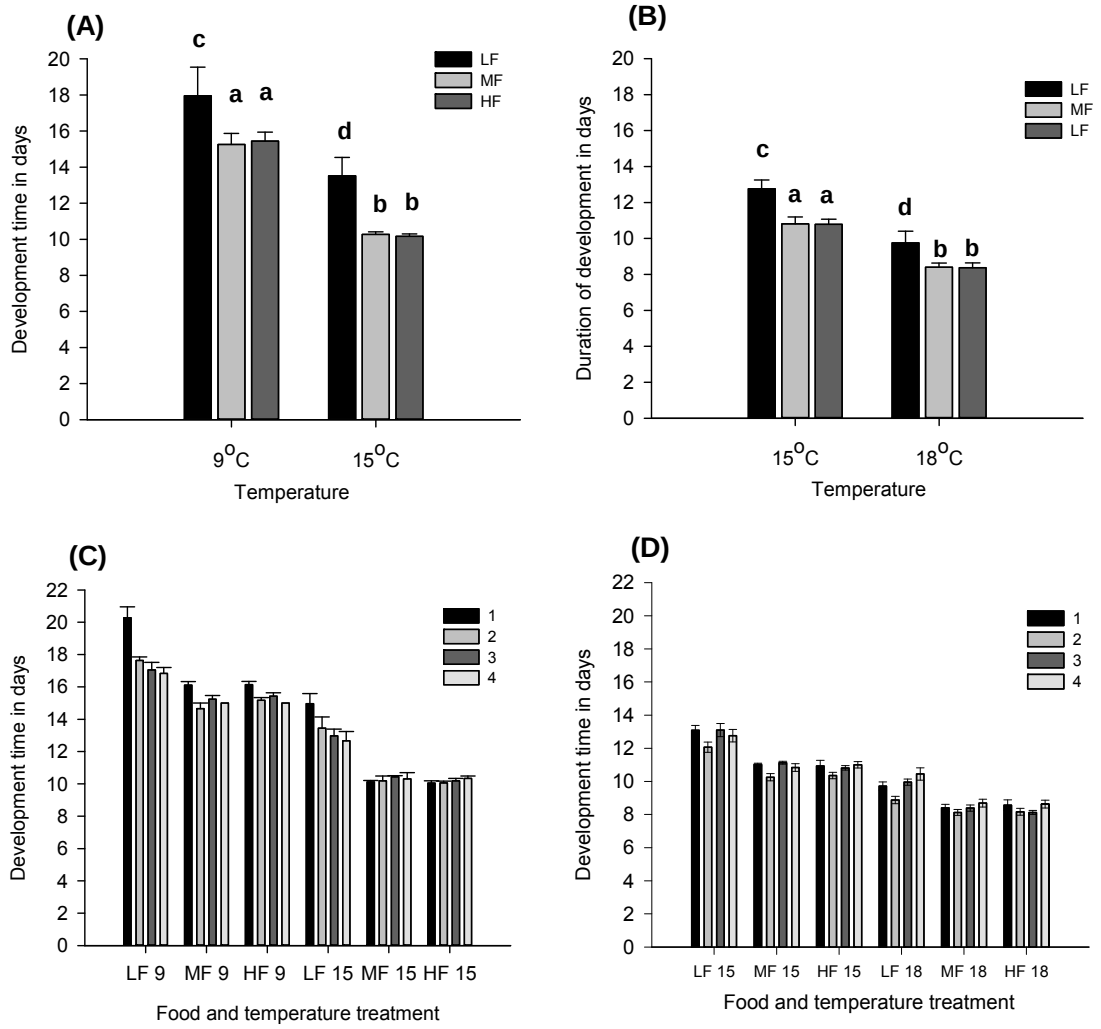

Fig. 2.10 Effects of food density and temperature for *Semibalanus balanoides* and *Austrominius modestus* Average duration of development to the cyprid stage for (A) *S. balanoides* and (B) *A. modestus;* and average duration of development to the cyprid stage for each parent(C) *S. balanoides* and (D) *A. modestus* at different food treatments and temperatures. Error bars: (A) and (B) SD based on replicate parents. (C) and (D):SD. Different lower case letters show significant differences ($p<0.05$) between food and temperature treatments for each species

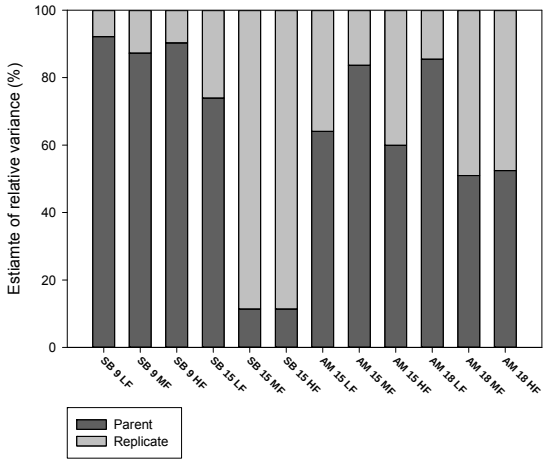

Fig. 2.11 Estimates of variance components for duration of development for *S. balanoides* (SB) and *A. modestus* (AM) under each experimental food and temperature treatment at the parent and replicate scale

Table 2.8 Mixed effects ANOVA on duration of development to the cyprid stage for larvae reared under different food and temperature treatments. Parents were considered a random factor. Food and temperature were fixed factors. (*) denotes that for *S. balanoides* significance level was judged at p < 0.01 as variances were heterogeneous (Cochran's *C*-test, p < 0.05)

Factor	SS	DF	MS	F	P
Experiment 2 –					
S. balanoides					
adut	29.53	3	9.84	82.79	≤0.0001
temp	719.32	1	719.32	261.55	0.0005
food	232.53	2	116.26	27.50	0.0010
adultXtemp	8.25	3	2.75	23.13	***≤0.0001**
adultXfood	25.37	6	4.23	35.56	***≤0.0001**
tempXfood	3.61	2	1.81	20.71	***0.0020**
adultXtempXfood	0.52	6	0.08	0.74	0.6226
Experiment 2 –					
A .modestus					
adut	10.52	3	3.50	57.68	≤0.0001
temp	203.97	1	203.97	381.68	0.0003
food	73.83	2	36.91	102.58	≤0.0001
adultXtemp	1.60	3	0.53	8.79	≤0.0001
adultXfood	2.16	6	0.36	5.92	≤0.0001
tempXfood	2.38	2	1.19	6.29	0.0337
adultXtempXfood	1.13	6	0.19	3.12	**0.0077**

Table 2.9 Mixed effects ANOVA on cyprid size for larvae reared under different food and temperature treatments. Parents were considered a random factor. Food and temperature were fixed factors.

Factor	SS	DF	MS	F	P
Experiment 2 –					
S. balanoides					
adut	1.88	3	0.63	25.87	≤0.0001
temperature	9.96	1	9.95	125.26	0.0015
food	17.78	2	8.89	41.35	0.0003
adultXtemp	0.24	3	0.08	3.29	0.0215
adultXfood	1.29	6	0.21	8.90	≤0.0001
tempXfood	0.29	2	0.15	2.23	0.1883
adultXtempXfood	0.4	6	0.07	2.73	**0.0142**
Experiment 2 –					
A. modestus					
adut	0.02	3	0.01	1.32	0.269
temperature	0.48	1	0.48	62.08	0.0043
food	3.59	2	1.79	502.87	≤0.0001
adultXtemp	0.02	3	0.01	1.58	0.1948
adultXfood	0.02	6	0.01	0.73	0.6286
tempXfood	0.04	2	0.02	1.92	0.2273
adultXtempXfood	0.07	6	0.01	2.35	**0.0319**

Variance components analysis of the data identified that variation in development time occurred mainly among parents for most of the experimental treatments, apart from *S. balanoides* at mid food/15°C and high food/15 °C where the greatest variation was among replicates (Fig 2.11).

2.3.2.4. Cyprid Size

For both species, the food and temperature conditions significantly influenced cyprid size (Table 2.9): the smallest cyprids were produced at the highest temperature and lowest food (Fig 2.12A-D). For *S. balanoides,* cyprids reared at 15°C displayed size reductions of 20%, 11% and 14% compared to the sizes attained at 9°C for low, mid and high food respectively (Fig 2.12A). Cyprids at the low food treatment were 18% smaller at 9°C and 24% smaller at 15°C than those reared at high and mid food (Fig 2.12A). The effect of temperature and food were dependent on parents (significant 3-way interaction: Table 2.9). SNK highlighted that the greatest degree of variation between parents was found at the combination of high temperature and low food. Variance components showed that the greatest degree of variability occurred among the parents at the low food level for the larvae of *S. balanoides* (Fig 2.13). This is not demonstrated at the high and mid food levels, where most of the variability is between the replicates. Cyprids from parent 1 were particularly small in size at the low food treatments compared to that of the other parents (Fig 2.12B). It appeared that an increase in temperature also had a more pronounced effect upon parent 1 larvae compared to other parents as their cyprid size is again reduced across all food treatments.

The magnitude of the effects upon *A. modestus* cyprid size also depended on an interaction between food, temperature and parent (Table 2.9) where parents displayed differing responses in cyprid size (Fig 2.12D). Cyprids were 8%, 4% and 10% smaller when reared at 18°C compared to 15°C for low, mid and high foods respectively (Fig 2.12B); cyprids at the low food treatment were 18% smaller at 15°C and 21% smaller at 18°C than at high and mid food for the same temperature.

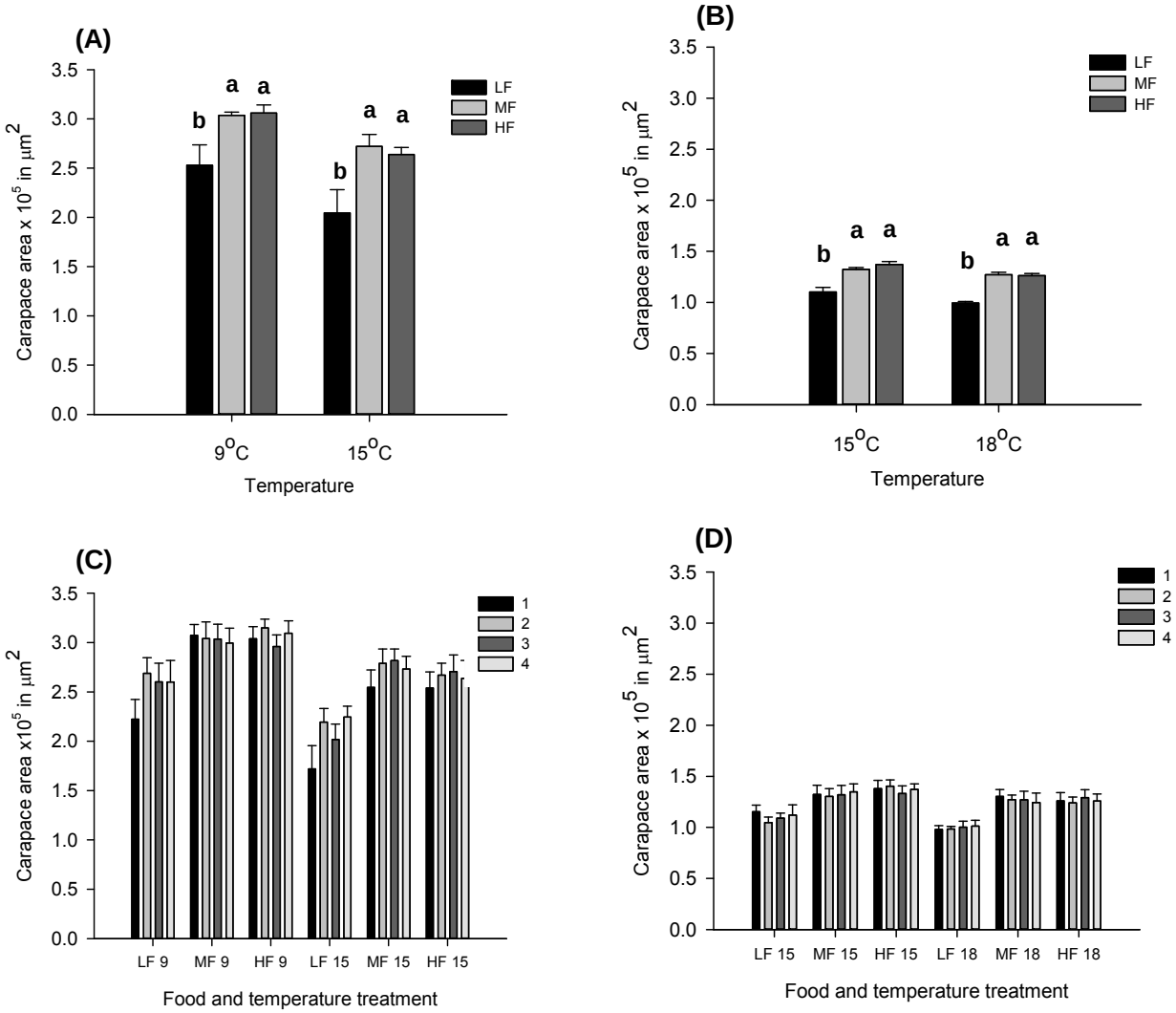

Fig. 2.12 Cyprid area for (A) *S. balanoides* and (B) *A. modestus;* and cyprid area of larvae from each parent(C) *S. balanoides* and (D) *A. modestus* at different food treatments and temperatures. Error bars: (A) and (B) SD based on replicate parents. (C) and (D):SD. Different lower case letters show significant differences ($p<0.05$)among food treatments for each species

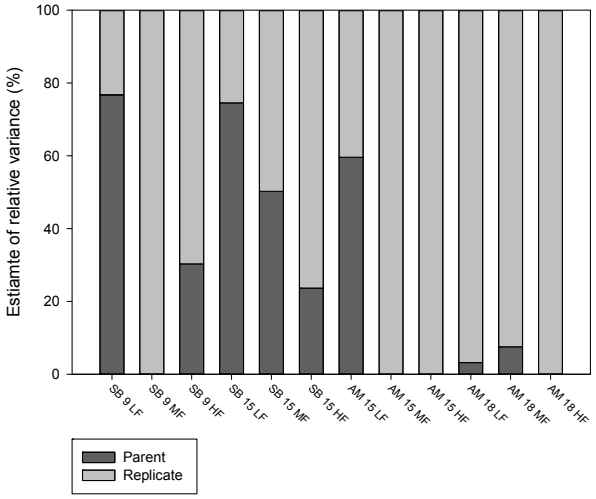

Fig. 2.13 Estimates of variance components for cyprid size for *S. balanoides* (SB) and *A. modestus* (AM) under each experimental food and temperature treatment at the scales parent and replicate

Table 2.10 Experiment-2: Parental body size and average larval size upon hatching by corresponding parent and respective response at the low food (LF) treatment at 15ºC

larval length in µm (±SD)	larval width in µm (±SD)	Label within experiment	basal length in µm	basal width in µm	Average duration of development at LF 15°C(±SD)	Average percentage survival to the cyprid stage at LF 15°C(±SD)
Experiment 2 – *S. balanoides*						
263.91 (±7.90)	154.43(±4.53)	Parent 1	8593.8	7500.0	14.96(±0.63)	64(±15.17)
275.58(±9.08)	158.83(±5.84)	Parent 4	6562.5	5781.3	12.66 (±0.57)	90 (±10)
280.65(±10.01)	157.60(±5.41)	Parent 3	7968.8	7734.4	12.97 (±0.41)	90 (±0)
285.35(±11.95)	173.50(±10.49)	Parent 2	10156.3	8046.9	13.45 (±0.69)	90 (±12.24)
Experiment 2 – *A.modestus*						
205.55(±7.87)	102.22(±5.80)	Parent 1	7700.0	7500.0	13.09 (±0.29)	84(±5.48)
210.87(±9.57)	105.25(±5.43)	Parent 4	8300.0	7900.0	12.76 (±0.37)	94(±8.94)
215.14(±10.59)	108.46(±4.89)	Parent 3	9300.0	8400.0	13.10 (±0.40)	96(±5.48)
219.68(±5.00)	109.47(±4.28)	Parent 2	9500.0	9200.0	12.06 (±0.31)	86(±8.94)

Table 2.11 ANOVA on larval lengths and widths at time of hatching among the different parents used in Experiment 2

Factor	SS	DF	MS	F	P
Experiment 2 – *S. balanoides*					
LARVAL LENGTH					
Parent	2548.64	3	849.55	13.64	**≤0.0001**
LARVAL WIDTH					
Parent	2155.11	3	718.37	14.81	**≤0.0001**
Experiment 2 – *A. modestus*					
LARVAL LENGTH					
Parent	1090.97	3	363.66	5.01	**0.0053**
LARVAL WIDTH					
Parent	324.53	3	108.18	4.11	**0.0132**

Variance components suggested that for *A. modestus*, the variability among parents was lower than that found in *S. balanoides*. The highest variation among parents was found at low food/15°C for *A. modestus* and at both low food treatments for *S. balanoides* (Fig 2.13).

2.3.2.5. Parental characteristics

For both species, stage I nauplii measured from each parent exhibited significant differences in their length and width (Tables 2.10 and 2.11). SNK tests highlighted that larvae from parent 1 were significantly smaller upon hatching than larvae harvested from other parents within the experiment for both species (average sizes shown in Table 2.10). As the low food /15°C treatment produced the most variability for both species, the larval characteristics and size of the parents were compared to rates of larval survival and duration of development to examine any potential relationships (Table 2.10). Basal length and width of parents included in the experiment did not appear to account for the variability seen between parental performances at the low food/15°C. For *S. balanoides*, larvae which were smaller in length and width upon hatching seemed to have poorer performance compared to larvae that were larger in comparison (Table 2.10). The size of the larvae upon hatching appears to have had no effect upon the performance of the *A. modestus* larvae.

2.4. Discussion

In this study, the responses of larvae to experimental conditions were consistent with the original hypothesis that a decrease in food abundance would have stronger implications for the settlement success of *S. balanoides* than *A. modestus*. The first experiments indicated that, when larvae experienced food limitation at a temperature of 15°C, there was a strong detrimental effect upon the performance of *S. balanoides;* this effect was not present for *A. modestus*. Therefore, experiments were repeated with an additional temperature level to investigate whether temperature was having an impact upon the strong response of *S. balanoides*. By

repeating this experiment with an additional temperature level, the second experiment provided support for the hypothesis that a higher temperature can reduce settlement success of *S. balanoides* and thereby exacerbate the effects of food limitation. There was no difference in performance between temperature levels for *A. modestus*.

Results demonstrated that larval responses to food availability and temperature can be ecologically important for the recruitment success of a species. This study, along with others (see review by Pechenik, 1987; Underwood and Fairweather, 1989), demonstrate how pre-settlement factors, such as food availability, can be crucial in determining densities of larvae settling and recruiting into a population. Recruitment is defined as the result of settlement plus early post-settlement mortality (Keough & Downes 1982; Connell, 1985); intense levels of mortality can occur within a 24 hour period immediately after settlement, which may be the result of factors such as energy reserves or ambient temperature (Gosselin and Qian, 1996). More recently, studies have recognized that pre-settlement factors can affect the quality of settling larvae and their potential for post-settlement survival and growth, showing that all settlers to a population are not equal (Thiyagarajan et al. 2002; 2003; 2005; Giménez, 2004; Marshall and Keough, 2006; Pechenik, 2006). Ultimately, this will determine whether a settler will recruit to a population. Juveniles obtained from low-quality larvae grow more slowly than those derived from high-quality larvae (Thiyaragajan, 2005). Slow-growing juveniles can be more vulnerable to physical factors, predation and competition (reviewed by Pechenik, 1987; Bertness et al. 1991; Gosselin and Qian, 1997) and, thus, are more likely to be wiped out of a population via these selective processes than faster-growing juveniles.

In the following sections, the responses of *S. balanoides* and *A. modestus* larvae to a low food environment are discussed, paying particular attention to their contrasting settlement responses. The possibility that a high temperature may exacerbate the negative effects exhibited by food-limited larvae is also examined. Throughout both sections, focus is placed predominantly on the physiological mechanisms that may explain the observed patterns.

2.4.1. Role of food concentration

Food concentration experiments demonstrated contrasting naupliar responses between the two study species to a low food environment and suggested that *S. balanoides* nauplii were more sensitive to a low food level than *A. modestus*. In the first experiments, low food rearing conditions decreased naupliar survival rates for *S. balanoides* but had no impact upon *A. modestus,* suggesting that an abundant food source is important for *S. balanoides* naupliar survival. A similar decrease in survival has been reported for *Balanus eberneus* in relation to food limited conditions (West and Costlow, 1987). Secondly, although low food conditions increased development time to cyprid for both species, this effect was much stronger for *S. balanoides* than *A. modestus*. Increased periods of naupliar development under food limited conditions have been observed for other species (e.g. *Balanus amphitrite*: Qiu and Qian, 1997; Desai and Anil, 2004, *B. eburneus:* West and Costlow, 1987, *B. glandula*: Hentschel and Emlet, 2000, *B. improvisus*: Lang and Marcy, 1982). Food concentration is known to influence naupliar feeding rates of *S. balanoides* and *A. modestus* and nauplii feed at higher rates in higher food concentrations (Yule, 1982). Therefore, low food levels can lead to decreased feeding efficiency and higher mortality rates (Scheltema and Williams, 1982) as there is less energy available to drive larval growth and development (Qiu and Qian, 1997).

Importantly, cyprid settlement success was very poor for *S. balanoides* under low food conditions; this effect was not present for *A. modestus.* Cyprids do not feed; therefore, the energy reserves they accumulate during the naupliar stages are important in determining their ability to attach and metamorphose successfully (Lucas et al. 1979; Tremblay et al. 2007). Metamorphosis is an energetically expensive process where cyprids may consume up to 30% of their own body organic carbon, and if the larvae do not have enough energy reserves they lose their competence to metamorphose (Lucas et al. 1979). It is well known that food limitation can result in lower larval energy reserves, causing reduced metamorphic success (West and Costlow, 1987; Qiu and Qian, 1997; Tremblay et al. 2007). In the present study, it appeared that larval feeding history had an impact upon energy

reserves of *S. balanoides* cyprids and reduced their capacity for competent metamorphosis resulting in poor settlement (West and Costlow, 1987; Tremblay et al. 2002; 2005; 2007).

Apart from average trends, variations in survival of larvae that hatched from different parents were important in *S. balanoides*, but not so in *A. modestus*. Although the quality (i.e. energy reserves) of cyprids was not determined within the scope of this study, it was evident that variability among parents was important in determining cyprid size and naupliar development for both species, particularly under food limited conditions. It is likely this variability would play a role in determining population patterns; for example, smaller cyprids have been shown to have a slower growth rates and reduced survival in the field (Qiu et al. 1997; Emlet and Sadro, 2006) and larvae which develop quicker would have the opportunity to settle upon the shore earlier. Parental variability could reflect genetic differences among parents or maternal effects of parental environment upon the larvae (e.g. intertidal height, density, food, temperature, age, size: Bertram and Strathmann, 1998; Marshall et al. 2008). The present experiment does not identify the source of this variability. Whatever the reason behind that variability it is clear that the capacity to tolerate food limitation varied among phenotypes produced by different parents and this also varied among species. Perhaps, there is higher variation in offspring phenotype in *S. balanoides* than in *A. modestus*. Alternatively, the results reflect the fact that phenotypes that are not food tolerant are manifested, in terms of development and survival, only under food limitation.

Observations from the second experiment suggested larval size at hatching may be an important component of the offspring phenotype in explaining parental variations in survival of *S. balanoides* larvae: larger larvae appeared to exhibit higher fitness under periods of food stress than smaller larvae. In marine organisms, offspring size is viewed as approximating maternal investment due to the positive relationship between offspring size and offspring fitness (Marshall and Keough, 2005). Larvae with higher biomass show higher survival and shorter duration of development (Bertram and Strathmann, 1998; Meidel et al. 1999; Giménez, 2002; Giménez and

Anger, 2003). To evaluate parental influences in larval quality, determination of initial larval biomass and elemental analysis of larvae upon hatching would be required; this issue will be addressed in Chapter 4.

2.4.2. Effects of temperature on food limitation

Thermal-dependent effects upon naupliar growth and development were present for both species. At higher temperatures, duration of development was shorter and cyprid sizes were smaller compared to respective food treatments at the lower temperature. A number of other studies upon barnacles have demonstrated similar results (West and Costlow, 1987; Hentschel and Emlet, 2000; Emlet and Sadro, 2006). Lower body size and increased development rates associated with increased temperatures are consistent with a phenotypically plastic response to temperature that occurs in a majority of ectotherms, known as the temperature-size-rule (TSR: Atkinson, 1994). The TSR refers to how, within a species, organisms develop faster at warmer temperatures, but will also mature at smaller body sizes (Zuo et al. 2012). This phenomenon can be explained by the rate of growth and the rate of development having different temperature dependence. For example, if development rates are more temperature sensitive than growth rates, then larvae will mature at a smaller size (Zuo et al. 2012). Food limited conditions are known to reduce cyprid size (West and Costlow, 1987; Emlet and Sadro, 2006). In this study, it appears that low food conditions increased the effect of the TSR as the smallest cyprids were produced under low food conditions at higher temperatures.

As previously discussed, at a low food level larvae of both species experienced an increase in development time. When the role of temperature was considered, there was an interactive effect of food and temperature for *S. balanoides*, at a higher temperature the effect of low food was proportionally greater than that observed at a lower temperature. Food concentration and temperature have previously been shown to jointly determine naupliar duration for *B. amphitrite* (Anil and Kurian, 1996; Anil et al. 2001). A thermal increase did not exert a similar effect upon *A.*

modestus; this suggested that *S. balanoides* may be less tolerant of low food and high temperature conditions.

Low food or a high temperature always reduced settlement success of *S. balanoides*. Environmental factors such as temperature can also have a significant effect on the amount of stored reserves in larvae as increased metabolic activities associated with thermal increases modify the allocation of energy (Anger et al. 1981b; Hodgson and Bourne, 1988; Fraser, 1989). For example, Thiyagarajan et al. (2003) demonstrated reduced larval attachment under increased temperature and reduced salinity conditions for *B. trigonus*; although larvae were able to rapidly develop successfully through to cyprid stage, less than 33% of cyprids were able to attach and complete metamorphosis compared to more successful rates at lower temperatures. Therefore, higher temperature may cause a reduction in cyprid energy reserves for *S. balanoides* resulting in increased rates of settlement failure, even under optimal food conditions. Whether this effect upon energy reserves occurs during energy storage (i.e. naupliar development) or is a direct effect of temperature upon cyprids prior to settlement is not clear.

Critically, the strong negative response of *S. balanoides* to low food becomes weaker at a lower temperature. Food and temperature may interact to determine settlement success for this species, but high variability among replicates (e.g. settlement ranged from 6-62% for low food at 15^{o}C) meant interactive effects were difficult to detect.

Differences in performance between the two study species may also arise due to contrasts in their physiological capabilities at different temperatures. All organisms live within a limited range of body temperatures, due to optimized structural and kinetic co-ordination of molecular, cellular and systemic processes (Portner and Farrell, 2008). Temperature extremes can cause functional constraints leading to a reduction in performance (Portner, 2001). Detrimental temperatures can cause insufficient oxygen supply which, coupled with high baseline oxygen demand at elevated temperatures, results in a reduction in performance (Monaco and Helmuth

2011; Portner, 2001). Temperatures of 15°C and above may not be suitable for *S. balanoides* larvae and such increased "costs of living" at a sub-optimal temperature may explain why higher temperatures increase the effect of low food for *S. balanoides.* Clearly, it would be beneficial to further study physiological mechanisms of *S. balanoides* in order to elucidate its thermal optimum and improve predictions of potential effects associated with climate change.

Previous work by Harms (1987) has demonstrated how temperature can exert an influence upon energy assimilation in *A. modestus*. When cyprids were produced under the same optimum food conditions at different temperatures Harms (1987) estimated that the energy content of freshly metamorphosed barnacle was 100mJ at 12°C, 150mJ at 18°C and 130mJ at 24°C. This meant there was up to 50% more energy in cyprids reared at 18°C. In addition, dry weight and elemental composition (C, H, N) were all at their highest values at 18°C and the lowest biomass loss by exuviae was also found at this temperature indicating that this species has better energy assimilation at 18°C (Harms, 1987). In addition, as the nauplii and cyprids of *A. modestus* are considerably smaller than those of *S. balanoides* at the equivalent larval stage (Knight-Jones and Waugh, 1949, Crisp, 1962), one might expect that nauplii of *A. modestus* have a lower food requirement to develop from hatching through to cyprid successfully. Therefore, it may be that *A. modestus* has lower energy requirements and/or increased energy assimilation levels than *S. balanoides*, particularly at higher temperatures, allowing larvae to tolerate lower phytoplankton levels.

2.4.3. Conclusions and perspectives

In conclusion, results of the present study were in agreement with the prediction that *S. balanoides* would be more sensitive than *A. modestus* to potential environmental changes associated with climate change (i.e. a reduction in the larval diet and an increase in temperature) and larval release of this species needs to be matched to an abundant food source, at optimum temperatures, in order to attain successful levels of settlement. Recent evidence has demonstrated that cues other

than phytoplankton can elicit larval release of *S. balanoides*. For example, turbid conditions associated with storm events can trigger synchronous larval release (Gyory and Pineda, 2011; 2012). Therefore, mismatches between larval releases of *S. balanoides* and their food sources may be more likely to occur than previously predicted; this may have important implications for the recruitment success of *S. balanoides* given that temperature increases, associated with climate change, are predicted to continue over the next 50 to 100 years (IPCC, 2007). In contrast, even under food limited conditions, moderate increases in temperature should not result in strong negative consequences for *A. modestus*. It is suggested that the observed disparities between the two species in this study may be related to differences in their physiological capabilities at different food concentrations and temperatures. These species may differ in their capacity to capture, ingest and convert food, or in the amount of reserves they have available for growth: these are critical factors in the survival and development of decapod crustaceans (Anger, 2001), and may also be important for barnacle larvae.

CHAPTER 3

Effects of temporal mismatches and temperature on the recruitment success of *Semibalanus balanoides* and *Austrominius modestus*

3.1. Introduction

Climate-driven phenological changes can result in differential shifts in the timing of events between predators and their target prey, causing a mistiming in offspring production relative to peaks of available food sources (Both and Visser, 2001; Parmesan and Yohe, 2003; Root et al. 2003) and consequent effects on survival rates for a range of species (Visser and Holleman 2001; Both and Visser, 2001; Phillipart et al. 2003; Durant et al. 2007). The mechanisms by which these phenological changes can govern recruitment success are proposed by the match/mismatch hypothesis (MMH) (Cushing, 1969; 1990).

The MMH states that recruitment variation in a population is caused by the relationship between the phenology of a predator/consumer and that of its prey/food species at the immediate lower level (Durant et al. 2007). A scenario where the peak abundance of a species coincides with the peak of its food source is described as a "match" and results in high survival and recruitment (Cushing, 1990). A "mismatch" situation occurs when there is a disruption in the temporal synchrony between peak abundances of a predator species and its food source. For example, offspring production may occur prior to or after the peak of food, resulting in varying degrees of food limitation for the predator species during different points of their development. Under such scenarios, the MMH predicts increased mortality and low recruitment (see Cushing, 1990; Both and Visser, 2001; Visser and Holleman, 2001; Beaugrand et al. 2003; Winder and Schindler, 2004; Durant et al. 2007; Moller et al. 2008).

In recent years, research has assessed the relationship between the temporal positioning of a mismatch and larval recruitment success of marine invertebrates (review in Cushing, 1990; Fortier and Gagne, 1990; Gotceitas et al. 1996; Philippart et al. 2003; Phillips, 2004; Bos et al. 2006). The timing of a mismatch may be important as, although early and late spawned/released larvae may exhibit a similar magnitude of temporal overlap with prey populations, the pattern of overlap differs in terms of food availability i.e. early-spawned larvae will experience low then high

food conditions compared to high then low food for later-spawned larvae. This temporal variability in the supply of food may influence recruitment success of larvae that are highly dependent on exogenous food sources (Lang and Marcy, 1982; Anger, 1987; Cushing, 1990; Starr et al. 1990). For example, larvae released prior to a peak of food abundance may be vulnerable to starvation due to a lack of available food upon hatching/release for first-feeding (Cushing, 1990; Durant et al. 2007).

Studies of fish larvae have demonstrated that patterns of mismatch are particularly important for recruitment success. Fortier and Gagne (1990) showed that spring-spawned herring larvae (*Clupea harengus*) hatching several weeks prior to the peak in plankton production had an improved survival rate when compared to larvae spawned later in the season. In a laboratory study, Gotceitas et al. (1996) examined the effect of temporal food availability upon larval survival and growth rates by exposing newly hatched Atlantic cod larvae to different mismatch scenarios (illustrated in Fig 3.1). Although their results showed that cod larval survival was reduced under all mismatch conditions, survival was improved when larvae experienced a low to high food environment when compared to a high to low food condition (Fig 3.1). Therefore, a mismatch in timing between a peak in fish larval abundance and that of their prey may not necessarily result in poor larval growth and survival. Instead, it appears that temporal positioning of a mismatch may be very important and larvae which advance spawning time (within limits) prior to the peak in prey availability can still attain optimal growth and high survival (Fortier & Gagne, 1990; Gotceitas et al. 1996).

The outcome of different temporal mismatches is not necessarily always predictable, as demonstrated by examination of larval development of the bivalve *Mytilus galloprovincialis.* Larvae reared under low then high food conditions were shown to have significantly increased levels of stored lipids and greater growth compared to those reared under high then low food conditions (Phillips, 2004), as expected from earlier studies on fish. However, when impacts of the larval food diet upon subsequent juvenile performance were examined, those which had experienced high then low larval food outperformed juveniles from the low then high larval food level

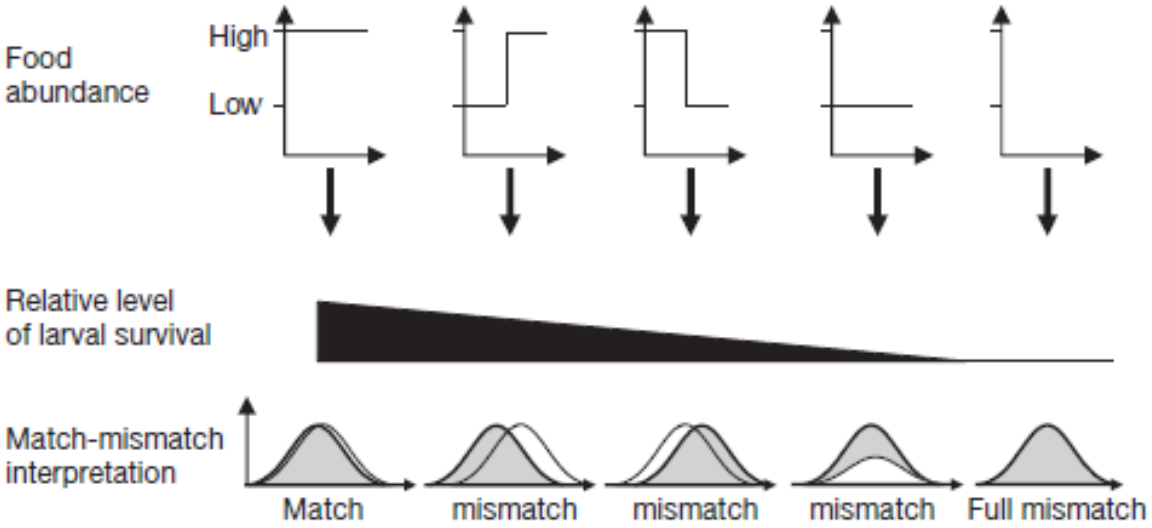

Fig. 3.1 Experimental example of laboratory induced match/mismatch conditions where different feeding conditions produced different Atlantic cod survival rates (Gotceitas et al. 1996)

in terms of growth and, more dramatically, the proportion of surviving individuals (Phillips, 2004). Thus, exposure to high food availability early in larval life was more beneficial for these bivalve juveniles than high food availability later in larval life. This outcome was surprising given the increased levels of lipids and greater growth observed for larvae reared under low then high food (Phillips, 2004). Therefore, it appears the timing of pulses of larval food may be crucial and can impact population dynamics at different life-stages for different species.

As presented in Chapter 2 of this thesis, the barnacle *Semibalanus balanoides,* which is believed to synchronise the timing of its larval release with the spring phytoplankton bloom (Barnes, 1956; Starr et al. 1991) experienced a stronger reduction in larval performance and, most importantly, post-settlement success than the exotic barnacle *Austrominius modestus* under the same conditions of food limitation. This suggested that settlement success depended on species-specific tolerance to food limitation. Although average food limitation influenced both larval survival and settlement for *S. balanoides,* the question remains whether the temporal positioning of a mismatch during larval development is important for larval survival and subsequent settlement for both species. Research upon various barnacle species has clearly demonstrated that food conditions experienced during larval development can determine the physiological condition of cyprids; since cypris larvae do not feed, their capacity to locate a suitable substrate, settle and metamorphose successfully is profoundly dependent on energy reserves accumulated through the preceding naupliar stages (West and Costlow, 1987; Jarrett and Pechenik, 1997; Hentschel and Emlet, 2000; Emlet and Sadro, 2006; Tremblay et al. 2007). Emlet and Sadro (2006) demonstrated how the effects of naupliar feeding history can persist through to the benthic life-stage and influence juvenile performance. In their experiments higher quality cyprids grew faster as juveniles and could survive better in the field than juveniles from lower quality cyprids that had their food ration reduced in the last feeding instar.

It is well documented that temperature strongly affects the rate of reserve utilisation (Anger et al. 1981b; Olson, 1985; Pechenik, 1990) with higher temperatures

increasing metabolism and energetic costs of living for ectothermic organisms, such as barnacle larvae (Desai and Anil, 2000); thus, when food is limited, responses to increasing temperatures are likely to lead to a decrease in fitness. Evidence in Chapter 2 showed that temperatures of 15^{o}C and above may diminish post-settlement success rates of *S. balanoides,* particularly under food-limited conditions. Although food availability and temperature are both known to regulate larval development and survival in marine invertebrates and have been well studied (reviewed by Pechenik, 1987; Olson and Olson 1989), relatively little is understood about the combined effects of temporal food variability and temperature on larval survival and settlement of barnacles (Emlet and Sadro, 2006). When the roles of food concentration, temperature and salinity on larval development of *B. amphitrite* were examined, results suggested that food availability and temperature jointly influenced energy allocation (Anil and Kurian, 1996; Anil et al. 2001). Given that different species exhibit different thermal tolerances and optima based on their natural environment (Portner, 2001; Monaco and Helmuth, 2011), the magnitude of species responses to increases in temperature would be expected to vary, particularly under food limited conditions. In addition, climate change scenarios predict an ongoing global warming with a further increase in sea water temperature of 2-3^{o}C over the 21st century (IPCC, 2007). Therefore, it is likely that larvae will be exposed to increasing sea temperatures in the future.

The aim of the present study was to investigate the effect of temporal mismatches upon larval performance of *S. balanoides* and *A. modestus* and evaluate whether elevated temperature can exacerbate this effect using manipulative laboratory experiments. To evaluate this, larvae were reared under varying food levels at different rearing temperatures and the rate of development, survival of larvae, cyprid size and subsequent metamorphosis rates of both species were observed. It is proposed that temporal shifts in larval food abundance will be critical in determining cyprid survival and the ability of these cyprids to settle and metamorphose successfully for both species. Barnacle larvae are particularly sensitive to food limitation during the last naupliar stage of their development (West and Costlow, 1987; Hentschel and Emlet, 2000). Therefore, it is predicted that larvae will

experience reduced settlement success under food conditions where larvae experience high then low food than under a scenario where they experience low then high food. Given the apparent general sensitivity of *S. balanoides* to food limitation it is predicted that any shifts in performance under mismatch conditions will be greater for this species compared to *A. modestus*. In addition, previous evidence has suggested that settlement success of *S. balanoides* is diminished at a temperature of 15^{o}C, thus, it is hypothesized that any decrements in performance under mismatch conditions will be more pronounced at a higher temperature for this species, but not for *A. modestus*.

3.2. Materials and Methods

3.2.1. Determining food concentrations.

Prior to the main experiment, a series of small scale experiments were conducted to deduce an appropriate range of food levels for experimental use, from optimal food concentrations which maximised survival to the cyprid stage to lower levels at which survival to cyprids was rare but which was high enough to support survival part way through larval development (larvae could survive for at least 8 days and develop to stage IV). This latter criterion was important in planning feeding experiments which varied food concentrations through larval development, for example a scenario where larvae switched from low to high food concentrations part way through development.

Previous results from a feeding trial for Chapter 4 indicated that a food concentration of 1 x 10^5 cells ml^{-1} resulted in high levels of survival to the cyprid stage and short development times in both species. Using this as the optimal (high) food concentration a feeding trial was conducted with a number of lower food levels: 4 x 10^4, 1 x 10^4, 4 x 10^3, 4 x 10^2 and 4 x 10 cells ml^{-1}. Larvae were collected and pooled from a number of adults and then reared as described in section 2.2.1. Five replicate x 100ml vessels containing 80ml of culture medium were used per food treatment with 10 larvae per vessel.

Results from this trial indicated that larvae were unable to survive to the cyprid stage at food levels of 1 x 10^4 cells ml^{-1} or below (Table 3.1) but at this food concentration they were able to survive for longer than 10 days and develop to the later stages of larval development (e.g. minimum stage IV) without mass mortality. Therefore, this concentration fulfilled criteria for use as a low food level.

3.2.2. Experimental design

To evaluate impacts of temporal mismatches and temperature upon performance of larvae, experiments were designed to rear larvae of *S. balanoides* and *A. modestus* under different food treatments at two different temperatures (Fig 3.2). Selected larval food treatments were: (1) low food throughout development (LF); (2) initial low food switched to high food at a pre-selected point (LF-HF); (3) initial high food switched to low food at a pre-selected point; (4) high food throughout development (HF). Food treatments where the food was switched were designed to mimic temporal mismatch scenarios where larvae are released prior to (treatment 2) or after (treatment 3) the peak of the phytoplankton bloom. Food treatment 1 represents a total mismatch scenario and treatment 4 is a match scenario. For each of the four experimental food treatments larvae were reared at two temperatures using the larval culturing procedure from section 2.2.1 until they reached the cyprid stage or had died. Larvae of *S. balanoides* were reared at 9°C and 15°C; *A. modestus* were reared at 15°C and 18°C, as illustrated in Fig 3.2. For each species there were 4 adults, 4 food conditions and 2 temperatures included within the design of the experiment, giving a total of 32 experimental treatments per species. Each of these treatments comprised of 5 replicates with x 10 larvae in each replicate giving n = 160 i.e. a total of 1600 reared larvae for each species.

Experiments were carried out in February 2012 for *S. balanoides* and in October 2012 for *A. modestus*. For each experiment, adults were collected from one location on the Menai Strait (53°13'16N, 04°09'47W) and left to hatch as described in section 2.2.1. For each experiment, 4 adults with ripe embryos were selected and released larvae from each adult used across the six food/temperature treatments.

Table 3.1 Survival rates of *S. balanoides* and *A. modestus* larvae reared in food trial under different low food concentrations of *S. costatum* (cells ml $^{-1}$)

	Survival rates					Average age when mortality occurred				
	4 x 10^4	1 x 10^4	4 x 10^3	4 x 10^2	4 x 10	4 x 10^4	1 x 10^4	4 x 10^3	4 x 10^2	4 x 10
S. balanoides										
9°C	90	0	0	0	0	10.0	13.4	13.6	12.4	12.3
15°C	86	0	0	0	0	9.33	9.0	9.1	8.2	8.1
A. modestus										
15°C	96	0	0	0	0	8.0	15.6	16.3	17.6	12.1
18°C	98	0	0	0	0	6.0	15.4	15.3	14.5	12.0

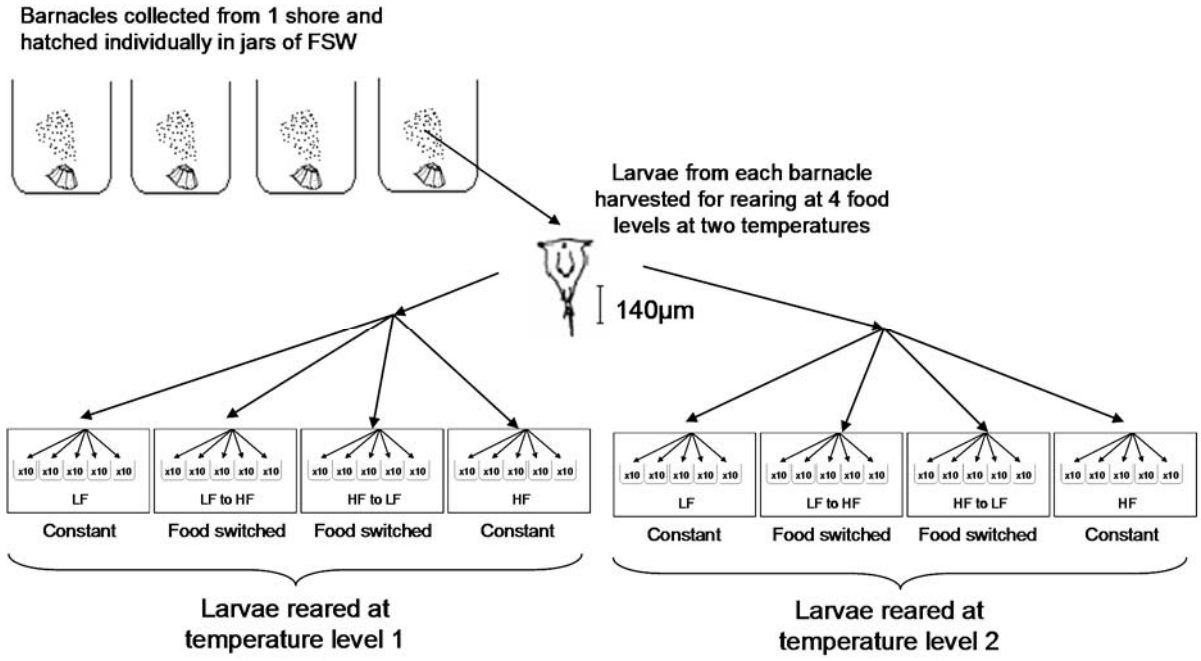

Fig. 3.2 *Semibalanus balanoides* and *Austrominius modestus*: Procedure for collection of larvae and experiment design. Larvae of each species were reared in a separate experiment.

Additional larvae were also harvested, preserved in ethanol and body length was later measured in order to provide an assessment of how larval development was related to initial larval and adult size. Larval performance within experiments was measured as duration of larval development to the cyprid stage, percentage survival to the cyprid stage, cyprid size and subsequent post-settlement survival.

In implementing the experiment a critical issue was the timing of the switch from HF to LF, and vice versa. This was chosen to represent a point which was approximately half-way through normal development at a HF concentration. Previous experiments have shown that development time to cyprid under HF for *S. balanoides* takes, on average, 16 days at 9^{o}C and 12 days at 15^{o}C. Therefore, in treatments where food needed to be switched from low to high or from high to low, this took place on day 8 at 9^{o}C and at 15^{o}C this switch occurred on day 6. Likewise, as larval development takes 11 days at 15^{o}C and 8 days at 18^{o}C for *A. modestus*, food was switched on day 6 at 15^{o}C and day 4 at 18^{o}C.

Once larvae had developed to the cyprid stage, cyprids from each adult reared under each food treatment and temperature condition were pooled together in one jar, giving four replicates per experimental treatment. Cyprids were kept in the incubator in FSW at the same temperature they were initially reared under. Ten cyprids from each adult at each experimental treatment were selected at random and photographed using a microscope camera GX-CAM 5. Photographs were subsequently analysed using Image J to calculate cyprid length, width and 2D cyprid area. Photographed cyprids were then returned to their cultures and overall settlement rates were observed for each experimental treatment. For this, a scraped clean rock was introduced as a settlement surface for *S. balanoides* cyprids, at the same time as the first cyprids, and subsequent settlement rates were observed; for *A. modestus*, no settlement surface was introduced and cyprids were left to settle upon the surface of the jar. On day 2, 4, 6 and 8 (after cyprids had been collected) the cyprid cultures were examined and the water was changed. During examination, numbers of metamorphosed cyprids upon the rocks were counted and any dead cyprids in the jar were removed.

3.2.3. Statistical analyses

The effects of the food treatments and temperature upon larval performance were analysed using GMAV5 (Underwood, 2002) The response variables were duration of development from hatching to the cyprid stage per replicate, percentage survival to cyprid per replicate, percentage settlement and mean cyprid size. These variables were analysed with orthogonal mixed model ANOVAs, with parent as a random factor (4 levels), and temperature (2 levels) and food (4 levels) as fixed factors. For some response variables there was a lack of replicates in some experimental treatments (due to poor larval survival) and therefore some treatments could not be included in some of the analyses. Cochran's test was used to test for homogeneity of variance and Student Newman Keul's (SNK) was used for *post-hoc* multiple comparisons. Where data transformation did not remove heterogeneity, non-transformed data were analysed using the ANOVA described, owing to a lack of alternative approaches. Thus, for data with heterogeneous variances, the critical value was adjusted to a more conservative level of $p < 0.01$. Here significant results should be interpreted with caution owing to an increased probability of type I error (Underwood 1997).

3.3. Results

3.3.1. Survival to cyprid stage

When both species were subjected to constant food conditions they exhibited a similar response; survival was highest under high food (>80%) and lowest at low food (<4% or 0% for *A. modestus* and *S. balanoides* respectively) (Fig 3.3A,B). Larval survival to cyprid for both species varied significantly among parents and depended upon larval food and temperature experience (significant interaction of parent x food x temperature: Table 3.2; Fig 3.3C,D). Patterns of survival under simulated mismatch conditions were considerably different for the two species. For *A. modestus,* survival rates under low-high food remained high (>80%) and were not significantly different to survival rates at constant high food (Table 3.2; Fig 3.3B). When the same comparisons to constant high food were made for *S. balanoides,* survival was significantly reduced at low-high food; under this treatment at 15^{o}C larval survival was particularly low at 30%. In contrast, at 9^{o}C survival was reduced, but only to

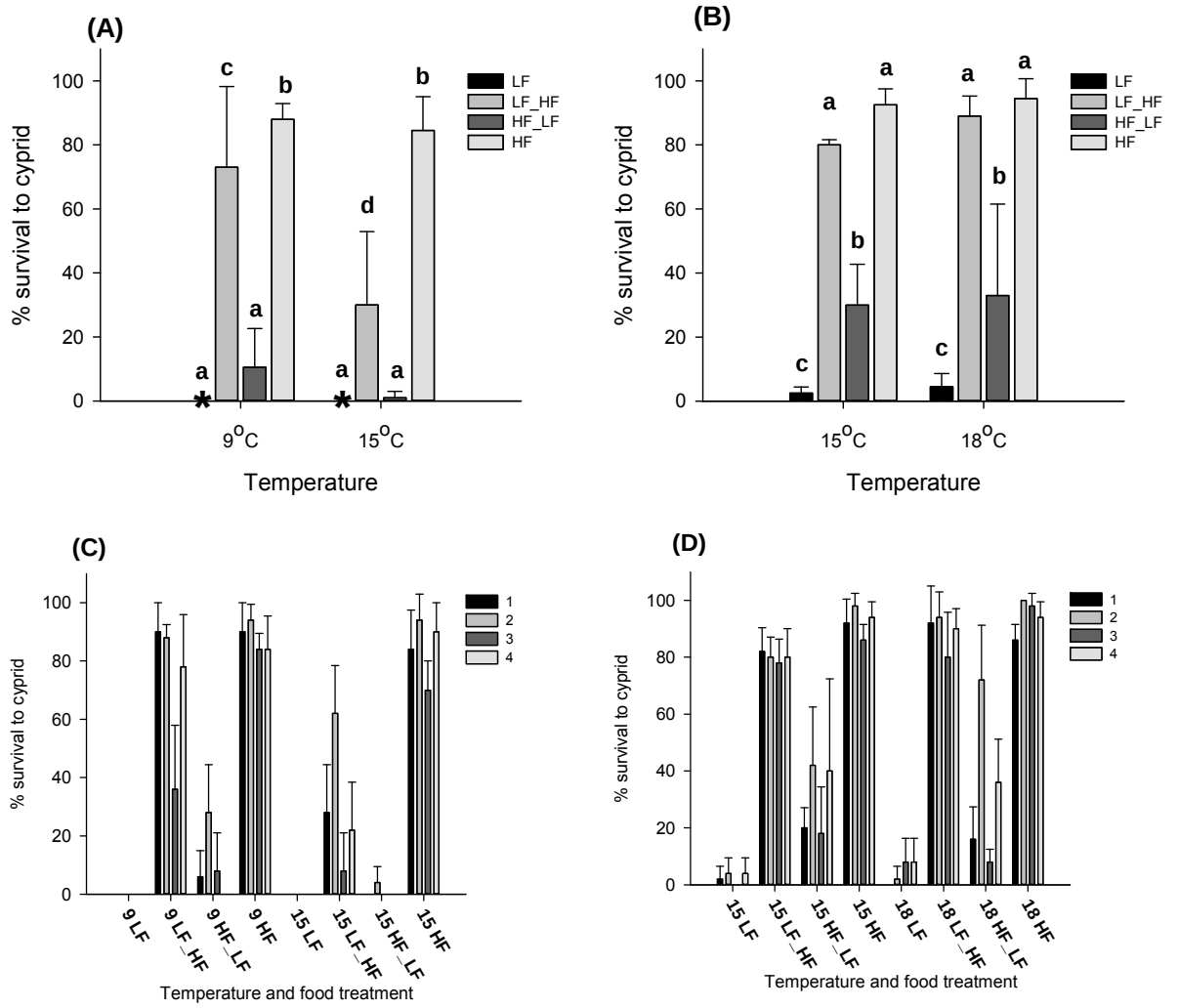

Fig. 3.3 Effects of food treatments and temperature for *S. balanoides* and *A. modestus* upon the average percentage survival to the cyprid stage for (A) *S. balanoides* and (B) *A. modestus*; and average percentage survival to the cyprid stage for each parent (C) *S. balanoides* and (D) *A. modestus*. Error bars: (A) and (B) based on replicate parents; (C) and (D) SD. * denotes no survival for treatment;different lower case letters indicate significant differences ($p < 0.01$) among food and temperature treatments

Table 3.2 Mixed effects ANOVA on percentage survival to the cyprid stage for larvae reared under different food and temperature treatments. Parent was considered a random factor. Food and temperature were fixed factors

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	6389.57	3	2129.86	23.75	≤0.0001
Temperature (T)	4902.90	1	4902.90	54.32	0.0052
Food (F)	135575.06	3	45191.69	75.66	≤0.0001
P x T	270.77	3	90.25	1.01	0.3922
P x F	5375.88	9	597.32	6.66	≤0.0001
T x F	6330.79	3	2110.26	9.51	0.0038
P x T x F	1997.40	9	221.93	2.48	**0.0123**
A .modestus					
Parent (P)	4322.83	3	1440.94	13.53	≤0.0001
Temperature (T)	894.85	1	894.85	4.73	0.1180
Food (F)	140654.78	3	46884.92	100.51	≤0.0001
P x T	568.02	3	189.34	1.78	0.1548
P x F	4198.22	9	466.46	4.38	0.0001
T x F	596.955	3	198.98	0.75	0.5501
P x T x F	2393.06	9	265.89	2.5	**0.0116**

73% (Fig 3.3A,B). When food was switched from high to low, survival was considerably reduced for both species (Fig 3.3A,B); at both temperatures, survival for *S. balanoides* was poor (≤10%) and was not significantly different to the zero survival at constant low food (Fig 3.3A), but a moderate percentage of *A. modestus* (30%) larvae developed to the cyprid stage.

SNK analysis suggested that most variability among parents manifested when *S. balanoides* larvae were reared under mismatch conditions (Fig 3.3A,C). In general, an increase in temperature seemed to increase variability in responses among parents, and under mismatched food treatments, detrimental responses to temperature were greater for some parents than for others (Fig 3.3C). When survival rates at low-high food were compared to those at constant high food (HF), only one of the adults exhibited a reduction in survival at 9^{o}C. In contrast, when the same food treatments at 15^{o}C were compared, most adults had a significantly reduced survival response.

The effects of mismatch conditions and temperature did not impact larval survival of *A. modestus* as strongly as it did *S. balanoides* (Fig 3.3A,B). SNK analyses highlighted that the main source of variation among the parents was when larvae experienced high to low food (HF-LF) at 18^{o}C, an effect that did not occur at 15^{o}C. Some parents also exhibited a significant increase in survival when temperature was increased under some of the food treatments (Fig 3.3D).

3.3.2. Final settlement success and cyprid settlement success.

For both species, highest final-settlement success (defined as the % of initial nauplii successfully settling and completing the transition to metamorphosed juvenile) occurred when larvae were reared at constant high food (>65%) (Fig 3.4A,B). Although both mismatch food conditions reduced final-settlement success of both species, these impacts were greater for *S. balanoides* than for *A. modestus* (Fig 3.4A,B). When compared to final-settlement at high food conditions, observations indicated that *S. balanoides* had reduced settlement survival under both mismatch food treatments (low-high, high-low food), with

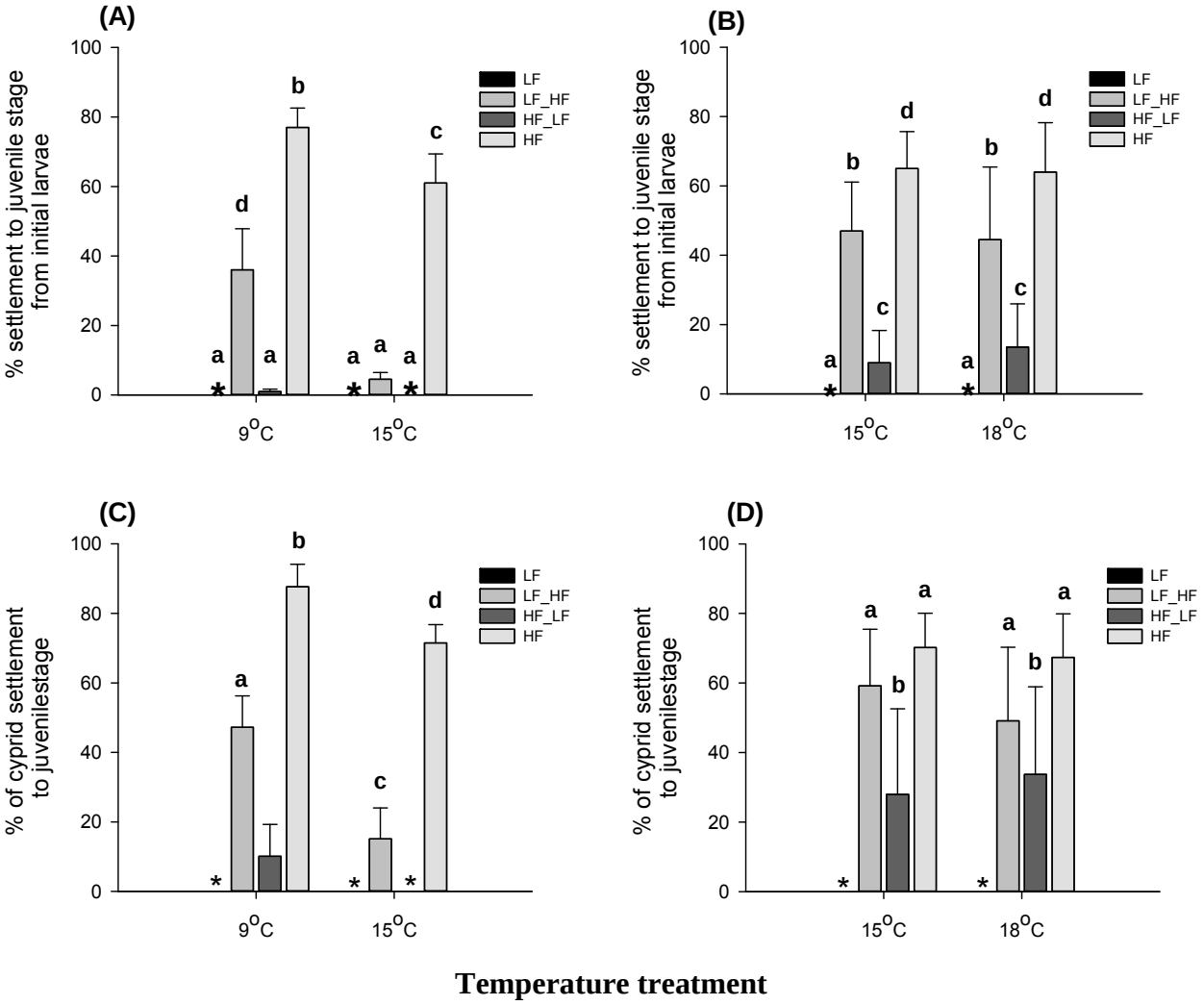

Fig. 3.4 Effects of food treatments and temperature for *S. balanoides* and *A. modestus* upon average post-settlement survival rates from initial larvae for (A) *S. balanoides* and (B) *A. modestus*; and average post-settlement survival rates from cyprids for (C) *S. balanoides* and (D) *A. modestus*. Error bars: (A-D) SD. (*) denotes zero survival; different lower case letters indicate significant differences ($p<0.003$) among food and temperature treatments; treaatments without lower case letters indicates that treatments were excluded from statistical analysis.

Table 3.3 ANOVA on settlement rates (as a proportion of initial larvae) for larvae reared under different food and temperature treatments. Food and temperature were fixed factors. (*) denotes that for *S. balanoides* significance level was judged at p < 0.01 as variances were heterogeneous (Cochran's *C*-test, p < 0.05)

Factor	SS	DF	MS	F	P
S. balanoides					
Temperature (T)	1176.12	1	1176.12	12.84	0.0015
Food (F)	25260.38	3	8420.12	91.9	≤0.0001
T x F	1322.37	3	440.79	4.81	0.0092(*)
A. modestus					
Temperature (T)	0.5	1	0.5	0	0.9543
Food (F)	21514.5	3	7171.5	48	0.0001
T x F	54.5	3	18.1667	0.12	0.9465

Table 3.4 ANOVA on settlement rates for cyprids reared under different food and temperature treatments. Food and temperature were fixed factors. For *S. balanoides* constant low food and high-low food were excluded from the analysis. For *A. modestus* constant low food only was excluded.

Factor	SS	DF	MS	F	P
S. balanoides					
Temperature (T)	2333.96	1	2333.96	13.57	0.003
Food (F)	9369.07	1	9369.07	54.47	0.000
T x F	255.23	1	255.23	1.48	0.247
A. modestus					
Temperature (T)	34.02	1	34.02	0.09	0.765
Food (F)	5857.90	2	2928.95	7.95	0.003
T x F	248.28	2	124.14	0.34	0.718

performance being particularly poor at 15°C. In treatments where settlement did occur (constant high, low-high, and high-low food), an increased temperature reduced final-settlement survival rates by 16%, 31% and 1% respectively). Levels of final-settlement survival at high-low food and low-high food were more successful for *A. modestus* than *S. balanoides* with average survival across temperatures at 47% for low-high food and at 12% for high-low food. For *A. modestus* final-settlement rates depended upon food treatment but not temperature (Table 3.3); settlement survival was significantly different among all the food treatments, apart from between high-low food and constant low food where survival was very low or zero.

ANOVA analysis indicated that for *S. balanoides* final settlement was dependant on an interaction between food treatment and temperature (Table 3.3). SNK analysis identified that increased temperature significantly reduced final-settlement survival rates for constant high food and low-high food treatments. The magnitude of this effect appeared greatest at the low-high food treatment as, when survival rates for low-high food were compared to those for constant high food, at 9°C survival was reduced by 40% whereas at 15°C this was reduced by 56% (Fig 3.4A). Survival for low to high food was very poor at 15°C (<5%) and did not differ significantly to the 0% recruitment for high to low food and constant low food. In addition, at 9°C survival rates for low to high food were significantly higher than those for high to low and constant low food.

Cyprid settlement (% of cyprids completing transition to juvenile) for *S. balanoides* was significantly influenced by food and temperature, but these two factors did not interact (Table 3.4). Cyprid settlement was shown to be significantly reduced at low-high food compared to rates observed at constant high food for *S. balanoides* (Fig 3.4C), and, for both these food treatments, settlement was significantly reduced at 15°C when compared to settlement at 9°C. At low-high food only 50% and 15% of cyprids were able to settle at 9°C and 15°C respectively (Fig 3.4C). No cyprids were produced under constant low food and very low numbers at high-low food (at 15°C) meant lack of replicates for analysis so these treatments were excluded from the analysis.

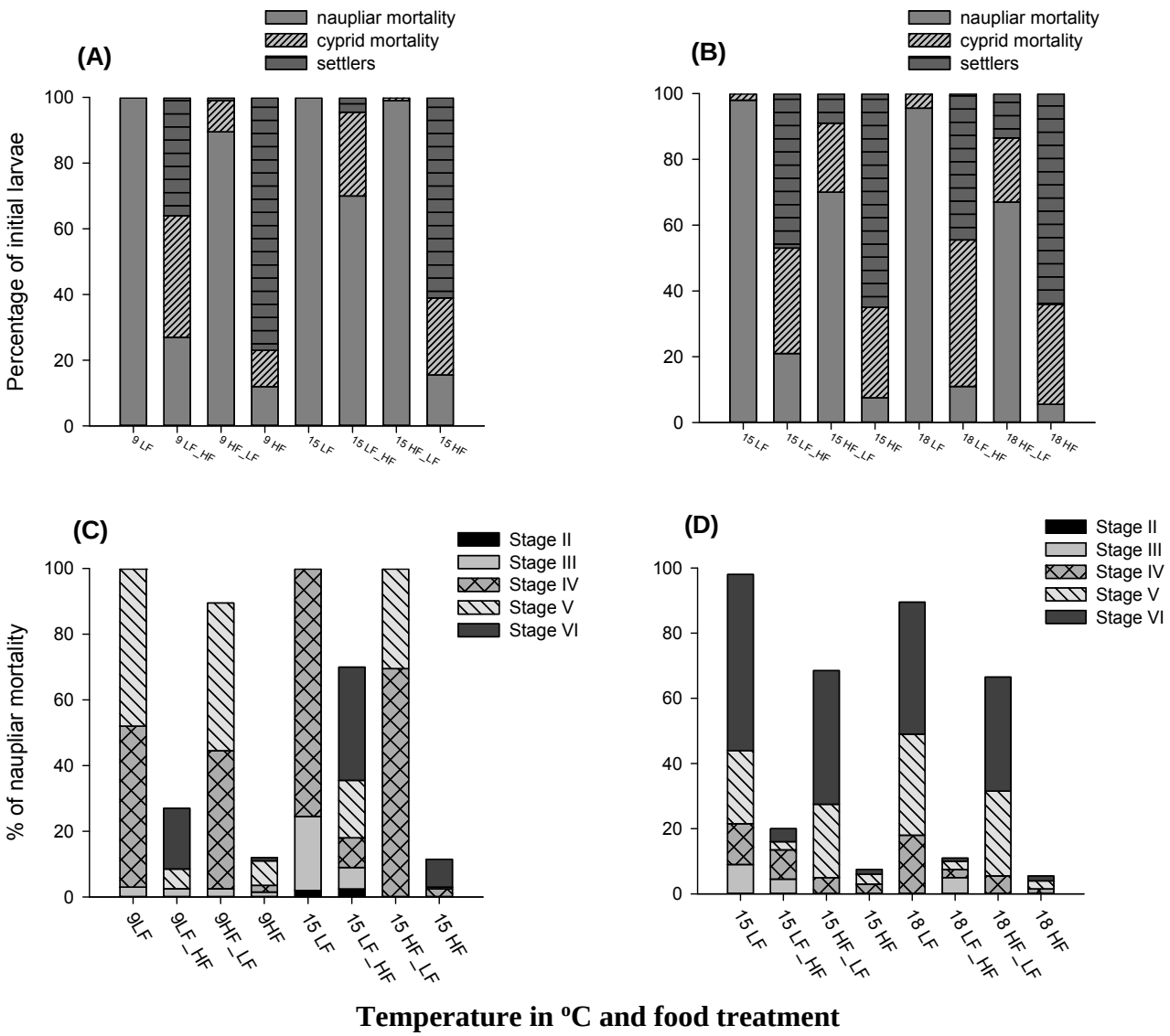

Fig. 3.5 Performance of larvae under different food treatments and temperature treatments for (A) *S. balanoides* and (B) *A. modestus;* and percentage of naupliar mortality for each stage for (C) *S. balanoides* and (D) *A. modestus*

Cyprid settlement for *A. modestus* was significantly influenced by food treatment but not by temperature (Table 3.4), settlement rates were similar at respective food treatments across both temperatures (Fig 3.4D). Although average cyprid settlement rates (across both temperatures) at low-high food (54%) were lower than those at constant high food (68%) (Fig 3.4D), these treatments were not significantly different from one another; compared to both of these treatments cyprid settlement at high-low food was significantly reduced (31%).

3.3.3. Larval mortality

Comparisons between larval and cyprid mortality (as proportions of initial larvae) indicated that patterns of mortality for *S. balanoides* under constant high food and low-high food were different at each temperature (Fig 3.5A,B). Naupliar mortality was the main cause of settlement failure for low-high food at 15^{o}C but not at 9^{o}C (Fig 3.5A). Cyprid mortality was high for the low-high food (both temperatures) and constant high food (15^{o}C only). This suggested that an increased temperature could exert an impact on cyprid mortality even under optimal food conditions. For *A. modestus* at constant high food and low to high food, cyprid mortality was greater than naupliar mortality (Fig 3.5B).

The main cause of settlement failure under constant low food and high-low food for both species was naupliar mortality prior to the cyprid stage (Fig 3.5A,B). For these treatments, percentages of overall mortality occurring during naupliar development were 70% or more for *A. modestus* and 88% or more for *S. balanoides*. This naupliar mortality appears to be related to experiencing a high then low food environment as, under low-high food and constant high food a proportion of larvae were able to develop to cyprid. Mass naupliar mortality under constant low and high-low occurred at different naupliar stages between species, suggesting that the food and temperature environments that nauplii experienced was particularly important in determining at which developmental stage mortality was most likely to occur (Fig 3.5C,D). For *A. modestus,* the majority of mortality occurred at stage VI at both temperatures. For *S. balanoides*, larvae appeared to die earlier on in their naupliar development as most larvae died at stage V, apart from low food at 15^{o}C, where most mortality occurred at stage IV.

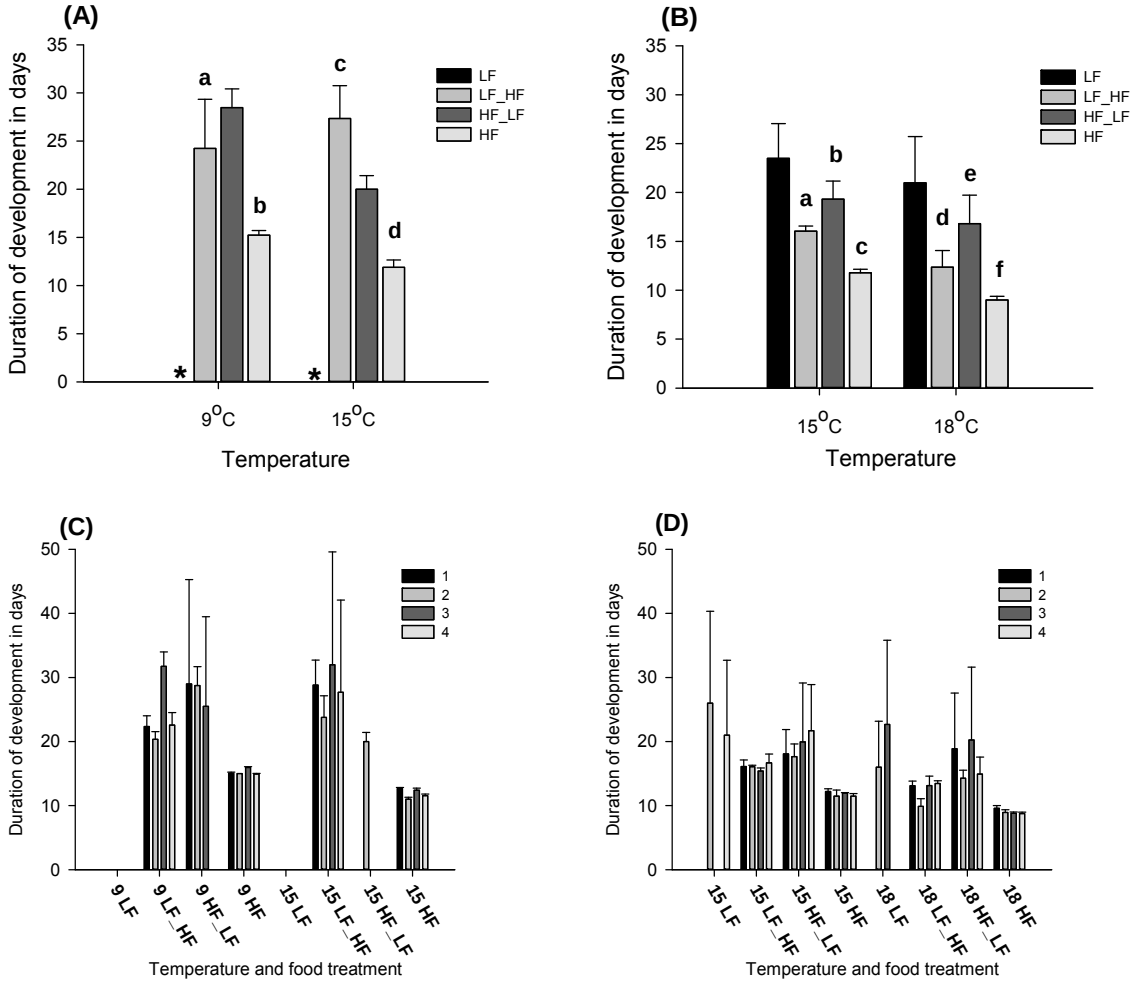

Fig. 3.6 Effects of food treatments and temperature for *S. balanoides* and *A. modestus* upon average duration of development to the cyprid stage for (A) *S. balanoides* and (B) *A. modestus*; and average duration of development to the cyprid stage for each parent (C) *S. balanoides* and (D) *A. modestus*. Error bars: (A) and (B) based on replicate parents; (C) and (D) SD. (*) denotes zero survival;different lower case letters indicates significant differences ($p < 0.05$) among food and temperature treatments; absence of lower case letter indicates that treatment was excluded from statistical analysis.

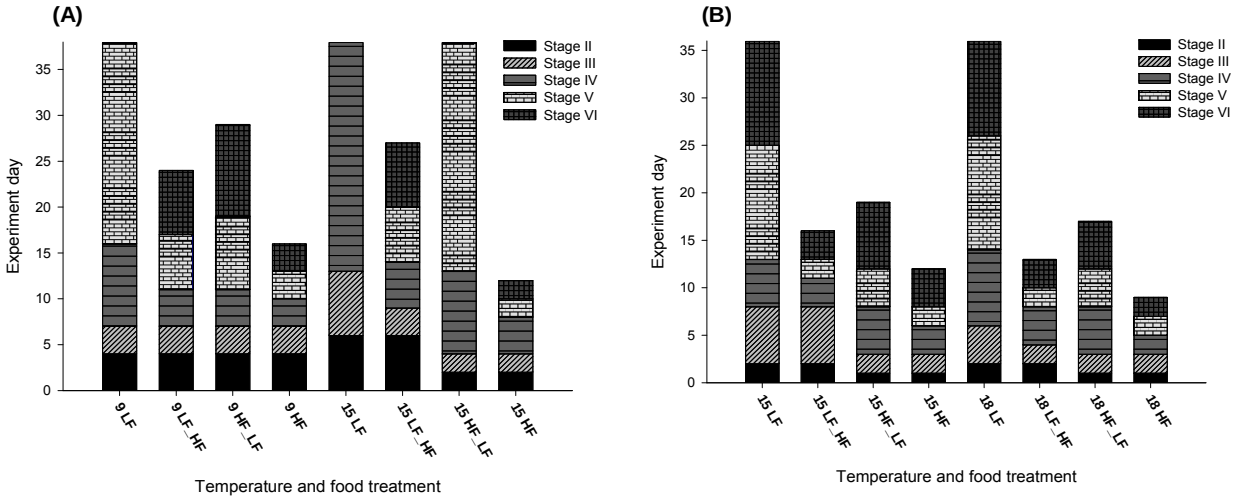

Fig. 3.7 Effects of food treatments and temperature for *S. balanoides* and *A. modestus* upon average duration of time spent at each naupliar stage for(A) *S. balanoides* and (B) *A. modestus*

3.3.4. Duration of development

Larvae from each species displayed similarities in their responses to experimental food treatments (Fig 3.6A,B). Development time was quickest when larvae experienced constant high food and, overall, larvae at low-high food developed quicker than those where food was switched from high to low (Fig 3.6A,B). For *A. modestus,* larvae at constant low food took the longest to develop and no individuals survived to the cyprid stage for *S. balanoides*. When larvae experienced a higher temperature their development time was shorter regardless of the food treatment, apart from *S. balanoides* larvae reared under low-high food (Fig 3.6A). Average responses of larvae from different parents can be seen in Fig 3.6 C,D.

ANOVA analysis conducted upon results from high food and low to high food alone (due to a lack of replicates in the other food treatments) highlighted that duration of development depended upon food and temperature (significant interaction for temperature x food; Table 3.5) but did not vary among parents. SNK analyses identified that temperature exerted a different effect upon these two food treatments. At an increased temperature and constant high food conditions, development time was shorter when compared to that at 9^{o}C. In contrast, at the low to high food condition, development time was longer at an increased temperature. Food limitation resulted in an increase in development times for nauplii when compared to groups reared under constant high food levels. For *S. balanoides* at low-high food development times were increased by 59% at 9^{o}C and 129% at 15^{o}C. In the high-low treatment development took 87% longer at 9^{o}C and 68% longer at 15^{o}C. In the latter treatment only 2 larvae survived to the cyprid stage and comparisons require caution.

Larval development times in *A. modestus* were dependent on food treatment and responses for temperature and parent were not significant at the $p < 0.01$ level (Table 3.5). SNK analysis revealed that development times among constant high food, low-high food and high-low food were all significantly different to one another (constant low food was removed from the analysis due to the lack of replicates). Development was always quicker when larvae of *A. modestus* experienced a higher temperature. However, the effects of food limitation on the duration of development tended to be stronger at higher temperature:

Table 3.5 Mixed effects ANOVA on duration of development to cyprid stage for larvae reared under different food and temperature treatments. Parent was considered a random factor. Food and temperature were fixed factors. Significance level was judged at $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$)

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	31.46	2	15.7	1.7	0.1969
Temperature (T)	6.16	1	6.2	2.62	0.2468
Food (F)	1439.08	1	1439.1	177.06	0.0056
P x T	4.70	2	2.3	0.25	0.777
P x F	16.26	2	8.1	0.88	0.424
T x F	191.36	1	191.4	1154.03	0.0009
P x T x F	0.33	2	0.2	0.02	0.9822
A .modestus					
Parent (P)	48.60	3	16.20	2.2	0.0951
Temperature (T)	214.77	1	214.77	12.57	0.0382
Food (F)	974.93	2	487.46	71.19	0.0001
P x T	51.24	3	17.08	2.32	0.0822
P x F	41.08	6	6.85	0.93	0.4781
T x F	5.95	2	2.97	0.23	0.8027
P x T x F	78.28	6	13.05	1.77	0.1165

Table 3.6. Mixed effects ANOVA on cyprid size for larvae reared under different food and temperature treatments. Parent was considered a random factor. Food and temperature were fixed factors

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	1.58	2	0.8	33.87	≤0.0001
Temperature (T)	27.62	1	27.6	1004.79	0.0010
Food (F)	1.33	1	1.3	33.12	0.0289
P x T	0.06	2	0.0	1.18	0.3109
P x F	0.08	2	0.0	1.73	0.1824
T x F	0.78	1	0.8	8.57	0.0996
P x T x F	0.18	2.0	0.1	3.93	0.0225
A .modestus					
Parent (P)	0.01	3	0.00	0.4	0.7557
Temperature (T)	0.21	1	0.21	24.09	0.0162
Food (F)	7.34	2	3.67	92.27	≤0.0001
P x T	0.03	3	0.01	1.52	0.2097
P x F	0.24	6	0.04	7.03	≤0.0001
T x F	0.01	2	0.00	0.15	0.8661
P x T x F	0.18	6	0.03	5.28	≤0.0001

development times at high-low food and constant low food were 64% and 100% longer at 15°C respectively, and 86% and 133% longer at 18°C than those at permanently high food densities. Development at low-high food treatments resulted in a similar increase (36-37%) in development time when compared to constant high food at both temperatures. Food conditions also affected the stage at which food conditions were switched. When food treatments were switched for *S. balanoides* (day 6 for 15°C and day 8 for 9°C) larvae experiencing initial high food (HF-LF) were at naupliar stage IV for both temperatures (Fig 3.7A); similarly, larvae which had experienced initial low food were at stage IV at 9°C. However, at 15°C they were still at stage II. At both temperatures, *A. modestus* larvae reared under initial low food had their food switched at stage III whereas under initial high food larvae were at stage V (Fig 3.7B).

3.3.5. Cyprid size

For both species, food and temperature conditions significantly influenced cyprid area (Table 3.6); the smallest cyprids were produced at a higher temperature and in the high-low food treatment (Fig 3.8A,B). Compared to sizes attained at 9°C, *S. balanoides* cyprids reared at 15°C displayed size reductions of 47%, 59% and 9% for constant high food, low-high and high-low food treatments respectively (Fig 3.8A). Compared to cyprids reared at constant high food, those at low to high treatment were 10% smaller at 9°C and 19% smaller at 15°C (Fig. 3.8A); for high to low food, these sizes were reduced by 69% at 9°C and by 25% at 15°C.

Parental effects on size varied among temperatures and food conditions (significant 3-way interaction: Table 3.6, constant high food and low to high food treatments only). SNK analysis indicated that variability among parents occurred when larvae were reared under low to high food conditions and/or the temperature was increased (Fig 3.8C). For all *S. balanoides* parents, cyprids reared under constant high food at 15°C were significantly larger than those at low to high food; however, at 9°C there was no significant difference in cyprid size between these food treatments for some parents (as seen in Fig 3.8C).

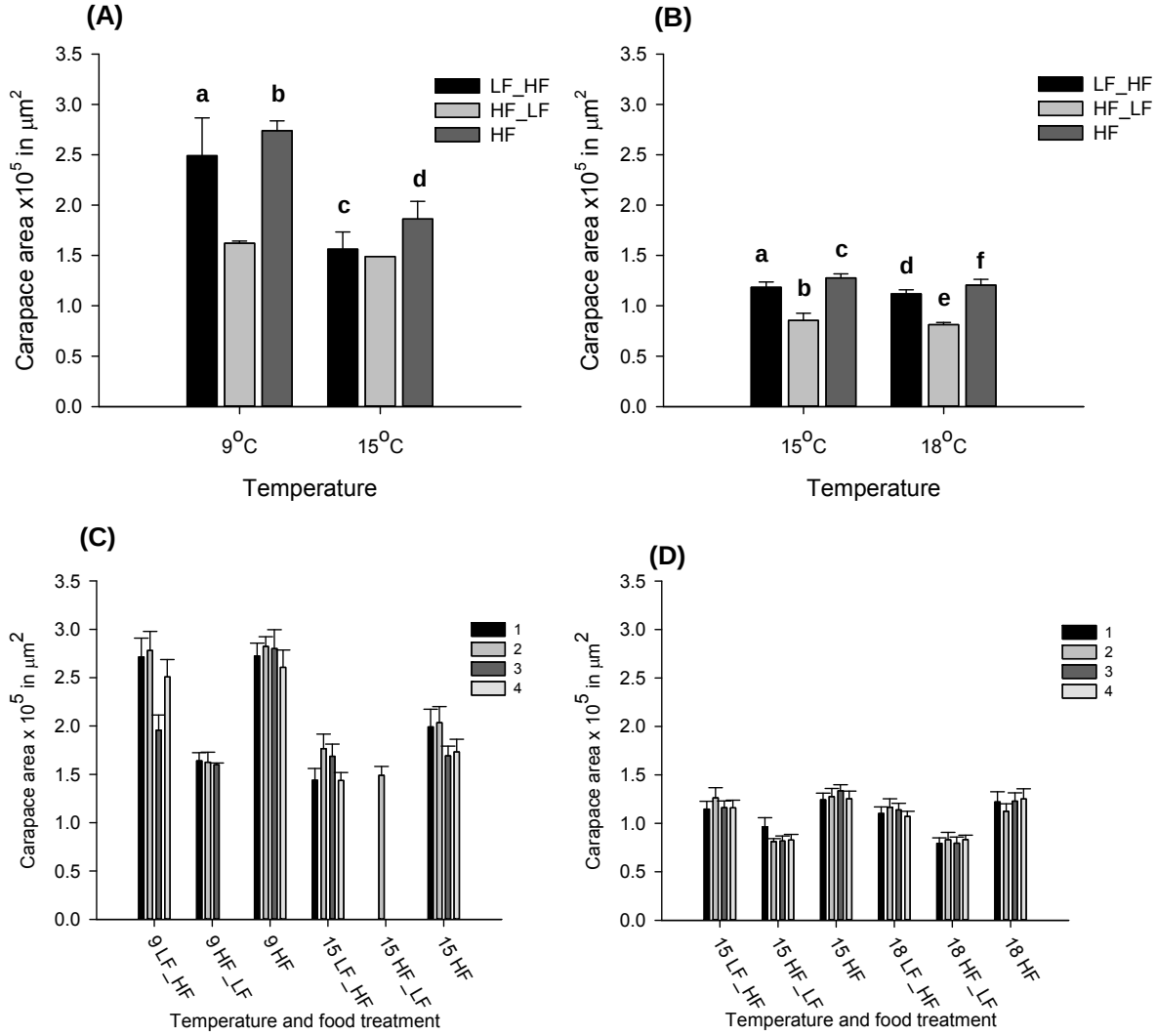

Fig. 3.8 Effects of food treatments and temperature for *S. balanoides* and *A. modestus* upon average cyprid size for (A) *S. balanoides* and (B) *A. modestus*; and upon average cyprid size each parent (C) *S. balanoides* and (D) *A. modestus*. Error bars: (A) and (B) based on replicate parents; (C) and (D) SD. Different lower case letter indicates significant differences ($p<0.05$) among food and temperature treatments;absence of lower case letter indicates treatment was omitted from statistical analysis.

Table 3.7 ANOVA analysis on larval lengths and widths at time of hatching among parents at stage I and stage II

species	larval stage	Larval size	factor	SS	DF	MS	F	P
S. balanoides	*stage I*	Length	parent	2279.79	3	759.93	41.33	0.0001
		Width	parent	1432.19	3	477.39	24.10	0.0001
	stage II	Length	parent	7064.49	3	2354.83	15.43	≤0.0001
		Width	parent	1950.07	3	650.02	5.94	0.0021
A.modestus	*stage I*	Length	parent	7064.49	3	2354.83	15.43	≤0.0001
		Width	parent	1950.07	3	650.02	5.94	0.0020
	stage II	Length	parent	3698.15	3	1232.71	10.90	≤0.0001
		Width	parent	571.047	3	190.34	3.89	0.0167

Table 3.8 Average larval size upon hatching by corresponding parent and respective response at the low food (LF) treatment at 15ºC

	Stage 1		Stage II			
Parent	length	width	length	width	% survival in treatment	Duration of development
S. balanoides					**15 LF-HF**	
1	263.29 (±4.41)	154.07 (±3.57)	419.39 (±10.04)	186.85 (±7.23)	28 (±16.4)	28.80 (±3.91)
2	266.85 (±5.45)	162.47 (±5.48)	428.11 (±11.05)	202.60 (±11.10)	62 (±16.4)	27.35 (±3.37)
3	246.88 (±2.60)	145.72 (±3.79)	400.51 (±13.57)	188.60 (±12.89)	8 (±13.0)	32.00 (±17.5)
4	257.87 (±4.20)	152.08 (±4.71)	436.28 (±14.26)	200.50 (±9.79)	22 (±16.4)	27.68 (±14.4)
A. modestus					**18 HF-LF**	
1	208.22 (±5.30)	103.47 (±3.70)	312.59 (±11.96)	140.55 (±7.98)	16 (±11.4)	18.9 (±8.7)
2	219.40 (±3.84)	109.77 (±2.46)	336.26 (±7.65)	145.07 (±5.67)	72 (±19.2)	14.3 (±1.3)
3	214.22 (±4.72)	107.46 (±4.79)	333.87 (±10.46)	150.59 (±6.79)	8 (±4.5)	20.3 (±11.4)
4	212.82(±5.75)	105.85 (±4.49)	321.68 (±11.70)	142.45 (±7.35)	36 (±15.2)	14.9 (±2.6)

ANOVA analysis upon constant high food, low-high food and high-low food for *A. modestus* indicated that cyprid size depended on an interaction between food, temperature and parent (Table 3.6). When compared to the constant high food treatment, cyprid size at low-high food and high-low food were reduced by a similar proportion at both temperatures; low-high cyprids were 8% smaller and high-low cyprids were 38% smaller. *A. modestus* cyprid size was reduced equally by 5% across all the food treatments when the temperature was increased. The magnitude of these effects varied among parents (Fig 3.8D.). For example, when temperature was increased, some of the parents experienced a decrease in cyprid size whereas others did not.

3.3.6. Relationships between larval performance and size at hatching

For each species, larvae measured at stage I from each parent exhibited significant differences among parents in their length and width (Table 3.7). This effect was also present among parents for larvae measured at stage II. SNK analyses highlighted that larvae from parent 3 were significantly smaller in length and width upon hatching than larvae harvested from other parents within the experiment for *S. balanoides* (average sizes shown in Table 3.8). As the low to high food treatment at 15^{o}C produced most variability for *S. balanoides*, rates of larval survival and duration of development for this treatment were compared to the larval sizes for each parent to examine any patterns. Within this treatment, larvae from parent 3 had the poorest performance which may be related to the smaller larval size upon hatching compared to other parents.

For *A. modestus,* stage I larvae were significantly smaller for parent 1 and significantly larger for parent 2 compared to the other parents. At stage II, larvae from parent 1 and 4 were significantly smaller than other parents. As a high to low treatment at 18^{o}C was believed to produce most variability for *A. modestus,* performance under this treatment was compared to these initial larval sizes. Although larvae from parent 1 did have a poor performance, it was shown that larvae of a larger size had experienced a poorer performance (Table 3.8).

3.4. Discussion

Results of the present study demonstrated that the timing of a mismatch was important in determining settlement success for both species. Responses of larvae to experimental conditions were consistent with the original hypothesis that reduced food availability during the later stages of larval development (high-low mismatch) would have greater negative impacts for both species. Importantly, as predicted, *A. modestus* exhibited a weaker negative response to both mismatch conditions when compared to *S. balanoides*. Temperature and mismatched food conditions were shown to have combined effects upon larval performance of *S. balanoides,* providing support for the hypothesis that impacts of mismatch conditions would be greater for this species at a higher temperature.

In the following sections, larval performance in response to temporal mismatches are discussed and the mechanisms driving the larval responses to these mismatch patterns are proposed. The combined impacts of elevated temperature and food limitation during different mismatch scenarios are considered and strong links are made between these factors and the weaker performance of *S. balanoides*. The greater impacts of the experimental treatments upon *S. balanoides* compared to *A. modestus* may be strongly linked to differences in larval physiology, as shown by differences in thermal tolerance; therefore, various mechanisms are proposed which may account for the observed differences between the species.

3.4.1. Consequences of temporal mismatches upon larval performance

In the present study, naupliar development, growth and survival of *S. balanoides* and *A. modestus* were influenced by temporal mismatches between larvae and their food source. Under low food levels, only limited amounts of energy would be available to drive larval growth and development, resulting in the observed longer developmental periods, smaller cyprid sizes and, at times, increased mortality. These results are consistent with previous studies (West and Costlow, 1987; Hentschel and Emlet, 2000; Emlet and Sadro, 2006). When performances for both mismatch scenarios were compared, naupliar responses (survival to cyprid, duration of development and cyprid size) of both species were consistent with outcomes from other similar studies (Van der Meeren and Naess; 1993, Gotceitas et al.

1996; Hentschel and Emlet, 2000; Phillips, 2004), where larvae subjected to a low food at early stages (low-high food) exhibited better larval performance than larvae exposed to low food at advanced stages (high-low). Nauplii could tolerate a low food level during the earlier naupliar stages (II and III) and continue to develop to the cyprid stage relatively successfully. In contrast, a low food level during the later stages of development considerably reduced larval survival. This was in agreement with other work that suggests barnacle nauplii are more sensitive to low food availability during the later developmental stages (West and Costlow, 1987; Anil and Kurian, 1996; Hentschel and Emlet, 2000).

Therefore, it seems that the degree of influence a mismatch had upon larval performance was related to the feeding environment that larvae experienced during the later stages (IV-VI). Food uptake generally increases through the naupliar developmental stages and varies with food concentration (Yule, 1982; Harms, 1987); as nauplii moult through each stage they become larger and a higher amount of food is required to drive development and growth and also to maintain increasing metabolic rates (Gillooly et al. 2001). It is likely that impacts of high-low mismatches were greater as, if larvae are food limited during their later stages of development (when energy demands are higher) they are more likely to experience a greater reduction of energy available for development and growth, resulting in smaller cyprids and longer development times (Hentschel and Emlet, 2000). The lengthening of naupliar period is viewed as an additional period necessary to accumulate enough energy to moult successfully to the next stage (Hentschel and Emlet, 2000; Emlet and Sadro, 2006).

The poor settlement rates (as a proportion of initial nauplii) associated with a high-low mismatch were, for the greater part, explained by the low proportion of nauplii surviving to cyprid. However, cyprid settlement rates (% of settlers from cyprid numbers not initial nauplii) from this treatment were also poor, indicating that a low food environment during the later stages, although allowing the cyprid stage to be reached, impaired the ability of cyprids to competently settle and metamorphose to the juvenile form. The amount of energy reserves a cyprid has varies with naupliar feeding history and depends on algal food quantity and quality (Thiyagarajan et al. 2002). More specifically, the feeding environment present during stage VI is thought to determine quality of cyprids as the majority of cyprid energy reserves are accumulated during this stage (West and Costlow, 1987; Hentschel and

Emlet, 2000). Therefore, it is likely that settlement failure of high-low cyprids is also linked to low food availability during the later stages of development. Cyprid metamorphosis to the benthic form is an energetically expensive process where cyprids may consume up to 30% of their own body organic carbon and, if the larvae do not have enough energy reserves, they lose their competence to metamorphose (Lucas et al. 1979; West and Costlow, 1987; Jarrett and Pechenik, 1997; Hentschel and Emlet 2000; Thiyagarajian et al. 2002, 2005; Emlet and Sadro, 2006; Tremblay et al. 2007). Presumably, under the high-low food mismatch in the present study, larvae would have been restricted in the amount of reserves they could accumulate during stage VI, which reduced the capacity of cyprids to be able to attach and metamorphose successfully (West and Costlow, 1987).

In addition, a low-high food mismatch seemed to impair cyprid settlement rates for *S. balanoides*. This was a surprising result given the relatively high survival rates to cyprid and that nauplii had experienced an abundant food source during stage VI allowing for optimal energy storage. It may be that food limitation during the early stages caused degradation of functional structures important for accumulating energy reserves during the later stages (Anger, 2001). It is also proposed that settlement failure may not be related to energy reserves and may be linked to the initial period of low food larvae experienced; Pechenik et al. (1998) suggest that some gene products needed for organogenesis or physiological function after metamorphosis may be transcribed early in development and stresses may interfere with the transcriptional or translational processes of these. If this hypothesis is correct, then even during early stages of development *S. balanoides* larvae may still be vulnerable to negative influences of poor larval nutrition.

3.4.2. Combined effects of mismatch and temperature on larval performance

Negative larval responses to mismatch conditions were stronger for *S. balanoides* at a higher temperature. Interestingly, a higher rearing temperature influenced cyprid size and duration of development of *A. modestus* but it did not exacerbate declines in larval survival and settlement. These findings provided support for the hypothesis that high temperatures can exacerbate the effects of a mismatch for *S. balanoides*.

As expected, for both species, larvae reared under elevated temperatures developed at a faster rate and matured at smaller size as observed in many other studies (Harms, 1984, Anil et al. 1995; Emlet and Sadro, 2006; O'Connor et al. 2007). Lower body size and increased development rates associated with increased temperatures may be explained by the rate of growth and the rate of development having different temperature dependence as explained by the temperature-size rule (TSR; Atkinson, 1994; Zuo et al. 2012). Although larvae of *S. balanoides* demonstrated improved performance under low-high mismatch compared to high-low mismatch at a low temperature, at a high temperature this was not so and growth, development and survival were poor under both mismatch conditions. At a higher temperature, it seemed that the pattern of mismatch did not matter for *S. balanoides* and settlement success was poor regardless of whether larvae experienced food limitation early or late in their development. This response provided further support for the hypothesis that higher temperatures can exacerbate the effects of a mismatch for this species. Previous studies upon *B. amphitrite* have shown that food availability and temperature can jointly determine energy assimilation and allocation during naupliar development and, thus, strongly influence development and growth of nauplii (Anil and Kurian, 1996; Anil et al. 2001). Elevated temperatures result in increased metabolism and energy demands (Anil and Kurian, 1996); therefore, during the low food period of a mismatch (where available energy is already depleted) even less energy would become available for growth and development if temperatures are high (Harms, 1987; Houde, 1987; Pechenik, 1987).

The greater impacts of elevated temperature upon low-high food for *S. balanoides* suggest that the low food levels were insufficient to accommodate increased energy demands even at the early stages of development. For example, when food treatments were switched at day 6 (15^{o}C) and day 8 (9^{o}C) *S. balanoides* larvae experiencing initial high food (high-low) were at stage IV for both temperatures; however, at the same point in time, larvae which had experienced initial low food (low-high)were at stage II at 15^{o}C and at stage IV for 9^{o}C. Given that larvae at the lower temperature under the same food conditions had moulted through to stage IV, it is evident that, at a higher temperature, low food conditions were not sufficient for larvae to moult through to stage III in the given time frame. This delay in moulting to the next naupliar stage may have occurred if energy is allocated first to survival,

then to moulting and finally to development, as is hypothesised for the development of shrimp larvae (Knowlton, 1974). These interpretations are also supported by the studies carried out on brachyuran larvae under starvation conditions (Anger and Dawris, 1981; Anger et al. 1981a).

Furthermore, it appeared that thermal-dependent food limitation (combined effects of low food and elevated temperature) during the early stages of larval development (i.e. low-high mismatch) caused a delayed impact upon naupliar mortality. Larval mortality was low during the low food period and it was expected that once the food treatment was switched to high food nauplii would successfully develop through to cyprid stage. However, mortality was unexpectedly high during stages IV and V despite high food availability. Similarly, *B. improvisus* nauplii displayed a delayed increase in mortality at stage IV when larvae were partially starved at stage II; Lang and Marcy, (1982) suggested that larvae failed to compensate for initial lack of food and exhausted a critical nutritional component which affected the condition of larvae during later stages. It is possible that *S. balanoides* larvae exhibit a similar stress response when reared under low-high food at a higher temperature. In addition, *B. amphitrite* exhibited increased larval mortality when exposed to food limited conditions at higher temperatures (Anil and Kurian, 1996).

Therefore, settlement patterns of *S. balanoides* (as a proportion of initial nauplii) were strongly determined by food treatment and temperature as the combined effects of both these factors considerably reduced naupliar survival and, subsequently, the number of cyprids available to settle. Assessment of *S. balanoides* cyprid settlement success (proportion of developed cyprids able to settle) revealed that elevated temperature reduced settlement; however, in this case, food and temperature did not interact. This effect was even present when cyprids had been reared under constant high food conditions. These observations were consistent with findings from chapter 2 where experiments showed that high temperature affected the capacity of *S. balanoides* cyprids to competently achieve settlement. This sensitivity of *S. balanoides* to elevated temperatures may be because temperature can affect the quality and quantity of cyprid energy stores; a higher temperature reduces the amount of reserves that the cyprids have due to increased metabolic activity (Fraser, 1989; Thiyagarajan et al. 2003). Whether the *S. balanoides* cyprid

settlement response is due to the effects of temperature during naupliar history i.e. a trait-mediated effect, or is a direct effect of temperature upon cyprids during the settlement process, cannot be deduced from this experiment and further research would be required to clarify this.

3.4.3. Differences between S. balanoides and A. modestus

The stronger response of *S. balanoides* larvae to low food periods associated with a mismatch may be because *S. balanoides* larvae have a higher food requirement than *A. modestus*, particularly at higher temperatures. As *S. balanoides* are considerably larger in size compared to *A. modestus* (Crisp, 1962; Knight-Jones and Waugh, 1949) it would not be unreasonable to expect that *S. balanoides* requires more energy to fuel growth and development. Results support this hypothesis as larval development and survival under constant low food conditions demonstrated that *S. balanoides* nauplii could not acquire the required energy demands to allow for growth and development beyond stage V at 9^{o}C and stage IV at 15^{o}C. In contrast, larvae of *A. modestus* were able to develop through to stage VI and a small percentage developed through to cyprid at both temperatures.

Notably, as the effects of a mismatch upon the general performance of *S. balanoides* were minimized at a lower temperature, it seems that temperatures of 15^{o}C and above are not an optimal temperature for larvae of this species. All organisms are specialised to live within the characteristic temperature window of their natural environment, so temperature extremes can cause functional constraints, and decrements in performance may occur (Pörtner, 2011). It has been suggested that the upper tolerance limit for larvae of *S. balanoides* is 20^{o}C (Barnes and Barnes, 1958). Larval rearing experiments by Harms (1984) were also in agreement with this limit as, despite using optimal food levels, naupliar mortality was 100% at 24^{o}C, and was shown to be higher at 18^{o}C than 12^{o}C. Therefore, higher temperatures appear sub-optimal for *S. balanoides*. In contrast, within the same study, Harms reported that *A. modestus* exhibited the lowest mortality at the highest temperature of 24^{o}C, implying that this species is suited to developing in warmer waters.

Cyprid energy reserves of both species will be influenced by feeding rates of the nauplius, which varies with body size, food concentration and temperature (Yule, 1982, Harms, 1987). In the present study, differences between the physiological efficiencies of the two species at different food concentrations and temperatures may account for their contrasting responses (Harms, 1987). Harms (1987) demonstrated how energy content of cyprids can vary under optimal food conditions depending on temperature, it was estimated that the energy content of cyprids was 98mJ at 12^{o}C, 142mJ at 18^{o}C and 83mJ at 24 ^{o}C. This meant there was up to 50% more energy in cyprids reared at 18^{o}C. Similarly, when the nutritional condition of *B. amphitrite* cyprids reared under high and low food treatments at 20^{o}C and 30^{o}C was assessed, overall it was found that those at 20^{o}C were in poorer condition than those at 30^{o}C; importantly, this was reflected in cyprid metamorphic capability where there was a shorter time frame in which poorer condition cyprids were capable of successfully metamorphosing (Anil et al. 2001). The studies of Harms (1987) and Anil et al. (2001) demonstrate that the assimilation of cyprid energy reserves seem strongly related to optimal thermal range of each species i.e. *B. amphitrite* is a tropical or sub-tropical barnacle which has an optimal rearing temperature of 25^{o}C (Anil and Kurian, 1996) and *A. modestus* is suited to temperatures of 18-24^{o}C (Harms, 1984; 1987; 1999). The implication here is that increased larval efficiency at higher temperatures for *A. modestus* may result in a greater tolerance to periods of food limitation at elevated temperatures when compared to *S. balanoides*. Further research on the precise relationship of cell concentration and temperature to the feeding efficiency of larvae of both species would be required to clarify this theory.

Larval survival and cyprid size of both species were shown to be subject to parental variability in response to food and temperature. For *S. balanoides* the greatest variability was present when larvae were subjected to a low-high mismatch or a high temperature. For *A. modestus* most variability occurred when larvae were reared at high-low food. Performance of *S. balanoides* larvae appeared to be linked to size of larvae upon hatching from the adult, with smaller larvae exhibiting a poorer performance than the larger larvae in terms of survival and duration of development. This response is important and provides additional support for findings from Chapter 2 which suggested that parental effects may be important in determining the density and quality of larvae that can settle upon the shore. A

number of other studies have shown that larvae with higher biomass show improved larval performance (Bertram and Strathmann, 1998; Meidel et al. 1999; Giménez, 2002; Giménez and Anger, 2003). This parental variability could reflect maternal effects of parental environment upon the larvae or genetic differences among parents (e.g. density, food, temperature, age, size: Bertram and Strathmann, 1998; Marshall et al. 2008). However, the source of this variability was not studied within the scope of this experiment.

3.4.4. Ecological consequences

Pre-settlement factors, such as food limitation during larval development, are known to have a strong influence on the abundance of larvae that can survive and settle into the benthic population (see reviews Pechenik, 1987; Giménez, 2004). In this study, dramatic differences in numbers of cyprids produced under different larval treatments and the subsequent capacity for these cyprids to successfully settle and metamorphose successfully emphasised the importance of larval history in determining recruitment success. Outcomes from experiments here predict that scenarios may lead to a decoupling of larval supply and settlement, as shown in the field by Jenkins et al. (2000). In the present study, periods of food limitation led to scenarios where despite a high supply of larvae (because of high survival to the cyprid stage) subsequent recruitment was low (due to the poor quality of the cyprids). Temporal patterns of food availability (associated with a mismatch) during the naupliar period seemed crucial in determining density and quality of settlers (West and Costlow, 1987; Thiyagarajian et al. 2002, 2005, 2007). As predicted, larvae which experienced high-low food environments (i.e. low food during the later stages of naupliar development) had decreased settlement success than larvae which experienced low-high food (i.e. initial low food during the earlier stages). Ultimately, it appeared that settlement success depended on the feeding environment present during the later stages (IV-VI) of larval development. Therefore, larvae released prior to a peak in food availability would experience greater settlement success than those released after the peak. Overall, experiments suggested that *S. balanoides* was more sensitive to mismatches than *A. modestus*; therefore, it is critical for *S. balanoides* to be matched to an abundant source of food to attain successful recruitment rates.

Importantly temperature exacerbated the detrimental impacts of a mismatch for *S. balanoides*, indicating that increases in temperature (≥15^{o}C) in conjunction with a variable food environment may have strong implications for the settlement success of *S. balanoides*. In contrast, even under mismatched food conditions, moderate increases in temperature did not result in strong negative consequences for *A. modestus*. It is evident that species-specific responses to food variability and temperature are complicated and can have strong implications for the settlement success of a species. It is likely that changes in size, age and lipid reserves of cyprids associated with these mismatch scenarios will have fitness implications for both species (Thiyagarajan el al. 2003; Emlet and Sadro, 2006; Pechenik, 2006) as naupliar history would also have a direct affect upon juvenile performance and post-settlement survival through trait-mediated effects i.e. quality of cyprids (Gimenez, 2004; Pechenik, 2006). No measures of larval quality were made within this study but assessment of how food availability and temperature interact to determine energy content in the critical cyprid stage would provide useful insight into the effects of a changing environment on recruitment success.

CHAPTER 4

Starvation tolerance and initial larval biomass of *Semibalanus balanoides* and *Austrominius modestus*

4.1. Introduction

In recent years, important changes in marine ecosystems have been linked with the changing global climate (see review Hoegh-Guldberg and Bruno, 2010). These changes have been documented across many habitats and range from biogeographic shifts in species distribution patterns to changes in species phenology (Southward et al. 1995; Beaugrand et al. 2003). In the pelagic realm one of the most important effects may be the observed shift in both the timing and abundance of phytoplankton food relative to peaks of larvae (Edwards and Richardson, 2004; Durant et al. 2005; Kirby et al. 2007; Richardson, 2008). Such shifts may lead lack of available food upon hatching which would be critical for the survival of many planktotrophic larvae that are dependent on exogenous sources for nutrition (Pechenik, 1987; Starr and Himmelman, 1990). Survival of larvae has long been considered as one of the most important factors determining recruitment into adult populations of marine organisms (Thorson, 1950; Pechenik, 1987). The timing of initial food availability is not equally important to all species (see review Olson and Olson, 1989); crustacean larvae in particular are believed to be very sensitive to starvation and the onset of feeding during early development is recognised as a critical period with extensive mortality occurring if proper food is unavailable (Anger and Dawirs, 1981a; Lang and Marcy, 1982; Pechenik, 1987). During periods of starvation, larvae would have to rely on limited amounts of endogenously stored nutrients for survival until they can encounter food (Anger, 2001).

During starvation, crustacean larvae experience three distinct phases of biomass degradation (Anger, 2001). Initially, energy-rich lipid reserves are preferentially metabolised. When most of these accessible lipid stores have been depleted, proteins are increasingly utilised causing the degradation of functional structures such as muscle and nervous tissue. In the final phase of starvation, prior to death, structural lipids may also be degraded causing irreversible physiological damage. In this condition, the larvae are described as having passed their point-of-no-return and do not recover even after re-feeding (Blaxter and Hempel, 1963; Anger and Dawirs, 1981a).

The point-of-no-return (abbreviated as PNR) is a concept first put forward by Blaxter and Hempel in 1963 when investigating the effects of starvation on fish larvae. They denoted a

threshold point during progressive starvation when 50% of the larvae were still alive but had lost their ability to feed and a supply of food afterwards could neither arrest mortality nor restore normal growth and development. The PNR_{50} for a species is therefore defined as the duration in days, of delayed first feeding, which causes irreversible effects that 50% of larvae cannot recover from. For example, after the PNR decapod larvae cease to develop, regardless of feeding conditions, as they have lost the ability to restore lost lipid levels due to irreversible physiological damage to the structures responsible for storing and absorbing lipids (Storch and Anger, 1983; Anger et al. 1985; Dawirs, 1986; Anger, 1987). Many studies have shown that critical points such as the PNR exist in the larval development of many crustaceans, (Anger and Dawris, 1981; Dawirs, 1984; Anger and Spindler, 1987; Anger, 1995; Giménez, 2002), fish (Blaxter and Hempel, 1963; Houde, 1987; 1996) and bivalves (His and Seaman, 1992; Moran and Manahan, 2004).

The PNR of different taxa can range from days to weeks and is largely dependent on the initial quantity of energy reserves in the newly hatched larvae and the rate these reserves are utilised in the environment (Anger and Dawirs, 1981a; Anger, 1995). Temperature has a strong influence on physiological processes and is the most important factor controlling metabolism of an organism, and thus, the rate of reserve utilisation (Anger et al. 1981a; Olson, 1985; Pechenik, 1990). As temperature increases, the PNR decreases due to quicker degradation of initial energy reserves (e.g. fish: Hempel and Blaxter, 1963; Houde, 1974; crustaceans: Anger and Dawirs, 1981a, 1981b; Dawirs, 1984). Therefore, the limits of starvation resistance can strongly depend on temperature, and differences among species are likely to arise due to different temperature optima (Anger and Dawirs, 1981a).

The existence and length of a PNR is particularly important in crustacean larval life cycles as larvae may experience poor recruitment during a period of low plankton productivity if larvae cannot survive until feeding conditions improve (Anger and Dawirs, 1981a; Hagen et al. 2001). Information on PNR and larval starvation can help to predict the probability of survival for different broods and contribute to a better understanding of factors controlling recruitment to populations (Yin and Blaxter, 1987). The distinction between the PNR and the time taken to starve to death is an important one for recruitment success as, although larvae do not die at the PNR point, once the PNR is passed, they will not recover and will ultimately die (Paschke et al. 2004, Meyer and Ottel, 2005). For example, in the crab *C. maenas* the

100% mortality rates under complete starvation does not occur until 12.7 days without food but the PNR_{50} is around 4 days (Dawirs, 1983).

Studies have shown that barnacle larvae can survive short-term starvation which, depending on the species, can also vary with temperature. For example, *Balanus improvisus* stage II nauplii starved at 15°C and 21°C showed 50% mortality after 4 days at both temperatures and total mortality at day 6 and day 7 respectively (Lang and Marcy, 1982). In contrast, at 20°C larvae of *B. amphitrite* could only withstand starvation for 3 days or less (Qiu et al. 1997), but have been shown to withstand increasing periods of starvation with decreasing temperatures (Desai and Anil, 2004). Barnacle larvae which are starved upon hatching will readily hatch to stage II within 24 hours as this first stage is lecithotrophic (Achituv et al. 1980), but without sufficient food nauplii will not moult to stage III (Lang, 1979; Lang and Marcy, 1982; Qiu and Qian, 1997). Although there is an increase in size of the nauplius in passing from stage I to II, because the former does not feed, there is a loss in weight between its release and the moult to stage II. Achituv et al. (1980) demonstrated that freshly hatched *S. balanoides* nauplii starved for 24 hours at 10°C will experience an estimated biomass loss of 0.234µg which is a considerable proportion (23%) of the initial weight of the newly-hatched larvae. During the life of the stage I nauplius, protein followed by lipid is the major metabolic substrate used, but both components are reduced in the same proportion (~52%) of that originally present. It is believed that much of this loss in biomass is a result of material metabolized to maintain the vigorous swimming activity stage I nauplii exhibit. Clearly, starvation even for a short time after hatching can have a strong impact upon the depletion of initial energy reserves for *S. balanoides*; there is currently no such data available for *A. modestus*.

Earlier studies in this thesis have shown that *A. modestus* has better larval performance than *S. balanoides* under food limited conditions. I have hypothesised that *A. modestus* may be more tolerant to starvation than *S. balanoides* and that this response may be linked to initial larval reserves of each species. This study aims to test this hypothesis by identifying the PNR and starvation tolerance of each species and to assess the initial energy reserves of each species, through elemental analysis. Carbon to nitrogen ratio (C:N) is often used as an index of lipid to protein ratio and is intended to reflect the relative proportions of energy storage (lipids) to those of musculature and support structures (proteins) (Anger, 2001). A decreased

C:N is thought to indicate lower levels of lipid storage (Anger, 2001). From previous evidence, it is predicted that *S. balanoides* will have a lower PNR and starvation threshold than *A. modestus*, which may be related to initial larval reserves. Due to the strong influence of temperature upon starvation limits, it is predicted that increases in temperature will reduce the PNR and starvation threshold of both species. *S. balanoides* experiences considerable biomass loss after only 24 hours of starvation at 10^{o}C (Achituv et al. 1980) and exhibits a decreased performance under elevated temperature conditions (chapter 2 and 3 of this thesis. Therefore, it is hypothesised that temperature increases will have a strong effect upon the starvation threshold in *S. balanoides*.

Previous chapters have also demonstrated the strong parental influence upon larval survival and development (Chapter 2 and 3). It has been suggested that this response may be due to variation in the initial energy content of larvae upon hatching, as this can result in various degrees of larval dependence on food availability within a species (see Giménez, 2002; Giménez et al. 2004). In order to investigate this hypothesis, I assess the initial reserves of larvae upon hatching from different parents. Subsequent assessment of larval performance under starvation conditions will examine whether intra-specific variability in energy reserves accounts for the differences observed among larvae in response to food limited conditions. Here, I predict that decreased initial larval reserves upon hatching will result in an earlier PNR and reduced starvation tolerance among parents of *S. balanoides* larvae.

4.2. Methods and Materials

4.2.1. Determining periods of delayed first feeding.

Prior to the main experiment, two preliminary trials were conducted with larvae from *A. modestus* to deduce appropriate starvation treatments for the experiment. This trial was important in identifying the starvation point at which larvae began to die, which is crucial is deciding the point in time to begin first feeding. The trial also identified at what point larvae began to suffer mass larval mortality to so that appropriate intervals of starvation could be used to detect the PNR_{50}. It was not possible to conduct trials upon *S. balanoides* due to time constraints associated with the seasonal availability of larvae for this species.

In both trials larvae were collected and pooled from a number of adults and then reared as described in *section 2.2.1*. When fed, an optimal food concentration of 1 x 10^5 cells ml^{-1} (as described in chapter 3) was used. Five replicate 80ml vessels were used per food treatment with 10 larvae per vessel. In the first trial, immediately after hatching, *A. modestus* larvae were subjected to increasing periods of starvation of 12, 24, 36, 48 and 60 hours before first feeding, after which time they were continuously fed. In the second trial larvae were exposed to 24, 72, 120, 144, 168 hours of starvation prior to feeding.

Results from these trials indicated that larvae were able to survive to the cyprid stage after 72 hours (3 days) of starvation without mass mortality occurring (Table 4.1). Nauplii were shown to experience 100% mortality by day 6 at both temperatures, so this was the longest starvation period in the experiment. Therefore, for the main experiment it was decided that increments of 24 hours of between starvation periods were appropriate and the first starvation period was set as 3 days. As no trials were conducted for *S. balanoides* it was decided to set the first starvation period as 2 days.

4.2.2. Experimental design

To evaluate impacts of periods of starvation upon the performance of larvae, experiments were designed to rear larvae of *S. balanoides* and *A. modestus* under different periods of first initial feeding at two different temperatures (Fig. 4.1A,B). Experiments comprised of treatments where larvae were subjected to different periods of initial starvation (beginning at hatching) followed by continuous feeding at an optimal food density of 1 x 10^5 cells ml^{-1} until nauplii moulted to cyprid or died (*S. balanoides,* 2-6 days; *A. modestus,* 3-6 days; Fig 4.1A,B.). Additionally, there were 2 control treatments where larvae were continually fed or starved from hatching (FC – fed control; SC – starved control). Larvae were reared under 2 different temperatures for each of these experimental starvation treatments until they reached the cyprid stage or died. Larvae of *S. balanoides* were reared at 9°C and 15°C; *A. modestus* were reared at 15°C and 18°C, as illustrated in Fig. 4.1A,B. For *S. balanoides*, 4 adults, 7 food conditions and 2 temperatures were included within the experiment giving 56 experimental treatments. Each of these treatments comprised of 5 replicates with x 10 larvae in each replicate giving n = 280 i.e. a total of 2800 reared larvae. For *A. modestus* there were 3 adults, 6 food treatments and 2 temperatures giving 36 experimental

Table 4.1 Average survival rates of *A. modestus* larvae to the cyprid stage after exposure to different periods of initial starvation prior to feeding (±SD)

	starvation time in hours					
A. modestus, Trial 1	0	12	24	36	48	60
15°C	94 (± 5.5)	92 (± 8.4)	90 (± 0)	94 (± 5.5)	90 (± 7.1)	94 (± 5.5)
A. modestus, Trial 2	0	48	72	120	144	168
15°C	92 (± 8.4)	90 (± 5.5)	82 (± 8.4)	68 (± 8.4)	12 (± 13.0)	0 (± 0.6)
18°C	94 (± 8.9)	94 (± 8.4)	84 (± 8.9)	56 (± 11.4)	8 (± 8.4)	0 (± 0)

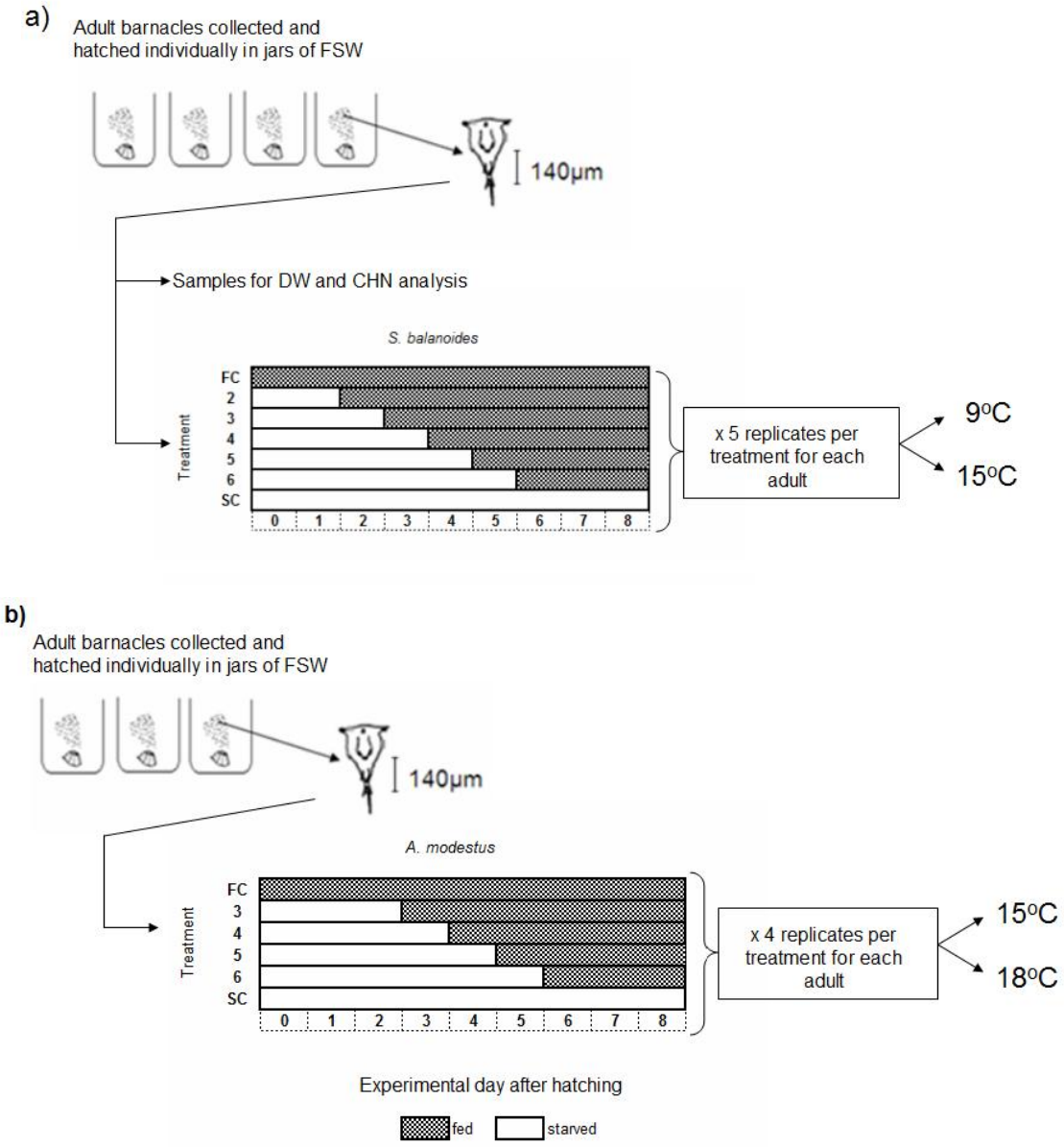

Fig. 4.1 Experimental design for point-of-no-return (PNR) experiments for a) *S. balanoides* (n = 280) and b) *A. modestus* (n=144). FC: continuously fed control; SC: continuously starved control; 2-6: days of starvation followed by continuous feeding.

treatments. There were 4 replicates (due to low brood numbers) with 10 larvae per treatment for each adult giving n= 144 and a total of 1440 reared larvae.

Using data from the treatments, median values for PNR could be estimated (PNR_{50}). PNR_{50} was defined as the time during naupliar development when mortality rates of an experimental treatment reached 50% of the continuously-fed control group (FC) to compensate for natural mortality. The PNR_{50} was calculated for FC treatments at both experimental temperatures to adjust for differences between survival rates at each temperature.

Experiments were carried out in February 2012 for *S. balanoides* and in October 2011 for *A. modestus*. For each experiment, adults were collected from one location on the Menai Strait (53°13'16N, 04°09'47W) and left to hatch as described in *section 2.2.1*. For each experiment, adults with ripe embryos were selected and released larvae from each adult used across the food/temperature treatments (4 adults were used across 14 treatments for *S. balanoides* and 3 adults across 12 treatments for *A. modestus* see Fig 4.1A,B). Freshly hatched larvae from each adult were collected to determine initial larval biomass (see *section 4.2.3*. and *section 4.2.4.).* Additional larvae were also harvested, preserved in ethanol and later body length and width were measured in order to provide an assessment of how larval development was related to initial larval size. Larval performance within experiments was measured as duration of larval development to the cyprid stage, percentage of nauplii surviving to the cyprid stage, cyprid size and settlement survival, which was calculated as percentage of metamorphosed juveniles relative to number of nauplii at the start of the experiment (final settlement success) and also as a percentage of developed cyprids (cyprid settlement).

Once larvae had developed to the cyprid stage, cyprids from each adult reared under each food treatment and temperature condition were pooled together in one jar giving 4 replicates per experimental food/temperature treatment. Cyprids were kept in the incubator in FSW at the same temperature they were initially reared under. Ten cyprids from each adult at each experimental treatment were selected at random and photographed using a microscope camera GX-CAM 5. Photographs were subsequently analysed using Image J to calculate cyprid length, width and 2D cyprid area. Cyprids were then returned to their

replicate vessels to observe overall settlement rates for each experimental treatment. For this, a scraped clean rock was introduced as a settlement surface for *S. balanoides* cyprids and subsequent settlement rates were observed; for *A. modestus*, no settlement surface was introduced and cyprids were left to settle upon the surface of the jar. On day 2, 4 and 6 (after cyprids had been collected) the cyprid cultures were examined and the water was changed. During examination, numbers of metamorphosed cyprids upon the rocks were counted and any dead cyprids in the jar were removed.

4.2.3. Determination of initial larval biomass for S. balanoides.

After hatching from the adult, samples of larvae were collected to measure initial larval biomass and elemental composition (dry weight, DW; carbon, C: nitrogen, N; hydrogen, H). Five sets of replicates with 400 larvae were counted into small glass crucibles containing FSW which were placed on ice in a dark environment to prevent larvae moulting to stage II. Using a pipette, each sample was then placed onto a 100µm mesh filter with absorbent paper underneath to blot the sample dry. Larvae were then rinsed with distilled water before being transferred into pre-weighed tin cartridges using fine tweezers. The cartridges were stored at -20^{o}C prior to being freeze-dried for 24 hours using a Super Modulyo freeze dryer (Mettler Toledo, Leicester, UK). Cartridges were then re-weighed on an electronic microbalance to the nearest 0.1µm to determine DW and then analysed for C, H, and N composition as a percentage of DW using a Flash EA 1112 CHNS-O Analyser (Thermo Scientific, MA).

4.2.4. Determination of initial larval biomass for A. modestus.

Due to smaller naupliar size and weight, around 1500 larvae of *A. modestus* were required per replicate for elemental analysis. Larval broods for an adult of *A. modestus* are much smaller in number (around 500 per brood) than *S. balanoides* so it was not possible to collect larvae from each adult used within the experiments. Therefore, following the start of the PNR experiment, adults were collected from the shore and pooled together into a 2 litre beaker containing FSW and left to hatch. Using a light source to attract the phototaxic larvae, these larvae were transferred to another beaker containing fresh FSW.

Previous trials of this sampling procedure had demonstrated that counting each of the 5 replicates was limited by time constraints and despite using cold water, ice and a dark environment, larvae would readily moult to stage II within a short time. To overcome this, two replicates x 1500 larvae were counted out individually and the remaining 3 or 4 samples were estimated. These calculations were made by estimating the larval density per ml within the beaker; this value was obtained by calculating the average amount of larvae per ml in 5 x 20ml samples that were collected from the larval culture using a Hensel-Stempel pipette. Well-mixed known volumes of larval culture were then extracted from the larval culture using Hensel-Stempel pipettes which would have contained an approximate larval density of 1500. Each sample was washed, stored, dried and analysed using the same procedure described for *S. balanoides.*

4.2.5. Statistical analyses

The effects of the food treatments and temperature upon larval performance were analysed using GMAV5 (Underwood, 2002). The response variables of percentage survival to cyprid per replicate, percentage settlement and mean cyprid size were analysed with mixed model ANOVAs. In the model for *S. balanoides*, parent was a random factor (4 levels), and temperature (2 levels) and food (6 levels) were fixed factors; for *A. modestus* a similar design was used but with 3, 2 and 5 levels for parent, temperature and food respectively. The continuously-starved controls (SC) were not included in any of these analyses. For some response variables there was a lack of replicates in some experimental treatments (due to poor larval survival) and therefore some treatments could not be included in some of the analyses. For the continuously-starved controls (SC), the average point in time when 50% and 100% mortality occurred for each parent were calculated and using a two-way ANOVA potential effects of parent and temperature upon mortality were tested. To examine differences in initial biomass reserves between species, comparisons for mean C :N ratio were evaluated using independent samples t-tests. The effects of initial biomass on larval performance among parents of *S. balanoides* were examined by comparing C, H, N (% DW) and C:N among larvae. Larval length and width among parents were compared for both species using a one-way ANOVA. Cochran's test was used to test for homogeneity of variance and Student Newman Keul's (SNK) was used for *post-hoc* multiple comparisons. Where data transformation did not remove heterogeneity, non-transformed data were

analysed using the ANOVA described, owing to a lack of alternative approaches. Thus, for data with heterogeneous variances, the critical value was adjusted to a more conservative level of $p < 0.01$. Here significant results should be interpreted with caution owing to an increased probability of type I error (Underwood, 1997).

4.3. Results

4.3.1. Survival to cyprid stage

With increasing time of initial starvation, a decreasing percentage of nauplii survived through to the cyprid stage at each temperature for both species Fig 4.2A,B). Under constant starvation conditions, no larvae survived beyond stage II (see *section 4.3.3*). Interestingly, at 15^{o}C, *S. balanoides* and *A. modestus* showed similar patterns of larval survival for respective starvation conditions (Fig 4.2 A,B). For both species, rates of naupliar survival to cyprid stage varied significantly among parents and depended on starvation period and temperature experience (significant 3-way interaction of parent x starvation x temperature; Table 4.2).

Patterns of survival for *S. balanoides* at 9^{o}C demonstrated that, when compared to the fed-control, while starvation periods of up to 3 days did not considerably reduce survival (<8%), beyond this point, survival was considerably reduced (>20%)(Fig 4.2A). An increase in temperature appeared to influence the effects of starvation as, at 15^{o}C, even short periods of starvation (2 days) resulted in a substantial decrease in survival (>28%), an effect that did not occur at 9^{o}C. Rearing larvae at a higher temperature did not significantly reduce survival rates in permanently fed larvae, in comparison, survival rates under conditions of starvation indicated that average survival rates at 9^{o}C were always greater (>17.5%) than those for the respective starvation treatments at 15^{o}C (Fig 4.2A).

Increasing periods of starvation also reduced survival rates for *A. modestus.* Larvae experienced a 30% decline in survival after 3 days of starvation (Fig 4.2B). After 5 or more days of starvation, temperature seemed to have an impact upon survival rates because survival at 18^{o}C was considerably decreased compared to observations at 15^{o}C (Fig 4.2, B). After shorter periods of starvation (≤ 4days) declines in survival rates remained proportionally similar, when compared to permanently fed larvae, at both temperatures.

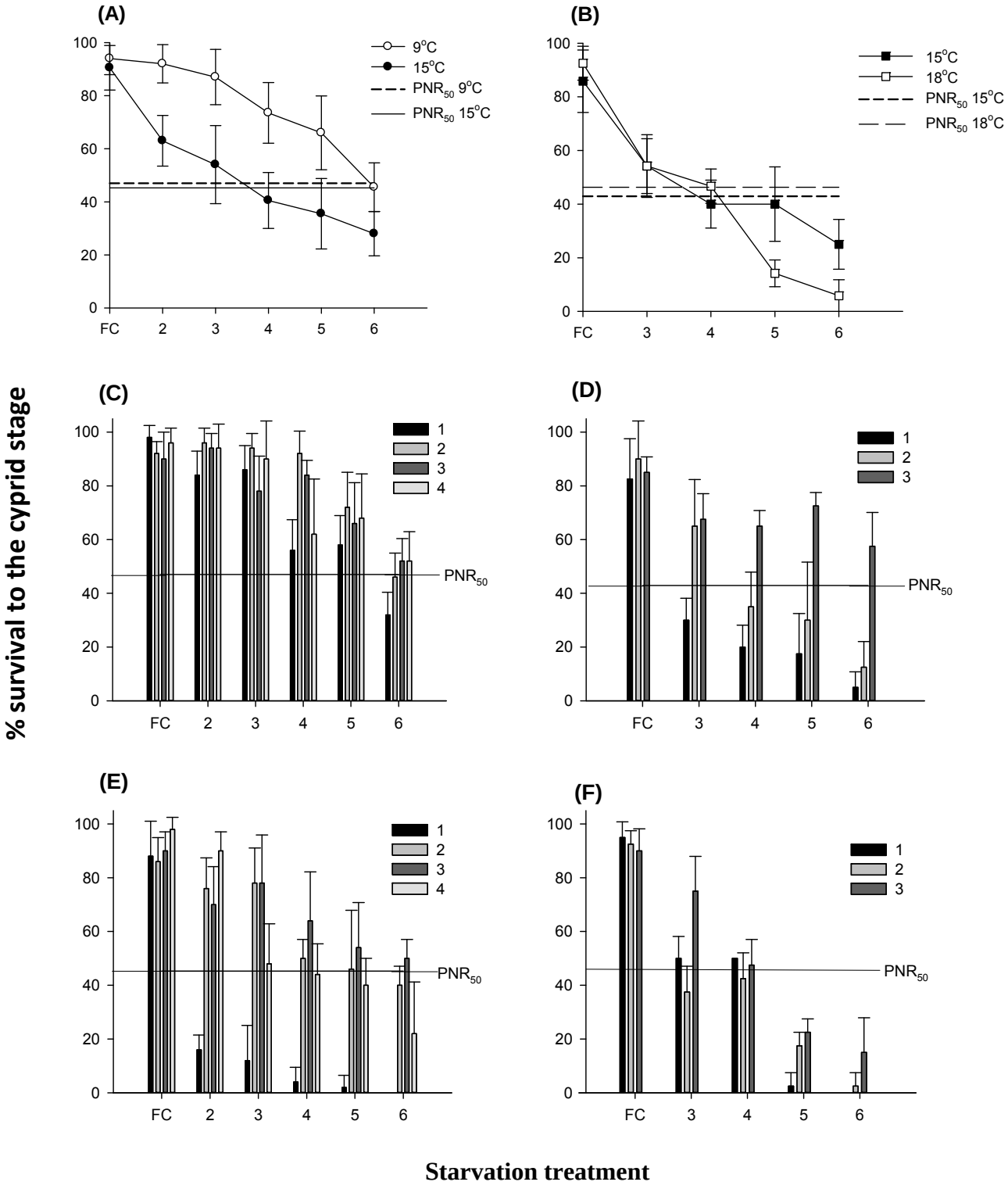

Fig 4.2 Effects of starvation period and temperature upon the average percentage survival to the cyprid stage for *S. balanoides* (A) and *A. modestus* (B); individual responses among parents for *S. balanoides* 9ºC (C) and 15ºC (E) and for *A. modestus* at 15ºC (D) and 18ºC (F) Error bars: (A) and (B) based on replicate parents; (C-F) SD. FC:continuously fed control; 2-6: days of starvation followed by continuous feeding. PNR_{50} value is derived from 50% of survival under FC conditions.

Table 4.2 Mixed effects ANOVA on percentage survival to the cyprid stage for larvae reared under different starvation and temperature treatments. Parent was considered a random factor. Starvation and temperature were fixed factors.

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	31854.58	3	10618.19	80.9	0.0001
Temperature (T)	34320.42	1	34320.42	7.87	0.0675
Starvation (S)	78803.75	5	15760.75	20.56	0.0001
P x T	13074.58	3	4358.194	33.21	0.0001
P x S	11497.92	15	766.5278	5.84	0.0001
T x S	6467.083	5	1293.417	2.34	0.0926
P x T x S	8287.917	15	552.5278	4.21	0.0001
A .modestus					
Parent (P)	12671.67	2	6335.833	61.65	0.0001
Temperature (T)	1203.333	1	1203.333	0.61	0.5173
Starvation (S)	77525	4	19381.25	34.3	0.0001
P x T	3961.667	2	1980.833	19.27	0.0001
P x S	4520	8	565	5.5	0.0001
T x S	5538.333	4	1384.583	2.61	0.1158
P x T x S	4246.667	8	530.8333	5.16	0.0001

Table 4.3 ANOVA on final settlement success rates (proportion of initial larvae achieving settlement) for larvae reared under different starvation and temperature treatments. Starvation and temperature were fixed factors

Factor	SS	DF	MS	F	P
S. balanoides					
Temperature (T)	7600.33	1	7600.33	35.41	≤0.0001
Starvation (S)	12361.67	5	2472.33	11.52	≤0.0001
T x S	820.67	5	164.13	0.76	0.5813
A.modestus					
Temperature (T)	630.21	1	630.21	3.68	0.0696
Starvation (S)	18648.75	4	4662.19	27.19	≤0.0001
T x S	418.75	4	104.69	0.61	0.6598

Table 4.4 ANOVA on cyprid settlement success rates (proportion of cyprids and not initial larvae) for cyprids reared under different starvation and temperature treatments. Starvation and temperature were fixed factors

Factor	SS	DF	MS	F	P
S. balanoides					
Temperature (T)	6320.81	1	6320.81	12.34	0.0012
Starvation (S)	3296.30	5	659.26	1.29	0.2909
T x S	1092.22	5	218.44	0.43	0.8271
A.modestus					
Temperature (T)	1566.60	1	1566.60	2.05	0.1674
Starvation (S)	12569.79	4	3142.45	4.12	0.0136
T x S	1115.78	4	278.95	0.37	0.8303

For both species, average responses of larvae from each parent are given in Fig 4.2C-F. For *S. balanoides*, SNK analyses indicated that the main source of variance among the parents occurred when larvae experienced periods of starvation at 15°C. There was also significant variability among parents among starvation treatments at 9°C, but these effects were only present after 4 days or more of starvation. SNK results for *A. modestus* suggested that most variability among parents was present under starvation conditions at 15°C.

Estimated PNR_{50} values at 15°C were 4 days for both species, indicating that they had a similar tolerance (Fig 4.2A,B). *S. balanoides* larvae under starvation conditions at 9°C were able to survive longer than those reared at 15°C (PNR_{50} 9°C = 6 days; PNR_{50} 15°C = 4 days; Fig 4.2A). For *A. modestus*, a higher temperature did not adjust the PNR_{50} value and this remained at around starvation Day 4 for larvae at 18°C (Fig 4.2B). PNR_{50} values among parents suggested a varied response to periods of initial starvation, particularly at 15°C for *S. balanoides* (Fig 4.2 C,E). Survival did not decrease below 50% for some parents; therefore, no statistical analysis was performed upon these estimates. Estimates for PNR_{50} among parents for *A. modestus* were considerably different at 15°C but not at 18°C (Fig 4.2 D,F).

4.3.2. Settlement success

Final settlement success (defined as the percentage of initial nauplii per starvation/temperature treatment that settled and metamoprhosed to juvenile successfully) decreased with increasing starvation periods for both species (Fig 4.3A,B).

In *S. balanoides* both starvation and temperature had significant independent effects (no interaction) upon rates of final settlement success (Table 4.3). Under fed control conditions at both temperatures, settlement was always significantly higher than settlement rates under any starvation conditions, indicating that even small periods of starvation reduced final settlement success even though survival to the cyprid stage may be high. Final settlement was always more successful at a lower temperature, regardless of starvation period (Fig 4.3A).

Final settlement rates of *A. modestus* were dependent on duration of starvation (Table 4.3). Although final settlement rates were reduced at a higher temperature for some starvation

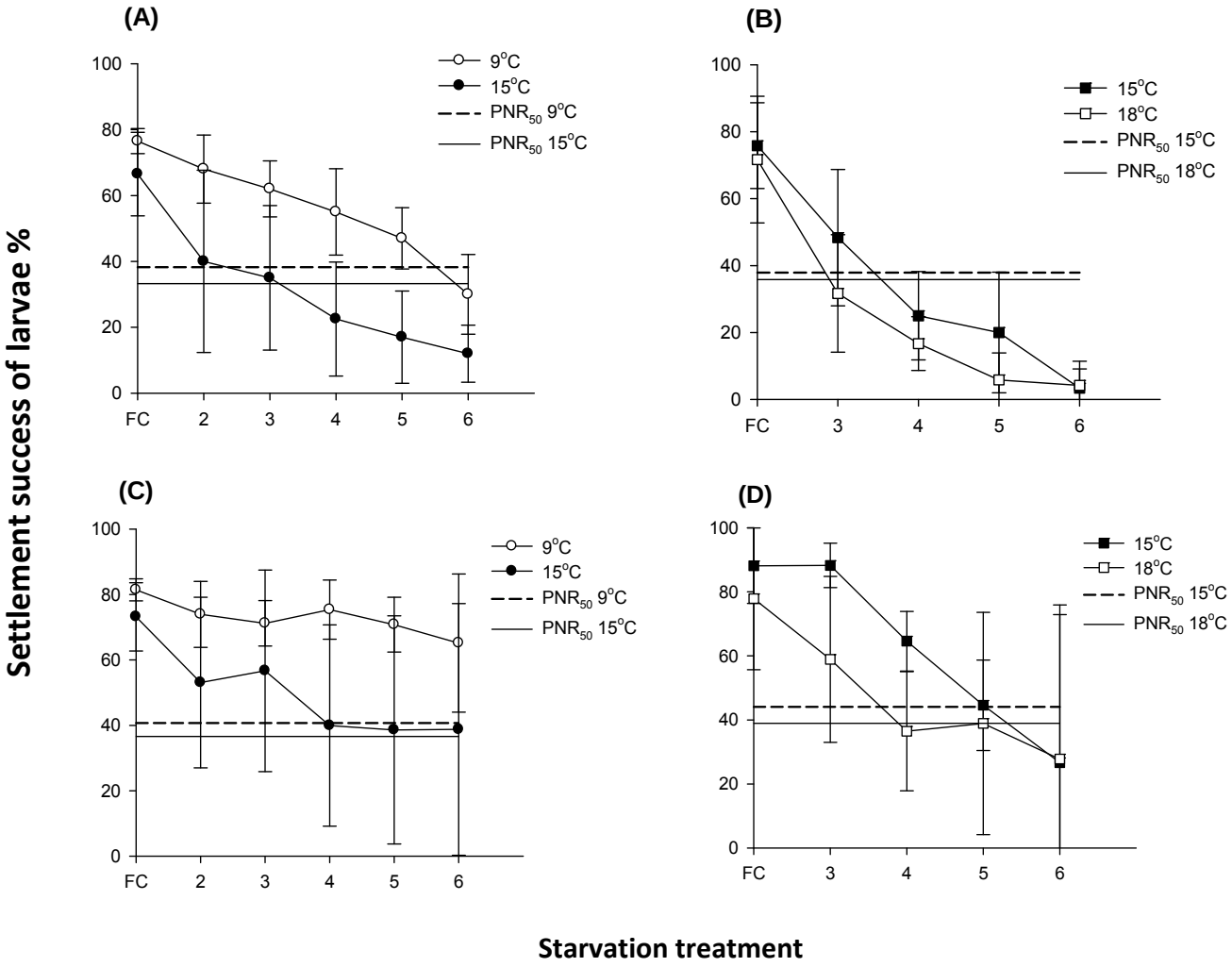

Fig. 4.3 Effects of starvation period and temperature upon average final settlement success (calculated as a proportion of initial nauplii) for larvae of *S. balanoides* (A) and *A. modestus (*B); and cyprid settlement success as a proportion of cyprids produced for *S. balanoides* (C) and *A. modestus* (D). Error bars based on SD. FC: continuously fed control; 2-6: days of starvation followed by continuous feeding. PNR_{50} value is derived from 50% of survival under FC conditions. PNR_{50} is derived from 50% of settlement under FC conditions

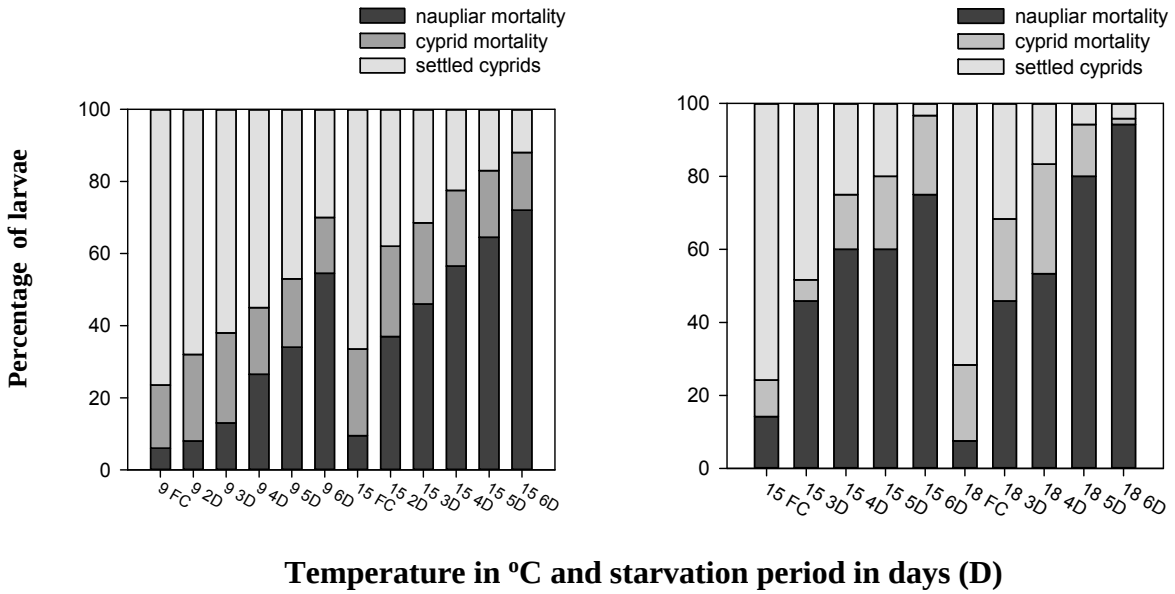

Fig.4 Performance of larvae in starvation and temperature experiments for *S. balanoides* (A) and *A. modestus* (B)

treatments (Fig 4.3B) temperature did not exert a significant influence (Table 4.3). For both FC conditions settlement success was over 70%, but was reduced to 48% at 15°C and 32% at 18°C after 3 days of starvation; by 6 days of starvation settlement was very poor at both temperatures (< 5%)(Fig 4.3B). SNK analyses identified that settlement was significantly decreased after longer periods of starvation (FC > 3 day > 4 day = 5 day = 6 day).

Cyprid settlement rates (determined as % of metamorphosed juveniles from cyprid numbers rather than as a % of initial larvae) for *S. balanoides* showed that periods of starvation did not have a significant influence upon cyprid settlement success as rates of cyprid mortality across all starvation treatments were similar to those experienced at fed-controls for both temperatures (Table 4.4). A higher temperature of 15°C significantly reduced cyprid settlement rates among all starvation treatments by 15-35% when compared to the respective starvation treatment at 9°C. Therefore, starvation was an important determinant of naupliar, but not cyprid, mortality for *S. balanoides* (Fig 4.4A).

Periods of starvation significantly influenced cyprid settlement of *A. modestus* (Table 4.4). Settlement remained high after 3 days starvation (88% and 59% for 15 and 18°C respectively) but considerably decreased after starvation periods of 5 days or more (<44%)(Fig 4.3D). SNK analyses revealed that settlement after 6 days of starvation was significantly reduced compared to fed-controls and 3 day starved larvae; no other treatments were significantly different from one other. A higher experimental temperature reduced cyprid settlement success for many starvation treatments, these reductions ranged from 5 to 29%, (Fig 4.3B) but statistical analysis revealed that cyprid settlement did not depend on temperature (Table 4.4).

Estimation values for PNR_{50} suggested that *S. balanoides* larvae at 9°C were able to complete final settlement more successfully than larvae reared at 15°C even after longer starvation periods (PNR_{50} 9°C = 6 days; PNR_{50} 15°C = 4 days; Fig 4.3A). For *A. modestus*, settlement was also increased at a lower temperature condition; PNR_{50} was reached after 4 days of starvation at 15°C, in comparison, at 18°C, survival decreased below PNR_{50} after 3 days of starvation (Fig 4.3B). When PNR_{50} cyprid settlement rates were examined, survival did not go below PNR_{50} at either temperature for *S. balanoides*. PNR_{50} occurred after 5 days of

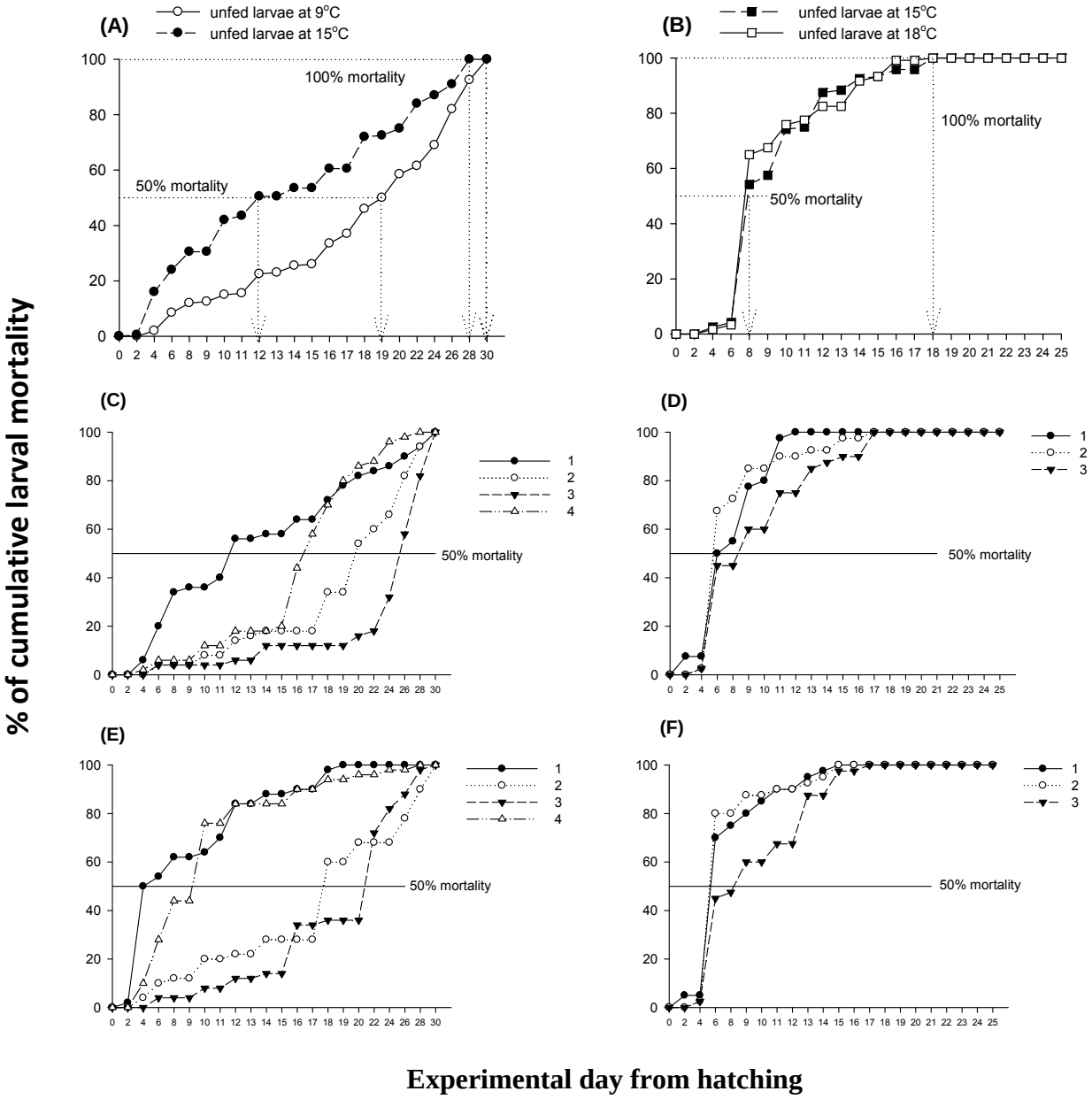

Fig.4.5 Larval mortality under constantly starved conditions for *S. balanoides* (A) and *A. modestus* (B), individual responses among parents for *S. balanoides* 9ºC (C) and 15ºC (E) and for *A. modestus* at 15ºC (D) and 18ºC (F)

Table 4.5 ANOVA on duration of time to reach 50% mortality for constantly starved larvae at different temperature treatments. Parent was a random factor and temperature was a fixed factor.

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	1391.48	3	463.83	181.00	≤0.0001
Temperature (T)	297.03	1	297.03	20.13	0.0207
T x S	44.28	3	14.76	5.76	0.0029
A.modestus					
Parent (P)	4.75	2	2.38	4.89	0.0202
Temperature (T)	0.04	1	0.04	1.00	0.4226
T x S	0.08	2	0.04	0.09	0.9182

starvation at both temperatures for *A. modestus,* suggesting that after an initial starvation period of this duration cyprids were unable to settle successfully.

4.3.3. Mortality under constant starvation

Larvae from each species responded differently when they were reared under continuously starved conditions (Fig 4.5A,B). *S. balanoides* larvae experienced 100% mortality after 28-30 days and, on average, 50% mortality occurred after 12 days at 15°C and 19 days at 9°C; this indicated a higher tolerance to starvation at a lower temperature (Fig 4.5A). Larvae of *A. modestus* seemed to have a lower threshold for starvation than *S. balanoides*; 100% mortality occurred after 16 and 18 days at 15°C and 18°C respectively. 50% mortality occurred after 8 days of starvation at both temperatures, suggesting a similar tolerance to starvation at both temperatures.

Comparisons of mortality patterns at different temperatures indicated that parental variation in the tolerance to complete starvation (Fig 4.5C-F). For *S. balanoides*, starvation tolerance among parents followed a similar pattern at both temperatures (parent 3 > 2 > 4 > 1; Fig 4.5C,E). The point in time where 50% larval mortality was reached was significantly reduced when larvae experienced a higher temperature; a significant interaction of parent x temperature indicated that this effect was more pronounced for some parents (Table 4.5). Compared to results at 9°C, starvation time until 50% mortality at 15°C was reduced by 55% and 45% for parents 1 and 4 respectively; in contrast, parents 2 and 3 were both reduced by 15%. This meant that parents 1 and 4 experienced mass mortality considerably sooner than the other parents (Fig 4.5C,E).

Larvae of *A. modestus* also exhibited a significant parental effect in starvation time until 50% mortality, but temperature did not have a significant effect (Table 4.5; Fig 4.5D,F). SNK analysis demonstrated that larvae from parent 3 had a higher tolerance to starvation than the others.

4.3.4. Duration of development

For both species at each experimental temperature, increasing periods of initial starvation increased the duration of development through to cyprid stage (Fig 4.6A,B). When starvation treatments at 9^{o}C were compared to the fed control treatment for *S. balanoides*, 2 days of starvation increased development time to cyprid by 22% and each additional day of starvation lengthened development period by a further 10% per day; for the same comparisons at 15^{o}C, development times were increased by 35% and 12-14% respectively (Fig 4.6A). Increases in development times under starvation conditions were considerably greater for *A. modestus* (Fig 4.6B). After 3 days of starvation development time was delayed by 68% and 104% at 15^{o}C and 18^{o}C respectively, and after 6 days of starvation the development period had increased by 91% and 144% (Fig 4.6B).

Increases in temperature reduced duration of cyprid development for *S. balanoides* larvae reared under experimental treatments (Fig 4.6A). For the fed control treatment, development time was faster at the higher temperature condition by 33% and 17% for *S. balanoides* and *A. modestus* respectively. When starvation treatments at the higher temperature were compared to the respective treatment at a lower temperature, development times were 22-25% faster for *S. balanoides* (Fig 4.6A). For *A. modestus*, FC larvae developed 17% faster at a higher temperature; however, under starvation conditions a higher temperature had little or no influence upon duration of development (1-7% faster) (Fig 4.6B). Average responses of larvae from each parent are given in Fig 4.6C-F.

4.3.5. Cyprid size

Average responses for both species indicated that higher temperatures reduced cyprid size and, that as periods of starvation increased, the size of cyprids decreased (Fig 4.7A, B). For *S. balanoides*, reductions in cyprid size in response to starvation and temperature varied significantly among parents (significant 3-way interaction of parent x temperature x starvation; Table 4.6; parent 1 not included in analysis due to low survival). Although increasing periods of starvation gradually decreased cyprid size for *S. balanoides*,

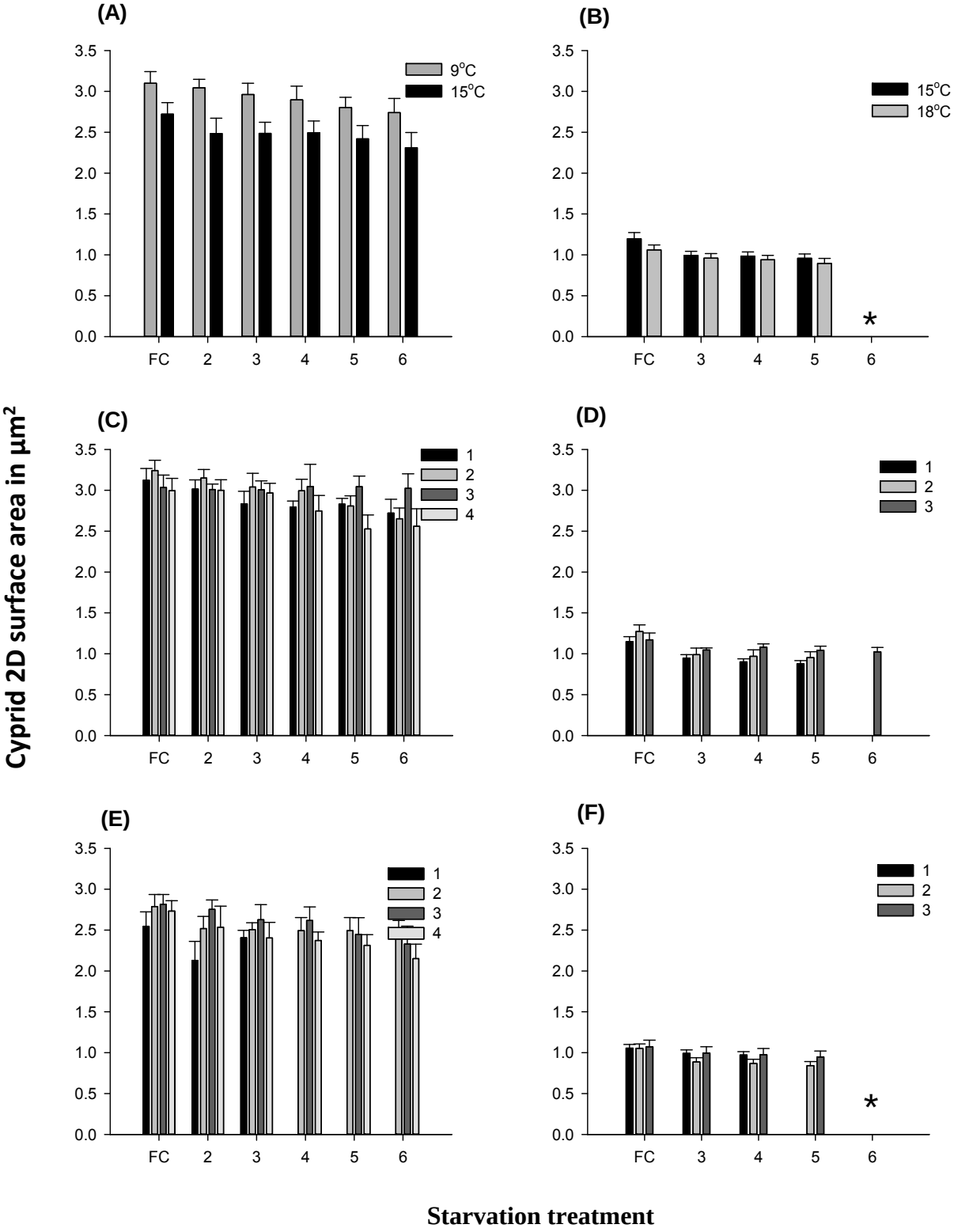

Fig. 4.7 Effects of starvation period and temperature on average cyprid size for *S. balanoides* (A) and *A. modestus* (B), individual responses among parents for *S. balanoides* 9ºC (C) and 15ºC (E) and for *A. modestus* at 15ºC (D) and 18ºC (F) Error bars: (A) and (B) based on replicate parents; (C-F) SD. FC: continuously fed control; 2-6: days of starvation followed by continuous feeding; (*) denotes no cyprids available to measure.

observations suggested that this response was stronger at a higher temperature (Fig 4.7A). When compared to FC treatments, sizes of starved (days 2-6) cyprids were reduced by 9-16% at 15°C; at 9°C the impact of starvation was weaker and size reductions ranged from 2-12%. Similarly, direct comparisons of cyprids from starved treatments at 15°C to their respective treatment at 9°C indicated that cyprids were 14-18% smaller at 15°C, under FC conditions this difference was only 11% (Fig 4.7A). SNK analysis indicated that, for all parents, a higher temperature always significantly reduced the size of *S. balanoides* cyprids, regardless of starvation treatment. However, larvae from different parents responded differently to the starvation treatments at each temperature (Fig 4.7C,E). For example, larvae from parent 3 displayed no significant differences among any of the starvation treatments at 9°C but other parents showed a significant decrease in size after 4 or more days of starvation. In addition, at 15°C, some of the parents experienced a significant reduction in cyprid size after 2 days of starvation and others did not.

A. modestus cyprids reared under starvation conditions also displayed a decrease in size relative to FC; cyprids were smaller by 15-20% and 12-16% at 15°C and 18°C respectively (Fig 4.7B). While an increase in temperature reduced cyprid size across all treatments for this species, it did not appear to increase the effect of starvation; a higher temperature reduced FC cyprids by 12% and starvation treatments by 7-8% (Fig 4.7B). Comparison of treatments indicated that parental effects were present for *A. modestus* in response to the experimental variables (significant two-way interactions for parent x starvation and parent x temperature) but that temperature and starvation did not interact with one other (Table 4.6). This analysis only included results for FC, Day 3 and Day 4 cyprids due to poor survival under other treatments. SNK analyses indicated that, under starvation conditions, *A. modestus* cyprids from one parent were always significantly smaller than those from other parents. At 18°C, significant differences were present among parents that did not occur at 15°C. For all parents, cyprids reared under starvation treatments were not significantly different in size from one another, but were significantly smaller than cyprids from FC (FC > 3 day = 4 day; Fig 4.7D,F).

Table 4.6 Mixed effects ANOVA on cyprid size for larvae reared under different starvation and temperature treatments. Parent was considered a random factor. Starvation and temperature were fixed factors

Factor	SS	DF	MS	F	P
S. balanoides					
Parent (P)	2.71	2	1.36	53.40	≤0.0001
Temperature (T)	15.61	1	15.61	555.17	0.0018
Starvation (S)	6.51	5	1.30	16.79	0.0001
P x T	0.06	2	0.03	1.11	0.3316
P x S	0.78	10	0.08	3.05	0.0010
T x S	0.30	5	0.06	0.37	0.8549
P x T x S	1.62	10	0.16	6.36	≤0.0001
A .modestus					
Parent (P)	0.11	2	0.05	14.62	≤0.0001
Temperature (T)	0.44	1	0.44	20.25	0.0460
Starvation (S)	1.04	2	0.52	15.82	0.0126
P x T	0.04	2	0.02	5.81	0.0037
P x S	0.13	4	0.03	8.91	≤0.0001
T x S	0.03	2	0.02	3.36	0.1393
P x T x S	0.02	4	0.01	1.39	0.2391

Table 4.7 Mean dry weight (DW) and elemental composition of carbon (C), nitrogen (N) and hydrogen (H) of freshly hatched stage I nauplius for *S. balanoides* (SB) and *A. modestus* (AM)

Species	**weight (µg)**	C (%)	**C (µg)**	N (%)	**N (µg)**	H (%)	**H (µg)**	**C : N**	**stage I length µm**	**stage I width µm**
SB	**1.030**	41.15	**0.424**	10.64	**0.109**	5.66	**0.067**	**3.87**	**276.37**	**161.09**
	± 0.037	± 0.62	± 0.003	± 0.13	± 0.013	± 1.14	± 0.012	± 0.028	±9.08	±5.84
AM	**0.241**	43.35	**0.105**	10.67	**0.026**	6.35	**0.015**	**4.06**	**217.64**	**108.85**
	± 0.006	± 1.757	± 0.002	± 0.497	± 0.001	± 0.297	± 0.000	± 0.015	±4.37	±2.32

Table 4.8 ANOVA on larval lengths and widths at time of hatching among the different parents used in Experiment 2

Factor	SS	DF	MS	F	P
S. balanoides					
LARVAL LENGTH					
parent	2548.64	3	849.55	13.64	**≤0.0001**
LARVAL WIDTH					
Parent	2155.11	3	718.37	14.81	**≤0.0001**
A. modestus					
LARVAL LENGTH					
parent	382.74	2	191.37	8.040	**0.0018**
LARVAL WIDTH					
parent	107.63	2	53.82	3.223	**0.0556**

Table 4.9 *A. modestus.* Parental larval size upon hatching by corresponding parent and responses for PNR_{50} and mortality under starved conditions at both experimental temperatures.

larval length in µm (±SD)	larval width in µm (±SD)	Label within experiment	PNR_{50} survival to cyprid at 15°C	PNR_{50} survival to cyprid at 18°C	50% mortality for unfed larvae at 15°C	50% mortality for unfed larvae at 18°C
A.modestus						
215.29(±4.74)	108.21(±4.82)	Parent 1	Day 3	Day 5	Day 8	Day 8
214.95(±5.80)	106.90(±4.53)	Parent 2	Day 4	Day 3	Day 8	Day 8
222.69(±3.89)	111.41(±2.50)	Parent 3	> Day 6	Day 5	Day 9	Day 9

4.3.6. Larval characteristics and elemental composition

The mean dry weight of larvae sampled at stage I for *S. balanoides* was 1.03µg ± 0.037; larvae were about 4 times heavier than a stage I *A. modestus* nauplius, which weighed 0.241µg ±0.006 (Table 7). Mean elemental composition (% of C, N, H) were similar for each species and did not differ significantly from one another (*t-test*; C: $p > 0.08$; N: $p > 0.8$; H: $p > 0.7$). Absolute weights for each element per individual larvae were considerably greater for *S. balanoides* than *A. modestus* (Table 4.7). However, these increases in elemental weight were proportional to the increased overall dry weight of *S. balanoides* i.e. there was approximately 4 times as much of each element. When carbon to nitrogen ratios (C:N) were compared among species, larvae of *A. modestus* had a significantly higher C:N ratio than *S. balanoides* (t-test, t=3.512, df = 4, $p = 0.025$).

Larvae from different parents varied significantly in size in both species (Table 4.8). For *A. modestus,* SNK analyses highlighted that larvae from parent 3 were significantly larger in length upon hatching than larvae from other parents within the experiment (average sizes shown in Table 4.9). Larvae from this parent had the improved performance to the smaller larvae under starvation conditions. Results for *S. balanoides* are given below in *section 4.3.7*.

4.3.7. Elemental composition among experimental parents of S. balanoides.

Larvae sampled from each parent were shown to vary significantly in their dry weight, elemental composition and size immediately after hatching (Tables 4.10 and 4.11). Comparisons among parents indicated that larvae from parent 1 had the least dry weight (6%-15% less than the other parents) and were the smallest in size. Larvae from parent 2 were the largest and had the greatest dry weight (Table 4.10). SNK results for parents were: weight; 2 = 4 > 3 > 1: length and width; 2 = 4 = 3 > 1). Larval C and N content varied significantly among parents (Table 4.10 and 4.11); larvae from Parent 4 had significantly less % C than all other parents (Table 4.10 and 4.11). When absolute weights of each element were calculated, parent 2 had significantly more C per individual larvae whereas parent 1 had the least (2 > 4 = 3 > 1: Table 4.10 and 4.11). A similar pattern was present when C:N ratios were compared; parent 2 had significantly higher C:N ratio than all other parents (SNK; 2 > 4 = 3 > 1 = 4)(Table 4.10 and 4.11).

Table 4.10 Mean dry weight (DW) and elemental composition of carbon (C), nitrogen (N) and hydrogen (H) of freshly hatched stage I nauplius for different parents of *S. balanoides* used within starvation experiment.

PARENT	**weight (µg)**	C (%)	**C (µg)**	N (%)	**N (µg)**	H (%)	**H (µg)**	**C : N**	**stage I length µm**	**stage I width µm**	PNR50 survival to cyprid at 15°C	50% mortality for unfed larvae at 9oC	50% mortality for unfed larvae at 15oC
1	**0.941** ± 0.017	41.54 ± 0.57	**0.391** ± 0.001	10.89 ± 0.12	**0.103** ± 0.005	6.70 ± 1.05	**0.063** ± 0.010	**3.81** ± 0.021	**263.91** ±7.90	**154.43** ±4.53	Day 2	Day 12	Day 4
2	**1.103** ± 0.052	41.84 ± 0.51	**0.461** ± 0.006	10.54 ± 0.09	**0.116** ± 0.023	6.57 ± 1.07	**0.073** ± 0.013	**3.97** ± 0.021	**285.35** ±11.95	**173.50** ±10.49	Day 6	Day 21	Day 17.5
3	**1.005** ± 0.053	41.20 ± 1.00	**0.414** ± 0.005	10.69 ± 0.20	**0.107** ± 0.017	3.00 ± 1.09	**0.063** ± 0.013	**3.86** ± 0.026	**280.65** ±10.01	**157.60** ±5.41	> Day 6	Day 25	Day 22
4	**1.071** ± 0.037	40.00 ± 0.62	**0.428** ± 0.003	10.45 ± 0.13	**0.112** ± 0.013	6.36 ± 1.14	**0.068** ± 0.012	**3.83** ± 0.028	**275.58** ±9.08	**158.83** ±5.84	Day 4	Day 17	Day 9
mean	**1.030** ± 0.037	41.15 ± 0.62	**0.424** ± 0.003	10.64 ± 0.13	**0.109** ± 0.013	5.66 ± 1.14	**0.067** ± 0.012	**3.87** ± 0.028	**276.37** ±9.08	**161.09** ±5.84	Day 4	Day 19	Day13

Table 4.11 One-way ANOVA for biomass measured as dry weight, nitrogen (N), carbon (C) and hydrogen (H) of freshly hatched stage I *S. balanoides* larvae collected from different parents

Factor	SS	DF	MS	F	P
Dry weight	0.08	3	0.03	14.35	0.0001
% N	0.57	3	0.19	9.73	0.0007
% C	9.72	3	3.24	6.58	0.0042
% H	0.58	3	0.19	0.16	0.9204
N µg/ind	0.00	3	0.00	10.24	0.0005
C µg/ind	0.01	3	0.00	17.10	≤0.0001
H µg/ind	0.00	3	0.00	0.74	0.5445
C:N	0.08	3	0.03	43.11	≤0.0001

Table 4.12 Survival times for starved crustacean larvae: C, copepodite: N, nauplius; Z, zoea.

	Stage	Temp. (“C)	Mortality (days) 50%	100%	Reference
Hyas araneus	Z I	12	9	14	Anger & Nair, 1979
Calanus finmarchicus	C5	15	21	-	Dagg, 1977
Acratia tonsa	NI	15	-	2	Dagg, 1977
Crangon crangon	ZI	15	-	7	Paschke et al. 2004
Balanus improvisus	NII	15	4	7	Lang & Marcy, 1982
		21	4	6	Lang & Marcy, 1982
Balanus amphritrite	NII	24	-	3	Qiu and Qian, 1997
S. balanoides	NII	9	19	30	This study
	NII	15	12	28	This study
	NII	15	8	16	This study
A. modestus	NII	18	8	18	This study

When larval characteristics and elemental composition were compared to larval performance (Table 4.10), it appeared that some of these factors may account for the parental effects observed for *S. balanoides*. Larvae from parent 1 which were smallest in size (length and width) and weighed less (in terms of DW and C µg/ind) had the poorest performance; these larvae experienced the greatest mortality rates under starvation conditions, particularly at the highest temperature (Table 4.10). Larvae which had higher C:N ratios took longer to experience mass mortality levels than those which had lower C:N (Table 4.10 and Fig 4.5A for mortality rates).

4.4. Discussion

Based on results of chapter 2 and 3, I hypothesised that a higher capacity to tolerate lack of food and a higher amount of initial reserves in *A. modestus* may explain its higher tolerance to food limitation when compared to *S. balanoides*. The laboratory experiments on starvation tolerance and the elemental analysis of nauplius II did not support this hypothesis. First, at 15^{o}C, starvation tolerance did not differ among species even though this temperature had shown sub-lethal effects on larval development of *S. balanoides*. In addition, when starvation tolerance of both species was considered at the typical temperature they would experience in the field during their peak (i.e. 9^{o}C for *S. balanoides* and 15^{o}C for *A. modestus), S. balanoides* showed higher tolerance than *A. modestus*. In addition, elemental analysis identified rather similar proportions of C and N in both species, suggesting that the level of reserves do not differ. However, as expected the effect of temperature on starvation was strong in the case of *S. balanoides*; a higher temperature caused increased naupliar mortality and also had an influence upon cyprid ability to settle and metamorphose successfully.

My results are consistent with those obtained in similar experiments using crustacean larvae as model species. Periods of starvation caused a decrease in larval performance for both species and nauplii had lengthened larval development times, decreased cyprid sizes, reduced larval survival and settlement success relative to constant-fed control treatments; the longer the starvation period was, the stronger the effects were on larval performance. In partial starvation experiments involving stage II nauplii of *Balanus improvisus,* Lang and Marcy (1982) found that survival potential of nauplii was reduced by 50% after 4 days of starvation. Qui et al. (1997) found that survival of *Balanus amphitrite* larvae initially starved

for 2-3 days was >25% lower than in continuously fed controls and that 4-day initial starvation led to 100% larval mortality. There is currently no information available on PNR_{50} for other barnacle species hence, values here are compared to studies for PNR of other crustaceans; PNR_{50} values in this study for both species at 15^{o}C (4 days) were in a similar range to those observed for decapods, for example, the PNR_{50} for *C. maenas* is 3.8 days (Dawirs, 1981) and *C. crangon* is 3-5 days (Paschke, 1988). The initial starvation is believed to cause irreversible physiological or biochemical damage (Blaxter and Hempel, 1963; Dawirs, 1986; Anger, 1987) which prevents larvae from developing once feeding begins.

The results with regards to temperature were also consistent with previous studies. When the role of temperature under starvation conditions was considered, nauplii of both species developed at a faster rate and matured at smaller size, most likely due to the increased metabolic rates associated with increased temperatures and the "temperature-size rule" (Harms, 1984, Anil et al. 1995; Emlet and Sadro, 2006; O'Connor et al. 2007). Temperature plays a major role in larval development due to its strong influence upon metabolism (see review Pechenik, 1987; Anil and Kurian, 1996; Anil et al. 2001). Previous studies upon *B. amphitrite* have shown that food availability and temperature can jointly determine energy assimilation and allocation during naupliar development and, thus, strongly influence development and growth of nauplii (Anil and Kurian, 1996; Anil et al. 2001).

As with other barnacle species (Lang and Marcy, 1982; Scheltema and Williams, 1982) continuously starved stage II larvae could not moult to stage III unless they were fed. *S. balanoides* larvae could survive for longer periods of time under complete starvation than *A. modestus* and, when results are compared to starvation times for other crustacean larvae (Table 4.12), it is clear that both species can survive without food for longer than other species that have been studied. However, survival times when there is complete absence of food do not constitute a reliable measure of larval starvation resistance as these times are longer than the PNR, i.e, the point where larval tissues experience irreversible damage (Blaxter and Hempel, 1963; Paschke et al. 2004). For example, the PNR_{50} for *S. balanoides* and *A. modestus* were about 33% and 50% respectively of the days of total starvation necessary for 50% of the larvae to die. The nutritional vulnerability of a larva should reflect its general physiological condition or health, so values of PNR_{50} can provide useful

information and be used as functional condition indices, which may complement or sometimes even replace biochemical indices of larval fitness (Paschke et al. 2004).

In conclusion, my findings do indeed reflect the physiological capabilites of nauplius II larvae of *S. balanoides* and *A. modestus* to tolerate starvation. In what follows, I focus on the results with regards to my original hypothesis and refer to elemental composition and species-specific responses to food limitation and temperature. I first discuss the similarities in elemental composition, and then compare the PNR_{50} values as well as other indicators of larval performance. Finally, I discuss the effect of temperature and elemental composition on the species-specific responses to food limitation.

4.4.1. Elemental composition

Overall, nauplii of both species showed fairly comparable values of relative elemental composition (i.e. as percentages of total dry weight for Carbon (C), Nitrogen (N) and Hydrogen (H)). A study by Harms (1987) has estimated the composition of larvae at each stage (apart from stage I) and proportions of elements at the later stages appear to verify those in this study. There is a paucity of data for elemental analysis of *S. balanoides* so there is no data available to validate the findings here. Nevertheless, here, it was shown that both species were similar in terms of their elemental composition and the periods of starvation they could tolerate immediately after hatching were the same. Therefore, neither of these mechanisms could account for poorer performance of *S. balanoides* that has been seen in Chapters 2 and 3.

Elemental analysis of freshly hatched larvae in this study suggested that *S. balanoides* had a significantly lower C:N ratio than *A. modestus*. A decreased C:N is thought to indicate lower levels of lipid storage, as in the case of decapod larvae (Anger, 2001). Given that *A. modestus* did not have an improved performance under starvation conditions to *S. balanoides* it appeared that a higher C:N did not enhance larval tolerance to starvation in this instance. There are a few studies relating C:N ratios at hatching and starvation tolerance, but these focus on decapod larvae: for instance, Anger (1995) showed that in grapsid crabs of Jamaica, the capacity to tolerate starvation conditions of the first zoea is correlated with the C:N ratios, with lecithotrophic larvae having the largest C:N. Other studies, albeit focusing on

intraspecific comparisons, show PNR values (and thus starvation tolerance) are positively correlated to larval C or N content per individual (Giménez 2002; Pashke et al. 2004).

4.4.2. Larval performance

An initial hypothesis of this study was that *A. modestus* would be more tolerant to starvation than *S. balanoides* at 15°C, although *S. balanoides* has shown decreased performance under food limitation at this temperature (Chapter 2). Results identified that the PNR_{50} values (survival to cyprid and final settlement success) occurred at around the same time for both species at a temperature of 15°C (ca. 4 days). Importantly, the settlement success of *S. balanoides* cyprids was shown to be less vulnerable to the effects of initial starvation than that of *A. modestus*. Results identified that after 5 or more days of starvation, cyprids of *A. modestus* exhibited a strong decline in settlement success whereas cyprid settlement of *S. balanoides* did not reach PNR_{50}.

If one was to compare larval performance under starvation conditions at a seasonal temperature that is more "typical" for a species (i.e. 9°C for *S. balanoides* and 15°C for *A. modestus*) then *S. balanoides* could be described as being more tolerant to starvation than *A. modestus*. Therefore, it is difficult to argue that differences in the capacity to tolerate food limitation in these species is due to differences in their amount of initial reserves and how these are utilised during periods of starvation at the early larval stage (nauplius II). However, results were consistent with the original hypothesis that an increase in temperature would have stronger effects on *S. balanoides* than on *A. modestus*: these outcomes are discussed in the next two sections.

4.4.3. Effects of temperature and starvation on S. balanoides

Importantly, larval survival and settlement (as a proportion of initial larvae) for *S. balanoides* were clearly temperature-dependent and PNR_{50} values indicated that larvae were capable of tolerating longer periods of initial starvation at lower temperatures. This later occurrence of irreversible nutritional stress showed that *S. balanoides* had a stronger tolerance to starvation at a lower temperature. Therefore, if *S. balanoides* did mismatch larval release

with the diatom bloom, larvae would have a better chance of recovering from the initial food limitation if this occurred at a low temperature.

Under starvation conditions, larval survival would depend on the extent of initial energy reserves and larval energy demands until the larvae can encounter food (Anger and Dawirs, 1981a). Elevated temperatures increase larval metabolism and energy demands and thus, strongly control reserve utilization (Anger et al. 1981a, Harms, 1987); under starvation conditions a higher temperatures would cause a faster degradation of initial larval energy reserves and larvae are more likely to suffer irreversible starvation (Hodgson and Bourne, 1988; Anil and Kurian, 1996). Results here showed that a higher temperature caused an increase in naupliar mortality under all starvation conditions; hence, an increase in temperature can be detrimental to larvae experiencing starvation even for a short time. Previous studies on starved fish and crustacean larvae have also shown how the relationship between increasing temperature and the earlier onset of irreversible starvation can have a detrimental influence on larval recruitment (Houde, 1987, 1996; Anil and Kurian, 1996; Desai and Anil, 2004).

Comparisons among the larval parents showed a positive relationship existed between initial larval reserves of *S. balanoides* larvae and larval performance. Larvae with the least biomass had the lowest C:N and exhibited a lower tolerance to starvation. This outcome provides evidence that larval susceptibility to starvation can coincide with intra-specific variation in larval biomass. Such relationships have been frequently described for inter-specific comparisons (for reviews see McConaugha, 1985; Anger, 2001) but relatively few studies have assessed intraspecific variability (for examples see Giménez, 2002; Giménez et al. 2004). These larval differences mean that factors such as starvation and temperature exert a selective pressure upon larvae and those with a lower biomass are more likely to suffer mortality. Ultimately, it seemed the initial quality of larvae had a strong influence on larval performance during the naupliar stages. This may also influence the quality of the settlers and subsequently determine their fitness post-settlement. However, neither of these hypotheses were examined within the scope of this study, the question still remains whether the initial advantage of increased energy reserves is fully retained throughout the later stages and increases the quality of the cyprids produced.

Temperature considerably reduced cyprid settlement and supported earlier findings (Chapter 2 and 3) which suggested that elevated temperatures during larval development can have a detrimental impact on the ability of *S. balanoides* cyprids to settle. This may be due to delayed stress-effects from experiencing starvation at an elevated temperature (Lang and Marcy, 1982; Pechenik et al. 1998) or may be attributed to direct temperature effects upon cyprid energy reserves that occur prior to, or during, settlement and metamorphosis to the juvenile form (Fraser, 1989; Thiyagarajan et al. 2003).

As long as nauplii of *S. balanoides* survived through to the cyprid stage, the initial starvation periods did not seem to compromise cyprid quality as they retained their competence to settle successfully. A similar response was reported for initially starved nauplii of *B. improvisus*, where larvae retained the capacity to metamorphose if they did reach the cyprid stage (Qiu et al. 1997; Qui and Qian, 1997). Cyprid quality may remain high as, after the initial starvation period, nauplii experienced a high food environment throughout their development, meaning they can accumulate optimum amounts of energy reserves during stage VI, allowing for competent settlement of cyprids (West and Costlow, 1987; Hentschel and Emlet, 2000; Thiyaragaran, 2002, 2005). This response to early food limitation is different to the effects of food limitation on settlement success seen earlier in Chapters 2 and 3. In these earlier experiments the effects of food limitation were shown during the settlement stage where cyprids were unable to complete the process of settlement due to their poor quality. Therefore, as long as nauplii can encounter an adequate food supply shortly after they are released, an initial period of food limitation should not compromise the quality of cyprids and rates of settlement success.

4.4.4. Effects of temperature and starvation on A. modestus

In contrast to *S. balanoides*, temperature did not have significant effects on larval survival or settlement success of *A. modestus.* Peak larval production and release for *A. modestus* occurs during the warmer summer months (June-September) where temperatures are 14^{o}C-16^{o}C (Crisp and Davies, 1955; O'Riordan and Murphy, 2000), but this species can be reproductive all year round (Crisp and Davies, 1955). Temperature variation may be less important for *A. modestus* as larvae may have adapted a more flexible thermal tolerance range than *S. balanoides*, which have evolved to release larvae once a year during the spring

when the water is colder. A hypothesis by Anger and Dawirs (1981) argues that a temperature range in which enzymatic and other biochemical processes function optimally due to adaptation (possibly genetic) may be superimposed on metabolic responses to temperature changes. This shows that the tolerance of *A. modestus* to temperature does not change much even if the temperature increase is above the normal average temperature (15 – 17^{o}C) in the Irish Sea or North Atlantic (CEFAS). Harms (1987) demonstrated *A. modestus* can perform well under optimum food conditions at temperatures of 18-24^{o}C. Therefore, predicted increases in global temperatures are unlikely to have a negative effect upon this species.

In the earlier food limitation experiments, cyprids of *A. modestus* were generally more robust to the effects of food limitation than *S. balanoides*, but in this experiment there was a negative effect of food limitation on cyprid settlement success after starvation periods of five days or more. The response of *A. modestus* was surprising as it was expected that cyprids would have sufficient energy reserves to settle successfully given the high food environment present prior to the cyprid stage (West and Costlow, 1987; Tremblay et al. 2007). It has been shown that stresses experienced during earlier larval stages can persist, even through metamorphosis, and cause a reduced fitness in later larval or even juvenile stages (Pechenik et al. 1998, Giménez, 2003, Giménez et al. 2004). For example, some gene products needed for organogenesis or physiological function after metamorphosis may be transcribed early in development and stresses, such as starvation, may interfere with the transcriptional or translational processes of these products (Pechenik et al. 1998). As such, late effects of early starvation may thus also occur in advanced stages causing delayed larval mortality (e.g. Lang and Marcy, 1982; Anger, 1987). This contrasting response of cyprid settlement for both species is an interesting result and demonstrates that effects of starvation seem to operate at different stages of their development.

Rather than increased tolerance to starvation it may be that larvae of *A. modestus* are adapted to feeding more efficiently under temperature elevated conditions, particularly under low food conditions. In the small scale experiments in chapter 3, which were used to determine the experimental low food level, larvae of *S. balanoides* were shown to die much sooner than those of *A. modestus* at very low food levels. It is suggested that the increased metabolic cost of feeding at a high temperature under low food conditions compromises the

energy contribution gained from feeding for *S. balanoides* (Monaco and Helmuth, 2011). The implication here is that larvae of *A. modestus* may have a lower energy budget than *S. balanoides* at higher temperatures and, thus, require less food to develop to cyprid and settle successfully, enabling *A. modestus* to survive in lower food environments at higher temperatures more successfully. More information on naupliar feeding rates at different food concentrations and temperatures would provide more insight into this hypothesis.

4.4.5. Conclusion

In summary, it appears vital that larvae of *S. balanoides* and *A. modestus* are released into the plankton when a food supply is readily available soon after hatching as periods of increasing starvation were critical in driving final settlement success of both species through reduced naupliar survival. Starvation periods greater than 5 days were shown to have a detrimental impact on the settlement capacity of *A. modestus* cyprids but were less important in determining the ability of *S. balanoides* cyprids to settle successfully, particularly at low temperatures. This indicated that, contrary to initial predictions, larvae of *A. modestus* were not more resistant to starvation than *S. balanoides*. Instead, it is proposed that larvae of *A. modestus* may be adapted to survive in lower food environments through adaptations such as a reduced energy budget at higher temperatures.

As stated by Anger (2001) the timing of initial food availability is not always equally important and, for *S. balanoides*, this depended strongly on temperature and variability in the quality of larvae upon hatching, both of which had strong implications in determining starvation resistance and subsequent settlement success for this species. Ultimately, elevated temperature had a strong negative effect upon starved larvae of *S. balanoides* but had a much weaker negative effect upon *A. modestus.* This difference is probably due to each species having different temperature optima which would influence rates of larval reserve utilisation under starvation conditions (Anger and Dawirs, 1981a). To optimise larval survival, larvae of *S. balanoides* need to be released into the plankton during periods of low temperatures to reduce the risk of larval mortality under starvation conditions. As temperature increases, associated with climate change, are predicted to continue over the next 50 to 100 years (IPCC, 2007) this may have implications for the survival of *S. balanoides* larvae that do not encounter food soon after hatching.

CHAPTER 5

Ingestion rates and partial energy budget of *Semibalanus balanoides* and *Austrominius modestus*

5.1. Introduction

Survival of larvae has long been considered as one of the most important factors determining recruitment into adult populations of marine organisms (Thorson, 1950; Pechenik, 1987). Spatial and temporal variation in the availability of food can lead to considerable differences in feeding opportunities for planktotrophic larvae (Olson and Olson; Qiu et al. 1997). Optimal survival strategies in these heterogeneous environments can depend on the capacity of larvae to feed and grow at a range of food concentrations. Food availability and temperature are both critical factors for the growth and development of marine larvae due to their strong influence upon the capacity of larvae to capture, ingest and convert food to fuel energetic requirements (Hodgson and Bourne, 1988; Olson and Olson, 1989; Anger, 2001).

The relationship between the consumption rate of a predator and the density of its prey is often referred to as the functional response (Holling, 1959; Jeschke et al. 2002; Kiørboe, 2008; Englund et al. 2011). The most commonly reported functional response in the literature is a type II response, where ingestion rates increase in proportion to prey density and typically saturate at high prey densities (Jeschke et al. 2002). A number of laboratory studies have illustrated this type of response when examining how ingestion rates of marine invertebrate larvae vary with food concentration (Frost, 1972; Dagg, 1977; Yule, 1984; Harms, 1987). Frost's investigations (1972) into the feeding behaviour of calanoid copepods concluded that nauplii fed more slowly in low algal concentrations but at an optimal rate above a certain algal concentration, thus balancing the energy expended on feeding to that gained by the food intake. Yule (1982) found a similar response when he investigated the mechanisms by which barnacle nauplii adjust their feeding rates in response to changes in algal concentrations. He demonstrated how stage IV nauplii of *S. balanoides* and *A. modestus* significantly increase the volume of water they filter with increasing food concentrations, up to a density of about 1 x 10^5 cells ml^{-1}; he deemed this food concentration to be the optimum feeding concentration as any further increases in food did not alter the feeding rate. Yule (1982) also studied the feeding rates of stage II nauplii of *A. modestus* across a range of food concentrations. He identified that nauplii will increase feeding rates until the maximum concentration of around 3 x 10^5 cells ml^{-1} is reached. At the

lowest food concentrations the rate of intake was limited by particle concentration and the feeding effort of the nauplius was not sufficient to obtain the maximum hourly ration. These naupliar feeding responses may thus be linked to larval food availability in such a way as to maximise the net energy gain to the nauplii, using a similar feeding strategy as those proposed by Frost (1972) and Lam and Frost (1976).

Previous studies in this thesis, examining the performance of *S. balanoides* and *A. modestus* nauplii under food limited conditions, have demonstrated that *S. balanoides* exhibit a poorer performance than *A. modestus* (Chapter 2 and 3). This work has also identified that an increase in temperature not only exacerbates the adverse effects of food limitation on larval performance of *S. balanoides,* but also has negative impacts on this species under optimal food conditions. Investigations in Chapter 4 have demonstrated that the better performances of *A. modestus* are not linked to starvation tolerance or initial energy reserves of the larvae, as these were similar between the two species. Therefore, this study aims to identify whether differences in ingestion rates, in relation to cell concentration, may account for the different responses of these two species. In addition, the results for feeding rates could be used to model and compare the energy budgets of both species over a range of food concentrations. Harms (1987) has previously calculated the energy budget at different temperatures (12, 18 and 24^{o}C) at each larval stage for *A. modestus* with an optimal food supply of 1 x 10^{5} cells ml^{-1} available throughout development. Thus, by integrating information of ingestions rates and metabolic losses, I aim to assess the amount of energy that nauplii have available for maintenance and growth; larval metabolic losses can be estimated using respiration rates from other studies (Lucas, 1980; Harms, 1987). It is predicted that optimum feeding conditions occur at lower food concentrations for *A. modestus*, thus, *A. modestus* will have higher ingestion rates than *S. balanoides* at lower food concentrations. Estimation of the energy budget will examine gains and losses of energy for both species. It is predicted that larvae of *A. modestus* may be adapted to survive in lower food environments by better balancing energy requirements for maintenance, growth and development under food limited conditions.

5.2. Method and Materials

Experiments were carried out in March 2012 for *S. balanoides* and September 2012 for *A. modestus*. For each experiment, adult barnacles were collected from intertidal locations along the Menai Strait, Anglesey, UK (53°13'16N, 04°09'47W) by scraping intact individuals from the substratum. Upon return to the laboratory, the adults were placed in a large 2 litre beaker filled with FSW and left to hatch for one hour. After this time, larvae which had aggregated at the surface were transferred with a pipette to one of two 2 litre beakers containing clean FSW. Each beaker was put into an incubator set at the temperature that the larvae would experience during the feeding experiment and left for 24 hours to moult to stage II. The first nauplius stage does not feed (Barnes and Barnes, 1958; Moyse, 1963).

The effect of food concentration on feeding behaviour was assessed by adding a suspension of algae to beakers and following the changes in cell concentration as the nauplii graze the suspension down. A single control beaker, containing a suspension of algae without the presence of nauplii was always used. Prior to the experiment, the appropriate volumes of algae and FSW required to produce the required experimental cell concentrations were calculated and all jars were prepared before adding the nauplii. Nauplii were counted and checked as being stage II using a stereo microscope before being added to the experimental jars.

5.2.1. Experimental design

Each experimental jar contained 50 larvae in 50ml (i.e. 1 larva per 1ml) of FSW at food concentrations of 4 x 10^3, 1 x 10^4, 5 x 10^4, 1x 10^5, 2 x 10^5 and 4 x 10^5 cells ml^{-1}. All food treatments were run at two different temperatures, 9°C and 15°C for *S. balanoides* and 15°C and 18°C for *A. modestus*. For each food/temperature combination there were 3 replicate jars containing larvae and 1 control jar with no larvae resulting in 36 jars with grazers and 12 control beakers without grazers per experiment in total. All jars were provided with a gentle air supply via small battery operated air pumps to keep algae suspended and were kept in an incubator on light/dark cycle of 12hr: 12hr, which ran from the start of the experiment.

Feeding behaviour was monitored over 48 hours at intervals of 4 x 12 hour periods. At each sampling stage, a 1ml sample was pipetted from each experimental jar, including controls, into a pre-labelled 1.5 ml Eppendorf tube and stored in a fridge. Each Eppendorf already

contained 0.02ml of Lugol's solution to preserve the phytoplankton. The algal concentrations in each jar were estimated using a haemocytometer. Two counts were made from each sample and the average used to calculate the ingestion rate per individual per hour via the method given below devised by Frost (1972). To account for the large difference in larval biomass between the two species (Knight-Waugh and Jones, 1949; Crisp, 1962, Chapter 4 of this thesis) ingestion rates were also converted into mass specific ingestion rates expressed as cells μg^{-1} hr^{-1} for each species by dividing the ingestion rate cells ind^{-1} hr^{-1} by the dry mass (DW) for each species (Yule, 1982; Harms, 1987)

5.2.2. Calculation of Ingestion Rates (Frost, 1972)

Ingestion rates, measured as number of cell ingested per individual larvae per hour, were calculated following Frost (1972), using cell counts of the control jars and counts for jars with larvae. The method considers that algal population will grow exponentially but will be under mortality by grazing. Therefore, differences between initial and final number of cells in each jar containing larvae is the result of grazing and growth; the ingestion is represented by the grazing constant.

First, the constant for algal growth, *k*, is calculated from the control jars, where grazing is zero, from the equation:

$$C_2 = C_1 \cdot e^{k(t_2 - t_1)} \qquad \text{Equation (1)}$$

where C_1 and C_2 are cell concentrations (cells ml^{-1}) in the control jar at time t_1 and time t_2.

Assuming that the algal growth rate is not modified by grazing rates the method uses *k* to derive the gazing rate in the jars containing larvae. For each jar with nauplii the grazing coefficient, *g*, was calculated by re-arranging terms of the equation defining the changes in the number of algal cells as a result of the balance of algal growth and grazing:

$$C_2^* = C_1^* \cdot e^{(k-g)\cdot(t_2 - t_1)} \qquad \text{Equation (2)}$$

where C_1* and C_2* are cell concentrations in a jar with grazing nauplii at times t_1 and t_2. Thus, $g = k - \ln(C_2^*/C_1^*)/t$

Using values of k and g the average cell concentration [C], for each grazer jar during time interval $t_2 - t_1$ is:

$$[C] = \frac{C_1^* \cdot e^{(k-g)\cdot(t_2-t_1)} - 1}{(k-g)\cdot(t_2-t_1)} \qquad \text{Equation (3)}$$

The true filtering rate (volume of water passing through the nauplius antennae per unit of time) cannot be directly measured. However, the volume of water swept clear by each individual nauplius, F, can be estimated from the grazing rate as:

$$F = V\,g/N \text{ (ml nauplii}^{-1}\text{ hr}^{-1}\text{)} \qquad \text{Equation (4)}$$

where V is the volume (ml) of the jar and N is the number of nauplii in the jar, "Volume swept clear" or "filtering rate" is defined as the volume of ambient medium from which cells are completely removed by nauplii to achieve the measured ingestion rate. Using F, the ingestion rate (I) is calculated as:

$$I = [C] \times F \text{ (ingested cells ind}^{-1}\text{ hr}^{-1}\text{)} \qquad \text{Equation (5)}$$

The effect of cell concentration upon ingestion rates is determined by plotting the ingestion rates [I] against the average cell concentration [C] for each period of grazing. The mass-specific ingestion rate (cells μg^{-1} hr^{-1}) is obtained by dividing the ingestion rate per individual with the biomass of the nauplii.

Using the values for ingested cells x ind hr^{-1} at a specific cell concentration, the amount of energy consumed per individual (in Joules ind^{-1} hr^{-1}) and per µg of nauplius biomass (joules μg^{-1} hr^{-1}) can be calculated using an energy conversion constant (one cell of *S. costatum* has the energy content of 0.67 µJ: Harms, 1987).

For the feeding periods 24-36 hours and 36-48 hours, ingestion could not be measured as estimations gave negative values for k in the majority of the control jars, indicating a

negative algae growth; jars with grazers present also had negative g values indicating no grazing effect. Therefore, data collected after 24 hours were removed from the analysis and feeding was only assessed over the initial 24 hours. Therefore, the number of cells of *Skeletonema costatum* ingested at each cell concentration during the feeding periods 0-24, 0-12, and 12-24 hours are further reported.

5.2.3. Curve fitting

During each feeding period, it was expected that both species would exhibit a positive relationship between cell concentration and ingestion rate, up to maximal rate, as shown by other studies (Frost, 1972, Yule, 1982; Harms, 1987). Different types of functions can be fitted to feeding rate data of this type (Jeshke et al. 2002; Kiørboe, 2008). Two functions were tested as potential descriptors for grazing response, the disc model (Holling, 1959) and the Ivlev model.

For the disc model the ingestion rate, I in response to the cell concentration follows

$$I = \frac{ax}{(b+x)} \qquad \text{Equation (6)}$$

The disc model has two constants, *a* is equal to the maximal feeding rate (*Imax*) and *b* is the cell concentration at which *Imax* is 50%. A lower value of *b* would mean that the feeding rate is reached at a lower food concentration.

The Ivlev function (1955), also used to describe type II functional responses in curve fitting and modelling (Mullin et al. 1975; Vidal, 1980; Yule.; 1982; Franks et al. 1986) is given by:

$$I = I_{max}\left(1 - e^{-aC}\right) \qquad \text{Equation (7)}$$

The Ivlev formulation has two parameters, the maximum ingestion rate (*Imax* per unit time $^{-1}$) and *a*, the rate at which saturation is achieved with increasing food levels (with increasing levels of concentration $^{-1}$) where *C* is the algal concentration The rate of feeding increase is dictated by the parameter *a*, such that a higher *a* corresponds to a lower prey density at which ingestion is satiated (maximal feeding).

In order to investigate differences in the feeding efficiency among species and temperature treatments, each curve was standardised by dividing the ingestion rate at each food concentration by *Imax,* which meant that at each food concentration the feeding rate could be expressed as a percentage of the maximum.

5.2.4. Energy loss and uptake in mJ

Harms (1987) states that the energy budget during the feeding period can be calculated using the formula

$$I = G + Ex + E + M \qquad \text{Equation (8)}$$

where G is the growth rate and M is Metabolism (which is estimated from oxygen uptake). Ex is the energy content of the exuviae, but it is not relevant in this analysis as the nauplii did not moult during the experimental period. The term E represents egestion and excretion (E).

By using information from the literature for the dry weight of nauplii and weight-specific oxygen uptake at a specific temperatures, M can be calculated and converted to energy loss by heat production (1 mg O_2 = 14.06 Joule). Information on respiration rates were available at 10^{o}C for *S. balanoides* and 18^{o}C for *A. modestus* (Lucas, 1980; Harms, 1987), which is around the optimum temperature for both species. No data were available for the respiration rates of the nauplii of both species at 15^{o}C and the energy budget could not be estimated at this temperature.

Using the values for I and M, energy available for growth (G in mJ. ind^{-1} hr^{-1} and mJ µg W^{-1} hr^{-1}) can be estimated, assuming that E is negligible, as

$$G = I - M \qquad \text{(Equation 9)}$$

5.3. Results

During all feeding periods (0-12; 12-24 and 0-24 hours, Figs 1-4) nauplii of both species exhibited greater food uptake with increasing cell concentrations and, in most conditions,

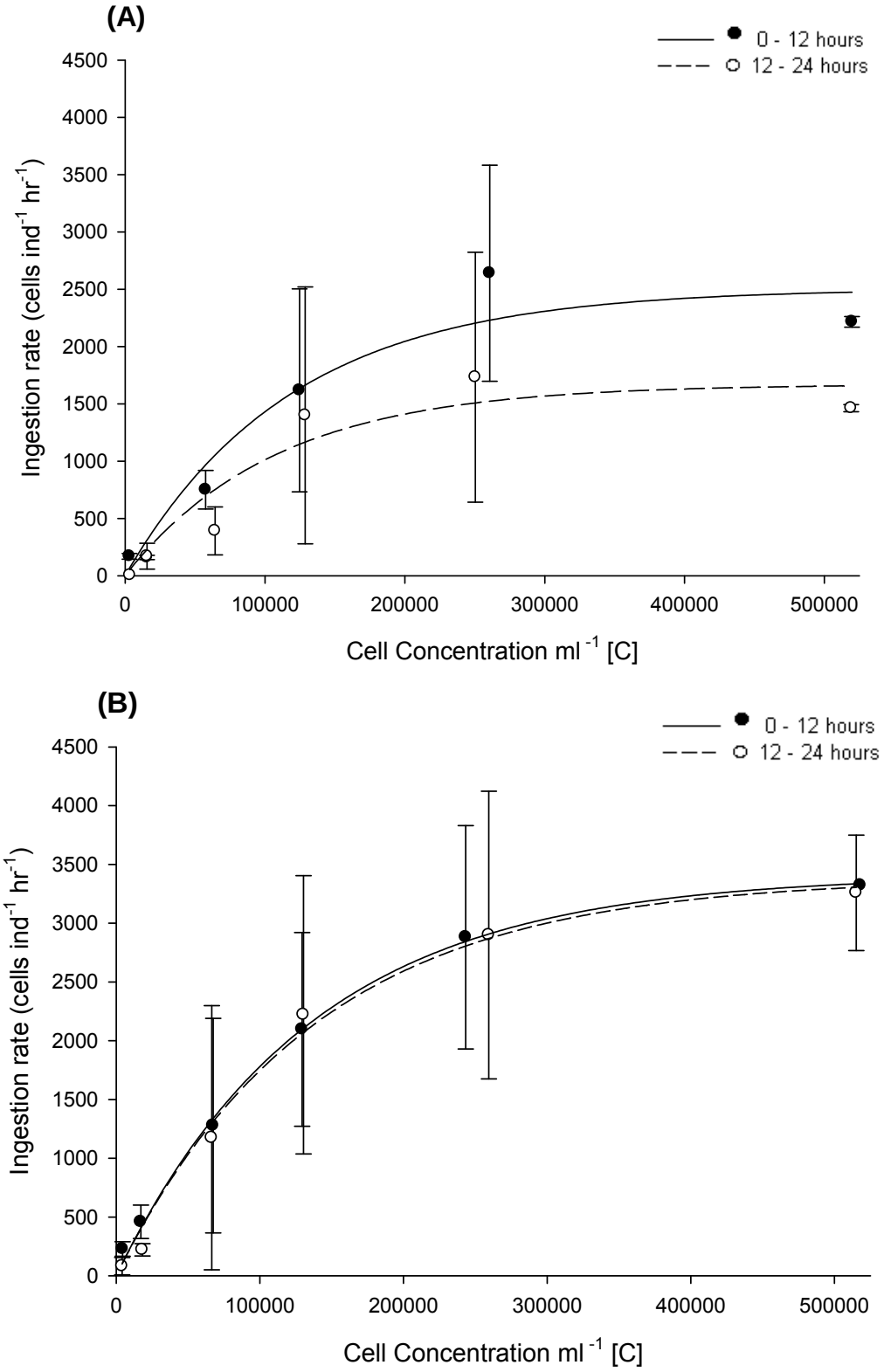

Fig. 5.1. Ingestion rates for stage II nauplii at different average food concentrations [C] (n=3) for *S. balanoides* at 9ºC (A) and 15ºC (B), (n=3) fitted curve parameters and r^2 values are given in Table 5.1. and Error bars; SD:

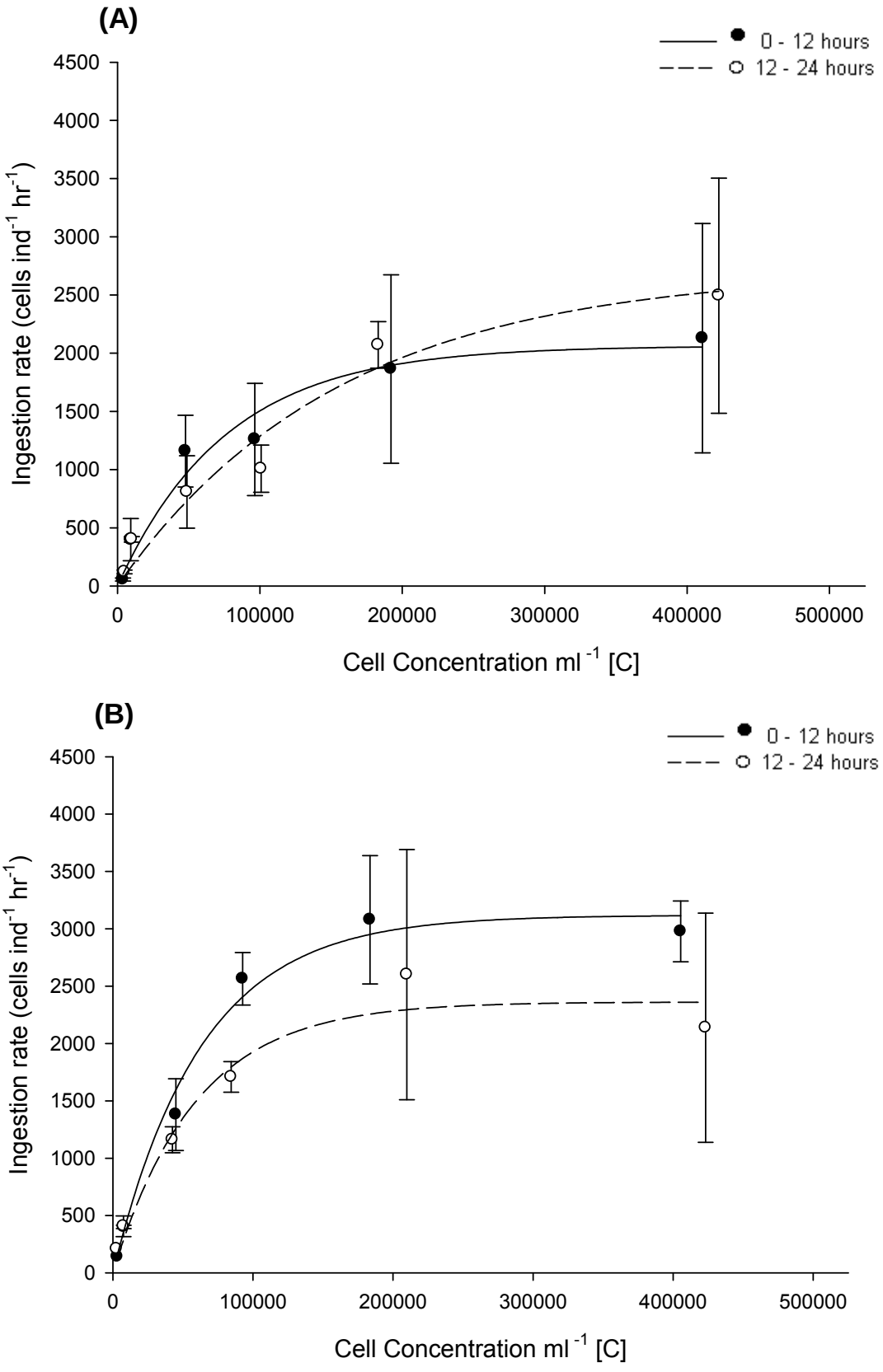

Fig. 5.2. Ingestion rates for stage II nauplii at different average food concentration[C] (n=3) for *A. modestus* at 15ºC (A) and 18ºC (B), fitted curve parameters and r^2 values are given in Table 5.1. Error bars; SD.

exhibited a tendency toward saturation, reaching a maximum ingestion rate at a food concentration of 2 x 10^5 cells ml^{-1} (Fig. 5.1-5.4). For both species, the Ivlev function (1955) was the best fit for feeding curves with r^2 values > 0.90. Curve parameters are given in Tables 5.1 and 5.2. By fitting the Ivlev function to the feeding curves this meant differences in feeding efficiency could be examined between species and temperature treatment by comparison of slopes (parameter *a*).

5.3.1. Ingestion rates (I) per individual (ind^{-1} hr^{-1}) at 0-12 and 12-24 hours

For both species at each cell concentration and temperature treatment, ingestion rates were generally greater during the first 12 hours of feeding than during the subsequent 12-24 hours. This was true for all treatments apart from *A. modestus* (15°C) at cell concentrations of 2 x 10^5 and 4 x 10^5 cells hr^{-1}, where ingestion rates were lower during the first 12 hours. Similarly, the values for *Imax* (Table 5.1) indicated that maximal ingestion rates were greater during the first 12 hour periods than during the subsequent 12-24 hour period for both species, apart from *A. modestus* at 15°C.

5.3.2. Ingestion rates (I) per individual (ind^{-1} hr^{-1}) 0-24 hours

The effects of temperature upon feeding behaviour were clear for both species; a higher temperature resulted in greater ingestion rates and a higher *Imax* value (Table 5.2) for both species. The maximal feeding threshold was reached at 2.5 to 3 x 10^5 cells ml^{-1} at 15°C and 18°C for *A. modestus* (Fig 5.3B) but for *S. balanoides* shifted from 2 x 10^5 cells ml^{-1} at 9°C up to a food concentration of 4 x 10^5 cells ml^{-1} at 15°C. At the lower cell densities (≤ 1 x 10^4 cells ml^{-1}), *A. modestus* nauplii feeding at 18°C had the greatest ingestion rates. However, for the higher food concentrations (≥ 1 x 10^5 cells ml^{-1}) *S. balanoides* nauplii at 15°C had the highest food uptake.

When the feeding behaviours of *S. balanoides* and *A. modestus* at 15°C were compared over a 24 hour period, *S. balanoides* always ingested more cells ind^{-1} hr^{-1} than *A. modestus*; for *S. balanoides* food uptake at the lowest food concentration of 4 x 10^3 cell ml^{-1} was greater by 23% and this gradually increased with increasing cell concentrations to 34% at 4 x 10^5 cells ml^{-1}.

Table 5.1 Curve parameters for ingestion rates (I) of *S. balanoides* and *A. modestus* derived from non-linear regression analysis using the function (I = *Imax* (1 – e^{-a*C}) where Imax = maximal ingestion rate; a = feeding efficicency; *C* = average cell concentration [C] at different temperatures during feeding periods of 0 – 12, 12-24 hours. Abbreviations SB: *S. balanoides*; AM: *A. modestus*

Species	Temp.	Feeding period (hrs)	Curve fitted in Fig.	*Imax*	±*SE Imax*	*a*	±*SE a*	R^2	F	P
SB	9ºC	0 -12	1A	2505.3	281.2	8.47E-06	2.67E-06	0.94	67.0	0.0012
SB	9ºC	12- 24	1A	1670.6	252.6	9.31E-06	4.01E-06	0.90	36.8	0.0037
SB	15ºC	0 -12	1B	3411.6	83.3	7.38E-06	4.61E-07	0.99	1476.4	<0.0001
SB	15ºC	12- 24	1B	3385.6	159.4	7.25E-06	8.87E-07	0.99	458.2	<0.0001
AM	15ºC	0 -12	2A	2062.8	160.6	1.31E-05	4.22E-06	0.96	100.7	0.0006
AM	15ºC	12- 24	2A	2711.1	333.1	6.43E-06	3.63E-06	0.96	92.8	0.0006
AM	18ºC	0 -12	2B	3118.3	139.2	1.59E-05	2.26E-06	0.99	308.4	<0.0001
AM	18ºC	12- 24	2B	2362.5	166.7	1.69E-05	3.87E-06	0.96	97.2	0.0006

Table 5.2 Curve parameters for ingestion rates (I) per individual (cells ind^{-1} hr^{-1}) of *S. balanoides* and *A. modestus* derived from non-linear regression analysis using the function (I = *Imax* (1 – e^{-a*C}) where Imax = maximal ingestion rate; a = feeding efficicency; *C* = average cell concentration [C] at different temperatures during the feeding period of 0- 24 hours. Abbreviations SB: *S. balanoides*; AM: *A. modestus*

Species	Temp.	Feeding period (hrs)	Curve fitted in Fig.	*Imax*	±*SE Imax*	*a*	±*SE a*	R^2	F	P
SB	9ºC	0 - 24	3A	2076.8	261.2	1.05E-05	3.79E-06	0.93	53.4	0.0019
SB	15ºC	0 - 24	3A	3570.4	67.9	7.91E-06	3.86E-07	0.99	2866.3	<0.0001
AM	15ºC	0 - 24	3B	2620.8	505.6	8.77E-06	4.22E-06	0.87	26.7	0.0067
AM	18ºC	0 - 24	3B	2952.2	333.1	1.13E-05	3.63E-06	0.94	59.9	0.0015

Table 5.3 Ingestion rates expressed as mJ per individual per hour (mJ x ind^{-1} hr^{-1}) for stage II nauplii of *S. balanoides* (SB) and *A. modestus* (AM) fed with different cell concentrations at two temperatures over a period of 0 – 24 hours

	mJ x ind^{-1} hr^{-1}			
	SB 9ºC	SB 15ºC	AM 15ºC	AM 18ºC
Cell concentration ml^{-1}				
4×10^3	0.06	0.07	0.06	0.09
1×10^4	0.14	0.18	0.15	0.21
5×10^4	0.57	0.78	0.62	0.86
1×10^5	0.91	1.31	1.03	1.34
2×10^5	1.22	1.90	1.45	1.77
4×10^5	1.37	2.29	1.70	1.96

The equivalent energy values for cells ingested (mJ x ind x hr^{-1}) indicated that energy uptake increased with increasing cell concentration and temperature (Table 5.3); greater ingestion rates at higher food densities meant that larvae had increased levels of energy uptake than those at lower food concentrations (Table 5.3). Therefore, at a temperature of 15°C, nauplii of *S. balanoides* had higher energy uptake (mJ x ind^{-1} hr^{-1}) than *A. modestus* (Table 5.3). An increase in temperature increased the maximal ingestion rate (*Imax*) for both species resulting in increased energy uptake (Table 5.2).

5.3.3. Mass specific ingestion rates (cells μg^{-1} hr^{-1}) 0- 24 hours

Mass-specific ingestion rates for *A. modestus* were considerably greater than those for *S. balanoides* at all food concentrations. (Fig 5.4 A;B: Table 5.4 and 5.5). The conversion to *µ*g W^{-1} shifted the maximal ingestion rates for both species (*Imax*); at a temperature of 15°C, the maximal feeding rate of *A. modestus* more than doubled that of *S. balanoides*. At the lowest food concentration *A. modestus* consumed 146% more food than *S. balanoides*, although this difference decreased with increasing cell concentration, the ingestion rate was still 124% greater at the highest food concentration. As a result, when energy uptake in the form of biomass was considered (mJ x µgW^{-1} hr^{-1}), *A. modestus* had a much greater mass specific energy uptake than *S. balanoides* (Table 5.5).

5.3.4. Feeding efficiency (a) using Ivlev's model (1955) for ind^{-1} hr^{-1} and µgW^{-1} hr^{-1}

The feeding efficiency (parameter *a*) is identical for individual or mass specific ingestion and therefore it can be used to describe feeding patterns across both of these units of measurement for each species at each temperature. A higher value for parameter *a* indicated that as food concentration increased there was faster increase in the ability of nauplii to capture and ingest that food.

Values for feeding efficiency indicated that *A. modestus* approached maximal feeding rates at a greater rate than *S. balanoides* (Table 5.3); at 15°C, feeding efficiency with increasing cell concentration was slightly higher for *A. modestus* than *S. balanoides* (Table 5.4; Fig 5.5). At the low food concentrations of 1 x 10^4 – 5 x 10^4 cells ml^{-1}, feeding rates for *A. modestus* were 2 - 4% higher than those of *S. balanoides*.

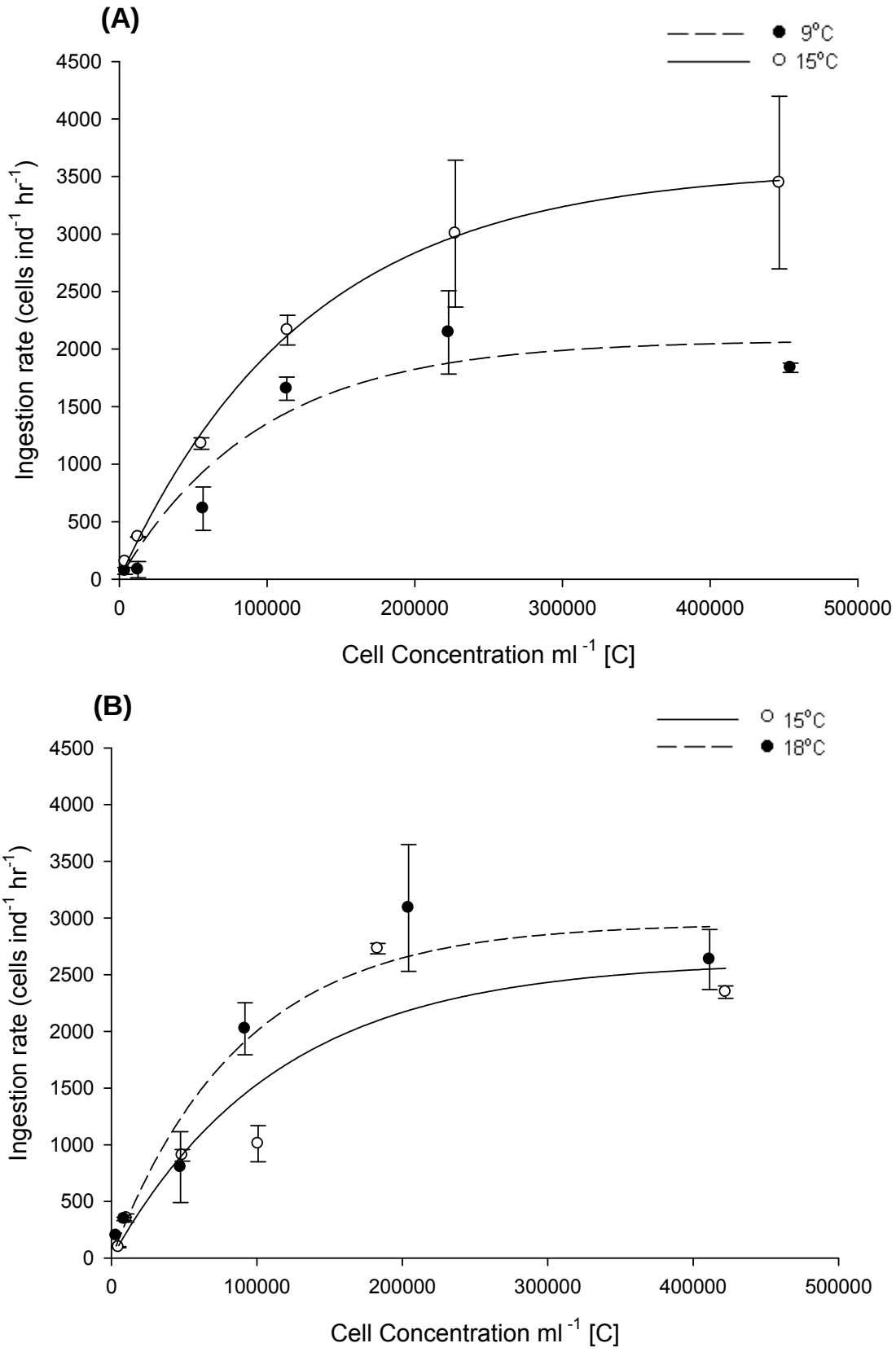

Fig. 5.3 Ingestion rates for stage II nauplii at different food concentrations and temperatures over the feeding period 0 -24 hours for *S. balanoides* (A) and *A. modestus* (B), fitted curve parameters and r^2 values are given in Table 5.2. Error bars; SD.

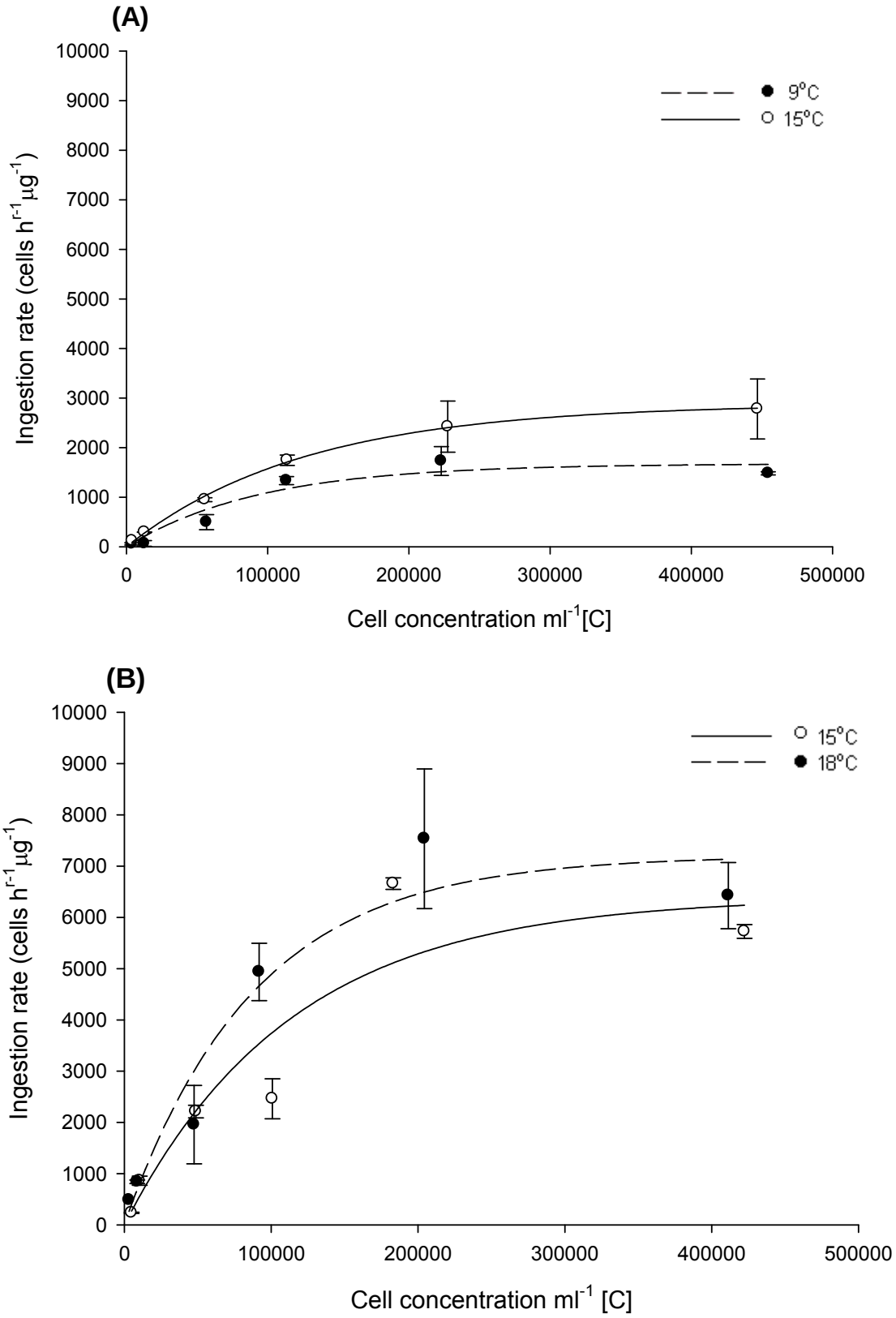

Fig. 5.4 Ingestion rates per µg of dry weight for stage II nauplii at different food concentrations and temperatures over the feeding period 0 -24 hours for *S. balanoides* (A) and *A. modestus* (B), fitted curve parameters and r^2 values are given in Table 5.2. Error bars ; SD.

Importantly, when nauplii of each species experienced the higher temperature condition, *S. balanoides* experienced a decline in feeding efficiency whereas *A. modestus* was shown to feed more optimally. At the higher temperature *S. balanoides* experience a 4 - 8% decline in feeding efficiency whereas *A. modestus* have a 3 - 8% increase in efficiency. Therefore, the optimal feeding temperatures for *S. balanoides* and *A. modestus* were 9°C and 18°C respectively (Table 5.5).

5.3.5. Partial energy budget for metabolism (M) and growth (G)

The proportion of ingested energy lost to metabolism per hour was calculated using the information in Table 5.6 for stage II nauplii of *A. modestus* (18°C) and *S. balanoides* (9°C). Energy losses by metabolism (M) per individual were higher for *S. balanoides* than *A. modestus*. The amount of energy available for growth (G) was calculated as the difference between energy ingested (I) and energy lost to metabolism (M) (as given by equation 8-9), assuming that E is negligible. For both species, the amount of energy available for growth (mJ x ind x hr^{-1}) increased with increasing cell concentration; larvae feeding at higher food densities had increased amounts of their consumed energy available for growth than those at lower food concentrations (Table 5.6). For *S. balanoides* nauplii feeding at the lower food densities (4 x 10^3; 1 x 10^4 cells ml^{-1}) amounts of energy available for growth were equal to, or less than, those used for metabolism; importantly, at the lowest food density, energy losses to metabolism resulted in a net loss of energy for growth (Table 5.6).

When energy losses were considered per unit of larval biomass, metabolic losses were greater for *A. modestus* than *S. balanoides*; at all experimental food concentrations *A. modestus* had considerably more energy available for growth than *S. balanoides*. At food levels of 1 x 10^4 cells ml^{-1}, *A. modestus* has almost 10 times more energy available for growth than *S. balanoides*; *A. modestus* had the same amount of energy available at 1 x 10^4 cells ml^{-1} as *S. balanoides* did at the higher food concentration of 5 x 10^4 cells ml^{-1}. At the lower food concentrations *S. balanoides* had little or no energy available for growth.

Table 5.4 Curve parameters for ingestion rates (I) per µg of biomass (µg W^{-1} hr^{-1}) of *S. balanoides* and *A. modestus* derived from non-linear regression analysis using the function (I = *Imax* $(1 - e^{-a*C})$ where Imax = maximal ingestion rate; a = feeding efficicency; *C* = average cell concentration [C] at different temperatures during the feeding period of 0- 24 hours. Abbreviations SB: *S. balanoides*; AM: *A. modestus*

Species	Temp.	Feeding period (hrs)	Curve fitted in Fig.	*Imax*	±*SE Imax*	*a*	±*SE a*	R^2	F	P
SB	9ºC	0 - 24	4A	1674.9	210.6	1.05E-05	3.7E-06	0.93	53.4	0.0019
SB	15ºC	0 - 24	4A	2879.4	54.7	7.91E-06	3.8E-07	0.99	2866.3	<0.0001
AM	15ºC	0 - 24	4B	6392.2	1223.3	8.77E-06	4.2E-06	0.87	26.7	0.0067
AM	18ºC	0 - 24	4B	7200.6	812.3	1.13E-05	3.6E-06	0.94	59.9	0.0015

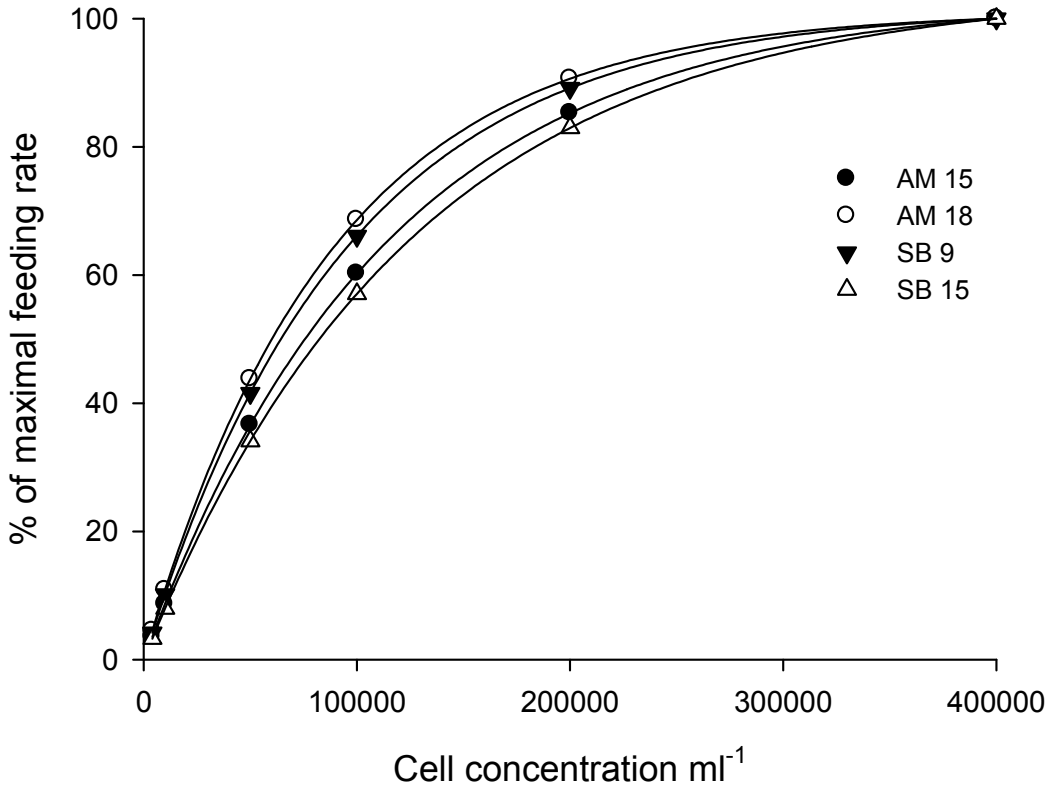

Fig. 5.5 The estimated rate at which nauplii reach their maximal feeding rate with increasing food concentration using parameter *a* fromTable 5.4

Table 5.5 Ingestion rates expressed as mJ per µg of dry weight (mJ x µg W^{-1} hr^{-1}) for stage II nauplii of *S. balanoides* (SB) and *A. modestus* (AM) fed with different cell concentrations at two temperatures over a period of 0 – 24 hours

	mJ x μg^{-1} hr^{-1}			
	SB 9°C	SB 15°C	AM 15°C	AM 18°C
Cell concentration ml^{-1}				
4 x 10^3	**0.05**	**0.06**	**0.15**	**0.21**
1 x 10^4	**0.11**	**0.15**	**0.36**	**0.52**
5 x 10^4	**0.46**	**0.63**	**1.52**	**2.09**
1 x 10^5	**0.73**	**1.05**	**2.50**	**3.27**
2 x 10^5	**0.99**	**1.53**	**3.54**	**4.33**
4 x 10^5	**1.11**	**1.85**	**4.16**	**4.77**

Table 5.6 Metabolic losses (M) and energy available for growth (G) for *S. balanoides* and *A. modestus*

Species	Temp.	Dry weight larva $^{-1}$ (µg)	Respiration rate (O_2 x µg W^{-1} h^{-1})	Cells ml^{-1}	mJ x ind^{-1} x hr^{-1}		mJ x μg^{-1} x hr^{-1}	
					Metabolism (M)	Growth (G)	Metabolism (M)	Growth (G)
SB Stage II	10°C	1.24	4.56	4 x 10^3	**0.08**	-0.022	**0.065**	-0.018
				1 x 10^4		0.060		0.048
				5 x 10^4		0.490		0.395
				1 x 10^5		0.827		0.666
				2 x 10^5		1.143		0.921
				4 x 10^5		1.291		1.041
AM Stage II	18°C	0.41	8.12	4 x 10^3	**0.047**	0.041	**0.115**	0.099
				1 x 10^4		0.165		0.403
				5 x 10^4		0.810		1.974
				1 x 10^5		1.295		3.159
				2 x 10^5		1.727		4.211
				4 x 10^5		1.910		4.658

5.4. Discussion

For both species, the dependence of ingestion rates upon cell concentration were consistent with a type II functional response that has been observed in a number of grazing zooplankton (Holling, 1959; Frost, 1972; Jeschke et al. 2002; Saiz et al. 2007; Kiørboe, 2008). Starting at low food densities, grazing rate first increases almost linearly until it gradually slows down to reach an upper limit (Holling, 1959; Jeschke et al. 2002). The upper limits of feeding are generally reached when ingestion rates become limited by the digestive capacity of the gut (Jeschke et al. 2002; Kiørboe, 2008).

General trends indicated that nauplii of both species fed at a greater rate during the first 12 hours of the experiment. This trend has been shown in other experiments with crustacean larvae (Frost, 1972; Yule, 1982); it is believed that starved animals can ingest food at unusually high rates as their gut is empty so they can process food at a quicker rate (Kiørboe, 2008). For most predators the maximal intake is limited by the rate of gut evacuation rather than the handling time of food (Jeschke et al. 2002). Another possibility is that grazing during the first 12 hours reduces the amount of food available during the later period of the experiment. However, at very high food densities this should not be an issue as, despite grazing behaviour, there would still be a high abundance of food available.

When Harms (1987) investigated the ingestion rates of *A. modestus* reared with 1 x 10^5 cell ml^{-1} at 12, 18 and 24^{o}C, he reported that stage II nauplii ingested 48,860 cells per individual during a 24 hour period at 18^{o}C. Yule (1980) reported that stage II nauplii of *A. modestus* ingested 3,176 cells hr^{-1} at food concentrations between 1.5 -2.5 x 10^5 cells ml^{-1} at temperatures of 18-20^{o}C, corresponding to 76,224 cells per day. In the present experiment, nauplii ingested 1,932 cells hr^{-1} at equivalent experimental conditions, which works out as 46,384 cells day^{-1}; these rates were in good agreement with those of Harms (1987) and Yule (1982). Nauplii of *S. balanoides* were shown to have greater ingestion rates per individual at all food levels when compared to *A. modestus;* however, nauplii of *S. balanoides* are considerably larger than those of *A. modestus* (Knight-Jones and Waugh, 1949; Crisp, 1962). Therefore, in the following sections results for ingestion rate and energy budget over the 24 hour feeding period are discussed in terms of mass-specific ingestion rates (unless otherwise stated) to standardise for body size across species.

5.4.1. Effect of food density

Under the same temperature conditions at equivalent food densities, mass specific ingestion rates were always much higher (>120%) for *A. modestus* than *S. balanoides*. Maximal intake rate is intimately coupled to the physiology of the digestive apparatus for most predators and ingestion rate eventually becomes limited by the digestive capacity of the gut (Jeschke et al. 2002; Kiørboe, 2008). Maximal ingestion rates for *A. modestus* were more than double that of *S. balanoides* providing support for the initial prediction that *A. modestus* nauplii would display higher ingestion rates than *S. balanoides* at lower food concentrations. This outcome suggests that *A. modestus* may be adapted to ingest and digest food at a faster rate than *S. balanoides*. Although *A. modestus* ingests more food than *S. balanoides* at each experimental food density, when these ingestion rates were standardised and examined as a proportion of the maximal feeding rate, both species were shown to exhibit similar increases in rates of feeding. These feeding rates were 2-4% higher for *A. modestus* and this may mean that this species would be able to consume higher amounts of food at lower food densities, but the difference here does not seem great enough to clearly explain why *A. modestus* performs better than *S. balanoides* under low food conditions at the same temperature.

Moyse (1963) has suggested that there is a correlation between the geographical distribution of a cirripede species and the diet of its larvae, with the larvae of cold water species developing well on diatoms while those for warm water species fared better on flagellates. Both species use their antennae for feeding and upon these appendages are structures called intersetules which enable nauplii to filter and capture food from the water column (Moyse, 1963; Stone, 1988; 1989). The intersetule distances will therefore influence what size particles will be captured and eaten. When Stone (1989) examined these feeding structures on stage II nauplii in detail, she reported that for *A. modestus* these distances are mostly 3-4 µm apart and for *S. balanoides* they are mostly 8µm. The size of the *S. costatum* cells that larvae were fed in this experiment were 7-8µm, thus, nauplii of both species should be able to able to capture the cells effectively. The smaller intersetule distances for *A. modestus* may result in the nauplii being more effective at filtering cells from the water resulting in higher ingestion rates, but this would also depend on the digestive capacities of the nauplii (Kiørboe, 2008).

Digestion strategies in crustacean larvae have been shown to reflect adaptations to variability in food density that may be an important factor in determining larval growth and survival (Le Vay et al. 2001). Le Vay et al. (2001) explored the hypothesis that larvae modulate the content of important digestive enzymes through their development to suit their dietary requirements; general trends indicated that species and developmental stages which are dependent on digestion of phytoplankton have higher trypsin content. The first three naupliar stages of *A. modestus* are typically herbivorous and Le Vay et al. (2001) found that during the early larval stages of development there were significantly higher amounts of the enzyme responsible for digesting phytoplankton present than during the later stages (where larval diet becomes more varied). A higher digestive enzyme content means that larvae can rapidly extract the more digestible components from a large number of food items, with a relatively low overall assimilation efficiency but high net energy gain. This strategy is well-adapted to an environment where food which is relatively low in energy content, enabling herbivores to maximise energy assimilation. (Le Vay et al. 2001). Differences in the larval capacity to ingest large amounts of cells observed between these two species may be related to their digestive enzyme content, but as there is currently no data available for enzyme content of *S. balanoides,* no comparisons can be made between the species at this time.

Results from previous chapters (Chapter 2 and 3), and other studies (Harms, 1984; 1987), have shown that at food densities of 1 - 4 x 10^5 cells ml^{-1} nauplii take the same duration of time to develop to cyprid for the respective species. In accordance with this, it was expected that ingestion rates in this study would increase with cell concentration and reach a maximal rate at around 1 x 10^5 cells ml^{-1} and would remain essentially unchanged despite further increases in cell concentration. However, for both species, the maximal ingestion rate was not reached until 2 x 10^5 cells ml^{-1}. Yule (1982) found a similar result and showed that the maximum rate is reached at around 3 x 10^5 cells ml^{-1}. It is likely that this higher than expected threshold is related to the early developmental stage of the nauplii and their ability to capture food. For many feeding larvae, food clearance rate is proportional to the length of the feeding structures (Strathmann, 1971) which increase with successive moults for cirripede larvae (Stone, 1986; 1988). As larvae get larger their antennules become larger and more efficient at catching the cells. For example, stage VI nauplii of *A. modestus* ingest double the amount of cells they consume at stage II (Harms, 1987). It is possible that

maximum ingestion rates occur at lower cell concentrations during the later stage of development.

5.4.2. Effect of temperature on feeding efficiency

An increase in temperature led to a higher maximal ingestion rate and increased ingestion rates at all food concentrations for both species. This response to temperature has previously been reported for *A. modestus* (Harms, 1987) and other crustacean species (e.g. Frost, 1972). Results show how a temperature of 15^{o}C can have detrimental effects on the performance *S. balanoides*; for this species, when feeding efficiencies at 9^{o}C and 15^{o}C were compared, feeding rates increased at a slower rate when the temperature was higher. In contrast, increases in feeding rates in response to food concentration more rapid with a higher temperature for *A. modestus*, indicating that that this species could feed more optimally at a higher temperature.

The poorer feeding performance of *S. balanoides* at a higher temperature may be linked to an effect that temperature has upon the functional response. Studies have identified that the functional response of a species can be limited either by its handling capacity or digestive capacity as some predators can handle prey faster than they can digest them and vice versa (Jeschke et al. 2002). Temperature can strongly influence the parameters of a functional response and lead to important changes in predator/prey interactions (Sentis et al. 2013). Physiological processes, such as digestion and handling time, can have different sensitivities to temperature and, although the nauplii may be capable of handling more food, their digestive capabilities may not allow additional processing of food (Jeschke et al. 2002). All organisms live within a limited range of body temperatures, due to optimised structural and kinetic co-ordination of molecular, cellular and systemic processes (Pörtner and Farrell, 2008) and it may be that 15^{o}C is at the limit of this range for *S. balanoides* larvae.

A number of studies with marine species of fish, crabs and bivalves have proposed the concept of oxygen and capacity-limited thermal tolerance (Pörtner et al. 1999; Frederich and Pörtner, 2000; Pörtner and Farrel, 2008) to explain the mechanism that regulates an organism's thermal optimum and performance window. This hypothesis states that detrimental temperatures bring about insufficient oxygen supply and transport within the

tissues of an organism leading to a decline in performance at elevated temperatures. As proposed in earlier chapters, it seems that *S. balanoides* and *A. modestus* have different tolerances to temperature and that temperatures of 15^{o}C and over can exert a detrimental physiological impact upon the performance of *S. balanoides*.

5.4.3. Partial Energy Budget – Metabolism (M) and Growth (G)

Potential amount of energy available for growth (G) was estimated by subtracting losses to metabolism (M) from the amount of energy ingested (I). A number of assumptions were implicit for constructing the partial energy budget. Energy losses include respired energy and losses to excretion; in this instance, energy losses to excretion were assumed to involve negligible energy loss (Gabbott and Bayne, 1973) and were not included in this budget. Metabolic losses were estimated by calculating the energy lost by respiration (M) and oxygen consumption rates were sourced from other literature (Lucas, 1980; Harms, 1987).

It is well documented that metabolic demands increase with increasing temperature (Hodgson and Bourne, 1988); therefore, as expected, a comparison of naupliar metabolic losses (M) at optimum temperatures for each species i.e. 10^{o}C for *S. balanoides* and 18^{o}C for *A. modestus* (Yule, 1982; Harms, 1984, 1987) indicated that *A. modestus* nauplii experienced a greater mass specific energy loss than *S. balanoides*. However, the high ingestion rates of *A. modestus* meant that energy losses to metabolism were low in comparison to amount of energy that remained available for growth (apart from at the lowest food concentration) if losses by egestion are considered negligible. Importantly, metabolic losses for *S. balanoides* at the lower food concentrations (≤ 1 x 10^4 cell ml^{-1}) were large in comparison to the amount of energy that the nauplii had consumed, which considerably reduced the amount of energy available for growth. Notably, at the lowest food concentration, metabolic losses were greater than the amount of energy ingested which meant the costs of living exceeded the gain from feeding.

Previous experiments have shown that larvae of *S. balanoides* and *A. modestus* can survive to stage V and VI respectively before suffering mass mortality at food concentrations of 1 x 10^4 cells ml^{-1} (Chapter 3). Therefore, one would expect that the rates of ingestion/energy uptake observed in this experiment are sufficient to sustain larval growth and development

through to these later stages (although this development occurs at a much slower rate than at higher food concentrations). As *A. modestus* survived to a later stage than *S. balanoides*, this supports the hypothesis that *A. modestus* has more energy available for growth under low food conditions than *S. balanoides*.

As nauplii of *S. balanoides* suffered considerable metabolic losses (relative to energy ingested) at low food concentrations under optimal temperature conditions, one would expect that at higher temperature these metabolic losses are greater. Increased temperatures lead to increased metabolic demands (Hodgson and Bourne, 1988; Gillooly et al. 2001) which would result in less energy availability for growth. Although feeding rates are known to increase with temperature, the associated exponential rise in metabolic costs with temperature can compromise the overall contribution of increased feeding rates shown by the organism (Levington, 1983; Sanford, 2002). Here, it is suggested that a greater proportion of this ingested energy would be lost at the higher temperature for *S. balanoides* (as compared to proportions lost at optimum temperatures). This assumption is based on evidence from previous chapters which shows that temperature modifies the stage to which larvae can develop under food limited conditions. For example, in Chapter 3, *S. balanoides* nauplii reared under the constant low food condition of 1 x 10^4 cell ml^{-1} survived to a later naupliar stage at the lower temperature than those at the higher temperature. This indicates that nauplii reared with low food concentrations at the higher temperature had less scope for growth and development. Unfortunately there is currently no data available for respiration of *S. balanoides* at 15^{o}C to validate whether energy losses to metabolism can explain the poor performance of *S. balanoides* in earlier experiments.

For *A. modestus*, Harms (1987) showed that, despite the increased energy demands linked with a higher temperature, the associated increase in ingestion outweighed this increased demand and energy available for growth actually increased with temperature. Harms (1987) also demonstrated that under optimal food conditions the net growth efficiency (proportion of assimilated food converted to growth) of *A. modestus* is scarcely influenced by temperature; during total larval development *A. modestus* can maintain a high (77-80%) net growth efficiency at 12, 18 and 24^{o}C. He proposed that this high net growth efficiency over this temperature interval (12-24^{o}C) is likely to be one of the pre-adaptations that has allowed for successful immigration of *A. modestus* around Europe. In his experiments, Lucas

(1980) showed that *S. balanoides* had an average net growth efficiency of 82% at the temperature of 10^{o}C. As this is believed to be the optimum temperature for larval development of *S. balanoides*, it is possible that at higher temperatures this efficiency may decrease resulting in a decline in performance.

In this study, the energy available for growth estimated is likely to be overestimated as ingested food also needs to be digested and assimilated before it can be combusted for growth (Jeschke et al. 2002; Kiørboe, 2008). In the process of transforming ingested food into final biomass there are losses of matter and energy, implying that the growth efficiency yield (proportion of ingested food converted to growth) will be considerably less than 100%. Empirically derived gross growth efficiencies for various species of zooplankton are typically in the range of 30-50% (Kiørboe, 2008). Furthermore, respiration rates used in this study may also have overestimated the energy available for growth as they were deduced from starved larvae; respiration rates of larvae undergoing starvation may be depressed in comparison with the rate of actively feeding larvae (Crisp, 1976). Larval metabolism is likely to vary with food concentration as larvae are more active when there are higher abundances of food, thus, less energy would be expended feeding at low concentrations than at higher ones (Yule, 1982). When food availability is very low or starvation periods are long, many planktonic organisms may turn down their cellular machinery and reduce metabolic rates; this may be considerably greater than anticipated. For example, during periods of starvation copepods reduce their feeding respiration to less than 25% of that of actively feeding individuals (Kiørboe et al. 1985).

5.4.4. Consequences for later naupliar stages.

The feeding patterns shown in this study are likely to change as the larvae moult through the subsequent naupliar stages; in his study, Harms (1987) identified that ingestion rates and thus energy uptake increases with larval stage. As larvae moult through the successive stages they will also increase in size and, thus, their metabolic demands will also increase. Referring to observations from chapter 2, it is known that larvae of both species can survive to the cyprid stage and metamorphose into juveniles successfully at a food density of 4 x 10^4 cells ml^{-1}. Therefore, in the laboratory, the minimum food density required to support larval development of both species falls between 1 x 10^4 and 4 x 10^4 cells ml^{-1} but, for *S.*

balanoides, this performance would strongly depend on temperature. It is well known that the energy reserves of cyprids can be compromised by food limited conditions during the final naupliar stage and can reduce cyprid capacity to settle and complete metamorphosis successfully (Thiyagarajan et al. 2002; Tremblay et al. 2007). In addition, the effects of poor cyprid quality can also carried through to the juvenile stage and affect post-settlement performance (Thiyagarajan et al. 2005; Emlet and Sadro, 2006). It is likely that an examination of the larval energy budget at this last naupliar stage at various food densities and temperatures would provide a deeper insight into the specific mechanisms of how these factors determine the quality of a cyprid and drive post-settlement survival.

5.4.5. Conclusions

In conclusion, it appeared that differences in the performance of these species at low food levels shown in previous chapters may be related to differences in their feeding capacity. The lower ingestion rates of *S. balanoides* resulted in less scope for growth and nauplii were more likely to experience instances where the cost of living exceeded energy uptake at low food concentrations. Larvae of *A. modestus* seem adapted to survive better in lower food environments and higher temperatures than *S. balanoides;* it is predicted that the responses of these species to climate warming will, to a large extent, reflect between-species differences in thermal optima. At higher temperatures, decreases in feeding efficiency combined with higher energy demands will reduce the amount of energy available for growth and development for *S. balanoides.* This would result in extensive larval mortality at low food densities and, at higher food densities, is likely to reduce the quality of larvae that can survive to the cyprid stage. For nauplii of *A. modestus* despite the increased energy demands at a higher temperature, associated increases in ingestion rates have been shown to outweigh this demand. Hence energy available for growth may actually increase with temperature (Harms, 1987) allowing nauplii to maximise the energy available for growth under a wide range of temperatures and food conditions. To optimise survival it seems that *S. balanoides* need to release larvae at low temperatures under abundant food conditions whereas nauplii of *A. modestus* are more flexible to increases in temperature under food limited conditions.

CHAPTER 6

GENERAL DISCUSSION

6.0. General discussion.

In this thesis, the potential effects of climate-driven changes in food availability and temperature upon marine invertebrates was considered by exploring the larval performance of *S. balanoides* and *A. modestus* in a range of different food manipulation and temperature experiments. Several aspects of larval performance were assessed. In terms of predicting recruitment success, the most important measurements were rates of naupliar survival to the cyprid stage and the proportion of cyprids that successfully settled (i.e. attached and metamorphosed). A summary of the significant impacts of food and temperature factors upon each species for all response variables observed during each experiment are illustrated in Fig 6.1.

Early studies (Barnes, 1956; 1957; 1962) correlated the recruitment success of *S. balanoides* with the timing of peaks and abundance of the spring diatom bloom. By contrast*, A. modestus* releases larvae all year round and peak in the summer, well after the spring peak of phytoplankton, when food levels are lower. Thus, assuming that both species use the same food sources, I hypothesised that larval success in *S. balanoides* should be more sensitive to low food concentrations than *A. modestus.* When the effects of constant food densities were examined (Chapter 2), a low food treatment showed no effect on the settlement success of *A. modestus* but cyprid settlement was considerably reduced for *S. balanoides*, particularly at the high temperature tested (15^{o}C).

Further investigations, simulating different mismatch scenarios, (Chapter 3) explored whether the timing of food availability was important for settlement success of both species, since previous work has demonstrated the importance of food availability during the final naupliar stage (West and Costlow, 1987; Hentschel and Emlet, 2000; Thiyagarajan et al. 2001; 2002). Results indicated that nauplii which experienced food limitation during their later stages of development (high-low food scenario) were more likely to experience naupliar mortality than those exposed to low food during the earlier naupliar stages (low-high food scenario). In addition, effects of naupliar food experience were carried over to the cyprid stage; the quality of the cyprids that did survive the periods of food limitation were reduced as a high proportion of these could not complete settlement (as shown by significant effects of low food upon cyprid settlement; Fig 1). Again, effects of food limitation

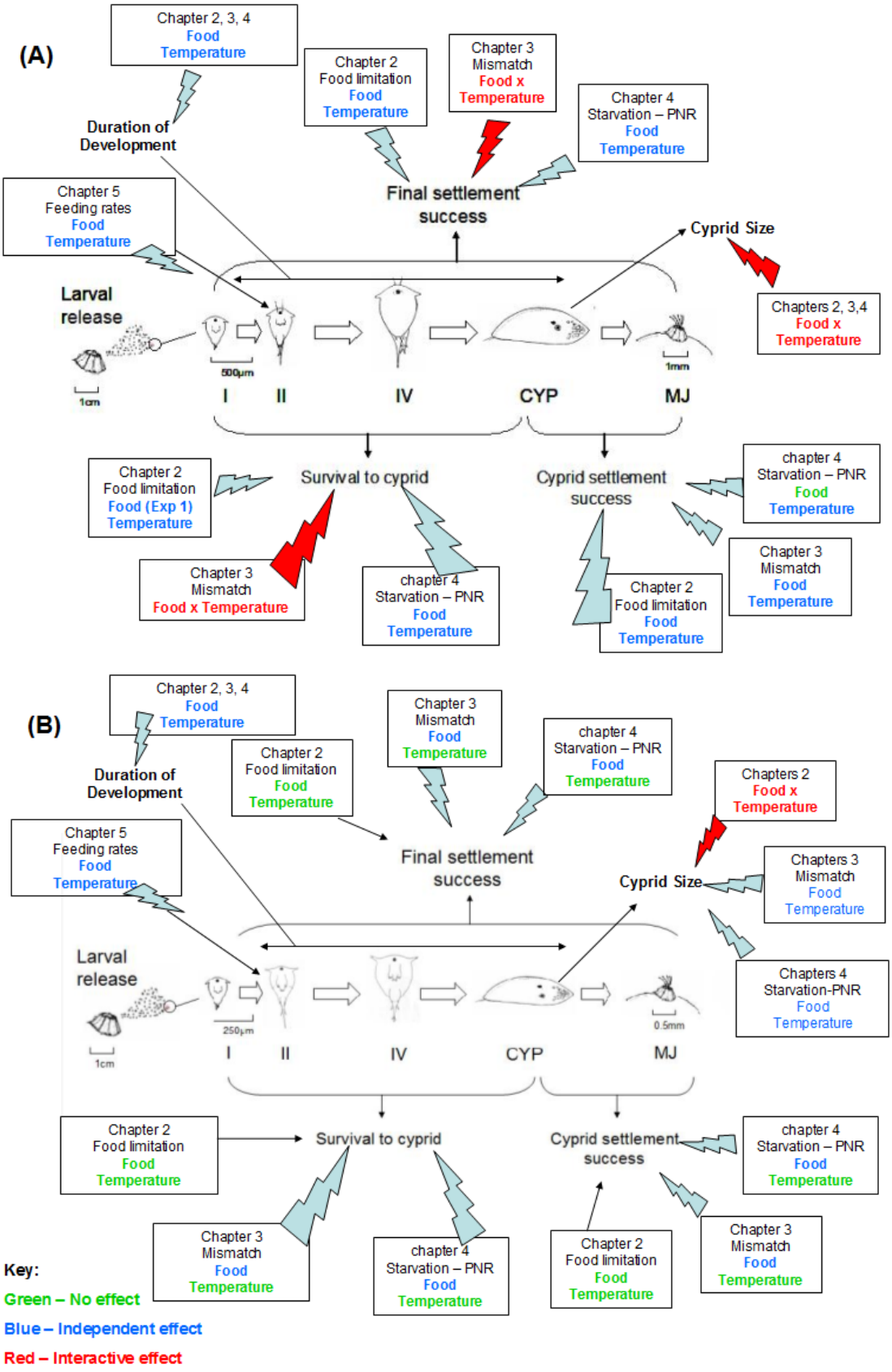

Fig. 6.1 Summary of the effects of food and temperature on response variables in each experiment for (A) *S. balanoides* and (B) *A. modestus.* Abbreviations CYP – cyprid stage; MJ – metamorphosed juvenile.

were stronger for *S. balanoides* than *A. modestus*, especially when larvae were reared at 15°C. At this temperature condition, the settlement success (relative to the number of hatched larvae) was always poor regardless of the timing of the low food period.

Exploration of larval tolerances to initial starvation periods (Chapter 4) indicated that differences in tolerances to starvation were not responsible for the higher performance of *A. modestus,* relative to *S. balanoides,* in food limited conditions. *S. balanoides* had a similar starvation tolerance to *A. modestus* at a temperature of 15°C and elemental analysis yielded that both species were very similar in terms of their mass-specific energy reserves (relative elemental composition - % carbon, nitrogen and hydrogen). Therefore, the higher performance of *A. modestus* under food limited conditions could not be explained by differences in initial energy reserves. Interestingly, when species are compared at or near the conditions most likely to be experienced by natural populations in the Irish Sea (*S. balanoides*; 9°C and *A. modestus*; 15°C), it was evident that *S. balanoides* could tolerate starvation for longer periods than *A. modestus*. Thus my work shows that *S. balanoides* has a strong capacity to tolerate periods of food limitation and, therefore could, cope with a mismatch of food, for example, if larvae were released prior to the spring bloom because of a high water turbulence event (Gyory and Pineda, 2012), as long as larval release occurs at low temperature.

Experiments on the feeding rates of stage II nauplii of both species (Chapter 5) identified that *A. modestus* exhibited higher mass-specific feeding rates, and thus energy uptake, than *S. balanoides* at all food concentrations. Notably, when the temperature increased from 9 to 15°C, *S. balanoides* experienced a decline in feeding efficiency whereas an increase from 15 to 18°C efficiency increased for *A. modestus*, Estimates of partial energy budgets at optimum temperatures (9°C for *S. balanoides* and 18°C for *A. modestus)* suggested that at low food concentrations the energetic costs of maintenance equalled or exceeded the energy uptake of the larvae for *S. balanoides*. In contrast *A. modestus* had about 4 times as much energy available than *S. balanoides*.

Overall, the results presented here are consistent with the hypothesis that *S. balanoides* larvae are more sensitive to food limitation than *A. modestus*. During early larval stages this difference appears to be based, but not restricted to, differences in mass-specific feeding

rates, in particular the maximum mass-specific feeding rates; tolerance to early starvation and amount of initial reserves do not explain the different responses to food limitation of *S. balanoides*. There were also differences in the capacity to tolerate food limitation at advanced larval stages; this was most likely related to differences between species in the balance of energy requirements for maintenance, growth and development under food limited conditions, but this was not verified in this study.

My work has shown how climate-driven changes may profoundly affect the abundance and distribution of a species through direct environmental effects on recruitment success. Changes in recruitment success are likely to alter important interactions, shift competitive balances and strengthen (or weaken) the interactions with competitors or predators. In what follows, firstly, I will discuss the limitations of this study before moving on to a section which proposes the ecological relevance of the study for populations. Thirdly, there will be a section on the proposed effects of climate change upon *S. balanoides,* which details a few different impacts upon the species. Lastly, I explore the potential application of this study.

6.1. Interpreting results of laboratory experiments in the context of population level processes: Limitations

The laboratory experiments reported here represent a way to find explanations for ecological patterns observed in the field, and patterns that may be observed in the future. However, the interpretation of laboratory experiments in an ecological context is not straightforward; for example, these studies do not account for community effects and are not truly representative of the natural environment. There are some limitations that have to be considered in order to avoid over-interpretation of results. Some of them concern differences in what can be actually observed in both the field and laboratory; others are related to the obvious differences of context existing between the field, where many factors vary simultaneously, and the laboratory, where the environment is highly simplified.

A main motivation of this thesis is the relationship between match-mismatch and recruitment observed in the field. Recruitment is defined as the result of settlement plus early post-settlement mortality (Keough and Downes, 1982; Connell, 1985). A larva is considered as “settled” at the moment it irreversibly attaches to the substratum, whilst a

settler is considered to be a "recruit" once it has survived the period up to observation (Keough and Downes, 1982). In this study, settlement success was defined from the number of individuals that survived for 6 days or more after settlement and metamorphosis. These small juveniles were observed feeding and had clearly survived the transition from benthic to juvenile form and were able to be defined as "recruits" within the scope of these experiments. In the field, intense levels of mortality can occur within a 24 hour period immediately after settlement (Gosselin and Qian, 1996) which may be the result of factors such as wave-exposure or desiccation that were not considered in the experiments. Clearly, during the studies presented here, settlers were not exposed to the same kind of environmental stresses and conditions that they would experience in the field, which would undoubtedly result in additional mortality to levels observed here. However, given the large differences in settlement success observed between the two species in this study, it would not be unreasonable to expect that the observed overall trend of one species outperforming the other would remain in a field situation.

Another important aspect to consider is the larval food environment. Typically, chlorophyll concentrations during the spring bloom peak between 10 - 18 mg m^{-3} in the Menai Strait (unpublished observations); for Irish coastal waters and offshore waters of the western Irish Sea, spring bloom chlorophyll can reach 23 and 16 mg m^{-3} respectively (Gowen and Bloomfield, 1996). The spring bloom is composed of many species; the larvae reared in these experiments were fed monocultures of *Skeletonema costatum* at a variety of cell concentrations. In the first experiment, using conversions from other literature (Starr et al. 1991; Desai and Kurian, 1996), it was estimated that the chlorophyll *a* content of the high, mid and low food cultures were 236, 108 and 23.6 mg m^{-3} respectively. Clearly these laboratory food levels are considerably in excess of food levels estimated for the field, but many studies have shown that prey levels necessary for growth and development in the laboratory are one to two orders of magnitude higher than average field densities (Olson and Olson, 1989). This paradox may be solved if consumers of plankton show a more generalistic feeding behaviour than previously assumed (Harms et al. 1991), for example, the fourth and fifth naupliar stages of *A. modestus* are known to be capable of feeding on small zooplankton (Wisely, 1960). Larvae may also attain successful growth and development from seemingly nutritionally low environments due to plankton patchiness, where they may frequently encounter patches of high food density (Gimenez and Anger, 2005). In the

mismatch food experiments (see chapter 3), chlorophyll *a* content of the high and low food levels are estimated as being 59 and 5.9 mg m^{-3} respectively. Therefore, these two food treatments were a good representation of high and low food conditions in the natural environment. The application of laboratory studies to the natural environment should be made with caution but I believe the patterns observed here can be tentatively applied to make predictions about the susceptibility of *S. balanoides* and *A. modestus* larvae to changes in environmental conditions as a consequence of climate change and how this may alter their recruitment success.

Clearly, the mono-algal rearing conditions do not represent the normal feeding conditions larvae would experience in the field. In their natural environment the available food would be more diverse and it is likely larvae would feed on a variety of phytoplankton plus small zooplankton during the later stages. A number of other studies have shown how both *S. balanoides* and *A. modestus* can both be reared on *S. costatum* (e.g. Harms, 1984; Stone, 1988; 1989) so it can be assumed that this food is nutritionally adequate and can be ingested and assimilated. Barnes (1962) has shown correlations between *S. balanoides* survival and high concentrations of *S. costatum,* but in the absence of *S. costatum* larval populations fail despite the presence of other species e.g *Chaetoceros*.

6.2. Ecological implications

In this study, the importance of shifts in food and temperature in determining the density and the quality of cyprids in determining settlement success was highlighted. Many benthic marine organisms rely on the dispersal of propagules to recruit to new populations and the supply and settlement of planktonic larvae are determinants of benthic invertebrate population dynamics (e.g. Menge, 2000). A number of studies (see review Pineda, 2010) have demonstrated a significant relationship between settlement input and recruitment. However, in recent years the assumption that all larvae reaching a settlement site are equivalent has been questioned (e.g. Tremblay et al. 2007. Larvae will experience different conditions during their planktonic life and may settle with radically different energy reserves. Differences in larval quality may thus modify a simple relationship between numbers of larvae arriving and those recruiting to the benthic population.

In this thesis, results from experiments that contained low food conditions (Chapter 2, 3) demonstrated scenarios where cyprid supply and recruitment were decoupled. For example, in chapter 2, high recruitment under low food conditions may be predicted for *S. balanoides* due to the high survival rates to cyprid. However, final settlement was low as cyprids were not able to survive the settlement process. Food limitation can result in reduced cyprid energy reserves where cyprids are unable to survive the energetically expensive process of metamorphosis through to settlement (Lucas et al. 1979; West and Costow, 1987; Qiu and Qian, 1997; Tremblay et al. 2007). Thus, the consequences of trophic mismatch on recruitment of species with complex life cycles can be manifested as increased larval mortality and as increased settlement failure.

The amount of energy a cyprid has varies depending on the quality and quantity of food it receives during its naupliar period. Poor cyprid settlement rates in this study were strongly linked with the presence of a low food environment during the later stages of development. This is because it is during the final naupliar stage that nauplii accumulate the reserves needed for metamorphosis (West and Costlow, 1987; Hentschel and Emlet, 2000). Settler quality appeared more affected in *S. balanoides* than in *A. modestus.* It is likely that these differences in quality, associated with trophic mismatches, may have fitness implications for post-settlement success as naupliar history can also have a direct affect upon juvenile performance and post-settlement survival through trait-mediated effects i.e. quality of cyprids (Thiyagarajan et al. 2003; Giménez, 2004; Emlet and Sadro; 2006; Pechenik, 2006).

Parental variability had an important effect throughout the thesis where larvae from some parents clearly outperformed others in response to food limitation and temperature. For *S. balanoides* these parental differences were linked with increased initial larval biomass. This variability could reflect maternal effects of parental environment upon the larvae or genetic differences among parents (Marshall et al. 2008). Intra-specific differences in survival and cyprid size may mean that parental effects may also be important in determining the density and abundance of settlers that recruit into a population. My results have two important implications: Firstly, if parental effects reflect genetic differences among offspring from different adults, my study suggests that trophic mismatch may result in erosion of the diversity of genes responsible for larval traits that determine larval capacity to survive under food limitation. If maternal effects (i.e. phenotypic plastic responses of the parental

phenotype to the environment) are underlying the parental effects, trophic mismatches may change the strength of selective pressures on parents.

6.3. Future impacts of climate change on *S. balanoides*: from physiology to populations

Climate changes can have a number of different direct and indirect effects upon species which are too innumerable to be covered within the scope of this discussion. Therefore, focus is placed upon a few potential impacts of climate change on *S. balanoides* through 1) the physiological effects of temperature upon performance 2) changes in turbulence 3) potential impacts upon the timing and quality of the spring phytoplankton bloom and 4) competition with other species.

6.3.1. Physiological effects of temperature

The magnitude of species responses to climate change under food limited conditions would be expected to vary, given that different species exhibit different thermal tolerances and optima (Pörtner, 2001; Monaco and Helmuth, 2011). For example, in this study the extent to which *S. balanoides* can exploit a match or tolerate a mismatch to a food source appears attributable to a suite of species-specific physiological processes that are regulated by temperature. Temperature has a fundamental effect on biological processes of an organism simply by its influence on molecular kinetic energy which determines the rate of cellular and systematic processes (Portner and Farrell, 2008; Hochachka and Somero, 2010).

One of the best ways to describe an organism's response to changing environmental conditions is through the use of physiological performance curves (Monaco and Helmuth, 2010). These thermal performance curves (TPCs) describe both an organism's physiological limits for survival and the temperatures under which that organism can survive and reproduce (Angilletta et al. 2002; 2003). TPCs follow a general shape: performance typically increases as temperature increases, reaches a maximum at some intermediate temperature (T_{opt}), and then, above the optimum, declines rapidly (Huey and Stevenson 1979; Huey and Kingsolver 1989, 1993; Angilletta et al. 2002; Angilletta, 2009). TPCs have great potential in helping to predict the responses of populations or species to climate change (e.g. Huey and Kingsolver, 2011). My experiments do not evaluate the TPCs for *S. balanoides* but do indicate

a reduction in performance at 15^{o}C, even under optimal food conditions, thus demonstrating that increases in seawater temperature in the spring time are likely to have a negative influence on this species.

Interactions between temperature and other abiotic and biotic factors can alter the relationship between temperature and performance, modifying the TPC (Pörtner and Farrell, 2008). A number of studies have highlighted the importance of food and temperature on naupliar development and survival during the naupliar period (e.g. Harms, 1984; West and Costlow, 1987), but most have studied these factors independently and relatively few have examined their combined effects (e.g. Anil and Kurian, 1996; Anil et al. 2001). TPCs must be affected by food limitation because food availability and temperature can jointly determine assimilation and allocation of energy in nauplii (Anil and Kurian, 1996) and reduce the energy available for growth and development (Harms, 1987; Houde, 1987; Pechenik, 1987). In Chapter 3 (Mismatch experiment) nauplii of *S. balanoides* reared under low-high food had relatively good survival to cyprid and cyprid settlement at a temperature of 9^{o}C, but at 15^{o}C there was poor naupliar survival and an almost complete failure of settlement. This highlights how important it is to examine the role of temperature in the context of food limitation experiments to get a more accurate prediction of species-specific responses.

Current climate change scenarios predict ongoing global warming with a further increase in sea water temperature of 2-3^{o}C over the 21st century (IPCC, 2007). It is therefore likely that larvae will be exposed to increasing sea temperatures in the future, causing a decline in recruitment of *S. balanoides*, regardless of food conditions. Repeated low recruitments on a large scale may eventually contract the southern margin of the species range as this can affect the size of the larval pool over subsequent years for the entire region, especially for isolated populations (Svensson et al. 2005). Studies in SW England suggest that extremely low levels of *S. balanoides* recruitment can occur quite frequently (Southward 1967, 1991; Jenkins et al. 2000). Given the warmer temperatures in this region, I propose that many southern *S. balanoides* populations may experience a future scenario of continuously low recruitment driven by reduced reproductive output and poor settlement success.

6.3.2. Consequence of turbulence for the larval food environment

Field studies suggest that the food conditions and the level of nearshore turbulence may be key factors affecting larval survival and settlement success. Over the years, field and laboratory research have proposed that larval release of *S. balanoides* is triggered by the spring phytoplankton bloom (Barnes, 1956, 1957, 1962; Starr et al. 1991), but recent evidence has demonstrated that cues other than phytoplankton can trigger larval release. For example, turbid conditions associated with storm events have been shown to elicit synchronous larval release (Gyory, 2010; Gyory and Pineda, 2011).

Although the precise mechanisms that trigger larval release may be in contention, the research presented in this thesis, along with a number of studies, shows that the successful recruitment of *S. balanoides* depends on matching larval release with the spring bloom (Crisp and Clegg 1960; Barnes 1957b, 1962, 1963; Connell, 1961; Hawkins and Hartnoll, 1982). There is currently a consensus that global warming will be accompanied by an increase in the magnitude and frequency of storms in the northern hemisphere (Goldenberg et al. 2001; Emanuel, 2005). This effect may trigger adults of *S. balanoides* to release their larvae prior to the peak in the phytoplankton bloom, so mismatches of larvae with their food sources may be more likely to occur than previously anticipated.

6.3.3. Climate driven variations in food availability

Diatom blooms in the spring have remained relatively fixed in time as they are dependent on light intensity, which is invariant to global warming (Sommer et al. 1986; Edwards and Richardson, 2004). However, cloud cover is expected to increase with climate change; reductions in global radiation (~10%; predominantly due to increased cloud cover) have already taken place in the last few decades ("global dimming"; Stanhill & Cohen, 2001; Liepert, 2002; Roderick & Farquhar, 2002). This "global dimming" could alter the timing and magnitude of phytoplankton blooms (Sommer et al. 2012; Winder et al. 2012), leading to mismatches in a temporal and abundance sense. Reduced light intensity has also been shown to reduce the cell size of phytoplankton which can have a strong effect on phytoplankton peak biomass (Sommer et al. 2012).

Temperature elevation has also been shown to have a strong negative influence on cell size and peak biomass of phytoplankton (Sommer et al. 2012; Winder et al. 2012). This is through effects of the temperature-size rule (TSR; Atkinson et al. 1994), where the average size of individuals is inversely related to temperature. These studies, along with others (e.g. Moran et al. 2010; Hoegh-Guldberg and Bruno, 2013) predict a gradual shift toward smaller primary producers and reduced productivity in a warmer ocean. Changes in phytoplankton abundance or productivity can lead to situations where, despite a match in time and space between a predator and its prey, recruitment success of the prey is unsuccessful or reduced owing to the fact that food is present only in limited amounts (Durant et al. 2005). Clearly there are a number of mechanisms associated with climate change that may have an effect on the timing, quality and magnitude of the spring phytoplankton bloom that may have consequences for the recruitment success of *S. balanoides*.

6.3.4. Indirect effects of climate change: competitive abilities

Complex and indirect effects of climate change also need to be taken into account if we are to accurately forecast the long-term effects of global warming (Poloczanska et al. 2008). For example, competition with other barnacle species, such as *A. modestus* and *Chthamalus spp*, may also mediate the recruitment success and distribution of *S. balanoides*. Given that these species are spatial competitors to *S. balanoides* they are likely to benefit from changes in SST where its distribution with *S. balanoides* overlaps, as they would perform better in the warmer temperatures. Under all of the climate scenarios investigated by Poloczanska et al. 2008, *S. balanoides* is predicted to virtually disappear from southwest England by 2050, while the abundance of the Chthamalid species will increase. Local population extinctions of *S. balanoides* have already been observed at some locations in southwest England (Southward et al. 1995, Miezskowska et al. 2006) suggesting that increasing SST will indeed lead to further extinctions along the southern edge of the species' current range.

6.4. Perspectives: a model system for other species?

The approach used in this study to examine the potential effects of climate change on *S. balanoides* and *A. modestus* could possibly be applied to other marine invertebrate species with complex life-cycles to explore how climate-driven changes may affect recruitment

success of other populations. It could be hypothesised that other boreal species will also be particularly susceptible to these climate driven changes in food supply, as they often spawn or release larvae in single pulses in synchronisation with the spring or autumn phytoplankton blooms (Starr et al. 1990; 1991). Therefore, as with *S. balanoides,* it is likely these species are dependent on matching to an abundant food source for recruitment success. If they become decoupled from their food source the number of settlers entering the population may be reduced, either through effects mediated upon larval density or larval quality.

As boreal species typically reproduce and release larvae during a single defined season, there would no chance for recruitment levels to the population to recover from the failure event for that year. In contrast, it may be that lusitanean species would be more likely to be able to tolerate low food periods; even if they do experience larval mortality, the release of multiple larval broods means there would be a number of other opportunities to add to the population throughout the larval season preventing catastrophic decline of the population. With global temperatures set to increase, this may amplify the current trend of coldwater adapted species decreasing in abundance and retreating poleward whereas warm water species are increasing in abundance and advancing.

6.5. Conclusions

In the study, climate-driven changes in the timing and abundance of food sources were shown to be critical for the recruitment success of *S. balanoides* and less important for *A. modestus*. Temperatures of 15^{o}C and above were sub-optimal for *S. balanoides* and tended to amplify the negative effects of food limitation. Both factors had strong effects on naupliar mortality and cyprid settlement success rates for *S. balanoides* and, thus, were important in determining the supply and quality of settlers to a population. High mortality during the larval stages can constitute a bottleneck for recruitment which can potentially have substantial effects on population dynamics and community structure. The improved performance of *A. modestus* may be related to adaptations which allow for higher mass-specific feeding rates which accommodate increased growth and development under food limited conditions. This study highlights that in order to get more accurate predictions of species-specific responses to climate change the interactive effects of different environmental factors at different life stages should be considered.

CHAPTER 7

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