Parental and environmental effects on the early life history of a tropical reef fish, *Amphiprion melanopus*

PhD thesis submitted by

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This thesis includes some collaborative work with my supervisor Dr Mark McCormick, Dr Kenneth Anthony and Dr Rebecca Fisher. While undertaking these collaborations, I was responsible for the project concept and design, carrying out the experiments, their analysis and interpretation and synthesis of the results into a format suitable for publication. My co-authors assisted financially, with editorial advice and technical instruction for experimental equipment.

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General abstract

Tropical coral reef fish larvae are characterised by high mortality, which is predominantly driven by size- and growth- selective processes. While recent studies of environmental correlates have explained 7 - 36 % of the variation in larval growth rate in wild populations, the majority of the variation in growth rate and recruitment remains unexplained. This thesis used a series of laboratory experiments to assess the contribution of environmental and parental influences on embryonic, larval and juvenile growth and development in a tropical marine fish species, *Amphiprion melanopus* (Pomacentridae).

Maternally determined egg size coupled with clutch micro-environment was important in determining initial offspring size. By sampling embryos and larvae for morphometric measurement and metabolic rate, we found that size differences between offspring within a clutch were related to the clutch design. Eggs on the periphery of newly laid clutches were 2% smaller than eggs from the centre, and this size difference increased throughout embryonic development. Larvae hatched from the clutch periphery were 6 – 8 % smaller than larvae hatched from the clutch centre. Embryos on the clutch periphery had 63 % lower rates of oxygen consumption. Changes in oxygen consumption throughout development were related to developmental changes within the embryo.

Given that the study species, like many demersal spawning fishes, has parental care of the eggs, we explored whether parental tending modified the oxygen microenvironment of the embryos, and subsequently, whether tending was modified according to ambient dissolved oxygen (DO), increasing metabolic demands of developing embryos and water temperature. There was a time lag of 1 second between fanning and increases in the amount of oxygen within the nest, demonstrating that DO is directly affected by parental tending. Males invested more time tending nests (40 % initially) than did females (20 – 30 %), and male investment increased to 70 % as embryo development progressed. Additionally, male fish adjusted fanning effort on a diel cycle as ambient DO fluctuated. The female's investment in nest tending was minor in comparison to

the males and did not change with ontogeny, with the exception of a small increase in activity just prior to hatching. Nest tending appears to be an important mechanism whereby males can invest in the survival of their offspring.

To determine the relative importance of maternal, paternal and environmental (specifically temperature) influences on early life history traits, we experimentally examined their interactive influences on larval growth, swimming ability and developmental rate using a full factorial (diallel) breeding design. There were strong paternal and maternal influences in size at hatching and metamorphosis, and surprisingly, paternal affects were responsible for 52 % of the variation in growth rate, while 30 % was attributable to the combination of temperature*female*male. We speculate this was due to the significant male contribution through their key role in nest tending. Pre-hatch egg size, post-hatching larvae size and size at metamorphosis all showed significant influences from male and female, and the interaction of these, while temperature had minimal influences on size at particular development stages. Temperature did, however, reduce developmental rate, increasing the time taken to reach metamorphosis by 50%. Larvae reared in water 25 $^{\circ}$C (3 $^{\circ}$C below ambient) were smaller than larvae reared at ambient temperature (28 $^{\circ}$C) at the same age (7 days after hatching, dah), and had slower critical swimming performance but took longer to metamorphose (mean: 8.9 ± 0.06 days at 28 ºC and 11.6 ± 0.09 days at 25°C). When this slower developmental time was factored in to size and swimming, fish reared at 25 $^{\circ}$C were larger at similar developmental age (11dah, pre-metamorphosis). This stage-specific size increase did not result in better performance as there was no difference in swimming ability immediately prior to settlement (11dah), despite slower swimming for larvae raised at 25 $^{\circ}$C, 7dah.

This thesis shows that position of an embryo within a clutch, maternally-determined egg size and subsequent parental care were important in influencing the condition and performance of marine fish embryos and larvae. Size advantages began in the embryonic stage due to maternal investment through gametogenesis and the allocation of endogenous reserves to the egg, and were enhanced throughout development. This thesis suggests that parental contributions to the embryo were important to size, growth and performance of

larvae, and may be the source of previously unexplained variation in larval growth and survival.

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General Introduction

Marine fish are characterised by high fecundity and almost equally high mortality. Despite the importance of larval survival to successful recruitment and replenishment of fish stocks, the processes underlying mortality are poorly understood. Commencing with Hjort's influential paper in 1914, nearly 100 years of research has related population-level recruitment (as the end-product of larval survival) to broad-scale environmental variation within the larval period (Houde 1974). The primary focus of such research was to predict the strength of recruitment by correlating larval growth (Tupper & Boutilier 1995) and condition (Cushing 1972, Theilacker 1978) to environmental variability such as food availability (Lasker 1975, Hunter 1981) and temperature (Brett 1967, Houde 1989a). These critical studies determined that variation in daily growth and mortality rates can lead to orders of magnitude difference in survival and recruitment (Houde 1989b, Cushing & Horwood 1994).

The multitude of factors that act on the early life history stages of marine fishes, affecting growth, condition and survival until recruitment, fall into three key categories: 1) genetic inheritance; 2) environmental influences; and 3) parental effects. Selection based on these factors operates on individuals, and is consequently expressed in populations. This thesis examines how parental and environmental influences are borne into the early life history traits of fishes within clutches in tropical marine fishes, investigating differences within and between clutches at all stages of development leading up to recruitment. Genetic inheritance is beyond the scope of the present study and will not be considered further here.

Environmental influences comprise a broad spectrum of factors that impact on growth and condition of larval fish, including abiotic influences, such as: temperature (Green & Fisher, 2004), salinity (Swanson 1996) and turbidity (Fiksen & Folkvord 1999); and biotic factors, such as: food availability (Green & McCormick 1999a), predators (Margulies 1989) and competition (Bystrom & Garcia-Berthou 1999). As it is not possible to examine all components of environmental variation that a developing fish might encounter, we have

selected the two most influential traits on fish metabolism in early development: temperature and dissolved oxygen (Rombough 1988, Jobling 1995), for examination in this thesis.

The majority of research on factors affecting the early life history of marine fishes and the recruitment/mortality relationship has focused on temperate northern hemisphere species, generally of commercial importance. Although these studies have provided a solid framework of theory for investigations into recruitment in tropical fishes, several critical environmental and biological attributes of tropical marine fishes differ from their temperate counterparts. Key developmental stages are much shorter (Green & McCormick 2001) and the larval stage finishes with a distinct habitat shift from plankton into the benthic reefal habitats of adults (see discussion in Bergenius et al. 2002). In the tropics, temperatures are higher and fluctuate less than temperate waters (McGregor & Nieuwolt 1998). For example, sea surface temperatures on the Great Barrier Reef, Australia, fluctuate from 4-6°C seasonally, and 1°C diurnally (McGregor & Niewolt 1998). The relative importance of temperature change in the tropics has been alluded to (Rombough 1997, Hunt von Herbing 2002), but rarely tested. Early development such as the larval phase is especially susceptible to temperature change (Jobling 1995, Rombough 1997), as it influences metabolism, growth and development (Meekan et al. 2003). Within this thesis, the effects of temperature change on growth, development and performance will be examined for the first time in a tropical marine fish.

While environmental influences have long been considered critical in determining variation in growth rate and survival (Hjort 1914), parental influences have received attention relatively recently (Blaxter 1969, Solemdal 1970). Parental effects are the “non-genetic influences derived from parental phenotypes or environments that have an impact on offspring phenotypes” (Heath & Blouw 1998, p178), although it is not always possible to separate the genetic influence from parental effects. Non-genetic *maternal* effects have been detected more frequently than *paternal* effects, and thus parental effects are generally referred to as ‘maternal effects’.

Maternal effects can occur through a variety of pathways including cytoplasmic inheritance, nutrition, transmission of pathogens and antibodies and

behavioural interactions with offspring (Bernado 1996). These effects may be manifested in many traits of offspring, including quality, quantity and behaviour. For example, the occurrence of diapause in flesh fly offspring is determined by the day length experienced by the mother (Denlinger 1998), and sprint speed in lizard offspring is determined by maternally aliquoted egg size (Sinervo 1990). Maternal influences have been identified as central in determining condition and survival of offspring in a large range of taxa including vertebrates (Bernado 1996), insects (Mousseau & Dingle 1991) and plants (Mazer & Wolfe 1998). The variation in propagule characteristics due to maternal effects occurs at all scales within a (meta)population, that is, between full siblings within clutches, between clutches from the same female, within species and between populations in fishes (Chambers & Leggett 1996) and frogs (Kaplan 1997).

While evidence of significant maternal influences on offspring phenotype and survival have been well documented both in plant and animal kingdoms, they are seldom considered as a source of variation in growth of marine fish larvae. Over three decades ago, maternal effects in fishes were noted as one of the most important characteristics in developing marine fishes, particularly in that the size of the female can influence the viability of offspring through conditions of incubation, fecundity and egg size (Blaxter 1969). However, it was not until recently fisheries biologists and ecologists have begun to consider that quantifying variation in the condition of individuals may be the key to understanding variable recruitment (rather than eliminating it as 'experimental noise' Falconer 1981). Subsequently, maternal effects have been examined relative to variation in offspring size, condition, viability and abundance, and strong relationships have been identified between attributes of the female and the life-history characteristics and body condition of her offspring, particularly at hatching. These mother/offspring relationships include: female size and egg size (Chambers & Leggett 1996, Benoit & Pepin 1999); female condition and egg yolk volume (Chambers et al. 1989, Kerrigan 1997); female condition and egg size (Chambers & Waiwood 1996); and female size, condition and age and egg and larval size (Marteinsdottir & Steinarsson 1998). Examination of maternal/parental effects is not commonplace, however, these studies have established parental effects as an aspect of individual history that is crucial for the comprehensive understanding of population dynamics of fish populations.

While these studies suggest that the maternal contributions to egg and early larval quality are important, the progression of these initials differences is rarely followed to determine maternal effects on the condition, growth and performance of late stage larvae and new recruits. Additionally, paternal effects are seldom considered and rarely identified, (with a few notable exceptions, e.g. Rakitin et al. 1999, Rakitin et al. 2001, Rideout et al. 2004), despite evidence that paternity can determine recruitment success (Knouft et al. 2003), hatching success, larval size, and yolk size (Rideout et al. 2004). While the egg is initially provisioned by the female through gametogenesis, the male parent contributes half of the genetic material, and the majority of the nest care in demersal brooding species (Clutton-Brock 1991).

Prior to the pelagic larval period (which has been the focus of most research in the last 100 years), is the embryo stage where offspring subsist on endogenous resources, provisioned by the mother. Female provisioning is a key source of variation in size of fishes at hatching (Kerrigan 1997). The embryo stage varies in length in tropical fishes from 24 - 48 hours for pelagic eggs from broadcast spawners, and up to 8 days for benthic eggs. For broadcast spawners, input into the early life history ceases after they have selected a suitable spawning site and released their clouds of gametes into the water column. Alternatively, benthic spawners attach their eggs to the substratum, often in discrete monolayered clutches and intensively guard these nest sites (Robertson 1991). While egg provisioning and spawning site selection are critical to benthic and pelagic eggs, benthic spawners can further influence their offspring survival through maintenance of the embryos' environment. Benthic eggs develop in the parentally chosen environment, which can differ markedly from the pelagic larval environment. It follows that different environmental processes may influence growth, condition and survival in benthic eggs than those which influence the pelagic larval stage. Due to the extended nature of embryogenesis in the 14 % of tropical species that develop from benthic eggs (Leis 1991), embryonic processes are important to subsequent larval growth and survival. Since growth trajectories are often established during early development, selective processes in the larval phase can act on growth variability derived from the benthic embryo phase and determine which individuals survive through to recruit as juveniles.

The species that is the focus of the present study, *Amphiprion melanopus* (Pomacentridae), is a benthic spawning reef fish with three distinct early life history stages: an 8-day egg stage, provisioned by the female and tended by both parents; an 8-day free swimming larval stage in the variable pelagic environment; and benthic juvenile where individuals compete within a size-selective hierarchy for limited space within their host anemones. This species provides a unique opportunity to separate factors affecting a tropical marine fish during its early life history stages, considering the interaction of paternal, maternal and environmental effects on growth development and performance at all life stages.

Recent studies of tropical species linking size at hatching and larval growth to post-settlement survival (Suthers 1998, Vigliola & Meekan 2002, McCormick & Hoey in press) found that both food and temperature contributed significantly to larval growth rates and survival (Booth & Hixon 1999, Meekan et al. 2003). While these studies have shown conclusive evidence that environmental variation influences growth in the larval stage, which in turn, is critical to survival and recruitment strength in tropical marine fishes, these relationships only explain part of the variation in growth. That is, only 7 - 36% of growth variation in larvae can be attributed to variation in water temperature, rainfall and wind in a Caribbean damselfish (Wilson & Meekan 2002). Due to an historic focus on larval biology as the key determinant of recruitment dynamics (see Houde 1987 and Robertson 1991 for discussion) and the logistic difficulties of sampling larvae and tracing parentage in wild populations, these correlations only address part of the lifecycle (the pelagic larval period), and therefore only answer part of the mortality/recruitment question. Broadly, this thesis investigates the causes and correlates of the remaining variation in growth of marine fish larvae by considering the relationship between parentage and environment for all stages of reef fish life cycle: eggs, larvae and juveniles.

This thesis examines some of the variability found within and between clutches of tropical reef fish and determine how parental influences interact with environmental conditions to influence the size, growth and performance of offspring from egg to juvenile. It further addresses the variability within and between clutches of fishes as they ultimately determine life history traits at the

population level, testing the key hypothesis *that variations in larval characteristics originate from parental influences and are subsequently modified by their environment.*

In order to test this hypothesis, I constructed 3 specific aims:

1. To determine the importance of the embryonic period to development and condition at hatching I investigated:
    - the structural and physiological changes an embryo undergoes through development in relation to changing oxygen requirements.
    - the relative variation in size and physiological measures such as oxygen consumption amongst embryos within a clutch;
2. To describe relative roles of male and female parents in nest tending in response to minor environmental variation, summarising the parental contribution to their offspring through nest tending behaviour;
3. To investigate how parental and environmental influences interact and affect embryo and larval and juvenile condition by differentiating between paternal, maternal and environmental contributions to larval condition and performance.

Each of these aims is comprehensively addressed in a chapter as detailed below.

This thesis commences by examining embryological details and progresses through the early life history stages of fish development with each ensuing chapter. **Chapter 1** provides some essential background material on embryo development and the concurrent general trend in embryological oxygen consumption. **Chapter 2** follows on by investigating how size differences of embryos within a clutch are related to oxygen consumption and the initial maternal aliquot; and are consequently the source of size variation in larvae at hatching. Further parental contributions throughout embryological development are considered in **Chapter 3**, through an examination of nest-directed tending behaviour in response to variable temperature and oxygen content of the water, and then related back to the changing embryonic oxygen requirements examined in Chapter 1. Chapter 3 explores the paternal contribution to embryonic development by examining the contribution of males to nest tending. In **Chapter 4** we further examined the effect of environmental factors on

performance of larvae through critical swimming trials, and whether decreased temperature decreased rates of growth and development. To elucidate how parental effects interact with temperature variation to influence egg and larval traits, we used a diallel (or full factorial) cross breeding experiment. This design enabled me to partition the variance in larval condition and swimming performance due to temperature change and to parentage (female and male) (**Chapter 5**). Details of the study species, *Amphiprion melanopus* (Pomacentridae) are included in each chapter, highlighting the features that make it a good model for each specific question addressed.

This dissertation is written as a series of stand-alone, though conceptually interconnected, publications (see Appendix for full list of publication details), tracing through the life cycle of the fishes from conception to metamorphosis. Each chapter considers how parental and environmental influences interact and affect each phase of development, and in turn, how this is expressed as growth, condition or performance of the offspring. The idea that variability in juvenile size and quality at recruitment may stem from embryonic or larval stages of development underpins this thesis. To this end, both larval and embryonic life stages are explored as sources of variability, and both stages are investigated in relation to environmental and parental influences. This thesis uses a series of laboratory experiments to manipulate parentage and environmental variables while holding all else constant to determine whether events and influences ***prior*** to the oft-sampled pre-settlement larval stage might be important in determining condition of larvae and recruits. No studies have yet addressed nest care, maternal aliquot and environmental variation to separate the sources of variability in larval growth and size and performance at metamorphosis, and as such, this thesis represents the first comprehensive attempt to consider all parts of the life cycle as sources of variation in larval growth.