

**AN EVALUATION OF GEOGRAPHIC VARIATION IN  
THE LIFE HISTORY AND BEHAVIOUR OF  
ANEMONEFISHES: A COMMON-GARDEN APPROACH**

**PhD thesis submitted by**

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## STATEMENT OF CONTRIBUTION OF OTHERS

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## Abstract

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The parameters of a species life history and performance do not vary independently. Co-varying traits provide the basis for hypotheses as to how individuals allocate energy, as populations genetically adapt to or phenotypically adjust to different environments. In coral reef fishes, the extent and underlying causes of such patterns across large geographic scales are poorly understood. Intraspecific phenotypic variation may be evident on a broad geographic scale, for example when comparing populations that are geographically isolated or those along a latitudinal cline. Such variation may be the consequence of local genetic variation due to natural selection, and/or it may reflect phenotypic and behavioural plasticity that is largely environmentally determined. Discriminating between these potential causes of local population differentiation is an important goal of evolutionary ecology. This study provides a comprehensive description of geographic life history variation (reproduction, growth, mortality) and performance (swimming ability) in a long-lived group of coral reef fishes (subfamily Amphiprioninae). It presents a unique evaluation of the degree to which life history and performance traits may be coupled, and determines whether these traits are environmentally induced *vs.* genetically determined by employing an integrated set of common-garden experiments.

*Chapter two* of this thesis describes a comparative field study that examined latitudinal differences in the life history characteristics of three species of anemonefishes (*Amphiprion melanopus*, *A. akindynos* and *Premnas biaculeatus*) among three locations, including Kimbe Bay in West New Britain, Papua New Guinea (5°30'S/150°05'E), and Lizard Island (14°40'S/145°28'E) and One Tree Island (23°30'S/152°05'E) on the Great Barrier Reef, encompassing 18° of latitude. Life

history theory predicts that populations from higher latitudes should exhibit larger adult body sizes, but smaller size at age (i.e. slower growth). Concomitant with slow growth, a delay in sexual maturation is expected, which should be compensated through the fecundity advantage of a larger size and longer lifespan. As predicted, maximum age increased with increasing latitude in all three species. *A. melanopus* always had a higher maximum age than either *A. akindynos* or *P. biaculeatus* at each location, reaching 38 years at the highest latitude location. At this location, *A. melanopus* also had a larger adult body size, slower growth, lower mortality, greater proportional age and size at maturity and sex change, larger egg size and higher batch fecundity than at either of the lower latitude locations, and thus conformed well to life history theory. For the other two species, maximum body size was smaller at the higher latitude location, there were no differences among locations in growth and mortality rates, and no consistent trends in the timing of maturation or sex change. Thus, while longevity varied with latitude as expected in all three species, some of the other life history traits examined did not. The divergence in magnitude and direction of life history patterns among these species suggests that there are processes beyond simple trade-offs that determine life history strategies. I discuss the hypothesis that in anemonefishes, selection for flexibility in life histories imposed by a highly specialised habitat and rigid social structure may oppose selection over latitudinal environmental gradients.

Geographic variation in life history phenotypes between populations of a species is often assumed to reflect genetic divergence caused by natural selection. The relative contribution of genetic and environmental sources of phenotypic variation has never been determined in coral reef fishes. Yet, distinguishing between these sources of variation is fundamental to understanding the ecological and evolutionary significance of geographic life history variation. A conventional way of demonstrating the genetic



basis of adaptive variation in the wild is to perform a common-garden experiment, in which individuals from different local populations are compared under identical environmental conditions established in a laboratory. This approach formed the basis of the following three chapters.

In *chapter three* I determined whether the latitudinal variation in maternal reproductive traits observed in the wild has a genetic basis or whether it represents a phenotypic response to the environment. Adult fishes of the three species were collected from three latitudes along the Great Barrier Reef (Lizard Island, Britomart Reef at 18°23'S/146°63'E and One Tree Island) and their reproductive performance was monitored in a common-environment for up to 3 years. Based on life history theory and previous field observations I predicted larger egg sizes, but lower egg numbers in populations from higher latitudes. However, size-specific egg size, fecundity and reproductive output did not differ among populations in any of the three species held under identical environmental conditions. Thus, any divergence in these traits observed among natural populations most likely results from environmentally induced plasticity, rather than genetic divergence.

The next chapter describes another common-garden experiment with a full-sib design to examine differences in early growth rates and growth plasticity and among the latitudinal populations described above. Temperature and food availability were manipulated under controlled laboratory conditions to determine whether there is intraspecific variation in growth plasticity in response to these two environmental factors. Given that environmental variability generally increases with latitude I expected that offspring from high-latitude populations should display greater levels of plasticity compared to their low-latitude counterparts. In all three species, mean growth rates differed significantly among populations and were consistent with the co-gradient

variation hypothesis (i.e. offspring from low latitudes grew faster than offspring from high latitudes). Demonstrated under common-garden conditions, this implies that population-level variation in the growth capacity of juvenile anemonefishes has a genetic basis and should therefore respond to natural selection. Furthermore, significant genotype x environment interactions suggest the evolution of different growth reaction norms in response to temperature and food availability at different latitudes, although the variation did not always follow the predicted pattern. Overall, these results provide evidence for adaptive genetic differentiation for growth rate and growth plasticity in the early juvenile stages of these latitudinal populations.

Swimming abilities of larval fishes are important to their survival, potentially affecting their ability to avoid predators, obtain food and control dispersal and recruitment patterns. To date, no attempt has been made to quantify geographic and population-level differences in swimming performance of coral reef fishes. The aim of *chapter five* was to determine if, and to what extent, there is heritable variation for swimming performance among populations of the three anemonefish species across a latitudinal gradient. Flume-based trials were conducted to measure maximum sustainable swimming speeds (or U-crit) as an estimate of aerobic capacity of laboratory-reared pre-settlement larvae. My results confirmed that these fishes have different intrinsic swimming capabilities, such that offspring from high-latitude populations out-performed those from lower latitudes in all three species. These consistent behavioural differences between populations in the laboratory may reflect adaptive specialisation in response to latitudinal changes in coral reef environments. Mean swimming speeds were inversely related to mean body sizes among populations, suggesting that observed patterns may arise from a trade-off with growth rate. This demonstrates that developmental attributes and performance are interrelated in a

complex fashion, with multiple selection pressures acting on multiple aspects of the phenotype, and that adaptation involves trade-offs among competing functions.

This study has shown that both phenotypic plasticity and genetic differentiation across environmental gradients may exist for important fitness-related traits in coral reef fishes. The high potential for gene flow and lack of obvious barriers to dispersal in these species does not appear to preclude local adaptation in life history and larval swimming capacity along a latitudinal gradient. These results are significant steps forward in resolving the evolutionary and ecological processes that explain the substantial variation in the life histories and behavioural abilities of coral reef fishes.

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# CHAPTER 1: General Introduction

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The evolution of population and species traits is founded on the premise that variation in phenotypes reflects adaptive changes in the gene pool of a population through the process of natural selection (Arnold 1983; Endler 1986). Because the fitness consequences of any particular phenotype are the result of complex interactions among an individual's genotype, morphology, behaviour, and the local environment, a phenotype best suited for one set of environmental conditions may not perform best in another (e.g. Stearns 1992; Schluter 1993). The resulting variability in organismal traits among animal populations and the extent to which they have diverged in response to local environmental conditions are core issues in evolutionary ecology (Endler 1977). Geographic variation, "the occurrence of differences among spatially segregated populations of a species" (Mayr 1963), has been the subject of extensive theoretical and empirical work (see Roff 1992; Stearns 1992; Charnov 1993 for recent reviews) and still retains its central role in modern debates on the nature of adaptation and speciation.

## 1.1. Geographic variation and life history strategies

### 1.1.1. *Life history theory*

A life history is a heritable set of "rules" that determines age-specific allocations of time and energy that an individual makes over its lifetime (Dunham et al. 1989). The rate at which an individual grows, for example, reflects proportional allocation of assimilated energy made to growth, maintenance, storage and reproduction (Dunham et al. 1989; Begon et al. 1996). Life history traits are intimately related to fitness and are therefore expected to evolve in response to local environments under the scrutiny of selection (Roff 1992; Stearns 1992; Bernardo 1994; Charlesworth 1994). A body of

theory has been developed which seeks to predict life history patterns that are driven by the effects of environmental conditions on age-specific schedules of growth, fecundity and mortality (reviewed in Roff 1992; Stearns 1992). Life history theory permits formulation of specific hypotheses regarding co-variation in major life history characters across latitudes, under the assumption that the process of selection favours those genotypes that generate the highest per capita rates of increase relative to other genotypes in the population.

### ***1.1.2. Environmental gradients***

Large-scale environmental gradients are common in nature and are recognised to exert a major effect on life history patterns (Mizera and Meszema 2003). The gradients of increasing water temperature from the poles to the tropics (Figure 1.1a) and decreasing temperature seasonality (Figure 1.1b) have received the greatest attention in the ecological literature (e.g. France 1992; Lyons and Willig 1999; Cardillo 2002). Temperature is perhaps the most important abiotic factor affecting the physiology and ecology of organisms (Johnston and Bennett 1996), especially for those that rely on external sources for their body heat (Bennett et al. 1992; Atkinson 1994; 1996). For ectothermic animals, temperature is known to have a significant effect on a multitude of traits, including metabolic rate, growth and overall size (Brett 1979; Rilling and Houde 1998). Thus, temperature is considered to be of fundamental importance in understanding biogeographical variation in life history patterns (Sebens 1987; Atkinson 1996). However, temperature gradients alone are not the universal explanation for clinal life history patterns. Along a latitudinal gradient there will typically also be changes in other abiotic and biotic factors such as photoperiod, habitat structure, food availability

and encounter rates with predators and competitors (Sale 1991; Caley 1995b; Yamamoto et al. 1999).

Species distributed across broad geographic ranges provide an opportunity to study intraspecific changes and co-variation in life history in response to these environmental gradients. General trends in life history parameters have been linked to latitudinal gradients in a wide variety of organisms, and include traits such as morphology (e.g. Forsman and Shine 1995), growth (e.g. Sand et al. 1995; Jensen et al. 2000; Pegg and Pierce 2001), reproductive characteristics (e.g. Iverson et al. 1993; Lardies and Castilla 2001; Hatle et al. 2002; Abookire and Macewicz 2003), larval development (e.g. Riha and Berven 1991), thermal sensitivity (e.g. Van Berkum 1988; Castaneda et al. 2004) and mortality (e.g. Frankino and Juliano 1999; Choat et al. 2003). Because similar patterns of life history variation have evolved repeatedly, independently, and even on different continents, it is believed that these patterns of phenotypic variation are generally adaptive (Endler 1986).

### ***1.1.3. Geographic variation in body size, growth and longevity***

Life history theory has been developed to analyse the relationship between the characteristics of an environment and the life history patterns of populations experiencing it. One general prediction is a trend towards larger body size at higher latitudes (Figure 1.1c), which is usually achieved by slower, prolonged growth over a longer life span (reviewed in Atkinson 1994; Mangel and Stamps 2001, Figures 1.1d and e). The observation that organisms developing in colder environments tend to grow bigger is known as Bergmann's rule, which was originally proposed to describe interspecific variation in endotherms (Bergmann 1847). Today, Bergmann's rule is more generally applied to describe an increase in the body size of a species as latitude

increases or ambient temperature decreases. The original adaptive explanation suggested that larger individuals possess smaller surface-to-volume ratios that are more conducive to conserving heat (Bergmann 1847; Ashton et al. 2000). Current evidence suggests that Bergmann's rule also extends to certain groups of ectotherms (Arnett and Gotelli 1999; Huey et al. 2000; Ashton and Feldman 2003; Morrison and Hero 2003), although heat conservation is likely not the source of selection in these organisms (Atkinson 1994; Atkinson and Sibly 1997; Partridge and Coyne 1997). Though the opposite trend also exists and is primarily mediated by season length rather than temperature *per se* (Mousseau 1997), clines in body size consistent with Bergmann's rule are common (Conover and Present 1990; Atkinson 1994; Atkinson and Sibly 1997; James et al. 1997).

#### ***1.1.4. Geographic variation in reproductive characteristics***

The inhibiting influence of low temperatures on ectotherm growth rates has follow-on effects on other important life history traits. If longevity increases with latitude, it follows that mortality rates are higher at lower latitudes (Stearns and Koella 1986; Hutchings 1993; Metcalfe and Monaghan 2003, Figure 1.1f). These combined effects of growth and survivorship, in turn, have a strong influence on reproductive characteristics such as age at maturity and level of reproductive effort (Charlesworth 1980; Kozlowski and Uchmanski 1987). As reproductive maturity is dependent on body size, populations with faster growth rates (low latitude) will reach the size required for reproduction sooner than those with slower growth rates and thus should be able to begin breeding at a younger age (Figure 1.1g). It has also been argued that populations suffering high levels of mortality (low latitude) should enter the reproductive populations as soon as possible to shorten generation length and ensure successful

propagation before death (Sutherland et al. 1986; Promislow and Harvey 1990; Shine and Charnov 1992; Abrams and Rowe 1996; Gasser et al. 2000).

Reproductive effort, on the other hand, is predicted to decrease with increasing latitude (Reznick et al. 1990; Bertschy and Fox 1999; Frankino and Juliano 1999, Figure 1.1i). The lower temperatures and shorter growing seasons associated with higher latitudes restrict the time available for energy accumulation. This, in turn, results in shorter breeding seasons and restricts the timing of reproductive bouts and the number of clutches that can be produced in a season. Further arguments involve age-specific mortality patterns and the trade-off between reproductive output and survival: at low latitudes, where adult survival rates are low, the proportion of assimilated energy allocated to reproduction should be high to compensate for the reduction in lifetime egg production resulting from reduced longevity (Reznick 1983; Case 2000; Gasser et al. 2000).

The next question is how reproductive effort should be packaged. The trade-off between egg size and clutch size for a given clutch volume influences the fitness of both the parent and the juvenile (Smith and Fretwell 1974). Larvae derived from large eggs generally enjoy an increased fitness because of their greater initial size, faster growth and lower mortality rates (reviewed in Miller et al. 1988). If larger young have lower mortality, the optimal offspring size increases with decreasing offspring survival (Sibly and Calow 1983; Lloyd 1987; Winkler and Wallin 1987). Theory predicts that high-latitude females trade fecundity for larger egg size and therefore increase survival of individual offspring in a less favourable environment (Sibly and Calow 1986; Yampolsky and Scheiner 1996, Figure 1.1k). The shorter breeding seasons, slower growth, older age at maturity, larger eggs, and fewer clutches expected in high-latitude populations therefore result in increased generation time and lower fecundity. In

summary, theory predicts that high-latitude populations are characterised by slower growth, greater adult body size and longevity, and lower mortality. Lower mortality selects for later maturation and decreased reproductive effort. Conversely, in low-latitude populations, the combination of earlier maturity and high relative fecundity help to compensate for the reduction in lifetime egg production resulting from reduced longevity and increased mortality.

#### ***1.1.5. Geographic variation in performance traits***

Whilst extensive geographic variation in life history traits has been demonstrated, the study of geographic variation in performance is still in its early stages and lags well behind research on other aspects of the phenotype (Endler 1986). Performance, such as swimming capacity, represents the co-adaptation of numerous morphological, physiological and behavioural traits, and is considered an integrated measure of an organism's fitness for a particular environment (Arnold 1983). Just as other traits, performance can vary geographically because it is subject to geographically varying conditions (Foster and Endler 1999). Whole organism performance evolves in response to the direct and indirect effects of selection acting on the suite of traits that augment performance (Arnold 1983). The outcome of selection can also be shaped by interactions or trade-offs with other components of the phenotype that contribute to fitness; yet, the link between life history and performance has only rarely been explored. Most research examining the evolution of performance has focussed on morphological, physiological and biochemical traits as potential targets of selection (e.g. Webb 1984; Jayne and Lauder 1993; Gibb and Dickson 2002). Likewise, studies on life history evolution have focused mainly on how selection acts on traits such as growth rate, fecundity and survival (e.g. Stearns 1992), without integrating any

measures of performance. In fish, two exceptions to this separation in focus are a study on the Atlantic silverside (*Menidia menidia*), showing that the evolution of swimming performance is determined by its trade-off with growth rate (Billerbeck et al. 2001), and the research by Ghalambor et al. (2004), demonstrating that the evolution of increased reproductive allocation in guppies (*Poecilia reticulata*) comes at the cost of reduced escape performance. These studies highlight the need to consider both life history and performance traits when examining phenotypic evolution and mechanisms of adaptive divergence.

## **1.2. The population comparative approach**

The study of geographic variation is implicitly comparative in nature and usually examines associations between character states of species or populations and the environmental factors to which they are exposed. Intraspecific comparisons can often provide clearer insights into the adaptive causes of differentiation than can comparisons among species (or higher taxonomic units) because populations often have been separated for less time than have species and are more likely to still reside in the habitats in which differentiation occurred (Charlesworth 1980; Harvey and Pagel 1991). Furthermore, populations tend to differ in fewer characteristics than do species, reducing the confounding effects of co-varying traits on the analysis (Harvey and Pagel 1991). Therefore, population comparison provides a valuable means of identifying possible causes of adaptive differentiation in wild populations.



### **1.3. Adaptive differentiation: genes and phenotypic plasticity**

Geographic variation in life history phenotypes between populations of a species is often assumed to reflect genetic divergence caused by natural selection. However, the relative contribution of genetic and environmental sources of phenotypic variation has rarely been properly determined, especially in vertebrates. Genetic divergence among populations is possible provided there is genetic variation in life history traits, and individuals with different traits vary in fitness (Ferguson and Talent 1993; Sinervo and Adolph 1994; Reznick and Travis 1996; Mousseau 2000). Alternatively, selection may produce population-specific phenotypic plasticity, described as the capacity of a single genotype to alter its phenotype in response to environmental change (Via and Lande 1985; Stearns 1989). In this case, selection acts on the pattern of condition-dependent expression of a trait, i.e. the norm of reaction. If reaction norms are primarily influenced by natural selection, then phenotypic plasticity is itself considered to be adaptive and may be geographically variable (Stearns and Koella 1986; Scheiner 1993; Via 1993). Distinguishing between genetic divergence and phenotypic plasticity is fundamental to understanding the ecological and evolutionary significance of geographic life history variation (Berven et al. 1979; Stearns 1980; Berven and Gill 1983). It is important to remember, however, that natural selection is not the only mechanism determining evolutionary change. Genetic drift, for example, alters the frequency of alleles and the predominance of traits in a population through entirely stochastic processes and results in changes that need not be adaptive. Such random events may account for some of the local genetic makeup of a population and depend, in part, on population size and connectivity (or lack thereof).

#### **1.4. Common-garden experiments**

As outlined above, both genetic and environmental factors contribute to phenotypic variance. Simply observing geographic differences among populations in the wild is therefore not meaningful in an evolutionary sense, until the genetic basis for the clines can be evaluated. One useful technique is the common-garden experiment, pioneered by plant ecologists many decades ago (reviewed in Conover and Schultz 1995). Individuals from different populations or locations are reared in a series of controlled environments that simulate the main environmental parameters likely to influence fitness in nature. A significant difference among populations in the mean or variance of traits in a common environment indicates that they are likely to have a genetic component and should therefore respond to selection (Gotthard and Nylin 1995; Arendt 1997). Differences in the slope of reaction norms indicate that the relative performance of each population depends on the environment (i.e. that there is a genotype x environment interaction). A comparison of field-collected and laboratory-reared populations can provide hints concerning the relative contribution of genetic and environmental factors to phenotypic expression in the wild (Mousseau and Roff 1989). Because of the logistic difficulties of performing common-garden experiments on vertebrates, most of these studies have involved plants and arthropods (e.g. Arnett and Gotelli 1999). However, they have been used to show genetically based population differentiation in other taxa including reptiles (e.g. Ferguson and Talent 1993) and fishes (e.g. Schultz et al. 1996).

#### **1.5. Local adaptation in coral reef fishes**

That fishes are capable of displaying adaptive genetic variation in life history, behavioural, morphological, or physiological traits across local populations has been

well established (Carvalho 1993). However, our knowledge of local adaptation in fishes comes almost entirely from temperate environments, and is mainly based on only three anadromous and/or freshwater families: the Salmonidae (reviewed in Taylor 1991), the Gasterosteidae (reviewed in Bell and Foster 1994), and the Poeciliidae (reviewed in Meffe and Snelson 1989). Species within each of these groups consist of numerous small demes and have unique migratory behaviours or habitat preferences that promote reproductive isolation, thus the results for these species may not be generally applicable. In marine species in general, and coral reef fishes in particular, the relative roles of local genetic differentiation, phenotypic plasticity, and large-scale fixed responses in determining life history and behaviour have been little studied and are poorly understood.

The potential for local adaptation is determined by the effective population size, the level of gene flow and the selection differential across environments (Holt and Gaines 1992; Adkison 1995; Lynch 1996). Levels of gene flow, in turn, will be heavily influenced by the spatial distribution of reproductive sites and the ability or likelihood of individuals returning to their native site. In reef fish species, where geographic barriers to gene flow are usually absent and early life stages are highly dispersive, the opportunity for local adaptation would therefore appear to be comparatively low (Edmands et al. 1990; Palumbi 1994; Mora and Sale 2002). However, while it has been assumed in the past that coral reef fish larvae are passive drifters with little control over their physical displacement, recent studies have shown that late pelagic stages have considerable sensory, behavioural, and swimming abilities (e.g. Leis et al. 1996; Stobutzki and Bellwood 1997; Fisher 2005). These abilities may influence the extent to which larvae can use active behaviour to modify their dispersal patterns, actively self-recruit or enhance their recruitment success (Sponaugle et al. 2002). Indeed, there is

increasing evidence of substantial self-recruitment to natal reefs in some damselfishes (Jones et al. 1999; Swearer et al. 1999; Jones et al. 2005). In addition, several molecular studies of reef fishes with high dispersal capacities have revealed genetic structuring over short geographical distances in the absence of any obvious physical barriers (Bell et al. 1982; Planes 1993; McMillan and Palumbi 1995; Shulman and Bermingham 1995; Planes et al. 1998; Taylor and Hellberg 2003; Rocha et al. 2005). Taken together, these relatively new findings challenge the assumption that long-distance dispersal and high levels of gene flow are the norm for coral reef fishes, and suggest that the potential for local adaptation may be much greater than previously believed, particularly in species that lay demersal eggs and have short larval durations (Palumbi 1996; Cowen et al. 2000; Schluter 2001).

Large-scale phenotypic variation of coral reef fishes has only recently begun to be examined, and latitudinal trends have been described for very few species. Whilst there have been no attempts so far to quantify geographic and population level differences in performance traits, there is a growing body of research examining geographic variation in life history traits of these reef-associated fishes. Although data for comparisons are still sparse, the emerging patterns suggest that coral reef fishes conform to some predictions of traditional life history theory, such as greater longevity, maximum size and survivorship at higher latitudes (Choat and Robertson 2002; Caldow and Wellington 2003; Choat et al. 2003; Robertson et al. 2005).

Most of these studies have implicitly assumed that the diversity of life histories observed in nature is adaptive and the outcome of natural selection acting to optimise populations to the prevailing environmental and demographic conditions. However, the relative contribution of genetic and environmental sources to trait variation has never been determined, even though this is essential when testing hypotheses involving

natural selection. As outlined earlier, common-garden experiments offer a unique opportunity to explore the genetic basis of phenotypic variation. However, the extensive facilities required, knowledge about how to culture progeny, and the time consuming management of large numbers of brood-stock are generally lacking which may explain why we still know very little about local adaptation in coral reef fishes.

## **1.6. Aims of the thesis**

The overall goal of this thesis was to evaluate the basis of latitudinal patterns in the life history and performance of a group of coral reef fishes using both comparative and experimental techniques. In particular, this work explored life history characteristics of anemonefishes and their plasticity across a wide geographic range. To date, no studies have used common-garden techniques to examine latitudinal life history variation in tropical reef fishes, and as such, this thesis represents the first comprehensive attempt to elucidate the ecological and evolutionary significance of geographic variation.

Three species of anemonefishes were the focus of this project: *Amphiprion melanopus* (Bleeker 1852), *Amphiprion akindynos* (Allen 1972), and *Premnas biaculeatus* (Bloch 1790). These species were chosen because they are common in the western Pacific region and they have broad, overlapping distributions that allow for the examination of demographic parameters across large regional scales (Figure 1.2). Anemonefishes are not a target of fisheries in the region, thus patterns in their demography can be examined without the confounding effects of variation in fishing effort among locations. All three species occur on the Great Barrier Reef, which was crucial for the establishment of captive breeding stocks at James Cook University. They also have several other characteristics that make them an ideal model group for this

study: they are long-lived, easy to collect in the field, and they readily breed in captivity. Furthermore, they lay comparatively large benthic eggs, have short larval phases, and their high growth and development rates facilitate successful experimental manipulation.

Fish populations were drawn from four locations: Kimbe Bay in West New Britain, Papua New Guinea (5°30'S/150°05'E); and Lizard Island (14°40'S/145°28'E), Britomart Reef (18°23'S/146°63'E) and One Tree Island (23°30'S/152°05'E) on the Great Barrier Reef, Australia (Figure 1.3). Among other factors, temperature regimes differ considerably along this large latitudinal gradient (Figure 1.4). On a yearly scale, tropical climates have higher, more constant mean temperatures, whilst temperate zones are characterised by lower mean temperatures and higher thermal variance. In my study species, natural populations at low latitudes therefore experience warmer and narrower ranges of temperatures than do populations at high latitudes.

## **1.7. Structure of the thesis**

*Chapter 2* examined patterns of co-variation in life history traits of *A. melanopus*, *A. akindynos* and *P. biaculeatus* among wild populations from three locations including Kimbe Bay, Lizard Island and One Tree Island. The aim was to evaluate whether changes in life history along a latitudinal gradient conform to predictions based on life history theory, and to examine co-variation among growth and reproductive characteristics. Specifically, I tested the predictions that populations from higher latitudes should display a combination of slower growth, greater adult body size and longevity, and lower mortality. Accordingly, maturation and sex change should occur later and reproductive effort should be reduced. At low latitudes, on the other hand, the converse pattern was expected. In anemonefishes, growth rates and

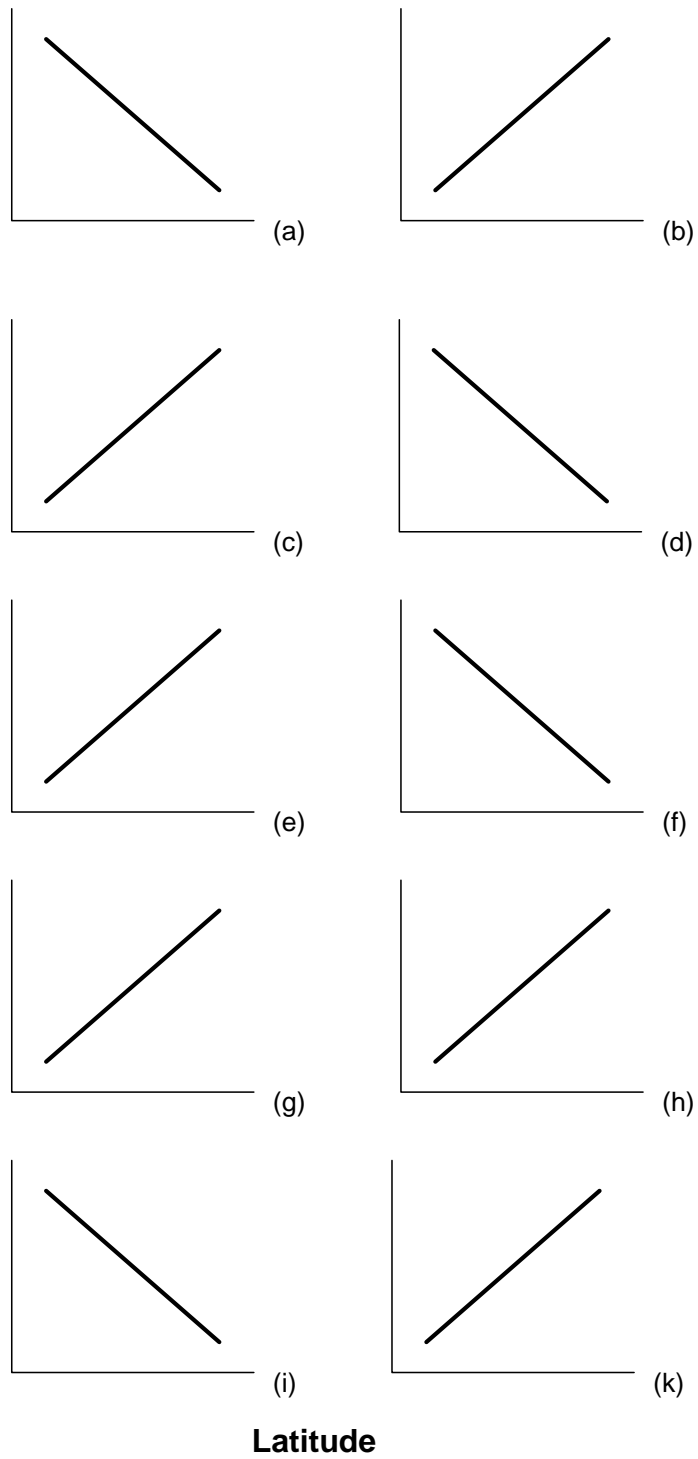
reproductive opportunity are socially regulated by size-based dominance hierarchies (Buston 2003b). The potentially strong influence of the social environment represents an important consideration when interpreting co-variation in life history traits in these species.

*Chapter 3* employed a common-garden experimental approach to determine whether differences in reproductive characters observed in the wild reflect phenotypic plasticity or have a heritable basis. I explored the possibility of adaptive latitudinal variation in egg size, fecundity and reproductive output by maintaining adults of the three study species in a common environment and by recording their reproductive activity over an extended period of time. I tested the specific prediction that females from higher latitudes produce larger eggs than females from low latitudes. Further, since egg size and number are usually opposing attributes, I predicted that populations in which females produce large eggs would be associated with lower fecundity and *vice versa*. I also predicted that populations from higher latitudes would have a lower total reproductive output compared to populations from lower latitudes.

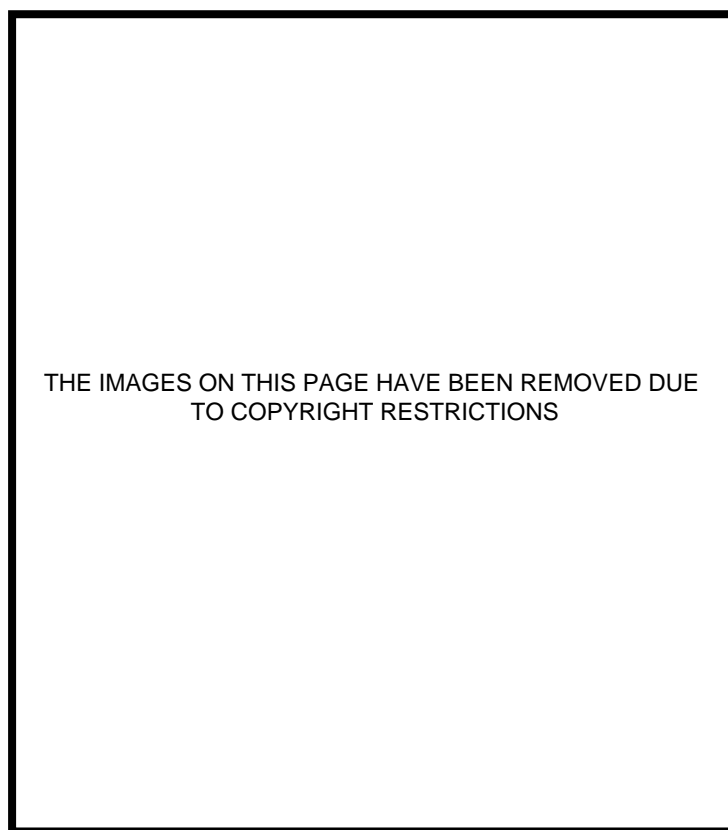
*Chapter 4* investigated whether there is evidence for adaptive genetic differentiation in growth rate and growth rate plasticity in the early life history of anemonefishes from different latitudinal populations. A common-garden experiment with a full-sib design was carried out to examine the effects of temperature and food availability on the growth and survival of juveniles raised from eggs from three locations along the Great Barrier Reef. The following specific questions were addressed: How does the capacity for growth respond to temperature and food availability? Is this response the same in all populations or are there different levels of plasticity among populations from different latitudes? And finally, is fast growth costly in terms of survival?

*Chapter 5* tested the extent of heritable performance phenotype-environment associations in anemonefishes from different latitudinal origins. This was achieved by performing swimming trials on progeny reared under identical conditions in the laboratory. In addition, it assessed whether there is any divergence amongst these populations in the thermal sensitivity of locomotor performance and examined potential trade-offs with other competing functions such as growth rate.

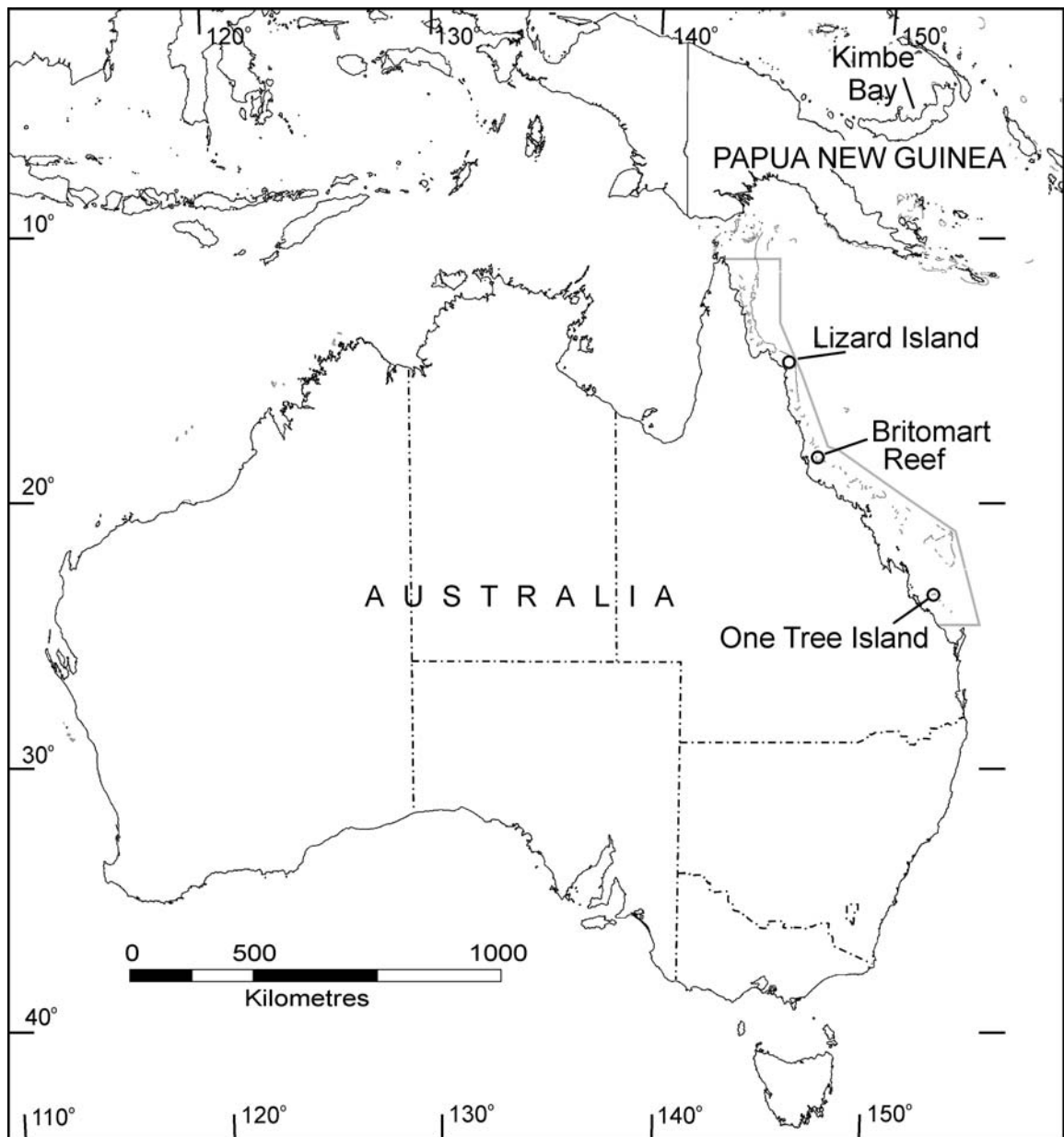




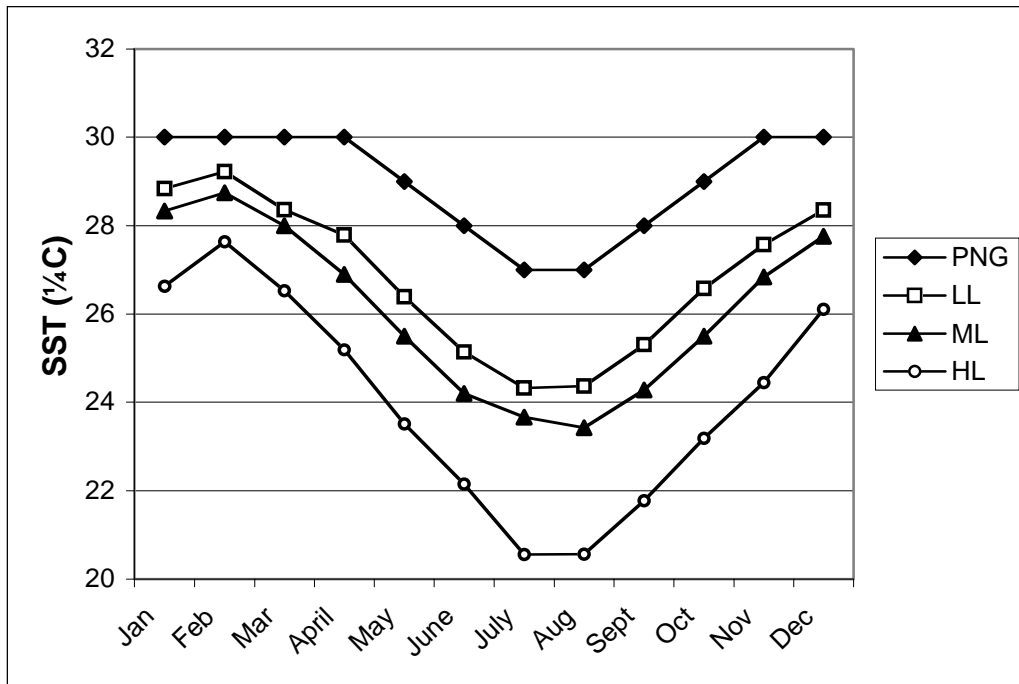
**Figure 1.1:** Predicted relationships between latitude, environmental characteristics and major life history traits according to life history theory: (a) mean temperature, (b) seasonality/environmental variability, (c) maximum size, (d) growth rate, (e) longevity, (f) mortality, (g) age and size at maturation, (h) age and size at sex change, (i) reproductive effort and (k) egg size.



**Figure 1.2:** Geographic ranges of the study species: (A) *Amphiprion melanopus*, (B) *Amphiprion akindynos* and (C) *Premnas biaculeatus* according to Fautin and Allen (1992).



**Figure 1.3:** Map showing the locations of the study sites in the western Pacific Ocean: Kimbe Bay (PNG), Lizard Island, Britomart Reef and One Tree Island (see text for detailed coordinates).



**Figure 1.4:** Seasonal changes in average sea-surface temperatures (SST) at each of the study sites. PNG = Kimbe Bay at 5°S, and along the Great Barrier Reef: LL = low latitude at 14°S, ML = mid-latitude at 18°S, and HL = high latitude at 23°S.

Monthly mean SSTs of the GBR sites were provided by the *Sea Temperature Monitoring Program*, courtesy of the Australian Institute of Marine Science, CRC Reef Research Centre and the Great Barrier Reef Marine Park Authority.

## **CHAPTER 2: Geographic variation in the life history traits of anemonefishes: are there latitudinal trends?**

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### **2.1. Introduction**

The life history of a species encompasses a range of characteristics including patterns of growth and mortality, size and age at maturity, and size- or age-specific fecundity. Interrelationships among these traits are predicted by a body of theory that assumes natural selection will optimise trade-offs among them, thereby maximizing fitness in a particular environment (Roff 1992; Stearns 1992). Many studies of geographic variation in life history traits have demonstrated latitudinal trends that can be attributed to environmental gradients. Bergmann's rule describes the commonly observed positive association between body size and latitude, a presumed trade-off in the effect of temperature on growth and development (Bergmann 1847; Rensch 1938; Mayr 1963; Ridley 1996; Futuyma 1998). The attainment of relatively larger adult sizes in colder environments is usually achieved by slower, prolonged growth over a longer lifespan (Stearns 1992; Atkinson 1994), which has follow-on effects on other key life history parameters. If longevity increases with latitude, it is expected that mortality rates should be higher at lower latitudes (Blueweiss et al. 1978; Hutchings 1993; Metcalfe and Monaghan 2003). The combined effects of growth and survivorship, in turn, have a strong influence on reproductive characteristics, selecting for later maturation at larger sizes and decreased reproductive effort at higher latitudes (Charlesworth 1980; Beverton 1992; Roff 1992). Many of these generalisations are well supported for terrestrial plants and animals, particularly over temperate latitudes.

In marine fishes, which are often distributed over broad latitudinal ranges, most studies have reported that individuals mature at larger sizes and/or older ages, attain larger adult body sizes and live longer at higher latitudes; latitudes which are typically associated with lower mean temperatures and shorter breeding seasons (Gilligan 1991; Atkinson 1994; Atkinson and Sibly 1997; Abookire and Macewicz 2003). Much of this work on latitudinal variation in the life histories of marine fishes, however, has been confined to temperate latitudes, where both growth and reproductive output can be quite limited by environmental conditions. In contrast, there have been few studies of latitudinal life history variation in tropical fishes that experience less extreme environmental gradients. The studies so far have reported greater longevity, maximum size, growth and survivorship with increasing latitude (Choat et al. 2003; Kritzer 2004; Walker and McCormick 2004) and greater reproductive seasonality at higher latitudes (Robertson 1991). While some of these studies show patterns consistent with those over temperate latitudes, few studies have simultaneously examined large numbers of life history traits that may co-vary across gradients.

Latitudinal variation in life histories of tropical reef fishes may be complicated further by the capacity of many of these species to change sex. For example, sex change may occur proportionally earlier with respect to total life span in environments where growth is more rapid and/or mortality rates are higher (Charnov 1982; Buston et al. 2004). Whether or not age at sex change is a life history invariant, there have been no comprehensive studies of latitudinal variation in age and size at sex change in tropical reef fishes in the context of its co-variation with other life history traits. Furthermore, social control of a variety of other life history traits (Cole and Robertson 1988; Warner 1988; Munday et al. 1998; Baroiller et al. 1999; Hobbs et al. 2004) has the capacity to affect latitudinal trends in life histories. This is especially likely to be so for

anemonefishes, which are highly habitat-specialised, and for which maturation and sex change from male to female (protandry) are socially controlled (Fricke and Fricke 1977; Moyer and Nakazono 1978; Fricke 1983). As growth, mortality, maturation and sex change are strongly dependent on social status (Fricke and Fricke 1977; Moyer and Nakazono 1978; Fricke 1979; 1983; Hattori 1991; Buston 2003b; 2003a), local effects leading to individual variation in these parameters may override the effects of latitudinal environmental gradients.

Here I investigated geographic variation in life history traits of three anemonefish species (*Amphiprion akindynos*, *A. melanopus* and *Premnas biaculeatus*) over 18° of latitude between Papua New Guinea and the southern Great Barrier Reef (GBR) to examine whether latitudinal trends, typical of many poikilothermic species, are expressed in these anemonefishes whose life history transitions are strongly controlled by their social environment. The life history traits examined included adult body size, growth, longevity, mortality, age and size at maturation and sex change, reproductive output, and their interrelationships. Specifically, I examined for these species whether slower growth, larger adult body size, greater longevity, lower mortality and lower reproductive output were associated with increasing latitude. The relationships of maximum body size and maximum age with maturation and sex change with respect to latitude were also investigated.

## **2.2. Materials and Methods**

### **2.2.1. Study species and sites**

Three anemonefish species were chosen, which differ in the extent of their north-south distributions. *Amphiprion melanopus* occurs from the southern Philippines to the southern GBR (Figure 1.2). *Amphiprion akindynos* has the narrowest distribution,

from the northern GBR to northern New South Wales (Figure 1.2). *Premnas biaculeatus* occurs from the Philippines to the northern GBR (Figure 1.2). For *A. melanopus*, three locations were compared: One Tree Island (23°30'S; 152°05'E) on the southern GBR, Lizard Island (14°40'S; 145°28'E) on the northern GBR, and Kimbe Bay (5°30'S; 150°05'E) in West New Britain, Papua New Guinea. *A. akindynos* does not occur in Kimbe Bay, therefore only the two GBR locations were compared. *P. biaculeatus* does not occur at One Tree Island, therefore only Kimbe Bay and Lizard Island were compared for this species. The main differences in abiotic conditions among the three geographic locations sampled include decreased average temperature and an increase in the degree of seasonality with increasing latitude (Figure 1.4). Both One Tree and Lizard Islands have distinct summer and winter seasons, with reproduction and recruitment of most fishes generally occurring only during the warmer summer months. One Tree Island has a shorter summer and cooler mean annual temperature (24°C) than Lizard Island (27°C). In Kimbe Bay, temperatures remain relatively constant (30-31°C), and for many species reproduction and recruitment occurs throughout the year. In addition, Kimbe Bay is outside the cyclone belt and so experiences less physical disturbance than locations on the Great Barrier Reef.

### **2.2.2. Fish collection**

Individuals of all three species were collected at Lizard Island in February and April 1997, October and September 2000, October 2002 and October 2003. *P. biaculeatus* and *A. melanopus* individuals were collected at Kimbe Bay in May 1997, and *A. melanopus* and *A. akindynos* individuals were collected at One Tree Island in August and December 2000, August, September and December 2001, and December 2002. Fish were anaesthetised using a 1:8 solution of clove oil in 70% ethanol and



captured using hand nets. Each fish was weighed and measured (SL) and its sagittal otoliths and gonads were removed for analysis.

### **2.2.3. Ageing and validation of the ageing technique**

A total of 294 individuals of *A. melanopus* (37 from Kimbe Bay, 160 from Lizard Island and 97 from One Tree Island), 170 individuals of *P. biaculeatus* (64 from Kimbe Bay and 106 from Lizard Island) and 195 individuals of *A. akindynos* (78 from Lizard Island and 117 from One Tree Island) were aged by the examination of their sagittal otoliths. Otoliths were sectioned by hand grinding and polishing both ends using 1200 grade glasspaper and lapping film to obtain a thin transverse section through the nucleus. Each otolith was then examined under a microscope (either 250x or 400x magnification) and the annual growth increments were counted. Daily increments were counted for individuals younger than one year of age. A total of four counts were done on separate occasions for each otolith. If age estimates were inconsistent, counts were repeated until two consistent counts were obtained. Otoliths with continually inconsistent counts and those with no clear rings were not included in the age and growth analyses.

To validate annual growth rings, several fish were injected with oxytetracycline hydrochloride dissolved in saline solution (at a dosage of 50mg OTH per kg of fish), to mark the otolith. The tetracycline mark is visible as a fluorescent yellow band when the sectioned otolith is viewed under ultraviolet light. Twenty-four individuals of *P. biaculeatus* were marked in Kimbe Bay during July 12-26, 2000. Each fish was captured as above, placed in a clear plastic bag and injected while anaesthetised. Fish were then returned to their anemones when fully recovered and the positions of the anemones were marked using small numbered buoys. Fish from marked anemones were

collected between January 24-26, 2002, and their otoliths removed and sectioned as above. Of the 12 of the injected fish collected, only 6 had visible tetracycline bands on their otoliths, and only two had annuli that were suitably clear for validation. Calculations according to Cappo et al. (2000) showed that for these two individuals otolith increments were indeed annual. In addition, the examination of otoliths from individuals that were bred and raised in captivity confirmed that increments are annual in all three species.

Age estimation using otoliths was possible for the majority of individuals of all three species from each location. Even otoliths from individuals at Kimbe Bay had increments that were sufficiently distinct, suggesting that relatively constant temperatures do not necessarily translate to uniform growth throughout the year. However, as reported in other studies (e.g. Caldow and Wellington 2003), samples from Kimbe Bay contained the highest proportions of otoliths without clear annual increments.

#### ***2.2.4. Geographic comparisons of longevity, growth and mortality***

In order to examine life spans of each species among locations, maximum age estimates were compared. In addition, a longevity estimate was calculated based on the mean age of the oldest 20% of individuals (*mean T<sub>max</sub>*) sampled in each population. If sample sizes are relatively small, longevity estimates that are based only on the maximum age recorded for a particular population may over-emphasise the age of outliers in local age distributions (Beverton 1992). Therefore, comparing *mean T<sub>max</sub>* estimates may provide a more robust and conservative result. A one-way ANOVA was performed on the *mean T<sub>max</sub>* estimates to determine differences between populations. Following a significant result for *A. melanopus*, a Tukey's multiple comparison post-

hoc test was performed. While 20% represents an arbitrary proportion of the population, it should be noted that an analysis of longevity using the oldest 10% of individuals in each population produced identical results.

Growth parameters were obtained by fitting the von Bertalanffy growth function (VBGF) to length-at-age data for each species from each location. The VBGF is defined as:  $L_t = L_\infty(1 - \exp^{-K(t-t_0)})$ , where  $L_t$  = length at age  $t$ ,  $K$  = Brody growth coefficient,  $L_\infty$  = asymptotic length and  $t_0$  = theoretical age at zero length. When fitted using non-linear least-squared residuals the von Bertalanffy curve represents the average growth of the population members. The VBGF was used because it describes growth better than polynomial functions (Chen et al. 1992).

Irrespective of sample sizes, the youngest and smallest fish are often underrepresented in data sets due to sampling biases. Because the VBGF parameter estimates are sensitive to the distribution of ages and sizes used (Ferreira and Russ 1994), growth curves were constrained using published (Thresher et al. 1989; Wellington and Victor 1989) and unpublished (Bay et al. in press) estimates of pelagic larval durations and sizes at settlement.

Growth curves were compared between populations of each species using the likelihood ratio test (LRT) following Kimura (1980). In an overview of various methods for the comparison of VBGF curves, Cerrato (1990) compared the suitability of the  $t$ -, univariate  $\chi^2$ , likelihood ratio, and Hotelling's T-tests. The latter two methods are generally considered better than the others because they consider all parameters of the von Bertalanffy equation at once, which is important as these parameters are strongly correlated and should not be compared individually. With an empirical comparison, Cerrato showed that the likelihood ratio test (Kimura 1980) often differs in outcome from the other methods, and that conflicting outcomes overwhelmingly resolve in

favour of the LRT. He concluded that the LRT is the most accurate of the procedures considered and it has therefore been chosen for this study.

The likelihood ratio method does not only test whether two or more curves are statistically different (i.e. are coincident curves), but also compares the parameters individually. Firstly, the best fitting curves for each data set are determined separately (termed base case), then the total residual sum of squares of the base case is compared sequentially with that obtained by adding various constraints. The order in which to impose these tests is to first assume the hypothesis that each data set can be best described by a single curve (coincident curves, e.g. each data set is effectively a sample from the same population). If a significant difference is found between the base case and the coincident curves, then it is sequentially assumed that single parameters are the same between the separate curves in order to identify which ones are different between the curves for each data set. For the von Bertalanffy curve these constraints are that a) the  $L_{\infty}$  values are equal, b) that the  $K$  values are equal, and c) that the  $t_0$  values are equal. In short, the likelihood ratio test calculates the total residual sum of squares separately for the base case and for the hypothesis of interest (e.g. the  $L_{\infty}$  values are equal). The ratio of the two likelihoods is then compared with the  $\chi^2$  distribution with degrees of freedom equal to the number of constraints, i.e. the number of parameters being assumed equal (Kimura 1980).

Mortality rates ( $M$ ), expressed as  $\text{year}^{-1}$ , were estimated for each population using age-based catch curves described by Beverton and Holt (1959) and Ricker (1975). This method assumes that annual recruitment rates remain constant at each location and involves the calculation of the natural log of the number of fish sampled from each age class, plotted against their corresponding age.  $M$  is estimated as the absolute value of the regression slope. As some of the sample sizes in this study were relatively small,

two consecutive age classes (i.e. ages 1 and 2, 3 and 4, and so on) were pooled for each population and catch curves fitted from the modal age class. The slopes of these catch curves were then compared among populations using ANCOVA (Zar 1999).

#### ***2.2.5. Gonad histology and estimation of batch fecundity and egg size***

Gonads were embedded in paraffin wax, sectioned to 5 $\mu$ m thickness and stained with haematoxylin and eosin. Gonad structure was examined under a high power microscope to determine the sex of each fish. Individuals with developing oocytes and no sign of mature male tissue were classified as females, individuals with undeveloped oocytes and mature male tissue (i.e. spermatozoa) were classified as males, and individuals with no mature male or female tissue were classified as juveniles.

Gonads from ripe females were not sectioned. Instead, a small section was taken from one lobe of each ripe gonad. This section was then weighed and the number of eggs it contained counted. Batch fecundity (number of eggs per female) was estimated for each ripe female by multiplying the number of eggs per gram of gonad by the weight of the gonad. Egg size was estimated by measuring the widths and lengths of twenty randomly chosen eggs from each gonad. Widths and lengths of each egg were averaged to obtain a single value of mean egg size for each fish. For *A. melanopus* and *P. biaculeatus*, sections were taken from the posterior, mid and anterior regions of the first 12 gonads for each species. Two separate one-way ANOVA's were then performed to determine whether there were any differences in egg number and egg size along the length of the gonad. No significant differences were found between regions for either species, therefore egg counts and measurements for the remaining ripe female gonads were taken from the mid-region of each gonad. After establishing that the relationship between batch fecundity and body weight was linear, the ratio of these two parameters

was calculated for each ripe female to control for differences in body size among locations.

#### **2.2.6. Geographic comparisons of reproductive parameters**

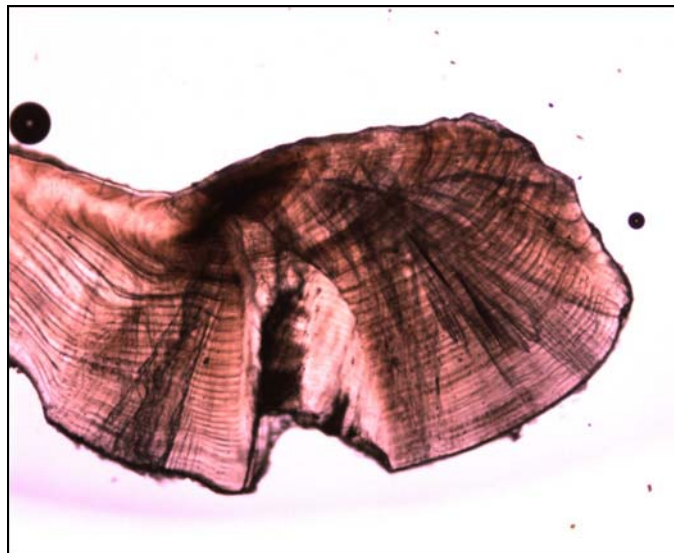
The percentage of individuals in each of three stages of maturity (juveniles/immature sub-adults, male and female) was plotted against several age and size classes, and these plots were used to compare the timing of maturation and sex change of each species among locations. I also determined the age and size classes at which greater than 50% of the non-female individuals (i.e. immature individuals and males) were mature males, to compare the timing of maturation among locations. These age and size classes were also converted to age or size ranges proportional to maximum age or size respectively. The age and size at sex change for each species at each location was estimated as the median age or size of the range of ages or sizes where males and females overlapped (following Warner and Robertson 1978). These median ages/sizes were also converted to an age or size range proportional to the maximum age or size.

Mean batch fecundity, mean egg size and mean batch fecundity-body weight ratio of *A. melanopus* were compared among the three locations using separate one-way ANOVAs. For *A. akindynos* and *P. biaculeatus*, t-tests were used to compare batch fecundity, egg size and fecundity-weight ratio between the two locations where these two species were collected. Batch fecundity data for *A. melanopus* and *P. biaculeatus* were transformed to  $\log_{10}x$  and egg size data for *A. melanopus* were transformed to  $\log_{10}(x + 1)$  to meet the assumptions of homogeneity of variances for analysis. I also used a series of correlations to test whether body weight or age is a predictor of batch fecundity for each species at each location.

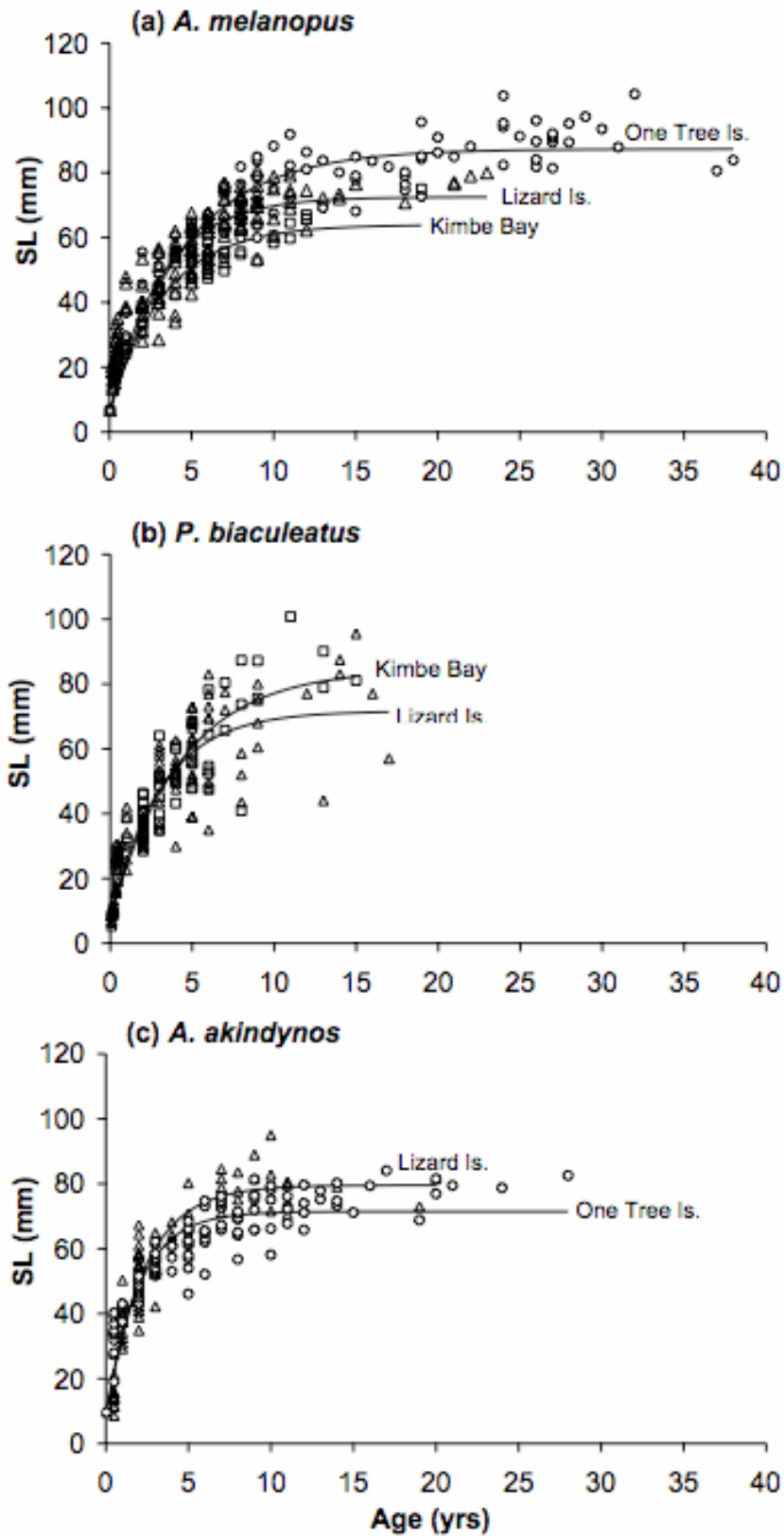
## 2.3. Results

### 2.3.1. Geographic comparisons of longevity, body size, growth and mortality

Maximum age increased at higher latitudes in all species, as predicted (Figure 2.2). The maximum age of both male and female *A. melanopus* increased with increasing latitude, from 12 and 19 years respectively at Kimbe Bay to 31 and 38 years respectively at One Tree Island (Table 2.1). Similarly, the maximum age of both males and females of the other two species, *A. akindynos* and *P. biaculeatus*, were higher at the higher-latitude location (One Tree Island and Lizard Island respectively). Overall, the annual increments show that all three species are relatively long-lived, the longest lived species at each location being *A. melanopus* (Table 2.1). The very oldest individual sampled was a 38-year-old *A. melanopus* female from One Tree Island. The otolith of the oldest *A. akindynos* sampled, a 28-year-old female from One Tree Island, is shown in Figure 2.1.



**Figure 2.1:** Sectioned sagittal otolith of the oldest (28 years) individual of *A. akindynos* collected from One Tree Island.



**Figure 2.2:** Von Bertalanffy growth functions fitted to size at age data of (a) *A. melanopus*, (b) *P. biaculeatus* and (c) *A. akindynos* at each location (O = One Tree Island,  $\Delta$  = Lizard Island,  $\square$  = Kimbe Bay). VBGf parameters are listed in Table 2.2.



**Table 2.1:** Observed maximum sizes (SL, in mm) and ages (in years) of males and females of each species at each location.

Species	Location	Max size (Males)	Max size (Females)	Max age (Males)	Max age (Females)
<i>A. melanopus</i>	Kimbe Bay	65.8	75.0	12	19
	Lizard Is.	76.9	80.7	15	23
	One Tree Is.	95.3	104.3	31	38
<i>P. biaculeatus</i>	Kimbe Bay	75.6	100.8	9	15
	Lizard Is.	56.5	95.5	13	17
<i>A. akindynos</i>	Lizard Is.	81.5	95.0	9	20
	One Tree Is.	74.7	84.0	11	28

When comparing mean 20% longevity estimates (*mean T<sub>max</sub>*) within species, not all these observed differences were statistically significant. *A. akindynos* showed significantly greater longevity at One Tree Island compared to Lizard Island (ANOVA,  $F = 7.58$ ,  $P = 0.009$ ), whereas in *P. biaculeatus* the difference between locations was non-significant ( $F = 0.03$ ,  $P = 0.864$ ). Following a significant result for *A. melanopus* ( $F = 105.42$ ,  $P < 0.001$ ) a Tukey's multiple comparison test revealed that the highest-latitude population at OTI had significantly greater *mean T<sub>max</sub>* estimates compared to Lizard Island and Kimbe Bay. However, these two latter locations did not differ significantly from one another ( $P = 0.982$ ).

*A. melanopus* conformed to the prediction that adult body size increases at higher latitudes, but the other two species did not. The observed maximum size of *A. melanopus* increased from 75.0 mm SL at Kimbe Bay to 104.3 mm at One Tree Island (Table 2.1). In contrast, the observed maximum size of both *A. akindynos* and *P. biaculeatus* was higher at the low-latitude location (Lizard Island and Kimbe Bay respectively), though for *P. biaculeatus* this difference in observed maximum size between locations was quite small (5mm).

Comparisons of VBGF parameters among/between locations showed that only *A. melanopus* conformed to the predictions of reaching a larger adult body size, at a slower growth rate, at higher latitudes (Table 2.2). *A. melanopus* from One Tree Island attained a significantly higher asymptotic size at a slower rate compared to Lizard Island; however, these VBGF parameters did not differ between the Lizard Island and Kimbe Bay populations (Figure 2.2, Table 2.3). Neither *A. akindynos* nor *P. biaculeatus* exhibited patterns consistent with my predictions, although for both species the overall growth trajectories differed significantly between the two locations compared. In both *A. akindynos* and *P. biaculeatus*, the primary differences in growth trajectories lay in  $L_{\infty}$ , but not in  $K$  (Table 2.3). Both species attained larger sizes at the lower-latitude location (Figure 2.2, Table 2.2), which is contrary to the predicted pattern, and within each species populations converged on their asymptotic sizes at similar rates (Table 2.3). Size at age data for all three species at each location fitted the VBGF much better than expected (Figure 2.2, Table 2.2), given the strong social control of growth that anemonefishes are known to exhibit.

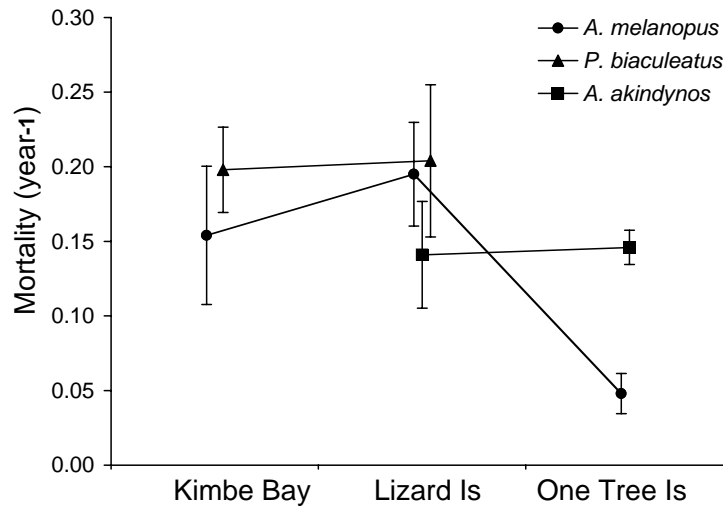
**Table 2.2:** Summary of Von Bertalanffy parameters for each species at each location.  $L_{\infty}$  is mean asymptotic SL (mm),  $k$  is the Brody growth coefficient,  $t_0$  is theoretical age at length zero (years) and  $n$  is the number of individuals.

Species	Location	$L_{\infty}$	$k$	$t_0$	$r^2$	$n$
<i>A. melanopus</i>	Kimbe Bay	63.97	0.30	-0.34	0.89	37
	Lizard Is.	72.53	0.32	-0.27	0.86	160
	One Tree Is.	87.32	0.22	-0.33	0.84	97
<i>P. biaculeatus</i>	Kimbe Bay	84.69	0.23	-0.43	0.76	64
	Lizard Is.	71.65	0.32	-0.36	0.78	106
<i>A. akindynos</i>	Lizard Is.	79.56	0.43	-0.26	0.88	78
	One Tree Is.	71.45	0.51	-0.25	0.82	117

**Table 2.3:** Results of likelihood ratio tests. RSS is the total residual sum of squares. L-O are comparisons between Lizard Island and One Tree Island, and K-L are comparisons between Kimbe Bay and Lizard Island.

	Base case	Coincident	$L_\infty$	$k$	$t_0$
<i>A. melanopus</i> (K-L)					
RSS	11284.3	13004.6	11482.2	11290.6	11374.6
$\chi^2$		28.2	3.5	0.1	1.6
$df$		3	1	1	1
$P$		0.0000	0.0629	0.7391	0.2080
<i>A. melanopus</i> (L-O)					
RSS	16666.1	19062.6	18727.6	17434.1	16978.0
$\chi^2$		34.8	30.2	11.7	4.8
$df$		3	1	1	1
$P$		0.0000	0.0000	0.0006	0.0284
<i>P. biaculeatus</i> (K-L)					
RSS	17305.4	18569.1	17722.8	17571.9	17451.3
$\chi^2$		12.1	4.1	2.6	1.4
$df$		3	1	1	1
$P$		0.0070	0.0429	0.1050	0.2295
<i>A. akindynos</i> (L-O)					
RSS	10097.1	13859.9	10896.3	10210.5	10108.3
$\chi^2$		62.4	15.0	2.2	0.2
$df$		3	1	1	1
$P$		0.0000	0.0001	0.1380	0.6401

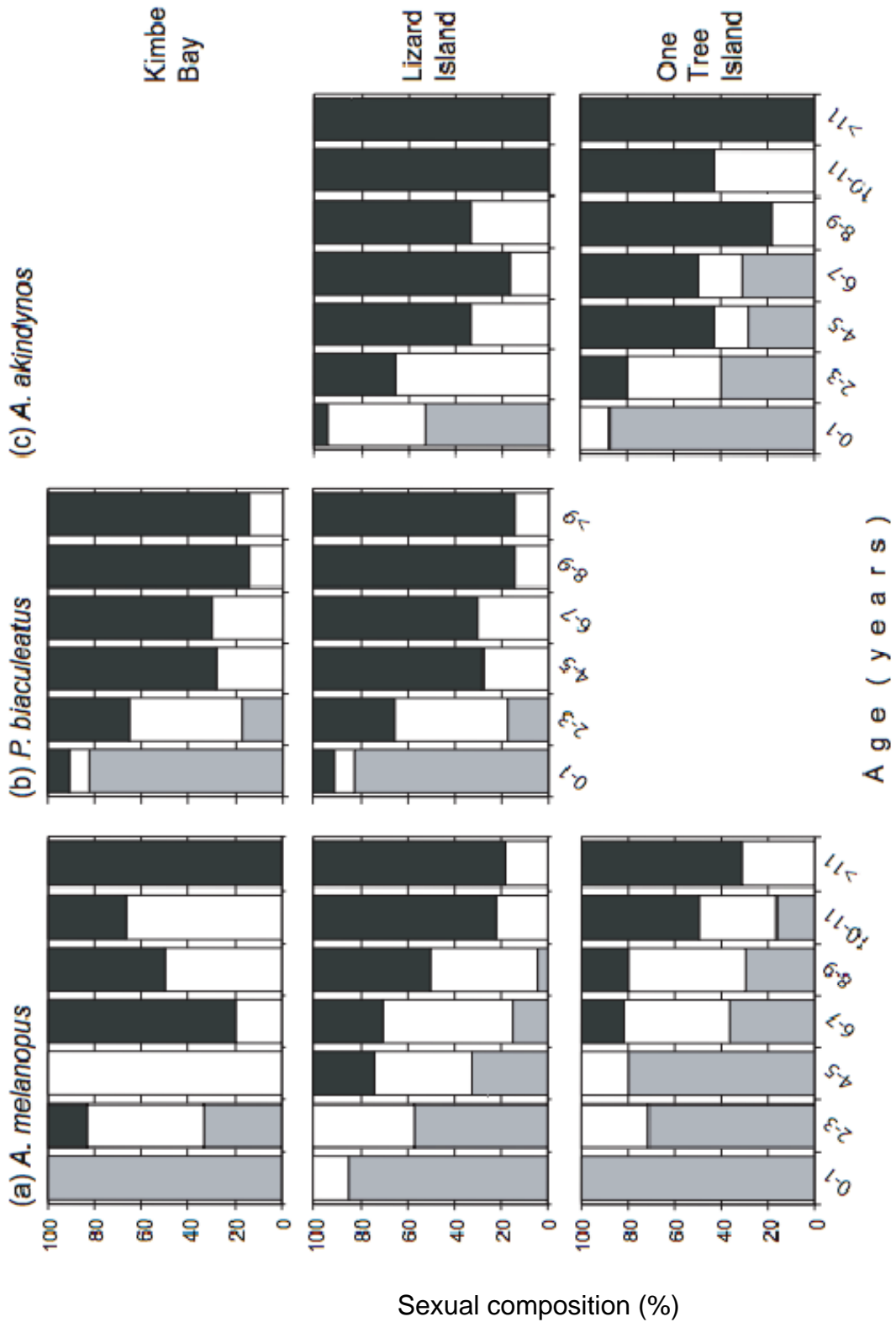
Again, only *A. melanopus* conformed to my prediction of lower mortality at higher latitudes. Mortality rates for this species differed significantly among locations (ANCOVA:  $F = 7.36$ ,  $P = 0.002$ ), with lower mortality at One Tree Island compared to both Kimbe Bay and Lizard Island, but there was no difference between Kimbe Bay and Lizard Island (Figure 2.3). For the other two species, mortality rates did not differ significantly between locations (ANCOVA, *A. akindynos*:  $F = 1.03$ ,  $P = 0.322$ , and *P. biaculeatus*:  $F = 0.10$ ,  $P = 0.762$ ; Figure 2.3).



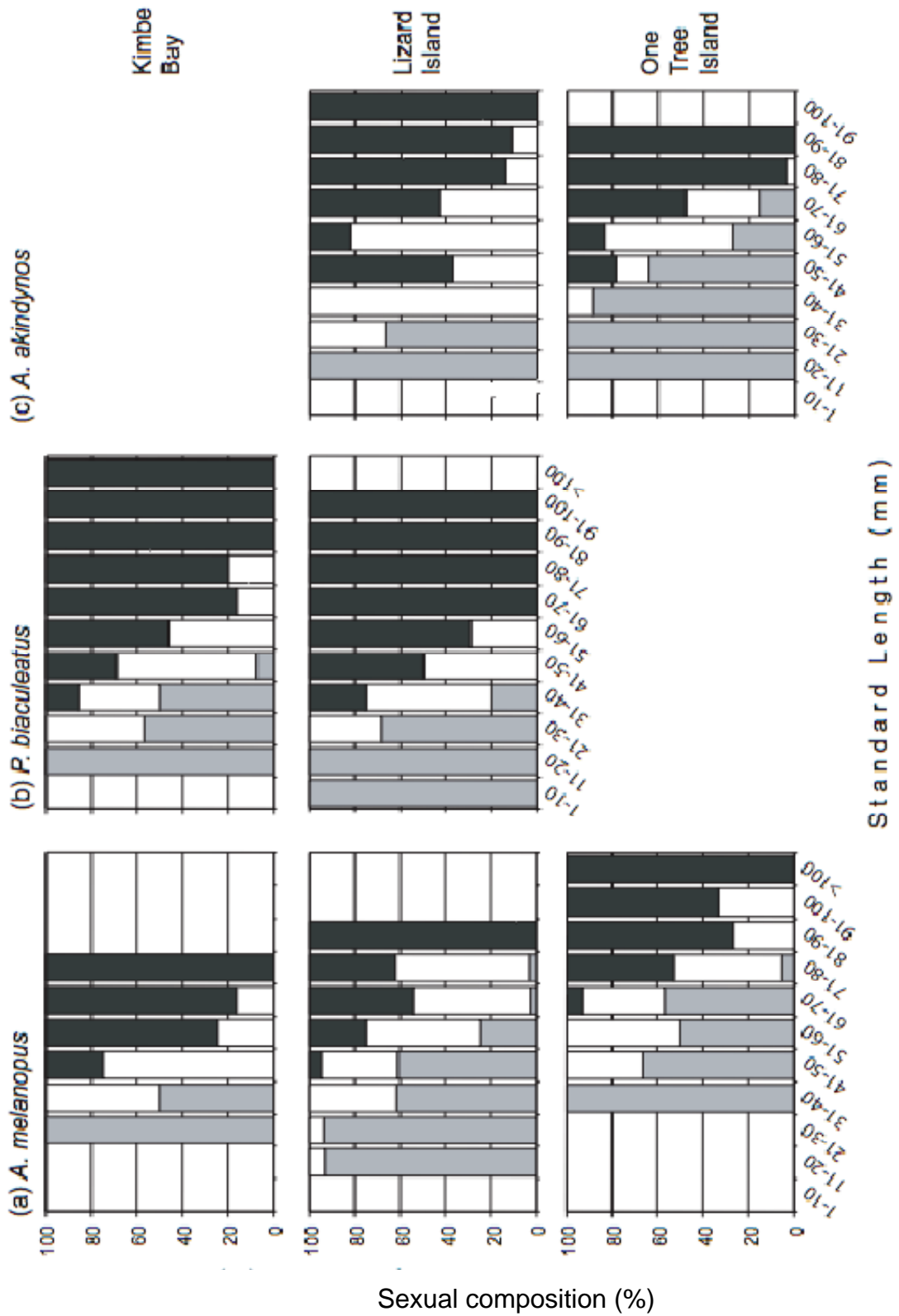
**Figure 2.3:** Mortality rates ( $\text{year}^{-1} \pm \text{SE}$ ) of each species at each location. Sample sizes are listed in Table 2.2.

### 2.3.2. Geographic comparisons of reproductive parameters

For all three species, the percentage of each sexual stage at each age or size class was highly variable, though for *A. melanopus* there was a trend for increasing percentages of juveniles and males in the older age classes, and larger size classes with increasing latitude (Figure 2.4 and 2.5). *A. melanopus* matured proportionally earlier (10.5-15.8% of maximum age) and smaller (41.3-53.3% of maximum size) at Kimbe Bay compared to Lizard Island and One Tree Island, which conforms to my predictions, but the pattern was reversed between the latter two locations (Table 2.4). *A. akindynos* also conformed to my predictions with respect to size at maturation, maturing at a larger proportional size at One Tree Island (60.7-71.4% of maximum size) compared to Lizard Island (32.6-42.1%), but proportional age at maturation was lower at One Tree Island (7.1-10.7% of maximum age) than at Lizard Island (10.0-15.0%), which is the reverse of what I predicted. For *P. biaculeatus*, proportional age and size at maturation were similar at both the locations compared (Figure 2.4 and 2.5, Table 2.4).



**Figure 2.4:** Sexual composition by age class for each species at each location (shaded bars: juveniles; open bars: males; solid bars: females).



**Figure 2.5:** Sexual composition by size class for each species at each location (shaded bars: juveniles; open bars: males; solid bars: females).

**Table 2.4:** The timing of maturation of each species at each location. Age/size at maturation is taken as the age/size class at which > 50% of non-female individuals are mature, and these age/size classes are also presented as percentages of the maximum age/size.

Species	Location	Age (y)	Maturation		
			% Max age	SL (mm)	% Max SL
<i>A. melanopus</i>	Kimbe Bay	2-3	10.5-15.8	31-40	41.3-53.3
	Lizard Is.	4-5	17.4-21.7	51-60	63.2-74.4
	One Tree Is.	6-7	15.8-18.4	51-60	48.9-57.5
<i>P. biaculeatus</i>	Kimbe Bay	2-3	13.3-20.0	31-40	30.8-39.7
	Lizard Is.	2-3	11.8-17.7	31-40	32.5-41.9
<i>A. akindynos</i>	Lizard Is.	2-3	10.0-15.0	31-40	32.6-42.1
	One Tree Is.	2-3	7.1-10.7	51-60	60.7-71.4

My prediction that size and age at sex change would be proportionally greater with increasing latitude was conformed to by *A. melanopus*, with sex change estimated to occur at 39.5% of maximum age and 73.7% of maximum size in Kimbe Bay, to 50% and 78.8% respectively at One Tree Island (Table 2.5). *P. biaculeatus* conformed to this prediction with respect to proportional age at sex change, with an increase from Kimbe Bay to Lizard Island, but proportional size at sex change increased from Lizard Island to Kimbe Bay (Table 2.5). *A. akindynos* conformed to my prediction with respect to proportional size at sex change (higher at One Tree Island than at Lizard Island), but had the opposite pattern with respect to proportional age at sex change (Table 2.5).

Batch fecundity, egg size and the batch fecundity-body weight ratio of *A. melanopus* differed significantly among the three locations (Table 2.6). For this species, mean batch fecundity at One Tree Island was roughly double that at both Kimbe Bay and Lizard Island (Figure 2.6a, Table 2.6), and egg size was significantly larger at One

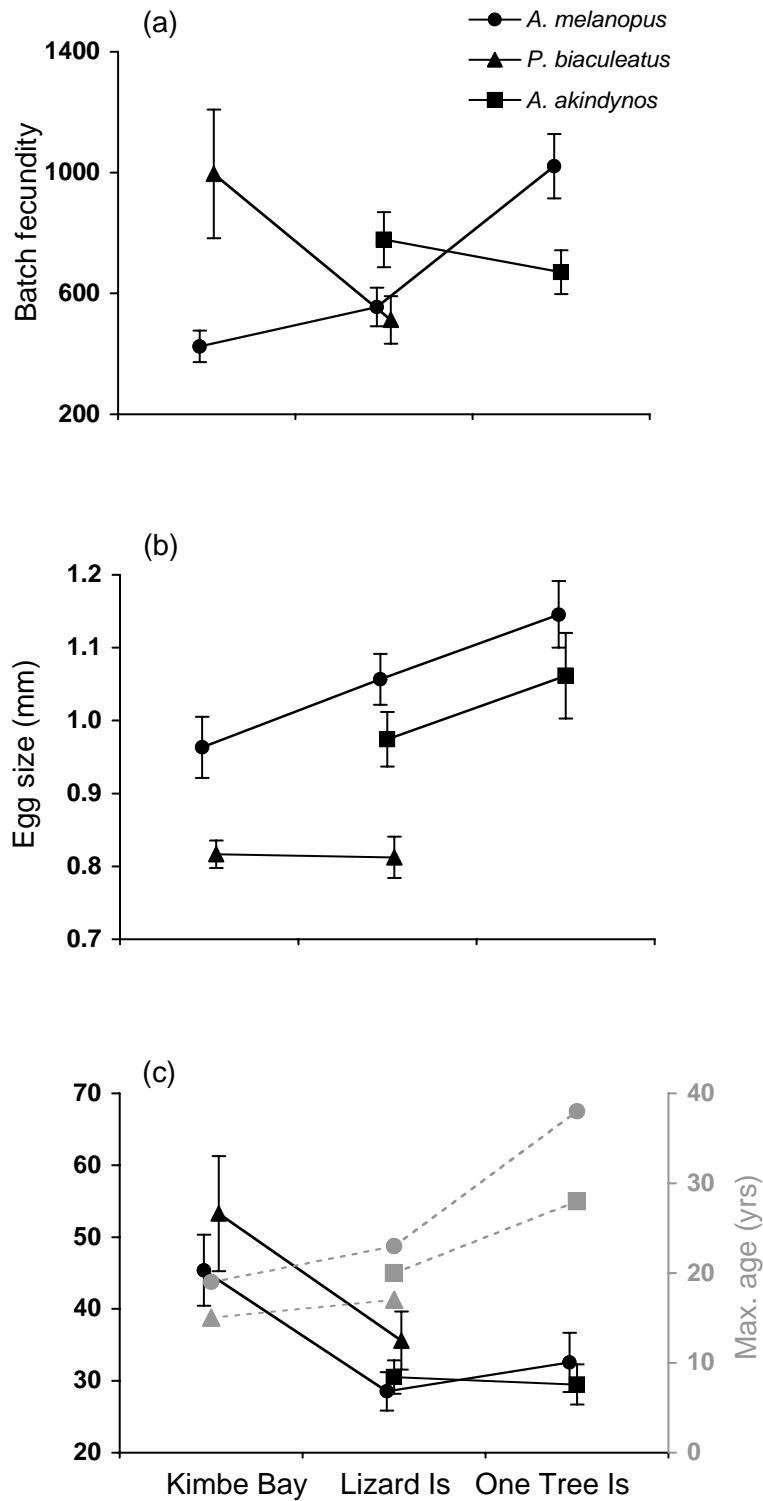
Tree Island than Kimbe Bay, but there were no significant differences in egg size between the other two pairs of locations (Figure 2.6b, Table 2.6).

Mean fecundity/weight was higher at Kimbe Bay than at both Lizard Island and One Tree Island, but did not differ between the latter two locations (Figure 2.6c, Table 2.6). Both batch fecundity and batch fecundity/body weight of *P. biaculeatus* were significantly higher at Kimbe Bay than at Lizard Island (two-tailed t-tests, batch fecundity:  $t = -2.417$ ,  $P = 0.002$ , fecundity/weight:  $t = 2.188$ ,  $P = 0.037$ , Figure 2.6a, c), but egg size did not differ significantly between locations ( $t = 0.176$ ,  $P = 0.861$ , Figure 2.6b). For *A. akindynos* there were no significant differences in any of these three parameters (batch fecundity:  $t = 0.875$ ,  $P = 0.388$ , egg size:  $t = -1.307$ ,  $P = 0.201$ , fecundity/weight:  $t = 0.283$ ,  $P = 0.799$ ) between Lizard Island and One Tree Island (Figure 2.6).

**Table 2.5:** The timing of sex change of each species at each location. Sex change is taken as the median of the age/size range at which mature males and females overlap, and this is also presented as a percentage of maximum age/size.

Species	Location	Age (y)	Sex change		
			% Max age	SL (mm)	% Max SL
<i>A. melanopus</i>	Kimbe Bay	7.5	39.5	55.3	73.7
	Lizard Is.	9.5	41.3	63.0	78.1
	One Tree Is.	19	50.0	82.2	78.8
<i>P. biaculeatus</i>	Kimbe Bay	5.5	37.7	57.8	57.3
	Lizard Is.	7	41.2	44.5	46.6
<i>A. akindynos</i>	Lizard Is.	5	25.0	61.8	65.1
	One Tree Is.	6.5	23.2	58.8	70.0





**Figure 2.6:** Reproductive parameters of each species at each location: (a) mean batch fecundity ( $\pm$ SE), (b) mean egg size ( $\pm$ SE), and (c) mean fecundity/weight ratio ( $\pm$ SE) and mean longevity.

**Table 2.6:** Summary of one-way ANOVAs and post-hoc comparisons of means for batch fecundity, egg size and batch fecundity-body weight ratio of *A. melanopus* at each location (OTI=One Tree Island, LI=Lizard Island and KB=Kimbe Bay). Batch fecundity data were transformed to  $\log_{10}x$ .

	<i>df</i> effect	MS effect	<i>df</i> error	MS error	<i>F</i>	<i>P</i>	Summary of post-hoc comparisons		
Batch fecundity	2	0.67	56	0.07	10.156	0.0002	KB <sup>a</sup>	LI <sup>a</sup>	OTI <sup>b</sup>
Egg size	2	0.13	56	0.03	3.960	0.0246	KB <sup>a</sup>	LI <sup>a,b</sup>	OTI <sup>b</sup>
Fecundity/ Weight	2	1259.6	56	263.6	4.778	0.0121	KB <sup>a</sup>	LI <sup>b</sup>	OTI <sup>b</sup>

Batch fecundities of *A. melanopus* and *A. akindynos* were positively correlated with both body weight and age at Lizard Island, but not at the other locations (Table 2.7). For *P. biaculeatus*, at both Kimbe Bay and Lizard Island, batch fecundity was positively correlated with body weight but not with age (Table 2.7).

**Table 2.7:** Results of correlations between batch fecundity and age/weight for each species at each location. *r* is Pearson's correlation coefficient.

Species	Location	Fecundity vs. Age		Fecundity vs. Weight	
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<i>A. melanopus</i>	Kimbe Bay	0.436	0.136	0.344	0.350
	Lizard Is.	0.701	<0.001	0.421	0.032
	One Tree Is.	-0.262	0.278	-0.185	0.450
<i>P. biaculeatus</i>	Kimbe Bay	0.364	0.547	0.784	0.012
	Lizard Is.	0.153	0.532	0.515	0.014
<i>A. akindynos</i>	Lizard Is.	0.491	0.039	0.708	0.001
	One Tree Is.	0.371	0.191	0.509	0.063

## 2.4. Discussion

Socially induced life history variation did not mask latitudinal trends in life history parameters at the population level: most of the life history characteristics measured here varied significantly among the 2 or 3 geographic locations examined. Some of these differences were consistent with latitudinal trends that have been observed for other fishes over temperate latitudes, but there were also considerable differences among species in the magnitude and direction of geographic variation.

*Amphiprion melanopus* most closely followed the predicted trends, i.e. the population at the highest-latitude location (One Tree Island) had lower growth and mortality rates, lived longer and grew to larger sizes compared to the 2 lower-latitude locations. In addition, individuals matured and changed sex proportionally later and at proportionally larger sizes at the two higher-latitude locations, and females produced larger eggs at the highest-latitude location compared to the lowest-latitude location. Females of this species also produced greater numbers of eggs at any one time at One Tree Island, but this was probably because they were larger, and when differences in body size were taken into account, Kimbe Bay females actually produced greater numbers of eggs per unit body weight than those at the two higher-latitude locations.

Life history characteristics of the other two species, *A. akindynos* and *P. biaculeatus*, were less variable than those of *A. melanopus* and some of the main differences between locations were the opposite to what I predicted based on life history theory. Although the higher-latitude population of each of these species lived longer (as predicted), growth and mortality rates did not differ between locations and, contrary to predictions, the lower-latitude population of each species attained larger body sizes. One possible explanation for the shorter-lived lower-latitude individuals of each species attaining larger adult body size is that there is a trade-off between large body size and

longevity in these species. For example, individuals in the higher-latitude population might stop growing earlier than those in the lower-latitude population to allocate more energy to somatic maintenance in order to live longer.

*A. akindynos* matured and changed sex at proportionally greater sizes at the higher-latitude location, which is consistent with predictions, but displayed the opposite pattern with respect to age at maturation and sex change. This is probably due to the fact that age and size classes at maturation and sex change were similar at each of the two locations compared, but maximum age was higher at One Tree Island, and maximum size was higher at Lizard Island. There was an overlap between locations in the range of proportional ages and sizes at maturation of *P. biaculeatus*, and this species displayed inconsistent patterns between locations with respect to age and size at sex change. Proportional age at sex change was higher at One Tree Island, as predicted, but proportional size at sex change was greater at Lizard Island. For *P. biaculeatus*, number of eggs per female was higher at the lower-latitude location, even when body size was controlled for. Egg size of *P. biaculeatus* and all reproductive parameters of *A. akindynos* did not differ between locations.

The main differences in reproductive parameters of both *A. melanopus* and *P. biaculeatus* were between the lowest-latitude location, Kimbe Bay, and the other two locations, Lizard Island and One Tree Island. In Kimbe Bay, *A. melanopus* females were smaller than those at the other two locations, but they produced a greater number of eggs for a given body size. *P. biaculeatus* females in Kimbe Bay were not only larger, but also produced more eggs for a given body size than those at Lizard Island. The annual temperature variation in Kimbe Bay does not exceed 1-3°C and most fish breed throughout the year, while the two southern locations experience seasonal climates. Although individuals at the higher-latitude locations are restricted to breeding

during the warmer months of the year, they are able to maximise their lifetime reproductive output by living longer and, in the case of *A. melanopus*, attaining a larger adult size. *A. melanopus* individuals from the two higher-latitude populations produce larger eggs, which are likely to produce better quality offspring with higher survival probabilities (Hutchings 1991; Yampolsky and Scheiner 1996; Einum and Fleming 1999). In contrast, populations in Kimbe Bay maximise their reproductive output by maturing and changing sex earlier, producing a greater number of eggs per unit body mass and reproducing throughout the year, to compensate for having short life spans.

It is possible that individuals in Kimbe Bay have shorter life spans and are smaller (in the case of *A. melanopus*) as a consequence of allocating more energy to reproduction at the expense of growth and somatic maintenance. The lack of between-location differences in reproductive parameters of *A. akindynos* might simply be due to the degree of seasonality not differing enough between the two southern locations where this species was sampled. Another possibility is that plasticity of life history traits varies among species depending on the size of their geographic ranges. *A. akindynos* has a much narrower geographic range than the other two species, only occurring on the Great Barrier Reef and northern New South Wales, and is therefore less likely to have high variability in its life history traits. *P. biaculeatus* has a broad geographic distribution and therefore one might expect the life history characteristics of this species to be variable; however, this species displays the highest degree of social control over life history traits due to its strict social structure (usually just one breeding pair per anemone) and highly aggressive nature (Fautin 1986; Srinivasan et al. 1999). *A. melanopus* also has a broad distribution, but in contrast to *Premnas* has very large, loose social groupings (Ross 1978; Fautin 1986), which might mean less social control of its

life history transitions, i.e. patterns of geographic variation might be expected to be similar to other small, non-anemone dwelling damselfishes.

Growth rates of anemonefishes are strongly influenced by social structure, with the female suppressing the growth of the smaller male and sub-adults. Removal of the dominant female is known to cause the male and sub-adults in the social group to rapidly increase in size (Fricke and Fricke 1977; Moyer and Nakazono 1978; Buston 2003b). Growth can also be controlled by the size of host anemone (Hattori 1991; Fautin 1992). For this reason, I initially expected that the VBGF might not fit the size at age data well, as variable growth patterns among individuals of a given age would act to inflate the error around this relationship. However, data for all three species at each location fit the VBGF relatively well ( $r^2$  values of 0.76 and over). Growth curves of *P. biaculeatus* at both locations had the lowest  $r^2$  values, which is probably again due to the fact that social control of growth in this species is the strongest. The size difference between males and females is also greater in this species than in other species of anemonefishes (Randall et al. 1997).

Another reason for the difference among the three species in the geographic variability of their life history traits is that the number of locations (i.e. three for *A. melanopus*, and just two for *A. akindynos* and *P. biaculeatus*) was small, and differences among locations may be influenced more by small-scale environmental variation rather than broader scale latitudinal trends. For example, Kimbe Bay is very sheltered and rarely experiences tropical cyclones, so any differences between this location and the other two locations might simply be due to the lack of environmental disturbance. Other studies have found that there can be significant variation in life history traits such as longevity, mortality rates and reproductive output even over small spatial scales within the same latitude, e.g. among reefs at different positions across the

east-west gradient of the Great Barrier Reef (Gust et al. 2002; Gust 2004). Another factor that may have affected my results is that sampling effort varied between/among locations and species. The sample of *A. melanopus* from Kimbe Bay (n=37) was particularly small, and this may have affected the power of the statistical tests used.

The results of this study add to an increasing body of evidence that coral reef fishes are much longer lived than previously thought (see also Choat and Axe 1996; Meekan et al. 2001; Caldow and Wellington 2003). In addition, this is the first study to use otoliths to obtain age estimates and examine size at age relationships for anemonefishes. Until now, only one study has estimated the age of an anemonefish (Moyer 1986), based on long-term observations of a single *Amphiprion clarkii* individual that reached 13 years before disappearing. Others have suggested longevity of up to 18 years for *A. frenatus* and 18+ years for *A. perideraion* (Fautin and Allen 1992). Here I show that anemonefishes can live for up to 38 years, which is much longer than the life spans of other similar sized damselfishes from the same location (One Tree Island, Fowler and Doherty 1992; Doherty and Fowler 1994). Anemonefishes are a popular target of the aquarium fish trade, and the fact that they are so long-lived has important implications for the recovery of overexploited populations.

## **CHAPTER 3: Evaluation of the basis of geographic variation in the reproductive traits of anemonefishes**

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### **3.1. Introduction**

Variations in life history characters across latitudes have been documented for a range of terrestrial, freshwater and marine animals (Stearns 1992). Among fishes, latitude correlates with intra- and interspecific differences in maternal reproductive traits such as egg size, relative fecundity, adult size, size and age at sexual maturation, length of spawning season and frequency of spawning (e.g. Bagenal 1978; Leggett and Carscadden 1978; Edwards 1984; Mann et al. 1984). Such latitudinal variation is expected because of the conspicuous environmental gradient and it is unlikely that any single phenotype will confer high fitness in all situations (Via et al. 1995).

Models of the evolution of progeny size generally start with two assumptions: (1) progeny fitness increases with increasing parental investment per offspring, and (2) there is a trade-off between the number of progeny a female can produce and the amount of resources allocated to each of them (e.g. Smith and Fretwell 1974; Roff 1992; Stearns 1992). Life history theory predicts that the optimal trade-off between egg size and number depends on the conditions experienced by offspring (Parker and Begon 1986). It follows that, for a given species in a given environment, selection will favour an optimal propagule size and fecundity will simply shift with total reproductive effort to match the optimal size (Smith and Fretwell 1974; Sargent et al. 1987). Where conditions are poor (e.g. a short growing season, lower average temperatures, lower food availability and predictability), selection should favour investment in fewer and larger progeny, that presumably have greater ability to survive, grow, and develop



(Sibly and Calow 1983; McGinley et al. 1987; Morris 1987; Winkler and Wallin 1987; Hutchings 1991; Winemiller and Rose 1993). Because growing seasons are shorter at high latitudes, egg size may be under strong latitudinal selection, as offspring need to develop to a critical size before the summer's end (Hurst and Conover 1998; Schultz et al. 1998). Similarly, the optimal allocation of resources to egg production (total reproductive output) should also vary with latitude due to local selection pressures. Theory suggests that overall reproductive effort should increase with decreasing latitude: at low latitudes, where adult survival rates are low, the proportion of assimilated energy allocated to reproduction should be high to compensate for the reduction in lifetime egg production resulting from reduced longevity (Reznick 1983; Case 2000; Gasser et al. 2000).

Most investigators assume that geographic clines in life history traits have a strong genetic component subject to natural selection. However, the observed variation may have at least two possible (non-exclusive) adaptive explanations. Either each environment has its particular selective regime and therefore selects for individuals with the appropriate phenotypic characteristics, which may differ among environments, i.e. local genetic adaptation due to natural selection (Mousseau et al. 2000). Alternatively, organisms may have phenotypically plastic life histories and exhibit traits that are imposed by the local environment (Via and Lande 1985). Simply observing geographic differences in life history traits in the wild is therefore not meaningful in an evolutionary sense, because both genetic and/or environmental factors contribute to phenotypic variance (Conover and Schultz 1995). For example, differences in egg size and fecundity among latitudes in wild populations may be purely environmentally driven and have no genetic component, since egg size and fecundity are affected by several environmental factors (Wootton 1998). Local adaptation can be revealed by

experiments where lineages are compared in a common environmental setting, where factors such as female condition, photoperiod, water temperature, and food availability etc. can be controlled.

The potential for local adaptation is determined by the interplay between gene flow and the selection differential across environments (Adkison 1995). In marine species, where geographic barriers to gene flow are usually absent and early life stages are highly dispersive, the opportunity for local adaptation would therefore seem to be relatively low (Hedgecock 1986; Warner 1997; Shulman 1998). Indeed, direct evidence of local adaptation in marine fish is extremely sparse. In most studies that have investigated latitudinal variation of life history traits in fish, the relative importance of genetic and non-genetic factors and the plasticity of individual traits or suites of traits remain unknown (Grant and Dunham 1990). One exception is the research by Conover and colleagues (1990; 1997), which documents adaptive latitudinal variation in physiological parameters in the Atlantic silverside (*Menidia menidia*). So far, very few studies have used the common-environment approach to explore the genetic basis of variation in reproductive traits in marine species in general and tropical fishes in particular.

There are a large number of empirical studies documenting considerable inter- and intraspecific variation in reproductive traits in fishes (Bagenal 1971; Kamler 1992; Brooks et al. 1997; Heath and Blouw 1998). Egg size variation within populations has been correlated with female size and season (Hendry et al. 2001), while geographic differences have been related to environmental factors such as temperature, salinity, length of growing season and offspring habitat quality (Solemdal 1967; Bagenal 1971; Fleming and Gross 1990; Tamate and Maekawa 2000). For example, latitudinal variation in egg size may be due to the temporal availability of larval food supply

(Pearse et al. 1991; Bauer 1992; Olive 1992). Since the temporal patterns of plankton productivity are negatively correlated with latitude (Bauer 1992), females at high latitudes should produce larger eggs that hatch with larger amounts of yolk reserves, which sustain the larvae in the plankton between pulses of productivity in the same season. Conversely, at lower latitudes, the higher temperature and more stable environment offers a high chance of survival for fish larvae, regardless of the investment per offspring; therefore a female should gain the highest returns by producing as many (small) eggs as possible. Although latitudinal variation in reproductive traits in marine fish has long been documented (e.g. Marshall 1953), the environmental and/or genetic basis of these patterns is largely unknown.

In the previous chapter, natural populations of *Amphiprion melanopus*, *Amphiprion akindynos* and *Premnas biaculeatus* were shown to exhibit a range of reproductive traits that varied among three locations along a latitudinal gradient of 18° between Papua New Guinea and the southern Great Barrier Reef. Some of the patterns found were in agreement with predictions of life history theory outlined above; for example, *A. melanopus* females produced significantly larger eggs at the highest-latitude location, and significantly greater numbers of eggs per unit body weight at the lowest-latitude location. Similarly, in *P. biaculeatus*, batch fecundity and reproductive effort were significantly higher at the lowest-latitude location.

Here I tested whether these observed differences in reproductive characteristics are likely to have a genetic basis, or whether they are phenotypic responses to the environment. This was achieved by a common-environment experiment, in which breeding stocks of different latitudinal populations were established in a common-garden setting and monitored for their reproductive activity over an extended period of time. By using this method I was able to control for environmental factors that may

affect reproductive performance, such as water temperature and depth, food availability and quality, habitat features, predation and social structure.

A possible problem with common-garden experiments is the elimination of persisting environmental influences on phenotypic performance that are a function of experience before the beginning of a trial (Conover and Schultz 1995). For example, fish collected from the wild and then tested in a common-environment may retain non-genetic morphological or physiological characters that were acquired in their native habitat (Conover and Schultz 1995). Using offspring from parents that were themselves raised in a common-environment can eliminate this possibility, but for long-lived and relatively late maturing species such as anemonefishes this is practically impossible. However, to account for this potential problem, one of the species investigated here (*A. melanopus*) was observed over three consecutive years. This allowed time for a possibly delayed phenotypic response to environmental conditions and thus an assessment of the degree to which these traits are regulated over time.

Another possible caveat when comparing populations from different latitudes is the possibility of local-scale variation at the habitat level (Sale 1998). Several studies have demonstrated that life history traits of reef fishes can vary over small spatial scales (Aldenhoven 1986; Choat et al. 1996; Gust et al. 2002; Robertson et al. 2005) due to local environmental factors. To control for such small-scale effects, one of the species investigated here, *A. melanopus*, was collected at two adjacent reefs within latitudes. Unfortunately, due to logistical constraints, spatial replication was not possible for the other two species.

Based on predictions of life history theory, and assuming that the previously observed life history variation in wild populations is adaptive, I predicted that egg sizes of populations from higher latitudes should be larger compared to egg sizes of

populations from low latitudes in a common-garden. Further, since egg size and number produced are usually opposing attributes, I predicted that populations with large eggs should have small egg numbers and *vice versa*. I also expected that populations from higher latitudes have a lower total reproductive output compared to populations from low latitudes.

Anemonefish are very suitable for this type of study: they are relatively abundant, easy to maintain in captivity and they readily spawn comparatively large benthic eggs on artificial substrates. Also, their broad distribution enables comparisons of phenotypic plasticity and local adaptation of fitness-related traits across a large latitudinal gradient. Here I discuss how the maternal reproductive traits of these fishes may vary with latitude. This study represents one of the first to experimentally test whether or not there are fixed differences in these traits in a group of long-lived coral reef fishes.

## **3.2. Materials and Methods**

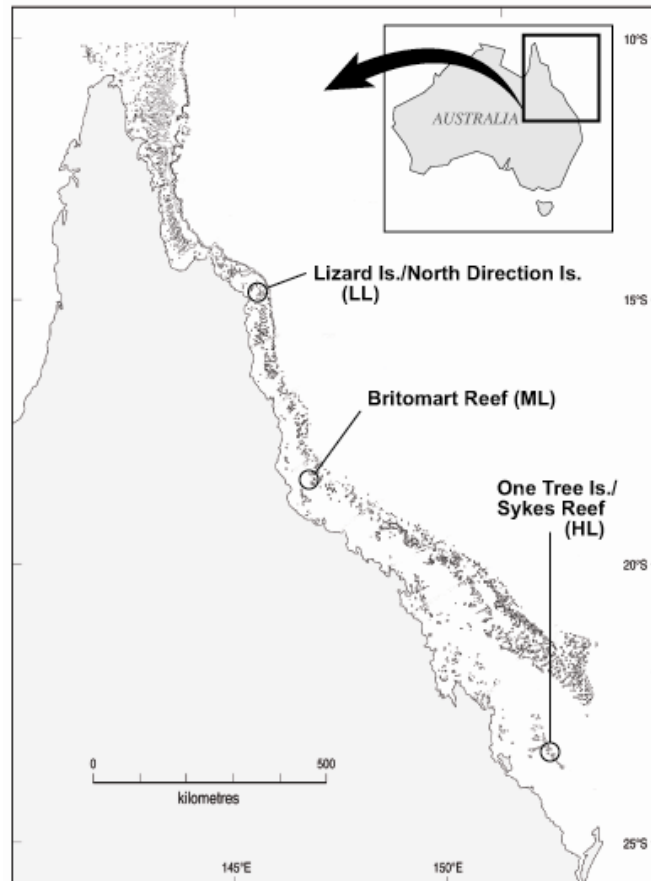
### ***3.2.1. Study species and study sites***

Three species of anemonefishes were used in this study: *Amphiprion melanopus*, *Amphiprion akindynos* and *Premnas biaculeatus*. These species inhabit shallow tropical and temperate reefs in the eastern Indian Ocean and Western Pacific. Detailed distribution maps are presented in *chapter 1* (Figure 1.2). Anemonefish are multiple batch spawners. Approximately every two weeks females attach several hundred to a few thousand eggs to the hard substrate under the tentacles of their host anemone. Both parents exhibit egg tending, although males typically provide more nest care than females (Thresher 1984). Tending continues for about 8 days until pelagic larvae are hatched.

For this experiment, adult breeding pairs were collected from three latitudes along the Great Barrier Reef: a low-latitude location (LL) at approximately 14°40'S, a mid-latitude location (ML) at approximately 18°23'S and a high-latitude location (HL) at approximately 23°30'S (Figure 3.1). These locations span a large latitudinal gradient of almost 9°, equalling a distance of over 1000km. Taking into account the possibility of smaller-scale variation within these latitudes, *A. melanopus* breeding pairs were collected from two replicate sites within the low and high latitude. These sites were Lizard Island and North Direction Island in the North and One Tree Island and Sykes Reef in the South. *A. akindynos* originated from single populations at the low latitude (Lizard Island), mid-latitude (Britomart Reef) and high latitude (One Tree Island). As the range of *P. biaculeatus* does not extend as far south as One Tree Island, this species was collected from single populations at the low (Lizard Island) and mid-latitude (Britomart Reef) respectively.

### **3.2.2. Common-environment experiment**

Breeding populations of *A. melanopus* were established between 1999 and 2002 and of *A. akindynos* and *P. biaculeatus* in 2001 and 2002. Fish were maintained outdoors under partly shaded, natural illumination in the James Cook University Marine and Aquaculture Research facilities, Queensland, Australia. Each breeding pair was housed individually in a circular aerated 100l plastic tank receiving flow-through seawater from the aquarium system. Prior to commencement of the experiment, adult fish were anaesthetised with clove oil, weighed for wet weight (g), and measured for standard length (mm). A period of several weeks was allowed for the fish to acclimatise before the common-environment experiment began. Each tank contained an anemone (*Heteractis magnifica*, Quoy and Gaimard 1833) and a halved terracotta pot serving as an artificial removable structure for shelter and egg spawning.



**Figure 3.1:** Map showing the geographic origin of breeding pairs along the Great Barrier Reef. The low-latitude location (LL) includes Lizard Island and North Direction Island, the mid-latitude location (ML) is Britomart Reef, and the high-latitude location (HL) includes One Tree Island and Sykes Reef.

Fish were fed to satiation once a day with a mixture of minced pilchard and squid. Reproductive parameters were recorded for *A. melanopus* over three consecutive reproductive seasons from 1999 to 2002, and for *A. akindynos* and *P. biaculeatus* over one reproductive season from September 2002 to February 2003.

### 3.2.3. *Egg and clutch measurements*

Terracotta pots of each breeding pair were checked daily for new egg clutches to determine spawning dates. Three randomly selected eggs per clutch were sampled on the day a clutch was laid and preserved in 10% formaldehyde. After 48 hours egg samples were transferred into 70% Ethanol and stored for later measurements. Lengths

and widths of eggs were recorded (to the nearest 0.01mm) using an Olympus SZ40 Stereo Microscope fitted with an ocular micrometer. Egg volume ( $\text{mm}^3$ ) was calculated as: egg length  $\times \pi$  (egg width/2)<sup>2</sup>. Fecundity was defined as the number of eggs in a freshly spawned egg clutch. New clutches were removed briefly from the tank and photographed using a Nikon F50 camera and slide film. Generally, oviposition occurred on the pots, though on rare occasions, fish oviposited on to the sidewall of the aquarium. For these clutches fecundity could not be measured. To estimate clutch sizes, slides were projected onto a whiteboard and eggs individually counted using a pen and tally counter. Clutch volume was calculated by multiplying the mean egg size per female by the number of eggs (fecundity) of each clutch. Total reproductive output was calculated as the sum of all clutch volumes of each female.

#### **3.2.4. Data analyses**

Egg size is affected by female body size in many taxa, including fish (reviewed in Roff 1992). If this is the case, then differences in egg size could be simple functions of maternal size. In all three species investigated here, however, egg size was not significantly correlated with maternal body size, indicating that egg size is independent of female size. Similarly, fecundity is almost universally positively correlated with female size in fish (Wootton 1998). This was also the case for *A. melanopus*, *A. akindynos* and *P. biaculeatus*. Therefore, fecundity was corrected for the body size of individual females using residual analyses (henceforth called relative fecundity).

In *A. akindynos* and *P. biaculeatus* analysis of variance (ANOVA) was performed to compare mean egg size, mean relative fecundity and total reproductive output between locations. In *A. melanopus* breeding pairs of two populations within each location were available, therefore regional variation was taken into account by



nested analysis of variance with populations nested within locations. Finally, linear regressions were used to test for relationships between egg size and relative fecundity. All analyses were performed using Statistica Version 6.2 (StatSoft Inc. Tulsa OK, USA).

### **3.3. Results**

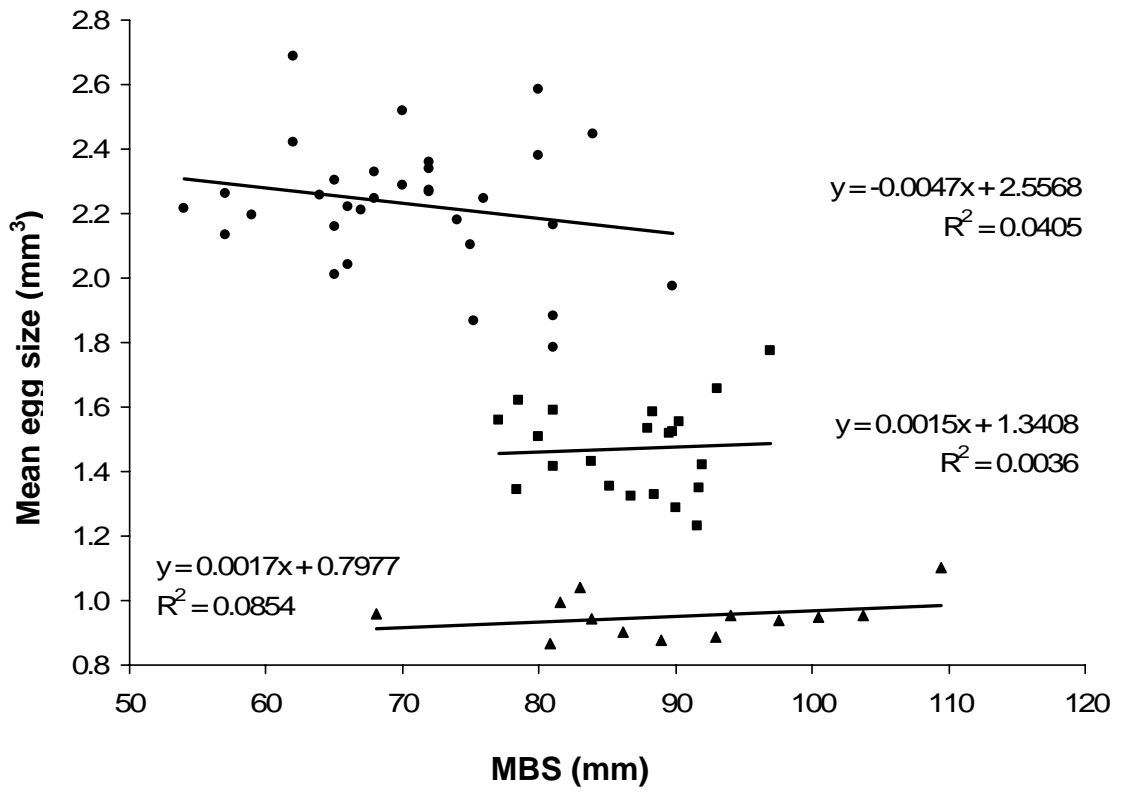
#### **3.3.1. Egg size**

##### *Egg size and maternal body size*

In all three species, the relationships between mean egg size and maternal body size (SL) were not statistically significant ( $F$  value range 1.268 – 0.056, all  $P > 0.05$ ), indicating that egg size is independent of female size in these species (Figure 3.2).

##### *Egg size and latitude*

A comparison of mean egg sizes between populations of each species provided no evidence for genetically based differences in this trait among latitudes. In *P. biaculeatus* mean egg sizes of the low-latitude (LL) and high-latitude (HL) populations were practically identical (ANOVA,  $F = 0.007$ ,  $P > 0.05$ ; Table 3.1). In *A. akindynos* there was more variation in mean egg sizes among the three populations; however, these differences were not statistically significant (ANOVA,  $F = 2.876$ ,  $P = 0.084$ ). Furthermore, the observed variation did not follow the expected linear latitudinal trend: the mid-latitude population (ML) produced the largest eggs, followed by the HL population and the LL population (Table 3.1). As in the other species, mean egg sizes of *A. melanopus* were not significantly different between locations or populations (nested ANOVA,  $F = 0.763$ ,  $P > 0.05$ ).



**Figure 3.2:** The relationships between mean egg size and maternal body size (MBS, standard length) in *P. biaculeatus* (▲), *A. akindynos* (■), and *A. melanopus* (●).

**Table 3.1:** Mean egg sizes ( $\pm$ SE) of *P. biaculeatus*, *A. akindynos* and *A. melanopus* from the respective locations along the Great Barrier Reef. LL: low-latitude (Lizard Island), ML: mid-latitude (Britomart Reef), HL: high-latitude (One Tree Island). In *A. melanopus* data of populations within locations were pooled (LL: Lizard Island & North Direction, HL: One Tree Island & Sykes Reef). N = number of breeding pairs observed.

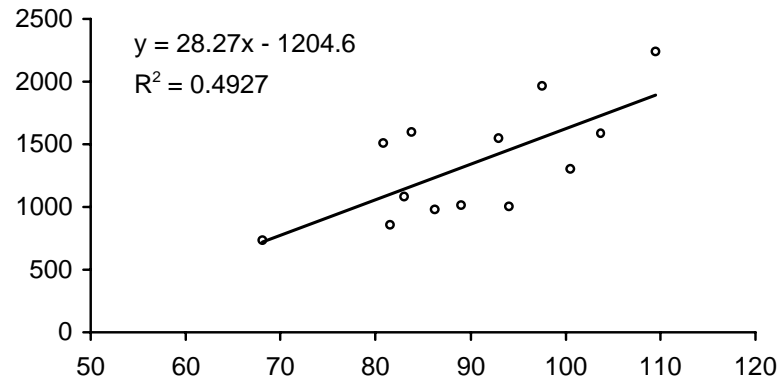
Species	Location	N	Mean egg vol. (mm <sup>3</sup> )	$\pm$ SE
<i>P. biaculeatus</i>	LL	8	0.953	0.029
	ML	5	0.950	0.015
<i>A. akindynos</i>	LL	9	1.406	0.039
	ML	6	1.569	0.056
	HL	5	1.460	0.064
<i>A. melanopus</i>	LL	24	2.222	0.041
	HL	13	2.251	0.071

### 3.3.2. Fecundity

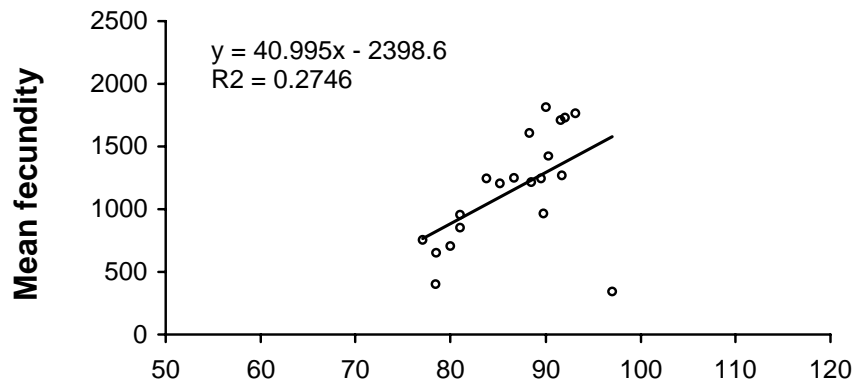
#### *Fecundity and maternal body size*

The present study confirmed that fecundity was significantly related to female size, a relationship commonly found in teleosts. Linear regressions between fecundity and maternal body size were significantly positive in all three species (*P. biaculeatus*:  $F = 10.683$ ,  $P = 0.008$ ; *A. akindynos*:  $F = 6.815$ ,  $P = 0.018$ ; *A. melanopus*:  $F = 29.322$ ,  $P < 0.001$ ; Figure 3.3). Maternal age may be an element influencing this relationship (Rana 1988), but unfortunately, brood stock used in the present study was of unknown age structure. To remove the effect of female size in further analyses, relative fecundity was determined using unstandardised residual fecundity from a regression of mean fecundity of each female on maternal body size.

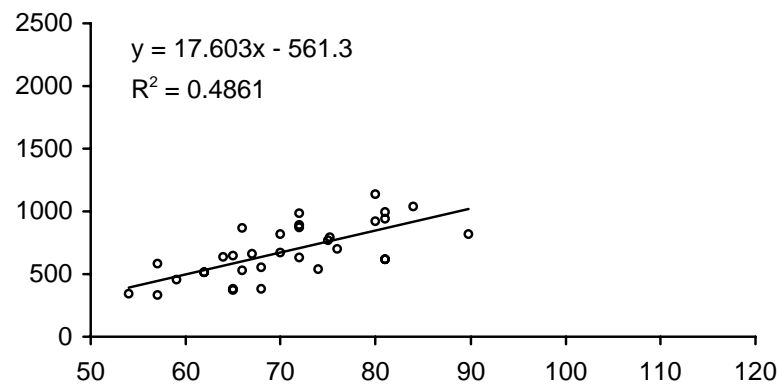
***P. biaculeatus***



***A. akindynos***



***A. melanopus***



**MBS (mm)**

**Figure 3.3:** The relationships between mean fecundity (egg number) and maternal body size (MBS, standard length) in *P. biaculeatus*, *A. akindynos*, and *A. melanopus*.

### *Fecundity and latitude*

As with egg size, a comparison of mean relative fecundity between populations of each species provided no evidence for fixed latitudinal variation in this trait. In *P. biaculeatus* relative fecundity did not differ between the two latitudinal populations (ANOVA,  $F = 2.534$ ,  $P > 0.05$ ; Table 3.2). In *A. akindynos*, variation in mean relative fecundity did not follow a latitudinal pattern, but mirrored the trend found in mean egg size: the mid-latitude population (ML), which produced the largest eggs, had the lowest relative fecundity value (Table 3.2). The HL population was intermediate in terms of both egg size and relative fecundity, followed by the LL population, which had the smallest eggs and highest relative fecundity (Table 3.2). However, none of these differences in relative fecundity among populations were statistically significant (ANOVA,  $F = 2.023$ ,  $P > 0.05$ ). Finally, *A. melanopus* also showed no significant differences in mean relative fecundity values, neither among locations nor replicate populations (nested ANOVA, Location:  $F = 2.899$ ,  $P > 0.05$ , Population(Location):  $F = 2.32$ ,  $P > 0.05$ ).

**Table 3.2:** Mean relative fecundity ( $\pm$ SE) of *P. biaculeatus*, *A. akindynos* and *A. melanopus* at the respective locations along the Great Barrier Reef. Relative fecundity was determined using unstandardised residual fecundity from a regression of mean fecundity of each pair on maternal body size (SL). In *A. melanopus* data of populations within locations are pooled (LL: Lizard Island & North Direction, HL: One Tree Island & Sykes Reef).

Species	Location	Mean fecundity ( $\pm$ SE)	Mean relative fecundity ( $\pm$ SE)
<i>P. biaculeatus</i>	LL	1460.9 ( $\pm$ 177.8)	105.3 ( $\pm$ 96.6)
	ML	1148.5 ( $\pm$ 134.0)	-168.5 ( $\pm$ 154.9)
<i>A. akindynos</i>	LL	1366.4 ( $\pm$ 126.6)	148.7 ( $\pm$ 96.8)
	ML	840.1 ( $\pm$ 139.4)	-234.3 ( $\pm$ 203.3)
	HL	1151.2 ( $\pm$ 230.2)	13.5 ( $\pm$ 124.2)
<i>A. melanopus</i>	LL	624.3 ( $\pm$ 40.9)	-37.1 ( $\pm$ 27.0)
	HL	836.5 ( $\pm$ 64.4)	99.0 ( $\pm$ 59.6)

### 3.3.3. *The relationship between egg size and egg number*

There is ample evidence in the literature of phenotypic trade-offs between egg size and fecundity from populations of a wide range of taxa; however, in the three species investigated here, no consistent pattern emerged. In *P. biaculeatus* the regression of mean relative fecundity on mean egg size showed no significant relationship between the two parameters in either of the two populations (LL:  $F = 0.088$ ,  $P > 0.05$ ; ML:  $F = 0.011$ ,  $P > 0.05$ ). In *A. akindynos* the expected negative correlation was evident only in the ML population ( $F = 9.16$ ,  $P = 0.04$ ), but no significant relationships were found in either the LL or HL populations ( $F = 1.145$ ,  $P > 0.05$  and  $F = 6.241$ ,  $P > 0.05$  respectively). In contrast, *A. melanopus* displayed positive phenotypic relationships between egg size and number in both the LL and HL locations (LL:  $F = 4.975$ ,  $P = 0.037$ ; HL:  $F = 6.383$ ,  $P = 0.039$ ).

### 3.3.4. *Total reproductive output*

Similar to egg size and fecundity, comparisons of total reproductive output between populations of each species did not provide support for my prediction of fixed differences among latitudinal populations. In *P. biaculeatus*, the mean reproductive output of the two populations during one reproductive season was very similar (Table 3.3) and not statistically significant (ANOVA,  $F = 0.09$ ,  $P > 0.05$ ). Compared to *P. biaculeatus*, overall reproductive investment was considerably higher in *A. akindynos*, with about 70% more output of egg mass during the same time period. However, among the three populations, mean total reproductive output did not differ significantly in *A. akindynos* (ANOVA,  $F = 0.588$ ,  $P > 0.05$ ). Variation was similar to the pattern found in fecundity: reproductive output was greatest in the LL population, followed closely by the HL population, whilst the mid-latitude population had the lowest reproductive

output (Table 3.3). In *A. melanopus*, total reproductive output (mean of three seasons) was again not significantly different between locations (nested ANOVA, Location:  $F = 1.834$ ,  $P > 0.05$ ; Population(Location):  $F = 0.095$ ,  $P > 0.05$ ).

**Table 3.3:** Mean total reproductive output (TRO) in cubic centimetres of *P. biaculeatus*, *A. akindynos* and *A. melanopus* from the respective locations. Total reproductive output was calculated as the sum of all clutch volumes of each pair. In *P. biaculeatus* and *A. akindynos* reproductive parameters were recorded over one reproductive season. In *A. melanopus* the means of three reproductive seasons are given.

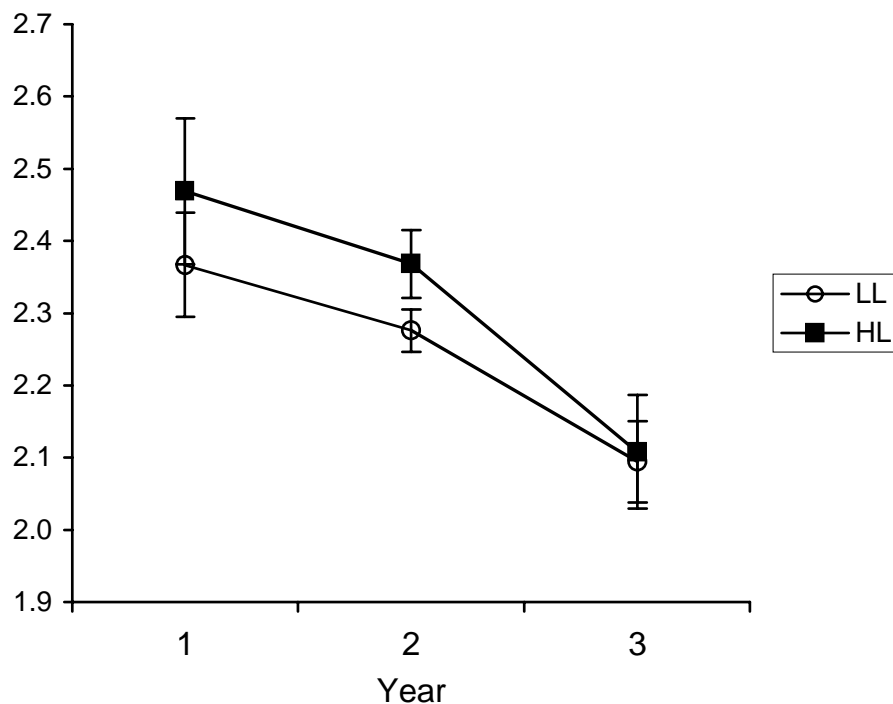
Species	Location	Mean TRO (cm <sup>3</sup> ±SE)	Range (cm <sup>3</sup> )
<i>P. biaculeatus</i>	LL	8.87 (± 1.95)	3.55 – 18.51
	ML	9.67 (± 1.22)	6.04 – 13.62
<i>A. akindynos</i>	LL	18.00 (± 3.66)	3.18 – 33.21
	ML	12.55 (± 2.92)	4.89 – 24.42
	HL	17.38 (± 4.77)	2.68 – 29.27
<i>A. melanopus</i>	LL	9.35 (± 1.74)	0.46 – 47.04
	HL	10.95 (± 3.16)	1.75 – 43.90

### 3.3.5. Temporal variation in egg size and fecundity

*A. melanopus* was the only species for which reproductive data was available for more than one year, and significant inter-annual variation in mean egg size was detected (nested ANOVA, year:  $F = 10.496$ ,  $P < 0.001$ ). Over the observation period of three years, mean egg sizes exhibited a significant continuous decrease in populations from both locations (Figure 3.4). This variation was not due to variation in temperature, as mean water temperatures in the aquarium system did not differ significantly among years (ANOVA,  $F = 1.067$ ,  $P > 0.05$ ). Furthermore, the possibility of a gradual decline in physical condition of these fishes can almost certainly be dismissed. Repeated measurements of length and weight of adults throughout the experiment were not possible, because these species are very susceptible to stress, and such invasive procedures would have interrupted their breeding activity. However, each day at

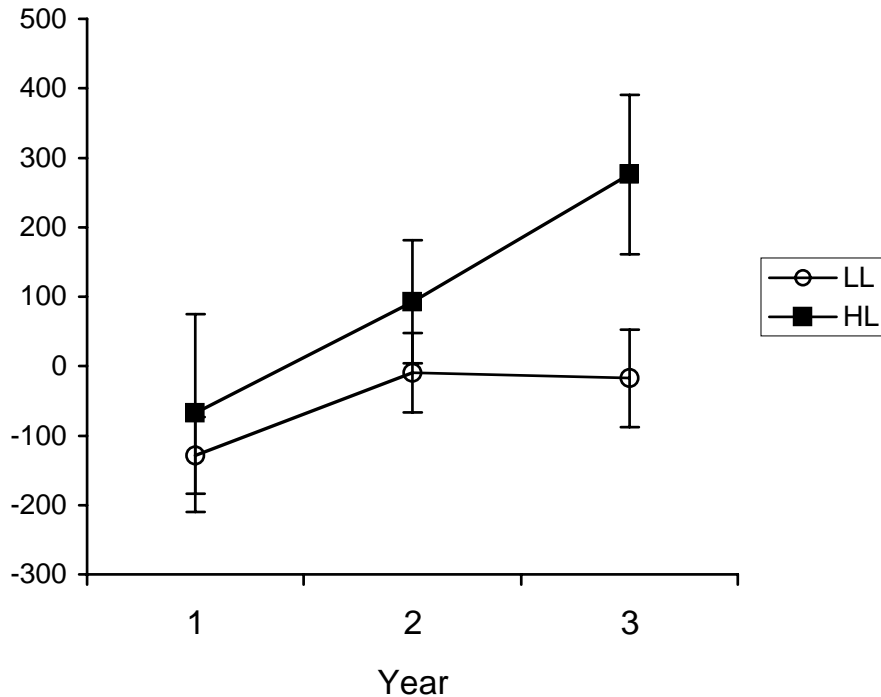
feeding time the health of all individuals was assessed by their appearance, activity and appetite, and there was no evidence of a gradual deterioration of their constitution over the duration of the experiment.

Temporal variation in mean relative fecundity of *A. melanopus* was not statistically significant (nested ANOVA, year:  $F = 2.045$ ,  $P > 0.05$ ). However, there was a trend for an increase in fecundity over time (Figure 3.5). Whilst the HL population showed a continuous increase in relative fecundity over the observation period of three years, the LL population only showed such an increase in the first two years and then stagnated in the third season (Figure 3.5).



**Figure 3.4:** Temporal variation in the yearly mean egg volumes ( $\text{mm}^3 \pm \text{SE}$ ) of *A. melanopus* from a low- (LL) and high-latitude (HL) location over three consecutive years. The year effect is significant (see text).





**Figure 3.5:** Temporal variation in the yearly mean relative fecundity ( $\pm$ SE) of *A. melanopus* from a low- (LL) and high-latitude (HL) location over three consecutive years. Relative fecundity was determined using unstandardised residual fecundity from a regression of mean fecundity (egg number) of each female on maternal body size.

### 3.4. Discussion

The results of the previous chapter have demonstrated that reproductive performance (i.e. egg size, fecundity and reproductive effort) can vary among anemonefish populations living at different latitudes. It is generally assumed that the values of life history characteristics expressed in nature are the outcome of long-term natural selection, acting to optimise the population to prevailing environmental conditions (Stearns 1977). However, there is growing recognition that the interpretation of spatial or temporal variation in life history traits requires partitioning of the sources of variation into environmentally induced components vs. variation caused by non-environmental, fixed factors (Berven and Gill 1983; Kaplan 1987; Sinervo and Doughty 1996). Here, I attempted to determine if the observed trait differences in the wild are

purely driven by environmental variation, or if they have a genetic basis, by measuring spawning performance of populations held in a common-garden. The lack of any differences in reproductive traits among populations held under identical environmental conditions suggests that the reproductive characteristics of these species are largely determined by environmental factors and do not have a (strong) genetic component.

#### **3.4.1. *Egg size and fecundity***

This study tested the prediction that, if there were fixed differences among populations from different latitudes, egg sizes would be larger and fecundity lower in fishes originating from higher latitudes held in a common-garden. However, no such differences were found in egg size or fecundity among populations. Numerous other previous studies have documented latitudinal trends in egg size (e.g. Fleming and Gross 1990; Tamate and Maekawa 2000) as well as fecundity (e.g. Paulson and Smith 1975; Leggett and Carscadden 1978; Fleming and Gross 1990; McGurk 2000) in fishes. However, similar to the inconsistent patterns found in *chapter 2*, the observed relationship between egg size and fecundity with latitude is quite variable, and the expected pattern of increasing egg size with latitude not always evident. For instance, Blaxter and Hempel (1963) showed that the mean egg size of herring differed widely between various areas in the North Sea: small eggs being produced in northern spawning populations, large eggs in southern. Similarly, both Fleming and Gross (1990) and Beacham and Murray (1993) found that egg size decreased and fecundity increased with latitude among Pacific salmon. On the other hand, egg size increases and fecundity decreases with latitude in masu salmon (Tamate and Maekawa 2000). Analogously, Miller et al. (1991) found that egg size increased with the northern limit of the latitudinal range in North American flatfish. However, in anadromous trout, L'Abée-

Lund and Hindar (1990) found no latitudinal gradient in egg size at all between 58° and 65°N in Norway. This is in agreement with Bagenal's (1971) assertion that egg size varies little over quite large distances. For tropical reef fishes, few comparative data is available. In five other species of damselfishes, sampled at two sites on the GBR (Lizard Island and One Tree Island), patterns were again inconsistent. As predicted, *Dischistodus pseudochrysopoecilus* had larger eggs at One Tree Island, but in *Chrysiptera biocellata* and *C. cyanea* the opposite was found. Two further species, *Pomacentrus rhodonotus* and *Dischistodus melanotus*, had virtually identical egg sizes at the two sites (Thresher and Brothers 1989).

What are possible reasons for the failure to find a direct relationship between reproductive characters and latitude? In order for selection to favour production of larger eggs at lower temperatures, the fitness advantage of producing them must outweigh the accompanying loss of fecundity. Maybe this situation does not exist in all fishes or temperature-mediated variation in egg size is a mere physiological response of little adaptive value (Van der Have and De Jong 1996; Van Voorhies 1996; Ernsting and Isaaks 1997). Alternatively, if reproduction is triggered by local water temperatures (Bye 1990), spawning may be seasonally delayed from lower to higher latitudes, but occur at similar water temperatures throughout the species range. Therefore, a latitudinal gradient would exist in the timing of spawning, but little or no gradient would be evident in egg characteristics or the duration of the embryonic period.

Further, genetic differentiation between these populations may be prevented by gene flow. The studies listed above, which have shown that fishes are capable of displaying variation in reproductive traits across local populations, have involved almost entirely only a few groups of freshwater and anadromous species (reviews by Taylor 1991; Carvalho 1993), where gene flow is restricted among populations through

land barriers and the tendency for “homing” to natal habitats. This genetic isolation, combined with different selective pressures among streams, has provided the opportunity for natural selection to shape population-specific differences in life history traits. However, many marine species, including anemonefishes, possess a benthic adult stage and pelagic larval stage. This pelagic phase in the early life history has high dispersal potential and geographic barriers to gene flow are generally lacking. Gene flow between populations is considered a strong constraint on local adaptation (e.g. Warner 1997), and it can therefore be assumed that gene flow results in the “averaging” of selection across wide proportions of a species’ range, thereby preventing local adaptation. However, it is also possible that the capacity for local adaptation exists, but it is overridden by selection for adaptive phenotypic plasticity. This may be the case if environmental factors that affect reproductive performance are so variable at each location that “choosing” a fixed egg size/fecundity combination would be maladaptive.

#### ***3.4.2. The relationship between egg size and number***

Optimality models of propagule size and number have a long history in evolutionary biology (Lack 1947; Smith and Fretwell 1974; Parker and Begon 1986; Lloyd 1987; reviewed in Clutton-Brock 1991). These models are an important foundation of life history theory, which predicts a trade-off between egg size and fecundity, as individuals have limited resources and both traits cannot be simultaneously maximised (Roff 1992; Stearns 1992). Thus, traits competing for resources from the same limited pool are expected to exhibit a negative correlation. It has been suggested that trade-offs between these two traits may be especially complex in organisms such as fishes, in which indeterminate growth is common and in which clutch size usually increases with body size (Blueweiss et al. 1978). Negative

correlations between egg size and fecundity have been shown for rainbow trout (Bromage et al. 1990), brown trout (L'Abée-Lund and Hindar 1990), and for 26 other families of freshwater and marine fishes (Elgar 1990). In contrast, the present study did not reveal consistent negative trade-offs within or among populations of the same species.

Possible explanations for the lack of negative correlations between egg size and number may be the following. First, trade-offs should ultimately exist at the genotypic level (Reznick 1985), but their expression in the phenotype may be masked by environmental influences such as maternal effects (e.g. Bernardo 1996). Second, increases in both resource acquisition and allocation may be possible under favourable environmental conditions (such as the ones in the common-garden experiment) due to a lowered cost of reproduction in a good habitat (Reznick et al. 2000). Third, offspring size and number may each co-vary with other traits and thus make the trade off between these two reproductive characters difficult to detect (Pease and Bull 1988). Fourth, trade-offs may be more difficult to detect in iteroparous species such as anemonefish, due to differences in age and thus, residual reproductive value (Fox and Czesak 2000). Also, trade-offs within individuals may not always translate into trade-offs among individuals, and a number of population-level studies have found positive correlations between traits that are expected to exhibit trade-offs (reviewed in Roff 1992; Stearns 1992). Such an example in fish is the study by Thorpe et al. (1984), who reported that egg diameter and fecundity were positively correlated in Atlantic salmon (*Salmo salar*).

### **3.4.3. Temporal variation in egg size and fecundity in *A. melanopus***

Studying interannual variation within populations is important because this provides clues as to the degree to which reproductive characteristics, such as egg size,

are being regulated or are stable. In the present experiment, an interesting between-year effect in the reproductive characteristics of *A. melanopus* was observed. Generally, egg size decreased and fecundity increased over a period of three consecutive years. While the decrease in egg size was significant and continuous in both locations, the increase in fecundity was only continuous in the HL populations, and fecundity remained constant in LL populations in the third year. This general temporal shift from large to small eggs and towards higher fecundity is consistent with the prediction that these two characters trade-off phenotypically and indicates that such trade-offs can be environmentally driven. It also suggests that the negative association commonly observed between egg size and number may evolve differently/independently between populations, whereby a change in egg size is not necessarily associated with a simultaneous change in fecundity.

The literature on annual variation in fish reproductive traits suggests that fish vary their reproductive effort in response to changes in their environmental conditions between years (Eldridge and Jarvis 1995; Lobon-Cervia et al. 1997). In the common-garden experiment, environmental factors such as food ration and quality (all fish were fed to satiation daily), competition, predation, and habitat quality were identical for all breeding pairs. Temperature, arguably the most important factor of all, was also the same for all individuals and did not vary significantly between years. Why then did fish produce smaller eggs over time? Laboratory studies have demonstrated that conditions of low food abundance can induce females to produce relatively larger eggs (Reznick and Yang 1993). Larger eggs generally yield offspring with a higher probability of survival, especially if conditions are poor (Miller et al. 1988). Conversely, Bagenal (1969) found that brown trout spawned more, but smaller eggs if they were fed during nine months prior to spawning, relative to unfed conspecifics. Similarly, in Atlantic

salmon, heavily fed females produce relatively small eggs (Jonsson et al. 1996). Therefore it is possible that the observed decrease in egg size is a response to the favourable, stable conditions in an energy-rich laboratory environment that is free of predation and competition. This is also in agreement with life history theory, which postulates that small eggs may be more advantageous in high-growth conditions. Furthermore, studies examining larval growth and development are frequently unable to detect an advantage to progeny developing from large eggs in benign/high-quality environments (Fox and Czesak 2000). The pattern also implies that egg size and fecundity in *A. melanopus* are plastic characters that can be adjusted to environmental conditions in relatively short periods of time. Caution should therefore be exercised when comparing populations on the basis of single year's data only.

# **CHAPTER 4: Geographic variation in juvenile growth and growth plasticity of anemonefishes: a common-garden experiment**

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## **4.1. Introduction**

Species with large ranges often display considerable geographic variation in life history traits such as growth rate, age at first reproduction and fecundity (Roff 1992; Stearns 1992). Much of this variation has been explained by large-scale clines in environmental factors such as temperature, seasonality or resource availability (Berven and Gill 1983; Sweeney and Vannote 1984; Sinervo and Adolph 1989; Dugan et al. 1994; Sibly and Atkinson 1994; Yamahira and Conover 2002). Understanding the processes leading to consistent geographic patterns in life histories remains an important area of theoretical and empirical research (Roff 1992; Stearns 1992).

The rate of somatic growth is one of the most fundamental life history traits in animals (Summerfelt and Hall 1987; Chambers and Miller 1995). It can be influenced by many biotic and abiotic factors, with temperature and food availability being particularly influential (Dutta 1994; Radtke and Fey 1996). Because there can be a large thermal gradient from lower to higher latitudes within the geographic range of widely distributed species, there is a potential for differences in growth rates among populations, as a result of different environmental conditions, selection for divergent rates, or a combination of the two. However, the relative importance of environmental and genetic factors acting on these growth rates, and their potential interactions, are poorly understood.



Two models have been proposed to account for the evolution of different growth rates along a latitudinal gradient. Co-gradient variation (*sensu* Levinton 1983) arises when environmental and genetic influences have the same effect on phenotypic expression, or in other words, there is a positive covariance between environmental and genetic sources of variation. This will result in a geographic pattern in which genotypes that grow relatively faster tend to be found in environments that promote fast growth (Conover and Schultz 1995). Alternatively, variation in growth can be countergradient (CnGV) with respect to environmental influences on growth (defined by Levins 1969; reviewed in Conover and Schultz 1995). In this case, environmental and genetic influences oppose one another across the latitudinal gradient, such that fast-growing genotypes are generally found in the environments that have an inhibitory effect on growth (i.e. in high-latitude environments). Countergradient variation is thought to arise when the phenotypes induced by the environment are the opposite of those favoured by natural selection in the same environment.

Both the co-gradient and countergradient models have gained empirical support. Co-gradient variation has been documented in several marine organisms, including polychaetes (Levinton 1983; Levinton and Monahan 1983), crustaceans (Lonsdale and Levinton 1985) and fish (Denit and Sponaugle 2004). In the latter example, juvenile grey snapper (*Lutjanus griseus*) grew faster at lower latitudes along the coast of the south-eastern United States. There is also evidence for countergradient patterns in ectothermal animals, including insects (Gotthard et al. 1994; James and Partridge 1995; Arnett and Gotelli 1999), amphibians (Berven et al. 1979; Riha and Berven 1991), reptiles (Ferguson and Talent 1993) and fish (Conover and Present 1990; Philipp and Whitt 1991; Nicieza et al. 1994; Schultz et al. 1996). The contrasting results of these

studies documenting both co- and countergradient variation preclude generalisations regarding latitudinal patterns in growth.

To understand the adaptive significance of intraspecific variation in growth rates we need to know the processes generating such variation in a trait, how it is connected to genetic variation, and how it interacts with other traits and the environment to determine fitness. Given the potential confounding influences of genetic and environmental sources of phenotypic variance, how can the existence of local growth adaptation be revealed? The most simple and direct approach to determining the genotypic contribution to phenotypic variation is the common-garden experiment (reviewed in Conover and Schultz 1995). In common-garden experiments, individuals from different populations or locations are reared in controlled environments, which involve the main environmental parameters likely to influence fitness in nature. Trait differences that persist among populations raised in a common environment suggest that the trait has a genetic component and can therefore evolve (Reznick and Travis 1996). Phenotypic plasticity in a trait is recognised when the expression of a trait varies with the environment in which the organism is reared (e.g. Stearns 1992), the graph (or slope) of this variation being considered as the trait's reaction norm. Genetic variation in plasticity is revealed by crossing reaction norms, or in other words, by genotype-by-environment (GxE) interactions (Via and Lande 1985; Stearns 1992; Day et al. 1994). Conversely, if genotypes have the same relative performance in different environments (parallel norms of reaction), then no GxE interaction is present and phenotypic plasticity cannot evolve. Differences in plasticity levels between populations are usually explained as an adaptive response to the degree of environmental variability experienced by populations.

In fishes, evidence for adaptive latitudinal variation in growth comes almost entirely from temperate taxa (e.g. Conover and Present 1990; Nicieza et al. 1994; Jonassen et al. 2000; Larsson et al. 2005). Whilst this previous work has provided support for both the co- and countergradient models, very little is known about such variation in coral reef fishes. Although there is a relatively new and growing body of research examining growth characteristics of tropical species across latitudinal gradients (Meekan et al. 2001; Choat et al. 2003; Williams et al. 2003; Ackerman 2004; Robertson et al. 2005), none of these studies have attempted to resolve the relative contribution of genetic and environmental sources to the observed patterns of growth, even though this is essential when testing hypotheses involving natural selection. This study is the first to experimentally examine the genetic basis of latitudinal variation in growth and growth plasticity in the early life history of coral reef fishes by using a common-garden approach.

Growth rates in the early life stages of coral reef fishes are influenced by a variety of environmental factors, of which temperature (Houde 1989; McCormick and Molony 1995; Wilson and Meekan 2002; Green and Fisher 2004) and food availability (Jones 1986; Forrester 1990; McCormick and Molony 1992; Booth and Hixon 1999; Green and McCormick 1999) are arguably the most important. Both these factors have a significant effect on metabolic rate (Houde 1989) and therefore on related physiological processes such as growth and development (Boehlert and Yoklavich 1983; McCormick and Molony 1995; Nicieza and Metcalfe 1997; Koumoundouros et al. 2001). Growth and size, on the other hand, will have a direct bearing on the survivorship of larval or juvenile reef fishes. The theory that larger and/or faster growing individuals have a higher probability of survival (i.e. the “growth-mortality” hypothesis, Anderson 1988) is now supported by several field studies (e.g. Booth and Hixon 1999; e.g. Bergenius et

al. 2002; Vigliola and Meekan 2002; Wilson and Meekan 2002), suggesting that fast growth not only provides a survival advantage in the plankton, but may also have the potential to influence post-settlement survivorship and thus recruitment in coral reef fishes. However, while there may be many ecological advantages to attaining a large body size quickly, especially in the more predation sensitive early life history stages, benefits of rapid growth can be countered by associated physiological costs (Mangel and Stamps 2001). Since energy and nutrients are budgeted among numerous functions in an organism, an increase in energy allocation to growth may decrease the energy available for maintenance or repair, and thus reduce survival (Sibly and Calow 1986; Mangel and Stamps 2001; Metcalfe and Monaghan 2003). Therefore, optimal growth strategies for juvenile fishes will depend on the nature of selection in a given environment, along with the relative costs and benefits of rapid growth.

The aim of this study was to evaluate the evidence for adaptive latitudinal variation in growth rate and growth plasticity across an environmental gradient in three species of anemonefishes. Specifically, I examined the relationship between growth rate and latitude to determine if patterns of growth are consistent with co-gradient or countergradient variation. In the case of co-gradient variation, fast-growing genotypes would be found in environments that promote fast growth (i.e. at low latitudes), whilst the opposite would indicate countergradient variation. Secondly, I wanted to assess whether there is population-level variation in growth plasticity in response to two environmental variables, temperature and food availability. Higher-latitude environments are generally characterised by greater spatial and temporal variability (Rudloff 1981), and I therefore expected greater levels of plasticity in higher-latitude populations. Finally, I wanted to investigate how growth rate affects survival, and if the proposed trade-off between these traits is apparent in the early post-settlement stage of

these fishes. To address these aims, a common-garden experiment was conducted involving laboratory-reared offspring of populations originating from three latitudes, spanning nearly 1,000 km along the Great Barrier Reef (GBR). Growth reaction norms in response to temperature and food availability were measured in controlled environments in order to determine the environmental and genotypic contribution to phenotypic variation.

## **4.2. Materials and Methods**

### **4.2.1. Adult brood stock**

The species examined here were *Amphiprion melanopus* (Bleeker, 1852), *Amphiprion akindynos* (Allen, 1972) and *Premnas biaculeatus* (Bloch, 1790). All three are members of the subfamily Amphiprioninae (Pomacentridae) and inhabit shallow tropical and temperate reefs in the eastern Indian Ocean and Western Pacific. Adult breeding pairs were collected from populations at three latitudes along the GBR: low latitude (LL) at approximately 14°40'S, mid latitude (ML) at approximately 18°23'S and high latitude (HL) at approximately 23°30'S (Figure 3.1). Breeding pairs of *A. melanopus* were collected from two populations each from within the low and high latitude. The low-latitude populations were Lizard and North Direction Islands, and the high-latitude populations One Tree Island and Sykes Reef respectively. *A. akindynos* was sampled from the low (Lizard Island), mid (Britomart Reef) and high (One Tree Island) latitude. *P. biaculeatus* was collected from the low (Lizard Island) and mid (Britomart Reef) latitude.

Breeding pairs were housed individually in 100L aerated plastic tanks at James Cook University and supplied with a constant flow of recirculated water at ambient temperature. These aquaria were housed under white opaque cloth canopies. Each

breeding pair was provided with an anemone (*Heteractis magnifica*) and a halved terracotta pot served as an artificial, removable substratum for shelter and as a surface for spawning. Fish were fed daily *ad libitum* with a mixture of chopped pilchard and squid.

#### **4.2.2. Larval rearing**

Larval rearing occurred from October to March over six consecutive summers (1997/98 to 2002/2003). Immediately before hatching, individual clutches of eggs were moved to a light- and temperature-controlled room and hatched in 100 L plastic tanks. These tanks were lined with a sleeve of black opaque plastic to reduce reflections off the inside tank walls. Larvae of these species are highly phototactic and will die from swimming into tank walls in response to such reflections. Water temperature in the tanks was maintained at 28 ( $\pm 1^\circ\text{C}$ ) and aerated with air stones. Fluorescent lights were used to simulate a 12-h daylight photoperiod.

Larvae were reared using “green water” techniques (Green and McCormick 1999), where cultured algae (*Nannochloropsis* sp.) are added to the tank during the day. The presence of the algae creates a light gradient in the tank, allowing larvae to avoid strong light by adjusting their depth. The algae also act as a food source for the zooplankton that were fed to the larvae. To maintain water quality, hatching tanks were gently flushed with seawater during the night. Larvae were fed cultured rotifers (*Brachionus* sp.) and brine shrimp (*Artemia*) nauplii, depending on species and size of fish larvae. Rotifers, at 120 micrometers wide, were suitable for young larvae of all three species. A gradual transition to *Artemia* nauplii (nutritionally equivalent to about 40 rotifers) was made after the first few days according to the feeding schedule in Table 4.1.

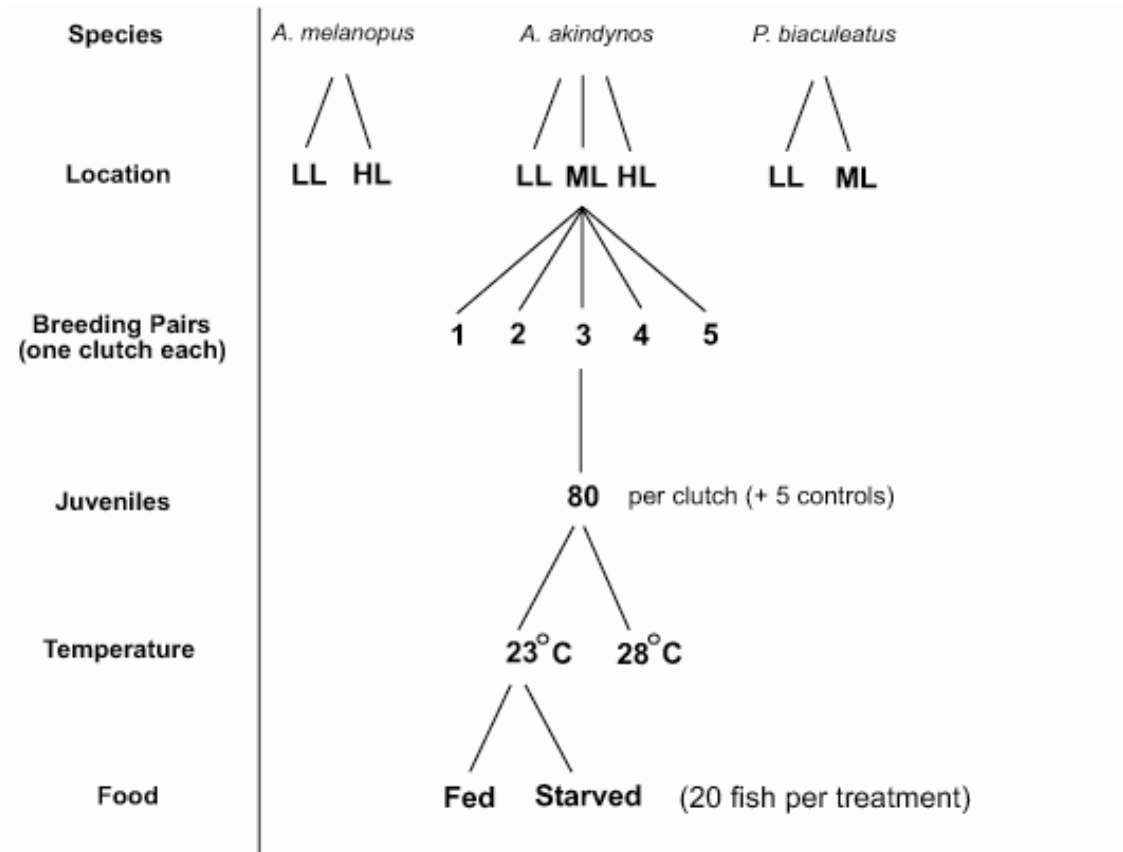
**Table 4.1:** Feeding schedule for larval fish of each species during rearing. Food densities are indicated in brackets and were maintained irrespective of the number of larvae in the rearing tank.

Species	Food type	
	Rotifers	Artemia
<i>A. melanopus</i>	Day 1-4 (~ 5 individuals/ml)	Day 3 – settlement (~ 3 individuals/ml)
<i>A. akindynos</i>	Day 1-9 (~ 15 individuals/ml)	Day 7 – settlement (~ 3 individuals/ml)
<i>P. biaculeatus</i>	Day 1-9 (~ 15 individuals/ml)	Day 7 – settlement (~ 3 individuals/ml)

#### 4.2.3. Experimental design

Food and temperature were manipulated in this experiment to test the effects of environmental factors on growth rate and survival of populations from different latitudes. Three to five days post-settlement, juveniles were moved into a grow-out facility with four experimental treatment combinations involving two temperatures and two feeding regimes (Figure 4.1). The factor temperature included two levels, “warm” (28°C ±1°C) and “cool” (23°C ±1°C), in order to simulate the thermal gradient that is found naturally across the latitudinal range of the breeding pairs. The factor food also included two levels, “fed” (fed once daily with *ad libitum* quantities of brine shrimp nauplii) and “starved” (fed once every third day with *ad libitum* quantities of brine shrimp nauplii) to simulate different levels of food availability. From each clutch reared that had sufficient juveniles, 85 were used in these trials. Five fish were randomly sampled at the start of the experiment to estimate starting body size, fixed in 10% formalin for two days and stored in 70% ethanol for later measurement. The remaining 80 individuals were randomly assigned to one of the four experimental treatments described above and were housed individually in 2L plastic containers with constant water circulation. Every 14 days, two specimens were randomly sampled from each treatment group prior to feeding, killed in ice water, fixed in 10% formalin for two days

and stored in 70% ethanol for later measurement. In this way, growth was recorded over a maximum period of 20 weeks. However, as a result of mortality during the experiment, this maximum experimental duration was not always reached.



**Figure 4.1:** Diagram of the experimental design. LL = low-latitude location, ML = mid-latitude location, HL = high-latitude location.

#### 4.2.4. Statistical analysis

Growth rates of juveniles were estimated by the regression slope of mass against age for each family in each treatment. These growth rates were then compared using analysis of covariance (ANCOVA). Latitude, temperature and feeding regime were analysed as fixed effects. Size at settlement (start of experiment) was treated as a covariate to control for variation that may be due to egg size-mediated maternal effects in larval traits (Sinervo 1990; Niewiarowski and Roosenburg 1993; Falconer and



Mackay 1996). In *A. melanopus*, which originated from two populations within each geographic location, populations were treated as nested and as a random factor (Lynch and Walsh 1998).

Measurement of genotype x environment interaction can be used to assess overall genetic variation in phenotypic plasticity (Via and Lande 1985; De Jong 1990; Gomulkiewicz and Kirkpatrick 1992). The evolution of phenotypic plasticity entails an increase or decrease in slope of a population's mean norm of reaction. In other words, if genotypes vary in their phenotypic response to rearing environments, the lines connecting the mean phenotype expressed in each environment (the norms of reaction) will have different slopes, with a flat slope indicating higher plasticity and a steep slope indicating lower plasticity (Gotthard and Nylin 1995). Geographic variation in phenotypic plasticity can be quantified in an ANOVA, where the statistical significance of genetic variation in slopes is assessed with the genotype (i.e. geographic location) x environment (i.e. temperature) interaction. If this interaction is significant, populations sampled from different geographic locations have different plastic responses to the environments and therefore genetically divergent norms of reaction. Details of the ANOVA-approach for estimating genotype x environment (GxE) interactions with full-sib quantitative genetic designs are discussed in Via (1987) and Falconer and Mackay (1996).

Survival was estimated as the number of days from when a clutch was introduced into the experiment until the last fish was sampled in each treatment (all fish not sampled had been subject to mortality). A factorial ANOVA was carried out to examine the effect of location, temperature and feeding regime on survival in the common-environment experiment. Finally, the relationship between growth rate and survival in each population was explored with regression analyses. All analyses were

performed using Statistica Version 6.2 (StatSoft Inc. Tulsa OK, USA). All assumptions of statistical tests were satisfied without data transformations unless stated otherwise.

#### ***4.2.5. Limits of the common-garden experiment***

Studies of genotype-by-environment interactions in natural populations are logistically difficult, which is the reason why most of the studies investigating GxE interactions for life history traits have involved plants and arthropods with short generation times (e.g. Hoffmann et al. 1995; Eckhart et al. 2004). Ideally, when using common-garden experiments to investigate population differences, comparisons should be carried out on progeny from stock that have themselves been reared in a common environment for at least two generations (Conover and Schultz 1995; Conover et al. 1997) to rule out the possibility of non-genetic differences arising from environmental effects. However, this was not feasible within the logistic constraints of this study, as anemonefishes are long-lived and resources were not available to maintain breeding pairs for such a long period of time. Here, fish were used from parents originating from the wild, thus previous environmental conditions experienced by the mothers may have influenced the results to a certain degree. In order to reduce these possible maternal contributions to offspring growth rates, size at settlement (determined from the initial controls taken at the start of the experiment) was statistically factored out, as size at settlement could influence subsequent growth rate and is likely to have some maternal contribution (Sinervo 1990).

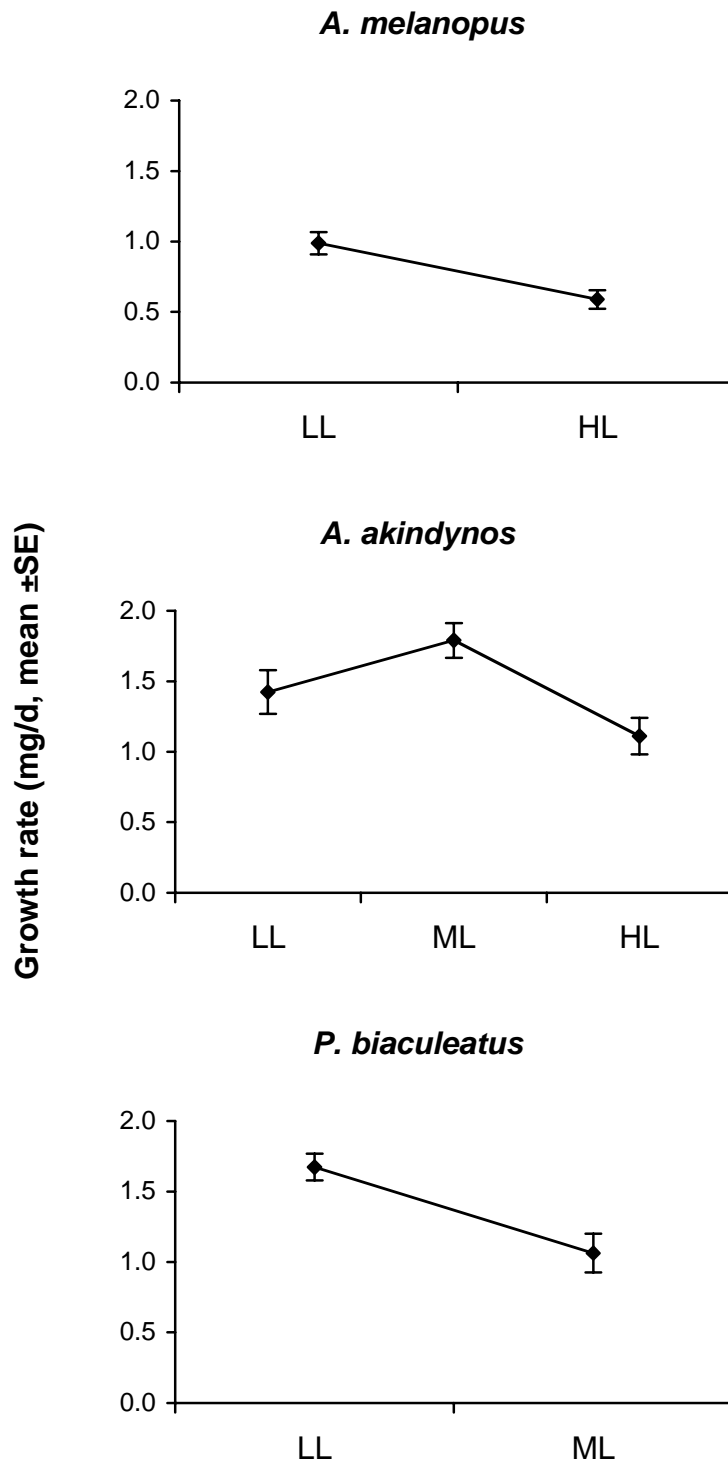
### **4.3. Results**

#### **4.3.1. Mean growth rates**

In all three species, mean growth rates of offspring differed significantly among populations from different latitudes (Table 4.2). Demonstrated under common-garden conditions, these significant differences implicate that population-level variation in the growth capacity of juvenile anemonefishes has a genetic basis and is not just environmentally determined. In both *A. melanopus* and *P. biaculeatus* overall mean growth rates were higher in the low-latitude populations (Figure 4.2), a pattern that is generally consistent with co-gradient variation. In *A. akindynos*, however, overall mean growth rate did not follow a linear latitudinal gradient: growth rate was highest in the mid-latitude population, followed by the low- and then high-latitude populations respectively (Figure 4.2).

**Table 4.2:** Results of ANCOVA examining the effects of population, food (“fed” or “starved”) and temperature (23°C or 28°C) treatments on juvenile growth rate of (a) *A. melanopus*, (b) *A. akindynos*, and (c) *P. biaculeatus*. The covariate was mean weight at settlement (mws). In *A. melanopus* populations were nested within locations and treated as random.

Source of Variation	<i>df</i>	Mean square	<i>F</i>	<i>P</i>
<b>(a) <i>A. melanopus</i></b>				
<i>Location</i>	1	0.099	12.36	0.0079
Population (Location)	2	0.006	0.453	0.6369
<i>Temperature</i>	1	0.220	17.29	< 0.0001
<i>Food</i>	1	0.391	30.69	< 0.0001
Location x Temperature	1	0.024	1.852	0.1762
Location x Food	1	0.000	0.000	0.9858
Temperature x Food	1	0.080	6.257	0.0138
Location x Temperature x Food	1	0.000	0.053	0.8183
mws	1	0.028	2.190	0.1417
Error	113	0.013	-	-
<b>(b) <i>A. akindynos</i></b>				
<i>Location</i>	2	2.223	11.14	< 0.0001
<i>Temperature</i>	1	9.411	47.17	< 0.0001
<i>Food</i>	1	3.142	15.75	0.0002
<i>Location x Temperature</i>	2	1.627	8.154	0.0008
Location x Food	2	0.394	1.974	0.1488
Temperature x Food	1	0.068	0.340	0.5625
Location x Temperature x Food	2	0.150	0.752	0.4762
mws	1	0.089	0.447	0.5067
Error	54	0.200	-	-
<b>(c) <i>P. biaculeatus</i></b>				
<i>Location</i>	1	2.697	79.98	< 0.0001
<i>Temperature</i>	1	10.65	315.9	< 0.0001
<i>Food</i>	1	0.985	29.22	< 0.0001
<i>Location x Temperature</i>	1	0.217	6.431	0.0156
<i>Location x Food</i>	1	0.169	5.004	0.0314
Temperature x Food	1	0.003	0.082	0.7767
Location x Temperature x Food	1	0.001	0.040	0.8426
mws	1	0.161	4.770	0.0353
Error	37	0.034	-	-

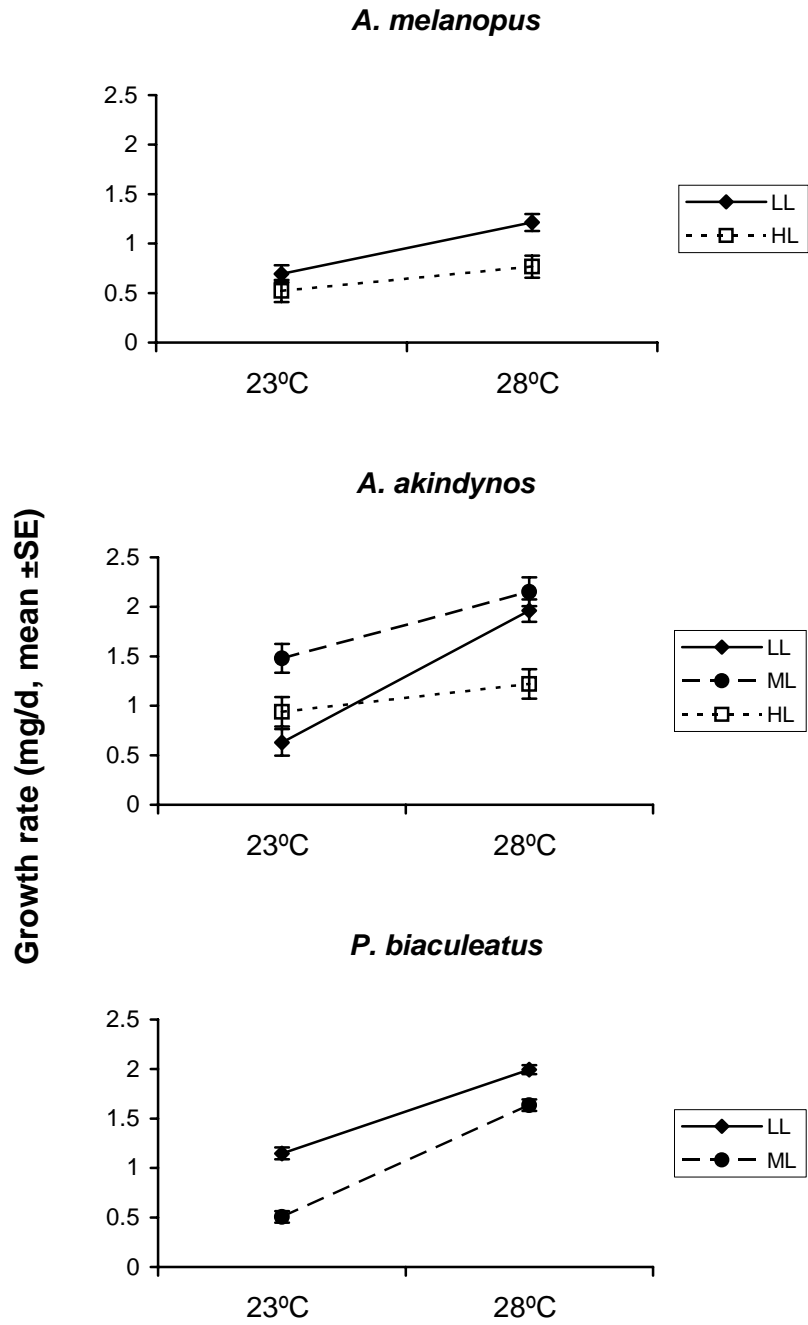


**Figure 4.2:** Mean growth rates (LS means  $\pm$  SE) of *A. melanopus*, *A. akindynos*, and *P. biaculeatus* offspring from the respective locations. LL = low-latitude location, ML = mid-latitude location, HL = high-latitude location.

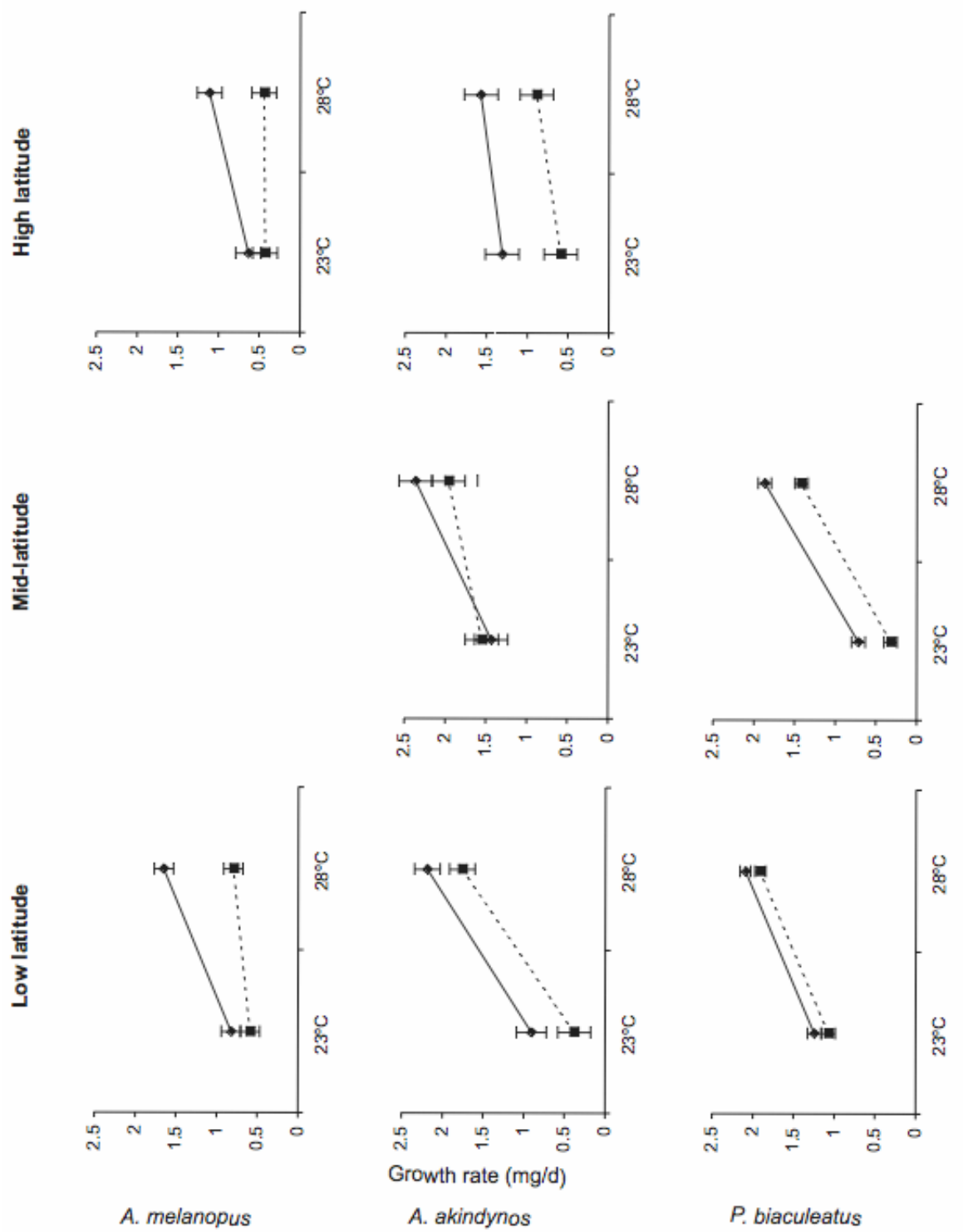
### 4.3.2. *Phenotypic plasticity in growth*

The main effects of location, temperature, and feeding regime were each significant in all three species (Table 4.2). The higher temperature (28°C) had an accelerating effect on growth rate in all populations and both food levels (Figure 4.3 and 4.4). Similarly, the higher food level (“fed”) enhanced development in all populations and both temperatures (Figure 4.4). However, levels of phenotypic plasticity in response to these environmental factors were clearly distinct among populations from different latitudes, as revealed by the different slopes of reaction norms. In response to temperature, both *A. akindynos* and *P. biaculeatus* displayed significant GxE interactions (Table 4.2), indicating that the growth-response to the warm and cool temperature varied among populations from different latitudes. *A. akindynos* showed a gradual increase in plasticity with latitude (Figure 4.3), which is in agreement with my prediction that high-latitude populations should evolve higher levels of plasticity due to the higher degree of environmental variability experienced by these populations. In *Premnas*, the location x temperature interaction was also significant. However, contrary to my prediction, the low-latitude population displayed greater plasticity compared to the higher-latitude population. In *A. melanopus*, growth plasticity in response to temperature varied as predicted, with a trend for higher plasticity at the higher latitude; however, the difference in slopes was not statistically significant (Table 4.2).

The only species that showed population-level variation in response to feeding regime was *Premnas*, as revealed by the significant location x food interaction (Table 4.2). Again contrary to my prediction, the low-latitude population had higher plasticity in response to food (Figure 4.4), such that low-latitude offspring was able to maintain



**Figure 4.3:** Growth reaction norms (LS means  $\pm$  SE) of offspring from the respective locations (LL = low-latitude location, ML = mid-latitude location, HL = high-latitude location) in response to the two experimental temperatures 23°C and 28°C.



**Figure 4.3:** 3-way interactions of ANCOVA, showing growth reaction norms of juveniles in the two experimental temperatures (23°C and 28°C) and feeding regimes (solid lines show the “fed” treatment and dotted lines show the “starved” treatment).



relatively higher growth rates even in conditions of low food availability. The higher-latitude (ML) population, on the other hand, was less plastic in response to food level and displayed a markedly lower growth rate under restricted food conditions.

#### 4.3.3. *Survival*

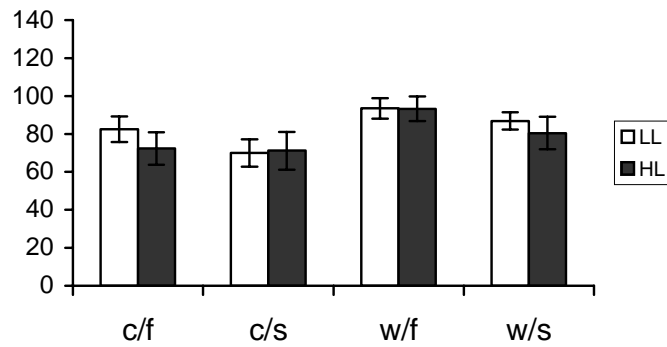
*A. melanopus* and *P. biaculeatus* displayed very similar patterns in survival, with no differences associated with latitude. In both species, ANOVA did not reveal any significant location x environment interactions (Table 4.3). The only factor affecting survival was temperature (significant in *P. biaculeatus* and nearly significant in *A. melanopus*), indicating that populations experienced higher mortality at 23°C than at 28°C, irrespective of their origin (Table 4.3, Figure 4.5). Low food availability only influenced survival to a very minor degree. In *A. akindynos*, survival varied significantly among locations (Table 4.3, Figure 4.5). The mid-latitude population clearly had the highest survival in all treatments. For the HL and LL populations survival was similar in the warm temperature. However, in the cold temperature survival was clearly lowest in the LL population (Figure 4.5). This reflects the fact that *A. akindynos* juveniles from the LL had comparatively high and early mortality in the cold temperature: a high proportion of juveniles died within the first three days of the experiment, and in three of the clutches there was even 100% mortality before the first sampling occasion.

Regressions of growth rate vs. survival of the three species from each location were each positive and significant in all cases except for the ML and HL population of *A. akindynos* (Table 4.4, Figure 4.6). Although the relationship is most likely not a linear one, a strong positive correlation between survival and growth rate in the context of this experiment was found.

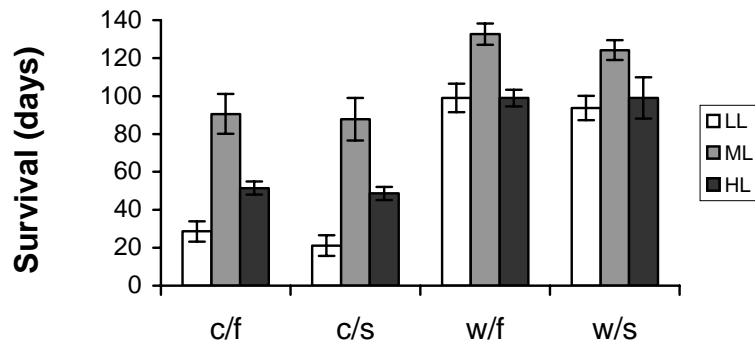
**Table 4.3:** Results of ANOVA testing for effects of population origin and experimental treatments on survival of (a) *A. melanopus*, (b) *A. akindynos*, and (c) *P. biaculeatus*. Survival is defined as the time (days) until total mortality of each family in each treatment was reached.

<b>Source of Variation</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>P</b>
<b>(a) <i>A. melanopus</i></b>				
Location	1	454.3	0.59	0.4434
Temperature	1	6256.7	8.15	0.0051
Food	1	2021.6	2.63	0.1074
Location x Temperature	1	10.0	0.01	0.9093
Location x Food	1	48.9	0.06	0.8012
Temperature x Food	1	61.3	0.08	0.7781
Location x Temperature x Food	1	567.1	0.74	0.3920
Error	116	768.0	-	-
<b>(b) <i>A. akindynos</i></b>				
Location	2	14438.5	48.54	< 0.0001
Temperature	1	48579.2	163.32	< 0.0001
Food	1	340.7	1.15	0.2888
Location x Temperature	2	1788.4	6.01	< 0.005
Location x Food	2	40.4	0.14	0.8732
Temperature x Food	1	0.1	0.00	0.9825
Location x Temperature x Food	2	29.5	0.01	0.9059
Error	60	297.4	-	-
<b>(c) <i>P. biaculeatus</i></b>				
Location	1	1611.8	1.65	0.2051
Temperature	1	28794.3	29.55	< 0.0001
Food	1	1080.3	1.11	0.2981
Location x Temperature	1	1374.8	1.41	0.2413
Location x Food	1	2.3	0.00	0.9616
Temperature x Food	1	68.3	0.07	0.7924
Location x Temperature x Food	1	11.3	0.01	0.9149
Error	44	974.5	-	-

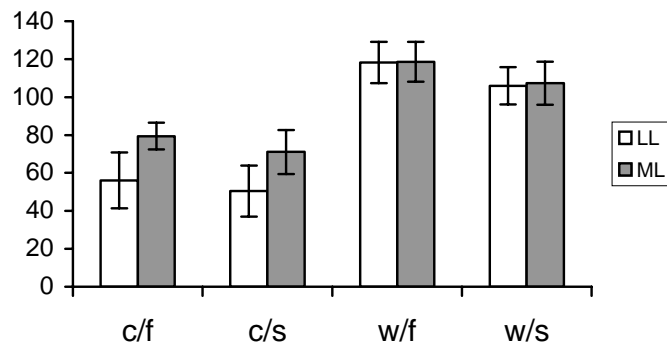
***A. melanopus***



***A. akindynos***

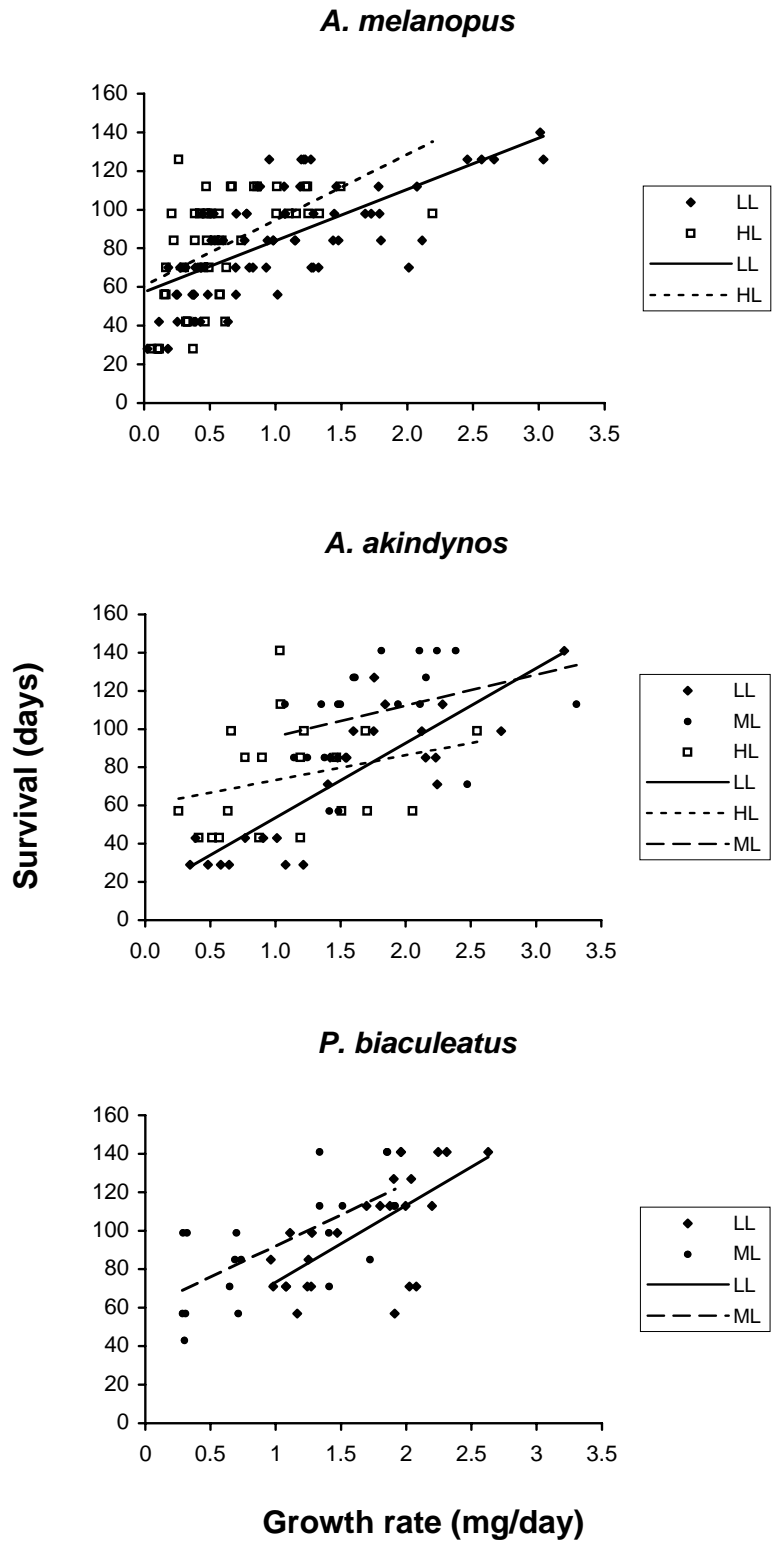


***P. biaculeatus***



**Treatment**

**Figure 4.4:** Mean survival ( $\pm$  SE) of the respective populations in each of the four experimental treatments (c/f = cold/food, c/s = cold/starved, w/f = warm/food, w/s = warm/starved). “Survival” is the time until total mortality, i.e. the number of days until the last juvenile of any particular clutch and treatment was dead.



**Figure 4.5:** The relationships between survival and growth rate in each species at each location. Symbols represent the value of a clutch within one of the four treatment groups and lines indicate the fit of a linear regression. Regression results are listed in Table 4.4.

**Table 4.4:** Regression results of the relationships between growth rate and survival in each species at each location. LL = low-latitude location, ML = mid-latitude location, HL = high-latitude location.

<b>Species</b>	<b>Location</b>	<b>r<sup>2</sup></b>	<b>r</b>	<b>P</b>
<i>A. melanopus</i>	LL	0.49	0.70	< 0.0001
	HL	0.36	0.60	< 0.0001
<i>A. akindynos</i>	LL	0.72	0.85	< 0.0001
	ML	0.11	0.33	0.155
	HL	0.07	0.27	0.252
<i>P. biaculeatus</i>	LL	0.44	0.67	< 0.001
	ML	0.47	0.69	< 0.001

#### **4.4. Discussion**

Variation in growth can have both genetic and environmental components, and knowledge of the relative contribution of these sources of variation may improve our understanding of how intraspecific differences in life histories evolve (Schultz et al. 1996). In coral reef fishes, the role of genetic influences on somatic growth among populations from different environments is poorly understood. In this first application of the common-garden approach I show that growth rate and growth plasticity of offspring from different latitudinal populations of anemonefishes have different mean values and reaction norms. Demonstrated under controlled conditions, these significant differences imply that population-level variation in the growth capacity of juveniles has a genetic component (i.e. is not just environmentally determined) and should therefore respond to natural selection (Arendt 1997).

##### **4.4.1. Geographic variation in mean growth rates**

In all three species, offspring of higher-latitude populations generally grew slower than offspring of lower-latitude populations. This growth pattern supports the co-gradient rather than the countergradient variation hypothesis (Conover and Schultz

1995). Co-gradient variation is expected to evolve when phenotypes induced by environmental influences and those favoured by natural selection are similar across habitat gradients (a situation also referred to as synergistic selection, Falconer 1990). My results therefore suggest that intraspecific differences in growth rates of anemonefishes may have evolved not only because of the direct effects of temperature on the physiology, but also as a consequence of the impact of the environment on population parameters such as longevity, sexual maturity and reproductive rate, which in turn determine the way in which natural selection acts on the life history. For example, at low latitudes populations generally have shorter generation times and higher mortality rates (Stearns 1992). The overall duration of reproductive activity is therefore reduced for these individuals and fast growth is essential to reach maturity as soon as possible to make up for the shorter lifespan (Sutherland et al. 1986; Gasser et al. 2000). Under these circumstances, the premium for fast growth in the early life history will be particularly high. At high latitudes, on the other hand, individuals are generally longer lived and maximise their fitness by delaying sexual maturity to an age at which a larger body size will result in increased fecundity (Atkinson and Sibly 1997). Slower growth will also allow these individuals to devote more resources to the prevention of ageing and ultimately reach an older age. Hence, at higher latitudes selection for rapid growth may be weaker because individuals can extend the period over which growth occurs prior to maturation. For anemonefishes, an optimal growth trajectory will therefore depend on factors such as the period over which growth is possible, and the relative contributions of adult body size and lifespan to fitness. The co-gradient pattern found in this experiment is consistent with the above predictions and suggests that fast initial growth is selectively favoured at lower latitudes.

An exception to a linear co-gradient pattern was the reduced mean growth rate of *A. akindynos* at the low-latitude location. In the case of co-gradient variation, mean growth rates should be highest in the north and decrease gradually with increasing latitude. However, in *A. akindynos* the highest mean growth rate was found in the mid-latitude population, followed by the LL and finally the HL population. The most likely explanation for this observation may be found in species' borders theory (reviewed in Hoffmann and Parsons 1991; Hoffmann and Blows 1994). The LL population of *A. akindynos* is relatively close to the edge of this species' distribution and is therefore probably affected by genetic and ecological processes that limit the capacity for local adaptation. As one moves from the interior towards the periphery of a species' range, the spatial distribution, dynamics and structure of populations change. Populations often become more patchy, isolated and transient, and their probability of extinction increases towards the edge of the range (Carter and Prince 1981; Maurer 1994; Brown et al. 1996). In addition, genetic diversity often declines at the periphery, where local populations are small and prone to bottlenecks, genetic drift and stochastic processes. The persistence of peripheral populations despite their assumed low fitness is usually attributed to immigration from central areas. This immigration is associated with gene flow, which involves genes that have been selected for different local environments. Sufficiently high rates of gene flow can swamp a local population with genes from outside, effectively preventing adaptation to local conditions (Dias 1996; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997). In *A. akindynos* it is therefore likely that gene flow from higher latitudes precludes the evolution of a higher growth rate in the marginal "sink" habitat, thereby trapping it in a state of maladaptation.

#### **4.4.2. *The role of phenotypic plasticity***

It has been shown that phenotypic plasticity is a heritable trait, which can respond to natural selection and evolve separately from the mean values of that trait (Thompson 1991). If a plastic response of a genotype to environmental conditions results in higher fitness under those conditions relative to a genotype that is insensitive to the environment, then plasticity is adaptive and can be selected for (Via and Lande 1985; Van Tienderen 1991; Gomulkiewicz and Kirkpatrick 1992; De Jong 1995; Ernande and Dieckmann 2004). This will result in evolutionary divergence in plasticity among populations (Donohue et al. 2000) and can have important consequences for local adaptation and the evolution of ecological niche breadth (Sultan 1987; Van Tienderen 1991; Whitlock 1996). If intraspecific differences in plastic responses are adaptive, they should be relatable to habitat differences, i.e. individuals that inhabit spatially or temporally heterogeneous environments should evolve a genetic constitution that allows phenotypic variability to adjust to a changing environment in order to maximize fitness. I therefore expected higher levels of plasticity in populations from higher latitudes, given that these populations experience a higher degree of environmental variability compared to their lower-latitude counterparts.

My results provided mixed support for this proposition. In response to temperature, genetic variation in plasticity was found in *A. akindynos* and *P. biaculeatus*, as indicated by the significant location x temperature interactions in these two species. *A. akindynos* conformed to my predictions nicely, with a gradual increase in plasticity with increasing latitude. Offspring of the low-latitude population achieved a high growth rate in the warm temperature, but performed poorly in the low temperature. This is consistent with the expectation that physiological specialisation maximises growth in the native (warm) environment at the expense of reduced growth efficiency



elsewhere (Schultz et al. 1996). The high-latitude population, on the other hand, had a flatter reaction norm, thus implying that these individuals are adapted to the colder and more variable temperatures they experience in nature. *P. biaculeatus* also had different levels of plasticity in response to temperature; however, in this species the low-latitude offspring showed greater plasticity compared to their higher-latitude counterparts, and thus did not support my prediction.

*Premnas* was also the only species that displayed population-level variation in response to food availability. As with temperature, however, plasticity was greater in the low-latitude population, i.e. the low-latitude offspring was able to maintain relatively higher growth rates under restricted feeding conditions. The fact that the higher-latitude population of *P. biaculeatus* failed to show the expected higher plasticity in response to both temperature and food availability might again be evidence of maladaptation at the range periphery, as Britomart Reef is relatively close to the southern limit of this species' distribution (Fautin and Allen 1992). It is possible that this marginal population does not have the genetic capacity to evolve optimal levels of plasticity and may therefore suffer in terms of fitness (DeWitt et al. 1998).

It is not clear why only *Premnas*, but not *Amphiprion*, showed genetic differentiation among populations with respect to food availability. It is possible that there are differences between these genera that affect sensitivity to food level, such as differences in food conversion efficiency, structural differences that affect feeding, or differences in the ability to ingest prey. For example, *Premnas* may possess morphological characters (i.e. gill-raker strength or gape width), which are more susceptible to food stress. Furthermore, behavioural plasticity may also vary among genera and most likely plays an important role in adaptation to resource variability. These explanations, however, are purely speculative and require further investigation.

#### ***4.4.3. The relationship between growth and survival***

The results of this study demonstrate a positive relationship between growth rate and survival in all species and populations, regardless of their latitudinal origin. These findings are consistent with a paradigm for larval and juvenile processes, which holds that larger and/or faster growing individuals will have a higher probability of survival, i.e. the “growth-mortality” hypothesis (Anderson 1988). It includes three functional mechanisms to explain why bigger size and/or faster growth may confer higher fitness in juvenile fish: the “stage-duration”, “bigger is better”, and the “growth-rate mechanism”, which are based on time, size and growth rate respectively. The “stage-duration” mechanism (Chambers and Leggett 1987; Houde 1987) focuses on the relationship between growth rate and larval stage duration. It proposes that faster developing larvae make the larval-juvenile transition at younger ages and therefore have a lower probability of mortality. The “bigger-is-better” hypothesis (Leggett and DeBlois 1994) states that, if mortality is size dependent, the larger individuals of a given age will have a lower probability of mortality compared to smaller individuals of the same age. And lastly, if the probability of mortality decreases with size, higher growth rates will minimize the time over which individuals experience higher mortality rates and therefore, faster growing fish will have a lower probability of mortality (i.e. the “growth-rate hypothesis”, Ware 1975; Shepherd and Cushing 1980). All three hypotheses involve the main argument that faster growing fish gain a size advantage with respect to predation or competition (Bailey and Houde 1989) and are now well supported in the literature (e.g. Meekan and Fortier 1996; Sirois and Dodson 2000; Bergenius et al. 2002; Wilson and Meekan 2002).

While these propositions may explain why bigger and/or faster growing individuals will have higher survival in the wild, the present study further suggests that

fast growing individuals also have an intrinsic advantage, given that the positive association between growth and survival was found in the absence of predation or competition. This experiment therefore helps to extend these ecological models to situations where predation or competition can be excluded as operative factors. The lack of the proposed trade-off between growth rate and survival implies that some individuals are generally superior and have more resources at their disposal to allocate to different aspects of the life history (Reznick et al. 2000). Thus, it appears that individual fish vary in their overall quality, such that those with a higher growth capacity also have a better overall condition and consequently a higher probability of survival in a controlled environment. These differences in individual quality of offspring are most probably mediated through maternal influences (Kerrigan 1997). Maternal effects are important determinants of offspring condition in a wide range of taxa (Bernardo 1996). A critical aspect of maternal provisioning is the size and composition of eggs (Chambers 1997): larger propagules generally produce larger and faster developing offspring with potentially far-reaching consequences for fitness and survival (reviewed in Mousseau and Fox 1998). Thus, there is the potential for larvae at the onset of the planktonic stage to be highly variable in their somatic development and quality. In the present study, the possibility of maternal effects was taken into account by statistically removing initial size differences of juveniles when calculating growth rates. However, it is possible that maternal influences also affect other condition factors of offspring (e.g. immune capacity, stress resistance, or biochemical composition) that may have contributed to the observed relationship between growth and survival (McCormick and Molony 1993). Overall, my results imply that bigger is indeed better for juvenile anemonefishes: not only are bigger and therefore faster growing individuals in a better overall condition with a greater inherent probability of survival, but larger

individuals will most likely also have a significant advantage in a natural situation by increased access to a greater size range of food, enhanced competitive abilities, and reduced susceptibility to predation (Blaxter and Hempel 1963; Bailey and Houde 1989).

In conclusion, the common-garden study presented here revealed intraspecific variation in the capacity for somatic growth in the early juvenile stage of anemonefishes. The different growth reaction norms in response to the same conditions of offspring from different latitudes suggest that growth and growth plasticity have a heritable genetic basis, although the influence of maternal effects cannot be entirely ruled out with the present experimental design. This is consistent with other findings of genetic control of growth rate in fishes (Schultz and Conover 1997), and implies that the different latitudinal populations have the capacity to evolve locally adapted life history strategies, despite a high potential for gene flow. The geographic pattern of growth was similar in all three species and varied according to co-gradient predictions. This finding is contrary to a considerable number of studies that have documented countergradient variation in growth in northern hemisphere temperate fishes (Schultz et al. 1996; Conover et al. 1997; Arendt and Wilson 1999; Imsland et al. 2000). Considering that coral reef fishes and their habitats display unique characteristics that differ substantially from those in temperate regions (i.e. in terms of seasonality or habitat complexity), it is likely that there are also fundamental differences between these environments in the way that selective processes influence growth strategies.

This study was a first attempt to experimentally examine the relative roles of genetic differentiation, phenotypic plasticity and large-scale adaptation in determining patterns of growth and survival in the early life history of coral reef fishes. Because of logistic constraints, sample sizes and geographic coverage were relatively limited; the conclusions drawn above must therefore remain suggestive for the time being. Further

studies in additional geographic locations and a greater range of species are required before the generality of the patterns found here can be accurately assessed.

# **CHAPTER 5: Experimental evaluation of geographic variation in the sustained swimming ability of larval anemonefishes**

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## **5.1. Introduction**

Many organisms exhibit extensive geographic variation in their life history traits in response to large-scale environmental patterns (Gould and Johnston 1972; Roff 1992; Stearns 1992). However, the degree to which such changes may also be associated with behavioural and performance traits has received little attention. Such behavioural changes may be expected because, just as other phenotypic traits, behaviour and performance are the expression of the combined effects of the genotype and environment. As such, behaviour can vary geographically because it is subject to geographically varying conditions, and hence to natural selection (Foster and Endler 1999).

Performance, such as locomotion or foraging, is an expression of the physiological and behavioural capabilities of an organism (Fuiman and Higgs 1997). It represents the co-adaptation of numerous morphological, physiological, and behavioural traits, and is considered an integrated measure of an animal's fitness for a particular environment (Nelson 1989). Multiple selection pressures are known to act on the suite of traits that influence performance. In aquatic animals, predation risk, foraging behaviour, and stream velocity have all been suggested as important factors affecting performance (e.g. Webb 1984; Lowe-McConnell 1987; Schluter and McPhail 1992; Videler 1993; Walker 1997).

The concept of trade-offs plays a central role in constraining the evolution of whole organism performance. While geographic variation is usually interpreted in the context of trade-offs between life history traits alone, clearly life history and performance traits may also be closely linked. For example, Kolok and Oris (1995) reported a trade-off between growth rate and aerobic swimming capacity in a minnow (*Pimephales promelas*). Instead of the expected positive correlation between growth rate and swimming speed, they found that faster-growing individuals had slower critical swimming speeds. The same trade-off was later demonstrated in studies with rainbow trout (Gregory and Wood 1998; 1999) and transgenic Coho salmon (Farrell et al. 1997). However, the links between these parameters on a geographic scale have received little attention.

Traditional approaches to the study of performance and behaviour have typically employed interspecific comparisons in which the characterisation of the species was based on single populations, and the patterns found were assumed to be invariant within the species (e.g. Bartholomew 1987). However, intraspecific comparisons of populations that display geographic variation in such traits can offer substantial insight into mechanisms of adaptive divergence. Relatively recent research on a diversity of behavioural traits in a wide array of taxa (e.g. Kroodsma and Canady 1985; Duijm 1990; Arnold 1992; Claridge and Morgan 1993; Herring and Verrell 1996) has provided evidence that geographic variation within species may be much more common than previously believed, and has recognised its value for elucidating evolutionary mechanisms (e.g. Dawson and Hamner 2003). However, only few of these studies have experimentally explored the causes of adaptive geographic variation in performance.

In fishes, there are two notable studies that demonstrate how local selection pressures may act on performance via trade-offs and interactions with other components

of fitness. Billerbeck et al. (2001) produced experimental evidence for the trade-off between growth rate and swimming performance in northern and southern populations of Atlantic silversides (*Menidia menidia*), thus confirming that locomotor capacity may be sacrificed in favour of rapid growth. The second study examined predator-mediated selection on reproductive allocation and escape performance in the live-bearing guppy (*Poecilia reticulata*), and found that females from high-predation localities attain faster acceleration and velocity compared to females from low-predation localities (Ghalambor et al. 2004), suggesting that predators have selected for increased escape performance. Despite the success of these studies in documenting adaptation in natural populations, the importance of genetic differences in behavioural or performance polymorphism in fish remains largely unknown.

Swimming performance is considered an important characteristic of fishes and other aquatic animals that can have a direct influence on patterns of distribution and survival. For example, research has found a strong relationship between swimming capability and among-habitat use by adult wrasses in both tropical (Fulton et al. 2001) and temperate (Fulton and Bellwood 2004) locations, suggesting that swimming capacity may be of general importance in shaping habitat use by fishes at a variety of spatial scales. Furthermore, maximal swimming performance may strongly influence the ability of a fish to obtain food, find a mate or avoid predators and unfavourable conditions (Videler 1993). Swimming capability is also of critical importance in juvenile and pre-settlement fish. Late-stage reef fish larvae have been shown by both laboratory and field methods to swim at high speeds, with the majority swimming substantially faster than average current speeds around reefs (Leis and Carson-Ewart 1997; Fisher 2005). They also exhibit remarkable endurance, being able to swim an average of 40.7 km (some up to 140 km), with a mean time to exhaustion of 83.7 hours



(Leis and Carson-Ewart 1997; Stobutzki and Bellwood 1997; Fisher and Bellwood 2002). In addition, there is emerging evidence that some larvae have good sensory abilities and may use olfactory cues (Sweatman 1988; Elliott et al. 1995; Lecchini et al. 2005), reef sounds (Leis et al. 2002; Simpson et al. 2004; Tolimieri et al. 2004; Lecchini et al. 2005) or solar orientation (Leis and Carson-Ewart 2003) to locate reefs. Recent demonstrations that self-recruitment of reef fish populations can be much higher than expected (Jones et al. 1999; Swearer et al. 1999; Cowen et al. 2000; Jones et al. 2005) suggest that these behavioural capabilities of larvae can strongly influence their dispersal, retention, and population connectivity.

At present, most researchers have examined swimming capabilities of larval reef fishes only at a single location within a single population or environment (e.g. Leis and Carson-Ewart 1997), assuming that larval behaviour does not vary among populations or different environments. However, one previous study by Leis and Carson-Ewart (2000) has shown that the swimming behaviour of pelagic larvae can vary with location. They observed settlement-stage larvae of four species of coral reef fishes in an atoll lagoon and in the open ocean and found that swimming behaviour varies among and within species between these two contrasting environments. Apart from this, no attempt has been made to quantify geographic and population level differences in swimming performance of coral reef fishes; yet, such information may be crucial for our understanding of the ecology and evolution of these species, considering the potential importance of larval behaviour for dispersal patterns, recruitment and replenishment.

The present study employs common-garden rearing to examine the extent of heritable swimming genotype-environment associations in three species of anemonefishes across a latitudinal gradient. Flume-based swimming trials were conducted to measure the maximum sustainable swimming speed as an estimate of the

aerobic capacity of pre-settlement larvae. Measurements of maximum sustained swimming speed are often correlated with other measures of swimming performance, including routine (Plaut 2000; Fisher and Bellwood 2003), sprint (Reidy et al. 2000), sustainable (Fisher and Bellwood 2002; Fisher and Wilson 2004), as well as *in situ* swimming speeds (Leis and Fisher 2004), and therefore provide insight into a variety of ecologically important abilities of larvae.

My first aim was to determine if, and to what extent, there is heritable variation for swimming performance among populations of anemonefishes from different latitudinal origins. If present, significant differences in swimming capacity should be detected between progeny from these populations raised under controlled conditions in the laboratory. Performance should evolve in response to the direct and indirect effects of selection acting on the suite of traits that augment performance (Arnold 1983). Along a latitudinal gradient there will typically be changes in a variety of environmental factors such as ambient temperature, encounter rates with predators and prey, or variations in the strength and direction of ocean currents. All these factors are likely to influence life history strategies in different environments and thus, the evolution of genetically differentiated populations can be expected (Reznick and Travis 1996). Given the reported trade-off between aerobic swimming capacity and growth rate, and the higher intrinsic growth rates in low-latitude populations of anemonefishes (*chapter 4*), I predicted faster swimming speeds in offspring from higher latitudes. Because resting metabolic rate increases with temperature, the absolute metabolic scope (the amount of ATP available for physiological work) will also vary with latitude (Clarke and Johnston 1999). Thus, at low latitudes, the allocation of energy to faster growth may limit the energy available for active metabolism and hence diminish locomotor capacity.

The second aim was to test whether there is a divergence amongst the populations in the thermal sensitivity of swimming performance. Variation in the efficiency of physiological processes in ectotherms as a result of changes in environmental temperature can affect survival and realised fitness through its influence on locomotion (Huey and Stevenson 1979). If different populations of anemonefishes have undergone thermal adaptation to their environment, I predicted that populations from cooler, high-latitude climates would perform better at cooler temperatures than those from warmer, low-latitude locations, and *vice versa* at warmer temperatures.

## **5.2. Materials and Methods**

### ***5.2.1. Brood stock and larval rearing***

The species used in this experiment were *Amphiprion melanopus* (Bleeker, 1852), *A. akindynos* (Allen, 1972) and *Premnas biaculeatus* (Bloch, 1790). All three are members of the subfamily Amphiprioninae (Pomacentridae) and inhabit shallow tropical and temperate reefs in the eastern Indian Ocean and Western Pacific. Brood stock were collected from three locations along the Great Barrier Reef, Australia: a low-latitude location (“LL”, Lizard Island) at 14°40’S, a mid-latitude location (“ML”, Britomart Reef) at 18°23’S and a high-latitude location (“HL”, One Tree Island) at 23°30’S, encompassing 9° of latitude and a distance of over 1000km. *A. melanopus* breeding pairs originated from Lizard Island and One Tree Island respectively. *A. akindynos* was collected from each of the three locations, and *P. biaculeatus* from Lizard Island and Britomart Reef.

Adult pairs of each species were kept individually in the covered outdoor facilities of the James Cook University Research Aquarium. They were housed in 100L aerated plastic tanks and supplied with a constant flow of recirculated water at ambient

temperature. Each pair was provided with an anemone (*Heteractis magnifica*) and a halved terracotta pot serving as an artificial, removable substratum for shelter and egg spawning. Fish were fed daily *ad libitum* with a mixture of chopped pilchard and squid. On the night they were due to hatch, egg clutches were transferred to an indoor rearing laboratory and hatched in 100 L plastic tanks held at 28°C ( $\pm 1^\circ\text{C}$ ). Larvae were reared using “green water” techniques following the methods of Green & McCormick (1999), where cultured *Nannochloropsis* sp. algae are added to the tank during the day. Larvae were fed with cultured rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) nauplii, according to the species-specific feeding schedule described in *chapter 4*.

### **5.2.2. *Swimming experiments***

Critical swimming speed (U-crit) was first introduced by Brett (1964) as a measure of the sub-maximum and mainly aerobic swimming performance of fishes. U-crit is an intermediate measure of speed, slower than burst speed, but faster than cruising speeds. For the measurement, a fish is forced to swim against an incrementally increasing flow of water until exhaustion. U-crit is arguably the best studied form of fish locomotion, as the water velocities necessary to exercise the fish are reproducible in the laboratory with a swimming chamber, and because fatigue is a discrete, identifiable endpoint.

In this study, offspring of the different latitudinal populations were tested in flume-based trials to examine the extent of heritable intraspecific variation in swimming performance of these species. If present, significant differences in U-crit should be detected between progeny of these populations raised under controlled conditions in the laboratory. The number of breeding pairs collected from each species and population is given in Figure 5.3. Of each breeding pair, one clutch was taken and raised as described

above. Swimming performance was estimated on pre-settlement fish near the end of the pelagic stage. The three species investigated here all displayed a conspicuous change in behaviour and pigmentation at metamorphosis (sensu McCormick et al. 2002). In the laboratory, this usually occurred ~ 8 days after hatching, when fish began associating with the side or bottom of the rearing tank. Swimming trials were performed one day prior to this average time taken to metamorphosis.

Of each clutch, 12 randomly selected larvae were swum in each of two temperatures (23°C and 28°C) to examine whether these populations diverge in their response to temperature change. Fish were acclimatised to the swimming temperatures over a 4-hour period prior to swimming. To begin a trial, a group was transferred to the flume and left to recover from handling stress for 15 minutes before the start of the experiment. Water velocity was then raised in stepwise increments of approximately 3 body lengths every 2 minutes until the fish were exhausted, following the protocol of Bellwood & Fisher (2001). Exhaustion is defined as the point at which the fish can no longer maintain position in the chamber against the current and is swept to the downstream mesh. As each fish in a group fatigued, the speed and time at exhaustion was recorded and used to calculate critical swimming speeds following Brett (1964):

$$U_{-crit} = U + (t/t_i \times U_i)$$

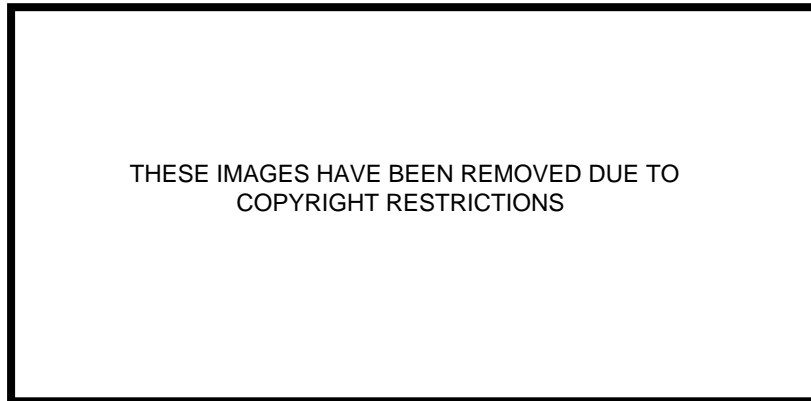
where  $U$  is the penultimate speed,  $U_i$  is the velocity increment ( $2 \text{ cm s}^{-1}$ ),  $t$  is the time swum in the final velocity increment, and  $t_i$  is the time interval for each velocity increment (2 min). Once all fish had fatigued they were removed, anaesthetised in chilled water, and fixed in 10% buffered formalin. After 24 h larvae were transferred to 70% alcohol and stored for later measurement of standard length and weight.

### **5.2.3. *Swimming chamber***

Swimming experiments were carried out using a multi-lane swimming apparatus designed by Stobutzki & Bellwood (1997), consisting of a Perspex chamber with 6 raceways, each 30 mm wide, 50 mm high and 390 mm long (Figure 5.1). A header tank with a T-piece diffuser ensured flow was evenly distributed into each channel. In addition, the mouth of each channel was fitted with a 40 mm long section of flow straighteners to minimise turbulence. During the swimming trials, the area where the fish swam was partially covered with a piece of black plastic to provide a stimulus for the fish to maintain their position. Water flow was generated by a 270 lpm 2.4 kW Onga<sup>TM</sup> pump, and a gate valve calibrated with a protractor controlled the volume, and therefore current speed. Calibration was carried out by recording the volume of water passing through the chamber in a unit time for different angles on the protractor and divided by the total cross-sectional area of the channels.

### **5.2.4. *Data analysis***

The relationship between swimming ability and body size was explored using regression analyses. Differences in body size of larvae from the different populations were examined using analyses of variance (ANOVA). Mean critical swimming speeds were compared among populations with nested factorial ANOVA based on the means of replicate clutches. This analysis was performed using both absolute U-crit and relative U-crit corrected for body size (SL). Size adjustment of swimming performance was done by regressing body length with absolute U-crit and by conducting additional analysis on the regression residuals. All analyses were performed using Statistica Version 6.2 (StatSoft Inc. Tulsa OK, USA).

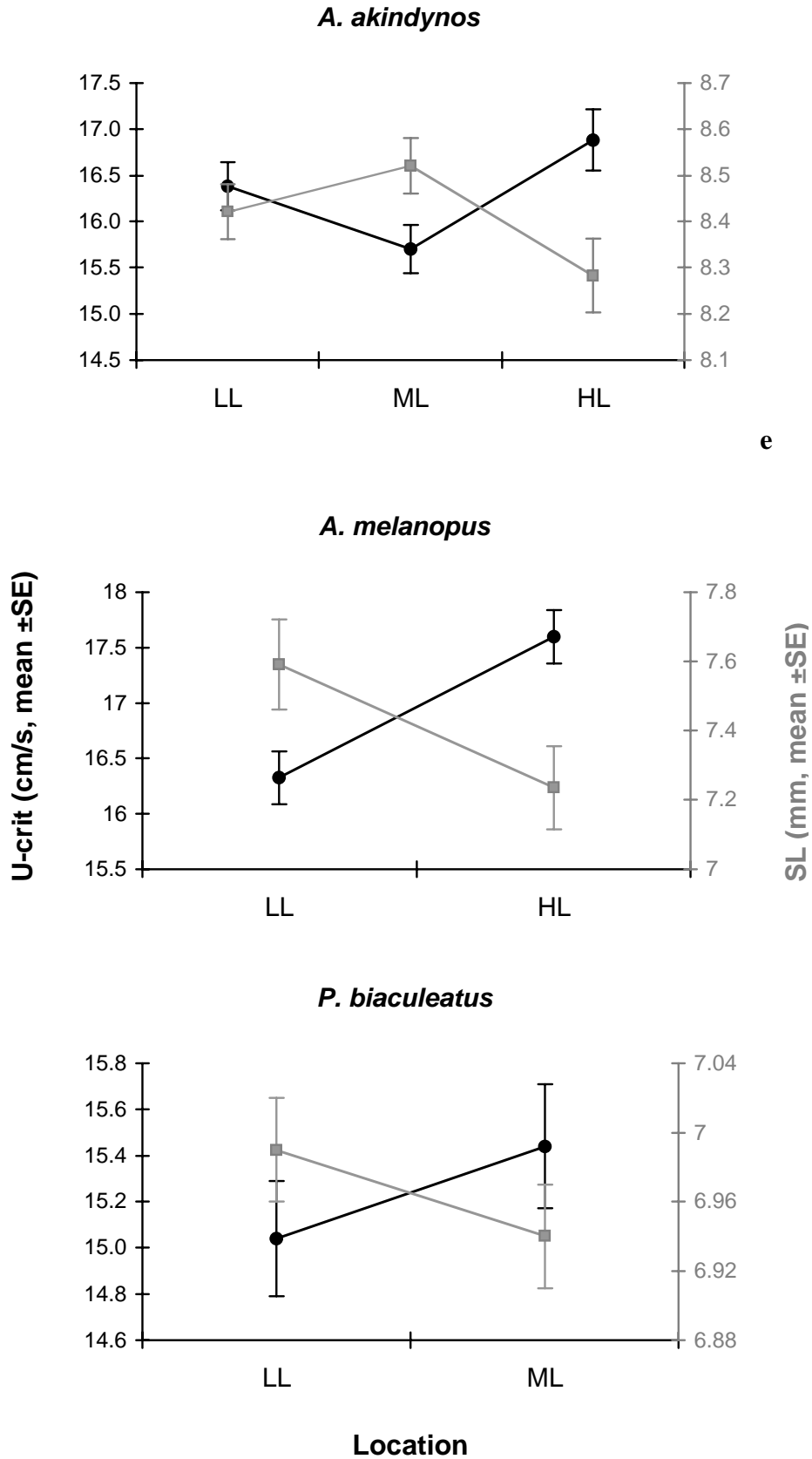


**Figure 5.1:** Swimming apparatus used in the present study: a) lateral view, b) top view. Arrows indicate the direction of water circulation (from Stobutzki and Bellwood 1997).

### **5.3. Results**

#### **5.3.1. *Effect of population origin and body size on swimming speed***

Mean absolute swimming speeds differed significantly among offspring of the different latitudinal populations (Table 5.1). Generally, offspring from higher-latitude populations out-performed those from lower-latitude populations in all three species (Figure 5.2).



**Figure 5.2:** Mean absolute swimming speeds (U-crit ± SE) and body sizes (SL ± SE) of the three species from the respective locations (LL = low latitude, ML = mid-latitude, HL = high latitude).



**Table 5.1:** Results of nested ANOVA testing for effects of population origin (location) and temperature on absolute U-crit, based on means from each treatment for each clutch.

Source of Variation	<i>df</i>	MS	<i>F</i>	<i>P</i>
<b><i>A. akindynos</i></b>				
<i>Location</i>	2	3.548	11.45	0.001
<i>Clutch (Location)</i>	13	5.661	18.28	<0.001
<i>Temperature</i>	1	4.278	13.81	0.003
<i>Location x Temperature</i>	2	1.377	4.45	0.034
Error	13	0.310	-	-
<b><i>A. melanopus</i></b>				
<i>Location</i>	1	8.128	10.94	0.011
<i>Clutch (Location)</i>	8	4.877	6.57	0.008
<i>Temperature</i>	1	0.047	0.06	0.808
<i>Location x Temperature</i>	1	1.105	1.49	0.257
Error	8	0.743	-	-
<b><i>P. biaculeatus</i></b>				
<i>Location</i>	1	0.895	5.45	0.044
<i>Clutch (Location)</i>	9	3.203	19.53	<0.001
<i>Temperature</i>	1	0.002	0.01	0.915
<i>Location x Temperature</i>	1	0.053	0.32	0.585
Error	9	0.164	-	-

In *A. akindynos*, mean swimming speeds did not follow a linear latitudinal gradient. High-latitude offspring swam fastest, followed by the low-latitude and finally the mid-latitude offspring (Figure 5.2). This pattern was inversely related to mean body size at the time of swimming trials: at the same absolute age, larvae of the mid-latitude location were largest, followed by the low-latitude larvae and finally the high-latitude larvae (Figure 5.2). These differences in body size were almost statistically significant (ANOVA,  $F = 2.98$ ,  $P = 0.052$ ) and confirm the pattern of post-settlement juvenile growth among the same latitudinal populations found in *chapter 4*. Linear regressions between mean U-crit and mean SL were significantly negative in all three populations (Table 5.2).

**Table 5.2:** Linear regression results of mean U-crit vs. mean fish length (SL) at the time of swimming trials, based on clutch means for each population.

Species	Location	r <sup>2</sup>	r	P	Regression equation
<i>A. akindynos</i>	LL	0.67	-0.82	< 0.01	y = 46.88 – 3.62*x
	ML	0.76	-0.87	< 0.01	y = 46.80 – 3.65*x
	HL	0.73	-0.85	< 0.01	y = 44.39 – 3.32*x
<i>A. melanopus</i>	LL	0.64	-0.80	< 0.01	y = 28.12 – 1.55*x
	HL	0.54	-0.74	< 0.05	y = 39.31 – 3.00*x
<i>P. biaculeatus</i>	LL	0.81	-0.90	< 0.001	y = 60.68 – 6.53*x
	ML	0.57	-0.76	< 0.05	y = 70.82 – 7.98*x

In *A. melanopus*, offspring of the high-latitude population swam significantly faster than offspring of the low-latitude population (Table 5.1, Figure 5.2). Conversely, mean body size of larvae from the low-latitude population was significantly larger (ANOVA,  $F = 5.97$ ,  $P = 0.015$ ) at the time of swimming compared to the mean body size of their high-latitude counterparts (Figure 5.2). As in *A. akindynos*, linear regressions of mean U-crit and mean SL were significant and negative in both populations (Table 5.2).

*P. biaculeatus* showed the same pattern as *A. melanopus*: offspring of the higher-latitude (ML) population swam significantly faster than offspring of the low-latitude population (Table 5.1, Figure 5.2), and mean U-crit and mean SL were significantly negatively correlated in both the low- and mid-latitude populations (Table 5.2). Low-latitude offspring were larger at 7 days after hatching than ML offspring; however, the difference was not statistically significant (ANOVA,  $F = 1.66$ ,  $P = 0.199$ ).

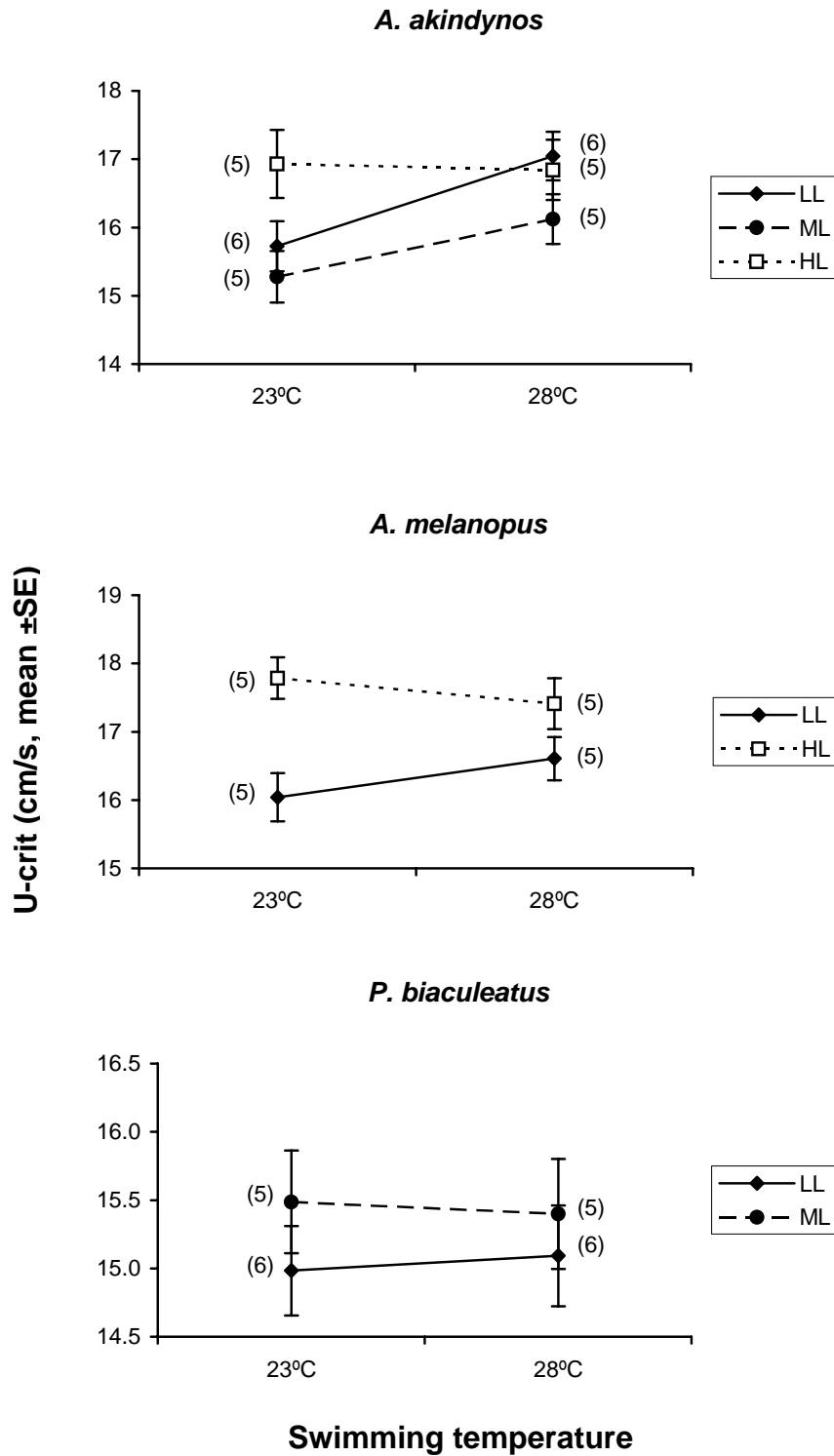
### 5.3.2. Effect of temperature on swimming speed

Temperature significantly affected swimming performance only in *A. akindynos* (Table 5.1). In addition, populations significantly differed in their response to the two experimental temperatures, as indicated by the significant location x temperature

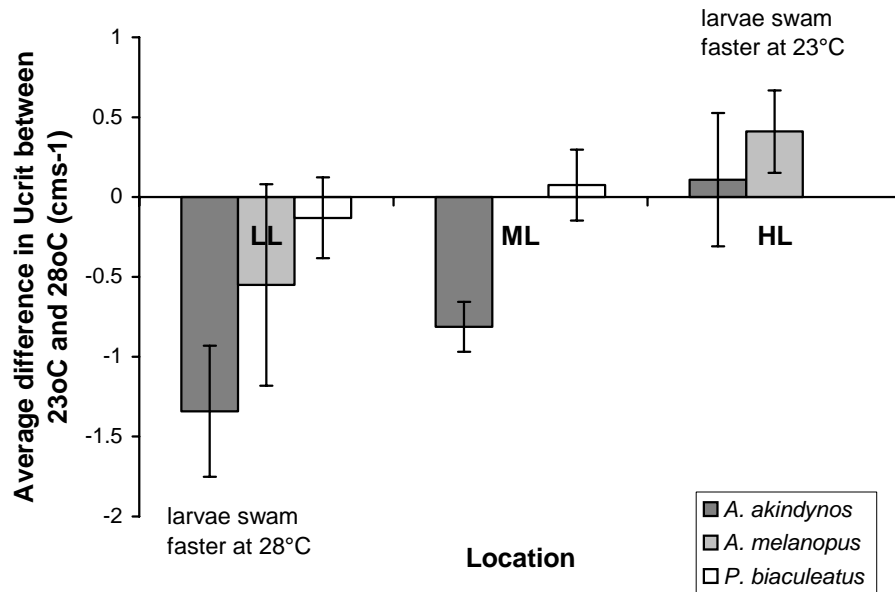
interaction (Table 5.1, Figure 5.3). Interestingly, temperature tolerance increased gradually with increasing latitude: low-latitude larvae achieved high swimming speeds in 28°C, but were not able to maintain their performance in 23°C. High-latitude larvae, on the other hand, were not only capable of maintaining a similar speed in both temperatures, but even swam slightly faster in 23°C, and mid-latitude offspring were intermediate between the two (Figure 5.4). In *A. melanopus* and *P. biaculeatus*, temperature had no significant effect on maximum sustained swimming speeds (Table 5.1). However, similar trends were found as in *A. akindynos*: in both species, low-latitude populations swam faster in 28°C than in 23°C, whilst the higher-latitude populations had a slightly better performance in the colder temperature (Figure 5.4).

### 5.3.3. *Size-adjusted U-crits*

When U-crit was size-adjusted for larval length (relative U-crit), most of the variation remained among populations. In *A. melanopus* and *P. biaculeatus* the same factors remained significant: the factors “location” and “clutch (location)” were significant, whilst the effect of temperature and the location x temperature interactions remained non-significant (Table 5.3). In *A. akindynos*, all factors remained significant except the location x temperature interaction (Table 5.3). However, with a  $P = 0.055$  it may be argued that this interaction is still “biologically significant”.



**Figure 5.3:** Interaction effect of geographic location and experimental temperature (23°C and 28°C) on the mean swimming performance (U-crit ± SE) of *A. akindynos*, *A. melanopus*, and *P. biaculeatus*. Numbers of clutches used in each treatment are shown in parentheses.



**Figure 5.4:** Average difference in swimming performance (U-crit  $\pm$  SE) between 23°C and 28°C in populations of the three species from the low-latitude (LL), mid-latitude (ML) and high-latitude (HL) locations.

**Table 5.3:** Results of nested ANOVA testing for effects of population origin and temperature on relative U-crit, based on means from each treatment for each clutch. Relative U-crit was determined using residual U-crit from regressions of absolute U-crit on larval length.

Source of Variation	<i>df</i>	MS	<i>F</i>	<i>P</i>
<b><i>A. akindynos</i></b>				
<i>Location</i>	2	5.670	13.73	<0.001
<i>Clutch (Location)</i>	13	9.709	23.51	<0.001
<i>Temperature</i>	1	3.601	8.72	0.011
<i>Location x Temperature</i>	2	1.504	3.64	0.055
Error	13	0.413	-	-
<b><i>A. melanopus</i></b>				
<i>Location</i>	1	9.573	14.25	0.005
<i>Clutch (Location)</i>	8	6.128	9.12	0.003
<i>Temperature</i>	1	0.037	0.06	0.821
<i>Location x Temperature</i>	1	1.124	1.67	0.232
Error	8	0.672	-	-
<b><i>P. biaculeatus</i></b>				
<i>Location</i>	1	2.189	11.64	0.008
<i>Clutch (Location)</i>	9	6.562	34.90	<0.001
<i>Temperature</i>	1	0.053	0.28	0.607
<i>Location x Temperature</i>	1	0.079	0.42	0.532
Error	9	0.188	-	-

## 5.4. Discussion

This study confirmed that offspring derived from adult anemonefishes from different latitudes have different innate swimming abilities, and represents the first demonstration of geographic variation in larval performance traits. Larvae reared from the most southerly populations out-performed those from lower latitudes in all three species. These consistent intraspecific differences in the laboratory indicate that these populations have undergone adaptive specialisation in response to latitudinal changes in coral reef environments. The fact that mean swimming speeds were inversely related to mean body size among populations of each species suggest that the observed patterns most likely arise from a trade-off with growth rate.

It has been shown previously that the latitudinal populations of the three species studied here have different intrinsic growth capacities (*chapter 4*). Reared in common environments, anemonefishes from lower latitudes generally grew faster than their high-latitude conspecifics, a pattern that was confirmed in the present study. These differences in growth rates should reflect the outcome of a complex mix of selective agents and their interactions with local environmental conditions. Given the potential costs and benefits of rapid growth and trade-offs in energy allocation to competing elements of fitness, growth rates are likely to be optimised, rather than maximised, in response to these functional trade-offs (Sibly et al. 1985; Abrams et al. 1996; Mangel and Stamps 2001). For example, potential costs to rapid growth in the early life stages of ectotherms may include lowered developmental stability (Leamy and Atchley 1985), reduced resistance to pathogens (Smoker 1986), and lower starvation endurance (Forsman and Lindell 1991; Gotthard et al. 1994). This study supports yet another explanation: that rapid growth at lower latitudes occurs at the expense of locomotor performance. My results demonstrated a negative relationship between body size and

swimming performance, i.e. swimming speeds of intrinsically fast-growing populations were lower than those of slow-growing populations and *vice versa*. This finding is consistent with recent studies of exercise physiology in fish (Kolok and Oris 1995; Farrell et al. 1997; Gregory and Wood 1999), and suggests that high rates of growth diminish swimming performance. A likely situation is the conflict in energy allocation between growth and activity: energetic demands for the synthesis of new somatic tissue may limit metabolic scope, thus reducing the energy available for locomotion (Fry 1971). There is a number of potential mechanisms underlying this trade-off, including changes in morphology associated with rapid growth (Taylor and McPhail 1985), excessive energetic inputs required for white muscle synthesis in fast growing fish (Valente et al. 1999), the metabolic cost of specific dynamic action associated with feeding (e.g. Jobling and Davis 1980; Alsop and Wood 1997), the effects of lower hematocrit on swimming performance (Gallaughier et al. 1995), and insufficient aerobic enzyme activity in fast-growing fish (Somero and Childress 1980; Pelletier et al. 1993). Taken together, it appears that the allocation of energy to growth may limit the energy available for active metabolism and hence reduce locomotor capacity. Small fish in early life stages may be particularly susceptible to such trade-offs due to their low metabolic scope, high weight-specific rates of metabolism, and poor anaerobic capacity (Billerbeck et al. 2001).

The foregoing discussion suggests that rapid growth occurs at the expense of reduced locomotor performance; however, it is also possible that differences in swimming ability are driven by divergent natural selection acting directly on performance traits, and that growth co-varies accordingly. A third option may be that both traits respond to other factors and vary independently of one another. From this study it is difficult to determine which of these traits is subject to direct selection, and

which is being compromised as a result of the proposed trade-off in energy allocation. Environmental factors that may impose direct selection on swimming performance (but are not necessarily correlated with latitude) include predation pressure and prey availability. Predation is thought to play a major role in regulating survival of pelagic larvae and, therefore, in recruitment (Chambers and Trippel 1997). Direct knowledge of predation on pelagic larvae is extremely limited, but it can be expected that fish with higher swimming speeds have a higher probability of escape given a predatory attack. For instance, Bailey (1984) found that escape speeds of five species of marine fish larvae closely predicted their ability to avoid invertebrate predators. Similarly, Lankford et al. (2001) demonstrated that a decrease in swimming performance was associated with increased mortality risk in juvenile Atlantic silversides exposed to a variety of natural predators. Predator abundance can vary among locations (Caley 1995a) and it is thus possible that differential predation pressure contributes to differential swimming abilities among populations as a result of predator-mediated selection. However, even if predation pressure is similar among locations it may be that the relative importance of fast growth *vs.* the ability to avoid predation varies across latitudes, i.e. that there is a change in balance of these two selective factors. For example, in low-latitude populations directional selection for fast growth is strong because maturity must be reached quickly to compensate for a reduced lifespan and increased mortality. Rapid intrinsic growth rate may therefore be favoured, even if this occurs to the detriment of swimming performance. In southern populations, on the other hand, there is less pressure for fast growth and predation may be a more immediate concern to larvae. Here, the duration of immature stages, when larvae are most vulnerable to predation, is longer, so the ability to avoid predation becomes relatively more important. This suggests that the conservative growth strategy found in high-latitude populations may



enhance fitness by reserving a larger proportion of metabolic scope for swimming performance, thereby improving predator avoidance ability.

Swimming is also central in hunting and prey capture (Hunt von Herbing et al. 2001). After conclusion of the yolk-sac period, the cruising speed of larvae becomes of major importance in their feeding ecology because it affects the frequency that larvae encounter prey. Differences in swimming performance may therefore relate to differences in prey availability among locations. High-latitude environments are temporally and spatially more variable than those at low latitude, and this may also apply to larval food abundance. Thus, performance may be selectively favoured in high-latitude populations, as they may require better swimming capabilities to find and feed on scarce prey. However, this prey density hypothesis remains largely speculative because the role of latitudinal variation in food supplies in the wild has not been investigated so far.

Yet another potential factor directly influencing swimming performance may be the strength and direction of ocean currents along the GBR. In outer shelf waters, during summer, the drift is almost entirely unidirectional southward longshore (Williams et al. 1984). Larvae from lower latitudes have the opportunity to settle long distances downstream of their natal reefs; however, this is not true for larvae from higher latitudes. These individuals may be in danger of being swept down too far into unsuitable habitats that do not warrant survival, and thus may be under stronger selective pressure to develop a greater swimming capacity in order to counter this dominant pole-ward flow.

Many teleost fish species may be exposed to changes in temperature, which may be relatively acute, for example during migration or tidal changes, or may be seasonal and long-term in nature. For fish larvae, a change in temperature may have a profound

effect on swimming performance. These effects are mainly attributed to the modification of the molecular kinetics and the rates of biochemical reactions that convert chemical energy into propulsive thrust (reviewed in Beamish 1978), and to the alteration of the physical properties of the water, which may affect a fish's movement (Johnson and Bennett 1995; Fuiman and Batty 1997). However, the maintenance of high swimming performance over a broad thermal range may be important for juvenile survival, since it affects behavioural attributes such as predator avoidance and prey capture (Fuiman 1991; Blaxter 1992; Temple and Johnston 1998; Ojanguren and Brana 2000).

The latitudinal populations examined in this study exhibited different thermal sensitivities in response to the two experimental temperatures. Generally, offspring from lower latitudes exhibited greater thermal sensitivity with comparatively faster swimming speeds in the warm temperature but reduced performance in the cold temperature. Contrarily, offspring from higher latitudes performed relatively well in both experimental temperatures and thus appeared to be less thermally sensitive. The capacity to compensate for acute temperature effects on locomotor performance via thermal acclimation are well known and documented in fish (e.g. Johnston and Dunn 1987; Beddow et al. 1995; Johnson and Bennett 1995; Fuiman and Batty 1997; Green and Fisher 2004), and should be especially important in populations living at higher latitudes where environmental temperature is more variable. On a yearly scale, the thermal variance in the tropics is considerably lower compared to temperate regions (Rudloff 1981). Evolutionary theory therefore predicts that tropical ectotherms should be adapted physiologically to this narrower range of temperatures, whereas temperate-zone ectotherms should be adapted to the broader range of temperatures they experience (Pianka 1966; Pielou 1975). Narrower tolerance ranges in the tropics have been shown

for fishes (e.g. Scholander et al. 1953; Brett 1970), amphibians (e.g. Feder 1982), and marine invertebrates (e.g. Vernberg 1984).

My results are consistent with this prediction. Although the “location x temperature” interaction was only significant in *A. akindynos* (and marginally significant when controlled for body size), a similar pattern was displayed by all species. Low-latitude populations swam faster in the higher temperature and therefore appear to possess specialist phenotypes that maximise peak performance in the temperature most commonly encountered in nature. The more temperate populations, on the other hand, performed well in both the high and low experimental temperature and therefore seem to be more generalist phenotypes that maximise performance breadth. Such a pattern would be expected if the thermal sensitivity of swimming speed had evolved to improve performance of fish in their natural habitat. Evolutionary theory further predicts that there should be a trade-off between specialist and generalist phenotypes, whereby the ability to perform well over a wide range of environments can be achieved only at the expense of maximal performance (“Jack-of-all-temperatures is a master of none”, Huey and Kingsolver 1989). Although this “Principle of Allocation hypothesis” (Levins 1968) underlies much of evolutionary theory, few studies have used the thermal sensitivity of ectotherm performance to investigate the generality of this evolutionary prediction. To date, empirical evidence remains inconclusive, as the suggested trade-off only occurs in some instances (Huey and Hertz 1984; Bennett et al. 1992; Gilchrist 1996). In this study, the overall higher performance of HL populations indicates that higher plasticity has evolved without the trade-off described above: not only have HL populations of all three species acquired the capacity to maintain their performance across a wider range of temperatures (i.e. lowered their thermal

sensitivity), but they also showed a superior overall performance compared to the LL populations.

This study is significant because it provides the first demonstration of population-level variation in the intrinsic swimming capacity of larval coral reef fishes. Given that swimming performance is negatively correlated with growth among populations in all three species, a trade-off between these two traits is a likely explanation for the observed patterns, although at this stage it is not clear whether swimming speed is being adjusted at the expense of growth, or *vice versa*, or some combination of these two factors. This demonstrates that developmental attributes and performance are interrelated in a complex fashion, with multiple selection pressures acting on multiple aspects of the phenotype, and that adaptation involves trade-offs among competing functions. It is therefore important to consider both developmental as well as performance traits when investigating adaptation in response to environmental change. For this, performance and behavioural specialisation in different environments must be further investigated throughout the ontogeny of these fishes and in additional locations.

## **CHAPTER 6: General Discussion**

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Our understanding of the life histories of coral reef fishes has been largely restricted to the description of pattern, and at most, quantifying variation in particular traits among locations (e.g. Meekan et al. 2001; Choat et al. 2003; Robertson et al. 2005). There have been few attempts to test life history theory or to reveal underlying processes, either by generating hypotheses based on relationships among life history traits, or by experimentally examining fitness-related traits under controlled conditions. Yet, phenotypic variation in the field can be difficult to interpret without studies of the underlying pattern of genetic differentiation (Conover and Schultz 1995). Intraspecific variation in life history traits may be as likely to represent phenotypically plastic responses to differences in local environmental conditions, as they are adaptive solutions to local selection pressures. Knowledge of the relative contribution of genetic and environmental sources to phenotypic expression observed in the wild is therefore an important prerequisite for a complete understanding of the ecological and evolutionary significance of geographic life history variation. This study is unique in that it is the first to not only describe life history patterns of populations in the wild, but also to experimentally examine the genetic basis of latitudinal trait variation in a group of long-lived coral reef fishes by using a common-garden approach.

### **6.1. Geographic life history variation in the wild**

Life history theory predicts faster initial growth rates, smaller maximum body sizes, reduced longevity, increased mortality, earlier maturation and increased reproductive effort in populations from low latitudes, compared to those from higher latitudes. Some of the geographic variation found in the natural populations examined

here was consistent with these predictions, and with latitudinal trends that have been observed for other fishes over temperate latitudes. However, while a number of generalisations were supported, there were also considerable differences among species in the magnitude and direction of geographic variation. The observed variation showed mixed, species-specific patterns, which cannot be solely explained by traditional theories, thus providing evidence for a complex interplay of a range of influences determining life history patterns in coral reef fishes.

All three species supported the prediction that longevity increases with latitude. *A. melanopus* always grew older than the other two species at each location, reaching an impressive age of 38 years at One Tree Island. In regard to other life history parameters, *A. melanopus* followed the predicted trends remarkably well, i.e. the population at the highest latitude location (One Tree Island) was characterised by slower growth, larger adult body sizes, lower mortality, greater proportional age and size at maturity and sex change, larger eggs and a lower fecundity/weight ratio. In *A. akindynos* and *Premnas*, however, not all patterns varied predictably with latitude. In these species, maximum body size was smaller at the higher latitude location, and there were no differences among locations in growth and mortality rates, and no consistent trends in the timing of maturation or sex change. Thus, while longevity varied with latitude as expected in all species, some of the other life history traits examined did not.

The divergence among these species in life history patterns suggests that there are processes beyond simple trade-offs that determine life history strategies. The most likely sources of these interspecific differences in life history patterns involve species-specific differences in social structures and geographic ranges. In site-attached species, where juveniles cohabit with adults in discrete social groups, patterns of growth and sexual development can be highly flexible and are often determined by local social

conditions (Francis 1992; Devlin and Nagahama 2002). In anemonefishes, maturation of subordinate juveniles is usually suppressed by the aggressive behaviour of larger members in the group and can only occur when the individual moves up in the social hierarchy, either through the disappearance of a dominant individual or through migration into another social unit (Fricke and Fricke 1977; Fricke 1979; Ochi 1989; Buston 2003b). If suitable habitats (i.e. anemones) are sparse, then juveniles may be forced to settle in existing groups of conspecifics, and growth is sacrificed in favour of an improved probability of survival. The social environment is therefore a major determinant of life history characteristics in these species.

*A. melanopus* has a broad distribution and lives in very large, loose social groupings of up to 30 or more individuals (pers. obs.). Among the three species studied here, *A. melanopus* has the least social control over its life history transitions, which may explain why its patterns of geographic variation most closely conform to evolutionary theory, and are similar to other small, non-anemone-dwelling damselfishes. *Premnas*, on the other hand, has the highest degree of social control over growth and reproduction due to a very strict social structure. This species does not live in social units with juveniles, but only occurs as solitary pairs in one particular actinian host (*Entacmaea quadricolor*). Furthermore, *Premnas* possesses a pronounced sexual dimorphism with males typically much smaller and brighter coloured than females. The lack of consistent latitudinal trends in this species may therefore be due to its rigid social structure, which possibly overrides any effects of environmental gradients in the wild. Finally, *A. akindynos* is intermediate in terms of its social structure, but has a much narrower geographic range than the other two species, only occurring on the Great Barrier Reef and adjacent Coral Sea. It can therefore be expected that this species

experiences less environmental variation across this limited distribution, and is therefore less likely to have high variability in its life history traits.

## **6.2. Reproductive performance is a phenotypically plastic trait**

In *chapter 3* I tested whether or not there is a genetic basis to the latitudinal variation in reproductive traits found in natural populations. This was achieved by examining the reproductive performance of breeding pairs from different latitudinal populations in a common-garden, in which environmental variables (including the social environment) were controlled and identical for all individuals. My results provided no evidence for genotypic variation in reproductive traits in these species: intraspecific comparisons of egg size, fecundity and reproductive output showed no differences among populations held in a common-garden for up to three consecutive years. This suggests that these parameters do not have a genetic basis, but are mainly regulated through environmentally induced plasticity. This apparent lack of genetic differentiation supports the notion that in marine species, where highly dispersive planktonic larvae potentially lead to high levels of gene flow, the opportunity for local adaptation is generally limited. Intraspecific differences in life histories will then be the result of phenotypic plasticity in accordance with local environmental conditions. However, it is also possible that the capacity of local adaptation exists, but it is overridden by selection for adaptive phenotypic plasticity in these traits. This may be the case if environmental variability (e.g. food availability) at each location is strong enough that choosing a particular egg size-fecundity trade-off would be maladaptive. The premises for arguing that plasticity can be adaptive, i.e. resulting in a phenotype that is able to respond to changing environmental conditions, are: 1) environmental variation has a significant influence on fitness, 2) traits respond to different



environments in a predictable fashion, and 3) the phenotypic response to environmental change has a genetic basis and is capable of responding to natural selection (Reznick 1990; Scheiner 1993). Environmental uncertainty in juvenile habitat among generations, coupled with size-dependent effects on fitness, should favour the evolution of plasticity in reproductive traits in anemonefishes. That is, selection should favour genotypes that optimise fitness by phenotypically altering life history traits in response to environmental change (Levins 1968). Such “individual optimisation” has been described in birds, for which clutch size appears to be dependent on individual ability to acquire food (Pettifor et al. 1988).

Further support for the conclusion that egg size and fecundity are phenotypically plastic is provided by *A. melanopus*, which showed significant temporal variation in these traits. Over a three-year period, reproductive effort remained the same, but egg size decreased and egg number increased in the common-garden. This indicates that such trade-offs may be environmentally driven, and I suggest that the shift towards smaller eggs is an adaptive response to the favourable, predictable conditions in an energy-rich laboratory environment, which is free of predation and competition.

### **6.3. Genetic variation for growth rate in the early life history**

In contrast to reproductive characteristics of adult fishes I found evidence for adaptive genetic differentiation for growth rate and growth rate plasticity in the early juvenile stages of anemonefish populations along a latitudinal gradient. In all three species, growth rates of laboratory-bred juveniles were significantly different among populations and followed a co-gradient pattern, with higher mean growth rates in lower-latitude populations. Demonstrated under common-garden conditions, this implies that population-level variation in the intrinsic growth rate of juvenile anemonefishes has a

genetic component and can therefore evolve (Reznick and Travis 1996). Furthermore, significant genotype x environment interactions suggest the evolution of different growth reaction norms in response to temperature and food availability at different latitudes. These results support the hypothesis that the patterns of intraspecific geographic variation in growth among locations are predominantly a result of natural selection with the potential for ecological and adaptive speciation in this system.

Growth rate may evolve in response to temperature not primarily because of the effects of thermal change on the physiology of individual organisms, but instead because of the impact of temperature on population parameters such as reproductive rate, which in turn determine the way in which natural selection acts on the life history. As such, the observed variation in growth may be explained by latitudinally dependent age-specific schedules of survival and reproduction (Charlesworth 1980), where the optimal strategy depends on the relative costs and benefits of rapid early growth in the respective local environment. The present study confirms that fast growth evolves where a minimum size must be reached quickly. At low latitudes, where turnover rates and mortality are high (Houde 1989; Stearns 1992), it is clearly adaptive to grow fast and invest in reproduction as soon as possible. This, in turn, reduces the reserves for later somatic growth and maintenance, resulting in relatively smaller adult body sizes and higher probability of mortality (i.e. shorter life span). These predictions were generally confirmed by field observations in these species, particularly in *A. melanopus*. At high latitudes, where spawning is often temporally confined and generation times are longer, offspring have different constraints. Here, populations are predicted to maximise fitness by delaying sexual maturation to an age at which their larger body size will result in increased fecundity and post-reproductive survival. Hence, there is little

selection for rapid growth, allowing individuals to devote more resources to the prevention of ageing and to ultimately attain a larger adult size.

In summary, the optimal growth trajectory will depend on factors such as the period over which growth is possible, the degree of flexibility in the timing of key life history events, and the relative contributions of adult body size *vs.* adult lifespan to overall fitness in the respective environments. This thesis has provided the first demonstration of population-level genetic variation in the early growth rates of long-lived coral reef fishes. It appears that these species have a genetically determined program of growth defining a framework within which external factors, such as the social environment, can cause modifications. For example, behavioural selection of social conditions that improve the probability of survival may result in a sacrifice of maximal growth in anemonefishes. Such circumstances may vary independently of latitude and mask the occurrence of directional selection within a particular habitat in the wild. Because data sets were small and no comparative information is available at this stage, conclusions about latitudinal variation of these early life history features must remain suggestive for the time being.

#### **6.4. Swimming performance of larvae differs among populations**

In the last part of this thesis I examined if, and to what extent, swimming performance of pre-settlement larvae differs among populations from different latitudes. My results confirmed that there is intraspecific variation in the intrinsic swimming capacity of these fishes, such that offspring from higher latitudes out-performed those from lower latitudes in all three species. This suggests that swimming ability is a heritable trait, with the potential to influence individual survival during the pelagic phase and settlement. However, performance did not follow a linear latitudinal gradient,

as seen in the three populations of *A. akindynos*. Given that mean swimming speed was inversely related to mean body size in all three species, a trade-off between swimming performance and growth capacity appears to be a likely explanation for the observed patterns.

It has been shown previously that a wide array of physiological and biological characteristics have a significant impact on intraspecific variation in swimming ability, including size (Koumoundouros et al. 2002), body morphology (Boily and Magnan 2002), food composition (McKenzie et al. 1998), growth rate (Kolok and Oris 1995) and muscle enzymatic activity (Farrell et al. 1991). Taken together, these findings demonstrate that the outcome of selection on different aspects of the organism is generally a compromise among competing influences. The outcome will be shaped by interactions or trade-offs among the different components of the phenotype that contribute to fitness, such as the proposed trade-off between growth rate and swimming performance described here. If the evolution of performance is viewed in the context of multiple selection pressures acting on multiple interacting traits, it becomes apparent that diverse perspectives and a multi-disciplinary approach are needed to better understand the conditions under which performance will evolve.

In terms of the overall significance of swimming performance in coral reef fishes, this study supports previous findings that late stage larvae are “effective” swimmers (sensu Leis and Stobutzki 1999), i.e. that they have the capacity to overcome average current speeds in a system like the Great Barrier Reef (e.g. Fisher 2005), and thus should be able to exert considerable control over their dispersal and settlement patterns. With overall mean  $U$ -crits of between  $15.22 \text{ cm s}^{-1}$  in *P. biaculeatus* to  $16.96 \text{ cm s}^{-1}$  in *A. melanopus* all three species exceeded the speed of an average net longshore drift along the Great Barrier Reef, which is in the order of  $10 \text{ cm s}^{-1}$  (Williams et al.

1984). Given the potential importance of swimming abilities to the successful settlement of highly site-attached fishes such as anemonefishes, along with their very short larval duration, small changes in swimming performance may be critical to both the survival and overall connectivity patterns of these populations. The large scale geographic and population level differences in swimming performance that I have observed here may therefore substantially influence the population ecology of these fishes. Clearly, more work needs to be done on further taxa to assess the importance and generality of the patterns found in this study. This refined knowledge of the behavioural abilities of larvae will then allow more sophisticated assumptions to be incorporated into oceanographic dispersal models (e.g. James et al. 2002), which will undoubtedly improve current efforts aiming to describe dispersal patterns of larval reef fishes.

## **6.5. Local adaptation in marine fishes**

In any benthically associated marine species, the relative roles of genetic differentiation, phenotypic plasticity and large-scale adaptation in determining life history patterns should be a function of the spatial and temporal heterogeneity of the selective environments, the degree of larval dispersal, and the response abilities of individuals (Warner 1997). If the local fitness consequences of a particular trait expression are strong and temporally consistent, local genetic differentiation can occur as long as the population is at least partially closed. Using a common-garden approach, this study shows that there is heritable variation for some fitness-related traits in anemonefishes, suggesting that these benthic species have the potential for local adaptation despite the lack of obvious barriers to dispersal. This adds to a large body of research showing intraspecific genetic divergence among fish populations (reviews in Powers 1987; Meffe and Snelson 1989; Taylor 1991; Bell and Foster 1994). These

studies span a diverse array of traits, taxonomic groups and environmental gradients, and thereby argue strongly for the ubiquity of local adaptation in fishes. However, marine taxa (and particularly tropical reef fishes) are underrepresented in this literature, and the degree of local adaptation to be expected in marine species remains largely unexplored.

Generally, the high capacity for gene flow in marine fishes is thought to inhibit local genetic differentiation. Indeed, several molecular studies have revealed little or no genetic structuring over geographic scales spanning hundreds to thousands of kilometres (reviewed in Avise 1994). However, it is important to note that the extent of gene flow sufficient to produce panmixia in neutral alleles does not rule out the evolution of local adaptation (Endler 1977; Holt and Gaines 1992; Strathmann et al. 2002). Geographic patterns of molecular traits cannot necessarily be used to map the patterns of fitness-related traits, as molecular genetic markers are assumed to be neutral to selection, and their relationship with genetic variation in adaptive traits, if any, is mostly unknown (Hard 1995; Lynch 1996; Storfer 1996). Nonetheless, my proposition that genetic differentiation may be prevalent in benthic marine fishes such as damselfishes is supported by two recent genetic studies. Firstly, Doherty and co-workers (1995) detected significant genetic differentiation among damselfish populations from the northern and southern regions of the GBR, despite pelagic larvae, numerous stepping-stones, and favourable current patterns. Secondly, Shulman (1998) showed that 44% of damselfishes (Pomacentridae) show genetic differentiation between populations across medium and large scales, in contrast to only 14% in other families. These findings suggests that damselfishes may have more restricted dispersal (and therefore gene flow) than other reef fishes, probably due to the demersal spawning and short planktonic durations, and it could be that their larvae use their mobility to retard,

rather than enhance, dispersal. My results agree with this suggestion; however, any definite conclusions about the capacity for local adaptation of fitness-related traits in these fishes require more rigorously controlled comparisons. Future studies focussing on the problem of local adaptation in marine fishes should take into account that more than just molecular genetics is needed to address these issues. As such, common-garden experiments should be employed more widely to explore the genetic basis of trait variation. Unfortunately, for many marine species it will be challenging (if not impossible) to perform experiments, as they are difficult to raise in captivity because of long generation times, complex life cycles, or obscure mating requirements.

## **6.6. Implications for conservation and management**

The issues discussed above and the findings of this thesis may have important implications for both management and conservation. The fact that there is heritable variation for at least some fitness-related traits in the species investigated here suggests that the dynamics of these traits are a consequence of natural selection, and thus may be adaptive. Such adaptive variation is beneficial to resource conservation because it constitutes direct evidence that species are capable of evolving in the face of environmental change, a necessary condition for the long-term persistence of populations (Ryman 1991). Flexibility for adaptation is especially important today, where reef systems are increasingly threatened by anthropogenic effects such as rapid climate change, or contamination of habitats with pathogens and pollutants. Furthermore, genetic variability may not only enhance fitness within a particular habitat, but also promote colonisation and range expansion by allowing persistence across a wider range of environments (Holt 1990; Hoffmann and Blows 1993; Davis and Shaw 2001). Therefore, if natural resources are to be managed wisely, the aim

should be to examine the geographic distribution of locally adapted traits and their covariance with environmental differences, and to maintain as much genetic variability as possible within a species. Another important finding of this study is that small coral reef fishes can be much longer lived than previously thought (close to 40 years). Anemonefishes are a popular target of the aquarium fish trade, and the fact that they are so long-lived makes them vulnerable to over-exploitation, because species exhibiting long life spans and low rates of natural mortality have relatively low sustainable fishery yields (Russ 1991). This clearly has profound consequences for ecosystem management and thus, studies of age and growth provide important information for the analysis of reef fish population dynamics.

## **6.7. Conclusions**

Overall, the present study demonstrates that the selective environment is often complex, with multiple selection pressures acting on multiple aspects of the phenotype, and that adaptation often involves trade-offs among competing functions. In natural populations of anemonefishes, some patterns of co-variation among life history traits conformed to predictions of life history theory, whilst others did not. One possible source of these non-latitudinal patterns may be that selection for flexibility in life histories imposed by their social hierarchy opposes selection over latitudinal environmental gradients. These complexities, as well as the potential for local scale variation, highlight the necessity to examine latitudinal relationships in many taxa and on different geographic scales, in order to identify robust trends in life history patterns. My results further suggest that there are fixed differences in some elements of the population ecology of these reef-associated fishes (such as growth rate in the early life history), while others are regulated by phenotypic plasticity (such as reproductive



performance of adult individuals). The observed shifts in mean phenotypic expressions of traits among the latitudinal populations most likely result from the nature of selection in each environment, along with the environmental and developmental constraints. More research is needed to further explore the means by which life history variation is generated in tropical reef fishes - only then can we begin to develop hypotheses encompassing a broader range of taxa and take further steps toward a conceptual framework of the reef fish community as a whole.

## References

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- Abookire AA, Macewicz BJ (2003) Latitudinal variation in reproductive biology and growth of female Dover sole (*Microstomus pacificus*) in the North Pacific, with emphasis on the Gulf of Alaska stock. *Journal of Sea Research* 50: 187-197
- Abrams PA, Leimar O, Nylin S, Wiklund C (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist* 147: 381-395
- Abrams PA, Rowe L (1996) The effects of predation on the age and size of maturity of prey. *Evolution* 50: 1052-1061
- Ackerman JL (2004) Geographic variation in size at age of the coral reef fish, *Thalassoma lunare* (Family: Labridae): a contribution to life history theory. PhD. School of Marine Biology and Aquaculture, Townsville
- Adkison MD (1995) Population differentiation in Pacific salmon: genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences* 52: 2762-2777
- Aldenhoven JM (1986) Local variation in mortality rates and life history estimates of the coral reef fish *Centropyge bicolor* (Pisces: Pomacanthidae). *Marine Biology* 92: 237-244
- Alsop DH, Wood CM (1997) The interactive effects of feeding and exercise on oxygen consumption, swimming performance, and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 200: 2337-2346
- Anderson JT (1988) A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *Journal of Northwest Atlantic Fisheries Science* 8: 55-66
- Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. *Quarterly Review of Biology* 72: 149
- Arendt JD, Wilson DS (1999) Countergradient selection for rapid growth in pumpkinseed sunfish: disentangling ecological and evolutionary effects. *Ecology* 80: 2793-2798
- Arnett AE, Gotelli NJ (1999) Geographic variation in life-history traits of the ant lion, *Myrmeleon immaculatus*: evolutionary implications of the Bergmann's rule. *Evolution* 53: 1180-1188
- Arnold SJ (1983) Morphology, performance, and fitness. *American Zoologist* 23: 347-361
- Arnold SJ (1992) Behavioural variation in natural populations. VI. Prey responses by two species of garter snakes in three regions of sympatry. *Animal Behaviour* 44: 705-719
- Ashton KG, Feldman CR (2003) Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* 57: 1151-1163
- Ashton KG, Tracy MC, De Queiroz A (2000) Is Bergmann's rule valid for mammals? *American Naturalist* 156: 390-415
- Atkinson D (1994) Temperature and organism size - a biological law for ectotherms? *Advances in Ecological Research* 25: 1-58
- Atkinson D (1996) Ectotherm life-history responses to developmental temperature. In: Johnston IA, Bennett AF (eds) *Animals and Temperature*, pp 183-204

- Atkinson D, Sibly RM (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* 12: 235-239
- Avise JC (1994) *Molecular markers, natural history, and evolution*. Chapman & Hall, New York
- Bagenal TB (1969) The relationship between food supply and fecundity in brown trout *Salmo trutta* L. *Journal of Fish Biology* 1: 167-182
- Bagenal TB (1971) The interrelation of the size of fish eggs, the date of spawning and the production cycle. *Journal of Fish Biology* 3: 207-219
- Bagenal TB (1978) Aspects of fish fecundity. In: Gerking SD (ed) *Ecology of Freshwater Fish Production*. Blackwell Scientific, Oxford, pp 75-101
- Bailey KM (1984) Comparison of laboratory rates of predation on five species of marine fish larvae by three planktonic invertebrates: effects of larval size on vulnerability. *Marine Biology* 79: 303-309
- Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology* 25: 1-83
- Baroiller J-F, Guiguen Y, Fostier A (1999) Endocrine and environmental aspects of sex differentiation in fish. *CMLS Cellular and Molecular Life Sciences* 55: 910-931
- Bartholomew GA (1987) Interspecific comparison as a tool for ecological physiologists. In: Feder ME (ed) *New directions in ecological physiology*. Cambridge University Press, Cambridge, pp 11-35
- Bauer RT (1992) Testing generalizations about latitudinal variation in reproduction and recruitment patterns with sicyonid and caridean shrimp species. *Invertebrate Reproduction and Development* 22: 193-202
- Bay LK, Buechler K, Gagliano M, Caley JM (in print) Intraspecific variation in the pelagic larval duration of tropical reef fishes. *Journal of Fish Biology*
- Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* 42: 485-508
- Beamish FWH (1978) Swimming capacity. In: Hoar WS, Randall DJ (eds) *Fish Physiology*. Academic Press, New York, pp 101-187
- Beddow TA, Van Leeuwen JL, Johnston IA (1995) Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *Journal of Experimental Biology* 198: 203-208
- Begon M, Harper JL, Townsend CR (1996) *Ecology: individuals, populations and communities*. Blackwell Scientific Publications, London
- Bell LJ, Moyer JT, Numachi K (1982) Morphological and genetic variation in Japanese populations of the anemonefish *Amphiprion clarkii*. *Marine Biology* 72: 99-108
- Bell MA, Foster SA (1994) *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford
- Bellwood DR, Fisher R (2001) Relative swimming speeds in reef fish larvae. *Marine Ecology Progress Series* 211: 299-303
- Bennett AF, Lenski RE, Mittler JE (1992) Evolutionary adaptation to temperature. *Evolution* 46: 16-30
- Bergenius MAJ, Meekan MG, Robertson DR, McCormick MI (2002) Larval growth predicts the recruitment success of a coral reef fish. *Oecologia* 131: 521-525
- Bergmann C (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Gottinger Studien* 3: 595-708
- Bernardo J (1994) Experimental analysis of allocation in two divergent, natural salamander populations. *American Naturalist* 143: 14-38
- Bernardo J (1996) Maternal effects in animal ecology. *American Zoologist* 36: 83-105

- Bertschy KA, Fox MG (1999) The influence of age-specific survivorship on pumpkinseed sunfish life histories. *Ecology* 80: 2299-2313
- Berven KA, Gill DE (1983) Interpreting geographic variation in life-history traits. *American Zoologist* 23: 85-97
- Berven KA, Gill DE, Smith-Gill SJ (1979) Countergradient selection in the green frog, *Rana clamitans*. *Evolution* 33: 609-623
- Beverton RJH (1992) Patterns of reproductive strategy parameters in some marine teleost fishes. *Journal of Fish Biology* 41: 137-160
- Beverton RJH, Holt SJ (1959) A review of the lifespans and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. *CIBA Foundation Colloquia on Ageing* 5: 142-180
- Billerbeck JM, Lankford TE, Conover DO (2001) Evolution of intrinsic growth and energy acquisition rates. I. Trade-offs with swimming performance in *Menidia menidia*. *Evolution* 55: 1863-1872
- Blaxter JHS (1992) The effect of temperature on larval fishes. *Netherlands Journal of Zoology* 42: 336-357
- Blaxter JHS, Hempel G (1963) The influence of egg size on herring larvae (*Clupea harengus* L.). *ICES Journal of Marine Science* 28: 211-240
- Blueweiss L, Fox H, Kudzma V, Nakashima D, Peters R, Sams S (1978) Relationships between body size and some life history parameters. *Oecologia* 37: 257-272
- Boehlert GA, Yoklavich MM (1983) Effects of temperature, ration, and fish size on growth of juvenile black rockfish, *Sebastes melanops*. *Environmental Biology of Fishes* 8: 17-28
- Boily P, Magnan P (2002) Relationship between individual variation in morphological characters and swimming costs in brook charr (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). *Journal of Experimental Biology* 205: 1031-1036
- Booth DJ, Hixon MA (1999) Food ration and condition affect early survival of the coral reef damselfish, *Stegastes partitus*. *Oecologia* 121: 364-368
- Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* 21: 1183-1226
- Brett JR (1970) Temperature. Animals. Fishes. In: Kinne O (ed) *Marine ecology*. Wiley, New York, pp 515-560
- Brett JR (1979) Environmental factors and growth. In: Hoar WS, Randall DJ, Brett JR (eds) *Fish Physiology*. Academic Press, Orlando, Florida, pp 599-675
- Bromage N, Hardiman P, Jones J, Springate J, Bye V (1990) Fecundity, egg size and total egg volume differences in 12 stocks of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture and Fisheries Management* 21: 269-284
- Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: what makes a good egg? *Reviews in Fish Biology and Fisheries* 7: 387-416
- Brown JH, Stevens GC, Kaufman DM (1996) The geographic range: size, shape, boundaries, and internal structure. *Annual Review of Ecology and Systematics* 27: 597-623
- Buston PM (2003a) Mortality is associated with social rank in the clown anemonefish (*Amphiprion percula*). *Marine Biology* 143: 811-815
- Buston PM (2003b) Size and growth modification in clownfish. *Nature* 424: 145-146
- Buston PM, Munday PL, Warner RR (2004) Sex change and relative body size in animals. *Nature* 428: 1

- Bye VJ (1990) Temperate marine teleosts. In: Munro AD, Scott AP, Lam TJ (eds) Reproductive seasonality in teleosts: environmental influences. CRC Press, Boca Raton, FL, pp 125-143
- Caldow C, Wellington GM (2003) Patterns of annual increment formation in otoliths of pomacentrids in the tropical western Atlantic: implications for population age-structure examination. *Marine Ecology Progress Series* 265: 185-195
- Caley JM (1995a) Reef-fish community structure and dynamics: an interaction between local and larger-scale processes? *Marine Ecology Progress Series* 129: 19-29
- Caley MJ (1995b) Community dynamics of tropical reef fishes: local patterns between latitudes. *Marine Ecology Progress Series* 129: 7-18
- Cappo M, Eden P, Newman SJ, Robertson S (2000) A new approach to validation of periodicity and timing of opaque zone formation in the otoliths of eleven species of *Lutjanus* from the central Great Barrier Reef. *Fishery Bulletin* 98: 474-488
- Cardillo M (2002) The life-history basis of latitudinal diversity gradients: how do species traits vary from the poles to the equator? *Journal of Animal Ecology* 71: 79-87
- Carter RN, Prince SD (1981) Epidemic models used to explain biogeographical distribution limits. *Nature* 293: 644-645
- Carvalho GR (1993) Evolutionary aspects of fish distribution: genetic variability and adaptation. *Journal of Fish Biology* 43 (Supplement A): 53-73
- Case TJ (2000) An illustrated guide to theoretical ecology. Oxford University Press, Oxford
- Castaneda LE, Lardies MA, Bozinovic F (2004) Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod. *Evolutionary Ecology Research* 6: 579-593
- Cerrato RM (1990) Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 1416-1426
- Chambers RC (1997) Environmental influences on egg and propagule sizes in marine fishes. In: Chambers RC, Trippel EA (eds) Early life history and recruitment in fish populations. Chapman & Hall, pp 63-102
- Chambers RC, Leggett WC (1987) Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1936-1947
- Chambers RC, Miller TJ (1995) Evaluating fish growth by means of increment analysis. In: Secor DH, Dean JM, Campana SE (eds) Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, pp 155-176
- Chambers RC, Trippel EA (1997) Early life history and recruitment in fish populations. Chapman and Hall, London
- Charlesworth B (1980) Evolution in age-structured populations. Cambridge University Press, Bristol, UK
- Charlesworth B (1994) Evolution in age-structured populations. Cambridge University Press, New York
- Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton
- Charnov EL (1993) Life history invariants. Oxford University Press, Oxford
- Chen Y, Jackson DA, Harvey HH (1992) A comparison of von Bertalanffy and polynomial functions in modelling fish growth data. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 1228-1235

- Choat JH, Axe LM (1996) Growth and longevity in acanthurid fishes; an analysis of otolith increments. *Marine Ecology Progress Series* 134: 15-26
- Choat JH, Axe LM, Lou DC (1996) Growth and longevity in fishes of the family Scaridae. *Marine Ecology Progress Series* 145: 33-41
- Choat JH, Robertson DR (2002) Age-based studies on coral reef fishes. In: Sale PF (ed) *Coral reef fishes: dynamics and diversity in a complex ecosystem*. Academic Press, New York, pp 57-80
- Choat JH, Robertson DR, Ackerman JL, Posada JM (2003) An age-based demographic analysis of the Caribbean stoplight parrotfish *Sparisoma viride*. *Marine Ecology Progress Series* 246: 265-277
- Claridge MF, Morgan JC (1993) Geographical variation in acoustic signals of the planthopper, *Nilaparvata bakeri* (Muir), in Asia: species recognition and sexual selection. *Biological Journal of the Linnean Society* 48: 267-281
- Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68: 893-905
- Clutton-Brock TH (1991) *The evolution of parental care*. Princeton University Press, Princeton, N.J.
- Cole KS, Robertson DR (1988) Protogyny in the Caribbean reef goby, *Coryphopterus personatus* - gonad ontogeny and social influences on sex-change. *Bulletin of Marine Science* 42: 317-333
- Conover DO (1990) The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. *Transactions of the American Fisheries Society* 119: 416-430
- Conover DO, Brown JJ, Ehtisham A (1997) Countergradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2401-2409
- Conover DO, Present TMC (1990) Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* 83: 316-324
- Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* 10: 248-252
- Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: open or closed? *Science* 287: 857-859
- Davis MB, Shaw RG (2001) Range shifts and adaptive responses to quaternary climate change. *Science* 292: 673-679
- Dawson MN, Hamner WM (2003) Geographic variation and behavioral evolution in marine plankton: the case of *Mastigias* (Scyphozoa, Rhizostomeae). *Marine Biology* 143: 1161-1174
- Day T, Pritchard J, Schluter D (1994) A comparison of two sticklebacks. *Evolution* 48: 1723-1734
- De Jong G (1990) Quantitative genetics of reaction norms. *Journal of Evolutionary Biology* 3: 447-468
- De Jong G (1995) Phenotypic plasticity as a product of selection in a variable environment. *American Naturalist* 145: 493
- Denit K, Sponaugle S (2004) Growth variation, settlement, and spawning of gray snapper across a latitudinal gradient. *Transactions of the American Fisheries Society* 133: 1339-1355

- Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. *Aquaculture* 208: 191-304
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution* 13: 77-81
- Dias PC (1996) Sources and sinks in population biology. *Trends in Ecology and Evolution* 11: 326-330
- Doherty PJ, Fowler AJ (1994) Demographic consequences of variable recruitment to coral reef fish populations: a congeneric comparison of two damselfishes. *Bulletin of Marine Science* 54: 297-313
- Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology* 76: 2373-2391
- Donohue K, Messiqua D, Pyle EH, Heschel MS, Schmitt J (2000) Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. *Evolution* 54: 1956-1968
- Dugan JE, Hubbard DM, Wenner AM (1994) Geographic variation in life history of the sand crab, *Emerita analoga* (Stimpson) on the California coast: relationships to environmental variables. *Journal of Experimental Marine Biology and Ecology* 181: 255-278
- Duijm M (1990) On some song characteristics in *Ephippiger* (Orthoptera: Tettigoniidae) and their geographic variation. *Netherlands Journal of Zoology* 40: 428-453
- Dunham AE, Grant BW, Overall KL (1989) Interfaces between biophysical and physiological ecology and the population ecology of terrestrial vertebrate ectotherms. *Physiological Zoology* 62: 335-355
- Dutta H (1994) Growth in fishes. *Gerontology* 40: 97-112
- Eckhart VM, Geber MA, McGuire CM (2004) Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. *Evolution* 58: 59-70
- Edmands S, Mohberg PE, Burton RS (1990) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Marine Biology* 126: 443-450
- Edwards RRC (1984) Comparisons of growth in weight of temperate and tropical marine fish counterparts. *Canadian Journal of Fisheries and Aquatic Sciences* 41: 1381-1384
- Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society of London B* 266: 2095-2100
- Eldridge MB, Jarvis BM (1995) Temporal and spatial variation in fecundity of yellowtail rockfish. *Transactions of the American Fisheries Society* 124: 16-25
- Elgar MA (1990) Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* 59: 283-287
- Elliott JK, Elliott JM, Mariscal RN (1995) Host selection, location, and association behaviours of anemonefishes in field selection experiments. *Marine Biology* 122: 377-389
- Endler JA (1977) Geographic variation, speciation, and clines. Princeton University Press, Princeton, NJ
- Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton, New Jersey

- Ernande B, Dieckmann U (2004) The evolution of phenotypic plasticity in spatially structured environments: implications of intraspecific competition, plasticity costs, and environmental characteristics. *Journal of Evolutionary Biology* 17: 613-628
- Ernsting G, Isaaks A (1997) Effects of temperature and season on egg size, hatchling size and adult size in *Notiophilus biguttatus*. *Ecological Entomology* 22: 32-40
- Falconer DS (1990) Selection in different environments - effects on environmental sensitivity (reaction norm) and on mean performance. *Genetic Research* 56: 57-70
- Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*. Longman Group, Harlow, England
- Farrell AP, Bennett W, Devlin RH (1997) Growth-enhanced transgenic salmon can be inferior swimmers. *Canadian Journal of Zoology* 75: 335-337
- Farrell AP, Johansen JA, Suarez R (1991) Effects of exercise training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* 9: 303-312
- Fautin DG (1986) Why do anemonefishes inhabit only some host actinians? *Environmental Biology of Fishes* 15: 171-180
- Fautin DG (1992) Anemonefish recruitment: the roles of order and chance. *Symbiosis* 14: 143-160
- Fautin DG, Allen GR (1992) Anemonefishes and their host sea anemones. Western Australian Museum, Perth, W.A.
- Feder ME (1982) Environmental variability and thermal acclimation of metabolism in tropical anurans. *Journal of Thermal Biology* 7: 23-28
- Ferguson GW, Talent LG (1993) Life-history traits of the lizard *Sceloporus undulatus* from two populations raised in a common laboratory environment. *Oecologia* 93: 88-94
- Ferreira BP, Russ GR (1994) Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus*, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. *Fisheries Bulletin* 92: 46-57
- Fisher R (2005) Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. *Marine Ecology Progress Series* 285: 223-232
- Fisher R, Bellwood DR (2002) The influence of swimming speed on sustained swimming performance of late-stage reef fish larvae. *Marine Biology* 140: 801-807
- Fisher R, Bellwood DR (2003) Undisturbed swimming behaviour and nocturnal activity of coral reef fish larvae. *Marine Ecology Progress Series* 263: 177-188
- Fisher R, Wilson SK (2004) Maximum sustainable swimming speeds of late-stage larvae of nine species of reef fishes. *Journal of Experimental Marine Biology and Ecology* 312: 171-186
- Fleming IA, Gross MR (1990) Latitudinal clines: a trade-off between egg number and size in Pacific salmon. *Ecology* 71: 1-11
- Forrester GE (1990) Factors influencing the juvenile demography of a coral reef fish. *Ecology* 71: 1666-1681
- Forsman A, Lindell LE (1991) Tradeoff between growth and energy storage in male *Vipera berus*, under different prey-density. *Functional Ecology* 5: 717-723
- Forsman A, Shine R (1995) Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Functional Ecology* 9: 818-828



- Foster SA, Endler JA (1999) Geographic variation in behavior. Oxford University Press, New York
- Fowler AJ, Doherty PJ (1992) Validation of annual growth increments in the otoliths of two species of damselfish from the Southern Great Barrier Reef. *Australian Journal of Marine and Freshwater Research* 43: 1057-1068
- Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. *Annual Reviews of Entomology* 45: 341-369
- France R (1992) The North American latitudinal gradient in species richness and geographical range of freshwater crayfish and amphipods. *American Naturalist* 139: 342-354
- Francis RC (1992) Sexual lability in teleosts: developmental factors. *Quarterly Review of Biology* 67: 1-18
- Frankino WA, Juliano SA (1999) Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. *Oecologia* 120: 59-68
- Fricke HW (1979) Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos*. *Zeitschrift fuer Tierpsychologie* 50: 313-326
- Fricke HW (1983) Social control of sex: Field experiments with the anemonefish *Amphiprion bicinctus*. *Zeitschrift fuer Tierpsychologie* 61: 71-77
- Fricke HW, Fricke S (1977) Monogamy and sex change by aggressive dominance in coral reef fish. *Nature* 266: 830-832
- Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ (eds) *Fish physiology*. Academic Press, New York, pp 1-98
- Fuiman LA (1991) Influence of temperature on evasive responses of Atlantic herring larvae attacked by yearling, *Clupea harengus* L. *Journal of Fish Biology* 39: 93-102
- Fuiman LA, Batty RS (1997) What a drag it is getting cold: Partitioning the physical and physiological effects of temperature on fish swimming. *Journal of Experimental Biology* 200: 1745-1755
- Fuiman LA, Higgs DM (1997) Ontogeny, growth and the recruitment process. In: Chambers RC, Trippel EA (eds) *Early life history and recruitment in fish populations*. Chapman & Hall, London, pp 225-250
- Fulton CJ, Bellwood DR (2004) Wave exposure, swimming performance, and the structure of tropical and temperate reef fish assemblages. *Marine Biology* 144: 429-437
- Fulton CJ, Bellwood DR, Wainwright PC (2001) The relationship between swimming ability and habitat use in wrasses (Labridae). *Marine Biology* 139: 25-33
- Futuyma DJ (1998) *Evolutionary Biology*. Sinauer Associates, Sunderland, MA
- Gallaugh P, Thorarensen H, Farrell AP (1995) Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respiration Physiology* 102: 279-292
- Gasser M, Kaiser M, Berrigan D, Stearns SC (2000) Life-history correlates of evolution under high and low adult mortality. *Evolution* 54: 1260-1272
- Ghalambor CK, Reznick DN, Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the trinidadian guppy (*Poecilia reticulata*). *American Naturalist* 164: 38-50
- Gibb A, Dickson KA (2002) Functional morphology and biochemical indices of performance: Is there a correlation between metabolic enzyme activity and swimming performance? *Integrative and Comparative Biology* 42: 199-207
- Gilchrist GW (1996) A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* 50: 1560-1572

- Gilligan MR (1991) Bergmann ecogeographic trends among triplefin blennies (Teleostei, Tripterygiidae) in the Gulf-of-California, Mexico. *Environmental Biology of Fishes* 31: 301-305
- Gomulkiewicz R, Kirkpatrick M (1992) Quantitative genetics and the evolution of reaction norms. *Evolution* 46: 390-411
- Gotthard K, Nylin S (1995) Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* 74: 3-17
- Gotthard K, Nylin S, Wiklund C (1994) Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* 99: 281-289
- Gould SJ, Johnston RF (1972) Geographic variation. *Annual Review of Ecology and Systematics* 3: 457-498
- Grant BW, Dunham AE (1990) Elevational covariation in environmental constraints and life-histories of the desert lizard *Sceloporus merriami*. *Ecology* 71: 1765-1776
- Green BS, Fisher R (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. *Journal of Experimental Marine Biology and Ecology* 299: 115-132
- Green BS, McCormick MI (1999) Influence of larval feeding history on the body condition of *Amphiprion melanopus*. *Journal of Fish Biology* 55: 1273-1289
- Gregory TR, Wood CM (1998) Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1583-1590
- Gregory TR, Wood CM (1999) Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) fed different rations. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 479-486
- Gust N (2004) Variation in the population biology of protogynous coral reef fishes over tens of kilometres. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 205-218
- Gust N, Choat JH, Ackerman JL (2002) Demographic plasticity in tropical reef fishes. *Marine Biology* 140: 1039-1051
- Hard JJ (1995) A quantitative perspective on the conservation of intraspecific diversity. In: Nielsen JL (ed) *Evolution and the aquatic ecosystem: defining unique units in population conservation*. American Fisheries Society, Bethesda, Maryland, pp 304-326
- Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford University Press, New York
- Hatle JD, Crowley MC, Andrews AL, Juliano SA (2002) Geographic variation of reproductive tactics in lubber grasshoppers. *Oecologia* 132: 517-523
- Hattori A (1991) Socially controlled growth and size-dependent sex change in the anemonefish *Amphiprion frenatus* in Okinawa, Japan. *Japanese Journal of Ichthyology* 38: 165-177
- Heath DD, Blouw DM (1998) Are maternal effects in fish adaptive or merely physiological side effects? In: Mousseau TA, Fox CW (eds) *Maternal effects as adaptations*. Oxford University Press, Oxford, UK, pp 178-201

- Hedgcock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science* 39: 550-564
- Hendry AP, Day T, Cooper AB (2001) Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *American Naturalist* 157: 387-407
- Herring K, Verrell P (1996) Sexual incompatibility and geographical variation in mate recognition systems: tests in the salamander *Desmognathus ochrophaeus*. *Animal Behaviour* 52: 279-287
- Hobbs JPA, Munday PL, Jones GP (2004) Social induction of maturation and sex determination in a coral reef fish. *Proceedings of the Royal Society of London B* 271: 2109-2114
- Hoffmann AA, Blows MW (1993) Evolutionary genetics and climate change: will animals adapt to global warming? In: Kareiva PM (ed) *Biotic interactions and global change*, pp 165-178
- Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives. *Trends in Ecology and Evolution* 9: 223-227
- Hoffmann AA, Parsons PA (1991) *Evolutionary genetics and environmental stress*. Oxford University Press, Oxford
- Hoffmann AA, Sgro CM, Lawler SH (1995) Ecological population genetics: the interface between genes and the environment. *Annual Review of Genetics* 29: 349-371
- Holt RD (1990) The microevolutionary consequences of climate change. *Trends in Ecology and Evolution* 5: 311-315
- Holt RD, Gaines MS (1992) Analysis of adaptation in heterogeneous landscapes: implications for the evolution of fundamental niches. *Evolutionary Ecology* 6: 433-447
- Holt RD, Gomulkiewicz R (1997) How does immigration influence local adaptation? A reexamination of a familiar paradigm. *American Naturalist* 149: 563-572
- Houde ED (1987) Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2: 17-29
- Houde ED (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fishery Bulletin* 87: 471-495
- Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308-309
- Huey RB, Hertz PE (1984) Is a jack-of-all-temperatures a master of none? *Evolution* 38: 441-444
- Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology and Evolution* 4: 131-135
- Huey RB, Stevenson RD (1979) Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist* 19: 357-366
- Hunt von Herbing I, Gallager SM, Halteman W (2001) Metabolic costs of pursuit and attack in early larval Atlantic cod. *Marine Ecology Progress Series* 216: 201-212
- Hurst TP, Conover DO (1998) Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): Size-dependent patterns and effects on recruitment. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1122-1130
- Hutchings JA (1991) Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* 45: 1162-1168
- Hutchings JA (1993) Adaptive life histories effected by age-specific survival and growth rate. *Ecology* 74: 673-684

- Imsland AK, Foss A, Naevdal G, Cross T, Bonga SW, Ham EV, Stefansson SO (2000) Countergradient variation in growth and food conversion efficiency of juvenile turbot. *Journal of Fish Biology* 57: 1213-1226
- Iverson JB, Balgooyen CP, Byrd KK, Lyddan KK (1993) Latitudinal variation in egg and clutch size in turtles. *Canadian Journal of Zoology* 71: 2448-2461
- James AC, Azevedo RBR, Partridge L (1997) Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* 146: 881-890
- James AC, Partridge L (1995) Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *Journal of Evolutionary Biology* 8: 315-330
- James MK, Armsworth PR, Mason LB, Bode L (2002) The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. *Proceedings of the Royal Society of London B* 269: 2079-2086
- Jayne BC, Lauder GV (1993) Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. *Journal of Comparative Physiology A* 173: 495-508
- Jensen AJ, Forseth T, Johnsen BO (2000) Latitudinal variation in growth of young brown trout *Salmo trutta*. *Journal of Animal Ecology* 69: 1010-1020
- Jobling M, Davis PS (1980) Effects of feeding on metabolic rate and the specific dynamic action in plaice, *Pleuronectes platessa*. *Journal of Fish Biology* 16: 629-638
- Johnson TP, Bennett AF (1995) The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *Journal of Experimental Biology* 198: 2165-2175
- Johnston IA, Bennett AF (1996) Animals and temperature: phenotypic and evolutionary adaptation. Cambridge University Press, Cambridge
- Johnston IA, Dunn JF (1987) Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In: Bowler K, Fuller BJ (eds) *Temperature and Animal Cells*. Symposia of the Society for Experimental Biology, Cambridge, pp 67-93
- Jonassen TM, Imsland AK, Fitzgerald R, Bonga SW, Ham EV, Naevdal G, Stefansson MO, Stefansson SO (2000) Geographic variation in growth and food conversion efficiency of juvenile Atlantic halibut related to latitude. *Journal of Fish Biology* 56: 279-294
- Jones GP (1986) Food availability affects growth in a coral reef fish. *Oecologia* 70: 136-139
- Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. *Nature* 402: 802-804
- Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. *Current Biology* 15: 1314-1318
- Jonsson N, Jonsson B, Fleming IA (1996) Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? *Functional Ecology* 10: 89-96
- Kamler E (1992) *Early life history of fish: an energetics approach*. Chapman and Hall, London, UK
- Kaplan RH (1987) Developmental plasticity and maternal effects of reproductive characteristics in the frog *Bombina orientalis*. *Oecologia* 71: 273-279

- Kerrigan BA (1997) Variability in larval development of the tropical reef fish *Pomacentrus amboinensis* (Pomacentridae): the parental legacy. *Marine Biology* 127: 395-402
- Kimura DK (1980) Likelihood methods for the Von Bertalanffy growth curve. *Fishery Bulletin* 77: 765-776
- Kirkpatrick M, Barton NH (1997) Evolution of a species' range. *American Naturalist* 150: 1-23
- Kolok AS, Oris JT (1995) The relationship between specific growth rate and swimming performance in male fathead minnows (*Pimephales promelas*). *Canadian Journal of Zoology* 73: 2165-2167
- Koumoundouros G, Divanach P, Anezaki L, Kentouri M (2001) Temperature-induced ontogenetic plasticity in sea bass (*Dicentrarchus labrax*). *Marine Biology* 139: 817-830
- Koumoundouros G, Sfakianakis DG, Divanach P, Kentouri M (2002) Effect of temperature on swimming performance of sea bass juveniles. *Journal of Fish Biology* 60: 923-932
- Kozlowski J, Uchmanski J (1987) Optimal individual growth and reproduction in perennial species with indeterminate growth. *Evolutionary Ecology* 1: 214-230
- Kritzer JP (2004) Sex-specific growth and mortality, spawning season, and female maturation of the stripey bass (*Lutjanus carponotatus*) on the Great Barrier Reef. *Fishery Bulletin* 102: 94-107
- Kroodsma DE, Canady RA (1985) Differences in repertoire size, singing behaviour, and associated neuroanatomy among marsh wren populations have a genetic basis. *Auk* 102: 439-446
- L'Abée-Lund JH, Hindar K (1990) Interpopulation variation in reproductive traits of anadromous female brown trout, *Salmo trutta* L. *Journal of Fish Biology* 37: 755-763
- Lack D (1947) The significance of clutch size. *Ibis* 89: 302-352
- Lankford TE, Billerbeck JM, Conover DO (2001) Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution* 55: 1873-1881
- Lardies MA, Castilla JC (2001) Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda: Pinnotheridae) along the Chilean coast. *Marine Biology* 139: 1125-1133
- Larsson S, Forseth T, Berglund I, Jensen AJ, Naslund I, Elliott JM, Jonsson B (2005) Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. *Freshwater Biology* 50: 353-368
- Leamy L, Atchley W (1985) Directional selection and developmental stability: evidence from fluctuating asymmetry of morphometric characteristics in rats. *Growth* 49: 8-18
- Lecchini D, Shima J, Banaigs B, Galzin R (2005) Larval sensory abilities and mechanisms of habitat selection of a coral reef fish during settlement. *Oecologia* 143: 326-334
- Leggett WC, Carscadden JE (1978) Latitudinal variation in reproductive characteristics of American shad (*Alosa sapidissima*): evidence for population specific life history strategies in fish. *J. Fish. Res. Board Can.* 35: 1469-1478
- Leggett WC, DeBlois E (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stage? *Netherlands Journal of Sea Research* 32: 119-134

- Leis JM, Carson-Ewart BM (1997) *In-situ* swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series* 159: 165-174
- Leis JM, Carson-Ewart BM (2000) Behaviour of pelagic larvae of four coral-reef fish species in the ocean and an atoll lagoon. *Coral Reefs* 19: 247-257
- Leis JM, Carson-Ewart BM (2003) Orientation of pelagic larvae of coral-reef fishes in the ocean. *Marine Ecology Progress Series* 252: 239-253
- Leis JM, Carson-Ewart BM, Cato DH (2002) Sound detection *in situ* by the larvae of a coral-reef damselfish (Pomacentridae). *Marine Ecology Progress Series* 232: 259-268
- Leis JM, Fisher R (2004) Swimming speed of settlement-stage reef-fish larvae measured in the laboratory and in the field: a comparison of critical speed and *in situ* speed 10th International Coral Reef Symposium, Okinawa
- Leis JM, Stobutzki IC (1999) Swimming performance of late pelagic larvae of coral-reef fishes: *in situ* and laboratory-based measurements. In: Seret B, Sire J-Y (eds) 5th Indo-Pacific fish conference. Societe Francaise d'Ichtyologie et Institut de Recherche pour le Developpement, Paris, France, Noumea, pp 575-583
- Leis JM, Sweatman HPA, Reader SE (1996) What the pelagic stages of coral reef fishes are doing out in the blue water: daytime field observations of larval behavioural capabilities. *Marine and Freshwater Research* 47: 401-411
- Levins R (1968) Evolution in changing environments. Princeton University Press, Princeton, NJ
- Levins R (1969) Thermal acclimation and heat resistance in *Drosophila* species. *American Naturalist* 103: 483-499
- Levinton JS (1983) The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. 1. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). *Biological Bulletin* 165: 686-698
- Levinton JS, Monahan RK (1983) The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. 2. Intraspecific comparisons between subspecies of *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). *Biological Bulletin* 165: 699-707
- Lloyd DG (1987) Selection of offspring size at independence and other size-versus-number strategies. *American Naturalist* 129: 800-817
- Lobon-Cervia J, Utrilla CG, Rincon PA, Amezcua F (1997) Environmentally induced spatio-temporal variations in the fecundity of brown trout *Salmo trutta* L.: trade-offs between egg size and number. *Freshwater Biology* 38: 277-288
- Lonsdale DJ, Levinton JS (1985) Latitudinal differentiation in copepod growth: An adaptation to temperature. *Ecology* 66: 1397-1407
- Lowe-McConnell RH (1987) Ecological studies in tropical fish communities. Cambridge University Press, Cambridge, UK
- Lynch M (1996) A quantitative-genetic perspective on conservation issues. In: Avise JC, Hamrick JL (eds) Conservation genetics: case histories from nature. Chapman and Hall, New York, pp 471-501
- Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer, Sunderland
- Lyons SK, Willig MR (1999) A hemispheric assessment of scale dependence in latitudinal gradients of species richness. *Ecology* 80: 2483-2492

- Mangel M, Stamps J (2001) Trade-offs between growth and mortality and the maintenance of individual variation in growth. *Evolutionary Ecology Research* 3: 583-593
- Mann RHK, Mills CA, Crisp DT (1984) Geographical variation in the life-history tactics of some species of freshwater fish. In: Potts GW, Wootton RJ (eds) *Fish Reproduction*. Academic Press, London, pp 171-186
- Marshall NB (1953) Egg size in Arctic, Antarctic and deep-sea fishes. *Evolution* 7: 328-341
- Maurer BA (1994) *Geographical population analysis: tools for the analysis of biodiversity*. Blackwell Scientific Publications, Oxford
- Mayr E (1963) *Animal species and evolution*. The Belknap Press of Harvard University Press, Cambridge
- McCormick MI, Makey L, Dufour V (2002) Comparative study of metamorphosis in tropical reef fishes. *Marine Biology* 141: 841-853
- McCormick MI, Molony BW (1992) Effects of feeding history on the growth characteristics of a reef fish at settlement. *Marine Biology* 114: 165-173
- McCormick MI, Molony BW (1993) Quality of the reef fish *Upeneus tragula* (Mullidae) at settlement: is size a good indicator of condition? *Marine Ecology Progress Series* 98: 45-54
- McCormick MI, Molony BW (1995) Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. *Marine Ecology Progress Series* 118: 59-68
- McGinley MA, Temme DH, Geber MA (1987) Parental investment in offspring in variable environments: theoretical and empirical consideration. *American Naturalist* 130: 370-398
- McGurk MD (2000) Comparison of fecundity-length-latitude relationships between nonanadromous (kokanee) and anadromous sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* 78: 1791-1805
- McKenzie DJ, Higgs DA, Dosanjh BS, Deacon G, Randall DJ (1998) Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. *Fish Physiology and Biochemistry* 19: 111-122
- McMillan WO, Palumbi SR (1995) Concordant evolutionary patterns among Indo-West Pacific Butterflyfishes. *Proceedings of the Royal Society of London B* 260: 229-236
- Meekan MG, Ackerman JL, Wellington GM (2001) Demography and age structures of coral reef damselfishes in the tropical eastern Pacific Ocean. *Marine Ecology Progress Series* 212: 223-232
- Meekan MG, Fortier L (1996) Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. *Marine Ecology Progress Series* 137: 25-37
- Meffe GK, Snelson FF (1989) *Ecology and evolution of livebearing fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey
- Metcalf NB, Monaghan P (2003) Growth versus lifespan: perspectives from evolutionary ecology. *Experimental Gerontology* 38: 935-940
- Miller JM, Burke JS, Fitzhugh GR (1991) Early life history patterns of Atlantic North American flatfish: likely (and unlikely) factors controlling recruitment. *Netherlands Journal of Sea Research* 27: 261-275
- Miller TJ, Crowder LB, Rice JA, Marshall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 1657-1670

- Mizera F, Meszyna G (2003) Spatial niche packing, character displacement and adaptive speciation along an environmental gradient. *Evolutionary Ecology Research* 5: 363-382
- Mora C, Sale PF (2002) Are populations of coral reef fish open or closed? *Trends in Ecology and Evolution* 17: 422-428
- Morris DW (1987) Optimal allocation of parental investment. *Oikos* 49: 332-339
- Morrison C, Hero J-M (2003) Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* 72: 270-279
- Mousseau TA (1997) Ectotherms follow the converse to Bergmann's rule. *Evolution* 51: 630-632
- Mousseau TA (2000) Intra- and interpopulation genetic variation: explaining the past and predicting the future. In: Mousseau TA, Sinervo B, Endler JA (eds) *Adaptive genetic variation in the wild*. Oxford University Press, New York, pp 219-250
- Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13: 403-407
- Mousseau TA, Roff DA (1989) Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variance in body size and diapause expression along a cline in season length. *Evolution* 43: 1483-1496
- Mousseau TA, Sinervo B, Endler JA (2000) *Adaptive genetic variation in the wild*. Oxford University Press, New York
- Moyer JT (1986) Longevity of the anemonefish *Amphiprion clarkii* at Miyake-jima, Japan with notes on four other species. *Copeia* 1: 135-139
- Moyer JT, Nakazono A (1978) Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. *Japanese Journal of Ichthyology* 25: 101-106
- Munday PL, Caley MJ, Jones GP (1998) Bi-directional sex change in a coral-dwelling goby. *Behavioral Ecology and Sociobiology* 43: 371-377
- Nelson JA (1989) Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. *Journal of Experimental Biology* 145: 239-254
- Nicieza AG, Metcalfe NB (1997) Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. *Ecology* 78: 2385-2400
- Nicieza AG, Reyes-Gavilan FG, Brana F (1994) Differentiation in juvenile growth and bimodality patterns between northern and southern populations of Atlantic salmon (*Salmo salar* L.). *Canadian Journal of Zoology* 72: 1603-1610
- Niewiarowski PH, Roosenburg W (1993) Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. *Ecology* 74: 1992-2002
- Ochi H (1989) Mating behaviour and sex change of the anemonefish *Amphiprion clarkii* in the temperate waters of southern Japan. *Environmental Biology of Fishes* 26: 257-275
- Ojanguren AF, Brana F (2000) Thermal dependence of swimming endurance in juvenile brown trout. *Journal of Fish Biology* 56: 1342-1347
- Olive PJW (1992) The adaptive significance of seasonal reproduction in marine invertebrates. *Invertebrate Reproduction and Development* 22: 165-174
- Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* 25: 547-572



- Palumbi SR (1996) Microspatial genetic structure and speciation in marine taxa with high dispersal abilities. In: Ferraris DJ, Palumbi SR (eds) *Molecular Zoology*. Wiley-Lass, New York, pp 101-113
- Parker GA, Begon M (1986) Optimal egg size and clutch size: effects of environment and maternal phenotype. *American Naturalist* 128: 573-592
- Partridge L, Coyne JA (1997) Bergmann's rule in ectotherms: is it adaptive? *Evolution* 51: 632-635
- Paulson AC, Smith RL (1975) Latitudinal variation of Pacific herring fecundity. *Transactions of the American Fisheries Society* 106: 244-247
- Pearse JS, McClintock JB, Bosch I (1991) Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. *American Zoologist* 31: 65-80
- Pease CM, Bull JJ (1988) A critique of methods for measuring life history trade-offs. *Journal of Evolutionary Biology* 1: 293-303
- Pegg MA, Pierce CL (2001) Growth rate responses of Missouri and Lower Yellowstone River fishes to a latitudinal gradient. *Journal of Fish Biology* 59: 1529-1543
- Pelletier D, Guderley H, Dutil J-D (1993) Does the aerobic capacity of fish muscle change with growth rates? *Fish Physiology and Biochemistry* 12: 83-93
- Pettifor RA, Perrins CM, McCleery RH (1988) Individual optimization of clutch size in great tits. *Nature* 336: 160-162
- Philipp DP, Whitt GS (1991) Survival and growth of northern, Florida, and reciprocal F1 hybrid largemouth bass in central Illinois. *Transactions of the American Fisheries Society* 120: 58-64
- Pianka ER (1966) Latitudinal gradients in species diversity: a review of concepts. *American Naturalist* 100: 33-46
- Pielou EC (1975) *Ecological diversity*. Wiley, New York
- Planes S (1993) Genetic differentiation in relation to restricted larval dispersal of the convict surgeonfish *Acanthurus triostegus* in French Polynesia. *Marine Ecology Progress Series* 98: 237-246
- Planes S, Parroni M, Chauvet C (1998) Evidence of limited gene flow in three species of coral-reef fishes in the lagoon of New Caledonia. *Marine Biology* 130: 361-368
- Plaut I (2000) Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. *Physiological and Biochemical Zoology* 73: 590-596
- Powers DA (1987) A multidisciplinary approach to the study of genetic variation within species. In: Feder ME, Bennett AF, Burggren WW (eds) *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp 102-134
- Promislow DEL, Harvey PH (1990) Living fast and dying young: A comparative analysis of life-history variation among mammals. *Journal of Zoology* 220: 417-437
- Radtke R, Fey DP (1996) Environmental effects on primary increment formation in the otoliths of newly hatched Arctic charr. *Journal of Fish Biology* 48: 1238-1255
- Rana KJ (1988) Reproductive biology and the hatchery rearing of tilapia eggs and fry. In: Muir JF, Roberts RJ (eds) *Recent Advances in Aquaculture*. Croom Helm, London, pp 343-406
- Randall JE, Allen GR, Steene RC (1997) *Fishes of the Great Barrier Reef and Coral Sea*. Crawford House Publishing, Bathurst
- Reidy SP, Kerr SR, Nelson JA (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *Journal of Experimental Biology* 203: 347-357

- Rensch B (1938) Some problems of geographical variation and species-formation. *Proceedings of the Linnean Society of London* 150: 275-285
- Reznick D (1983) The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology* 64: 862-873
- Reznick D (1985) Costs of reproduction: an evaluation of the empirical evidence. *Oikos* 44: 257-267
- Reznick D, Nunney L, Tessier A (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution* 15: 421-425
- Reznick D, Yang AP (1993) The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. *Ecology* 74: 2011-2019
- Reznick DA, Bryga H, Endler JA (1990) Experimentally induced life-history evolution in a natural population. *Nature* 346: 357-359
- Reznick DN (1990) Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): An experimental evaluation of alternative models of development. *Journal of Evolutionary Biology* 3: 185-204
- Reznick DN, Travis J (1996) The empirical study of adaptation. In: Rose M, Lauder G (eds) *Adaptation*. Academic Press, San Diego, California, pp 243-289
- Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. *Fisheries Research Board of Canada, Ottawa Bulletin* 191
- Ridley M (1996) *Evolution*. Blackwell Science, Cambridge
- Riha VF, Berven KA (1991) An analysis of latitudinal variation in the larval development of the wood frog *Rana sylvatica*. *Copeia* 1: 209-221
- Rilling GC, Houde ED (1998) Regional and temporal variability in growth and mortality of bay anchovy, *Anchoa mitchilli*, larvae in Chesapeake Bay. *Fishery Bulletin* 97: 555-569
- Robertson DR (1991) The role of adult biology in the timing of spawning of tropical reef fishes. In: Sale PF (ed) *The ecology of coral reef fishes*. Academic Press, New York, pp 356-382
- Robertson DR, Ackerman JL, Choat JH, Posada JM, Pitt J (2005) Ocean surgeonfish *Acanthurus bahianus*. I. The geography of demography. *Marine Ecology Progress Series* 295: 229-244
- Rocha LA, Robertson DR, Roman J, Bowen BW (2005) Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society of London B* 272: 573-579
- Roff DA (1992) *The evolution of life histories*. Chapman and Hall, New York, New York, USA
- Ross RM (1978) Territorial behaviour and ecology of the anemonefish *Amphiprion melanopus* in Guam. *Zeitschrift fuer Tierpsychologie* 36: 71-83
- Rudloff W (1981) *World-climates*. Wissenschaftliche Verlagsgesellschaft, Stuttgart
- Russ GR (1991) Coral reef fisheries: effects and yields. In: Sale PF (ed) *The ecology of fishes on coral reefs*. Academic Press, San Diego, pp 601-635
- Ryman N (1991) Conservation genetics considerations in fishery management. *Journal of Fish Biology* 39: 211-224
- Sale PF (1991) Habitat structure and recruitment in coral reef fishes. In: Bell SS, McCoy ED, Mushinski HR (eds) *Habitat structure: the physical arrangement of objects in space*. Chapman and Hall, London
- Sale PF (1998) Appropriate spatial scales for studies of reef-fish ecology. *Australian Journal of Ecology* 23: 202-208
- Sand H, Cederland G, Danell K (1995) Geographical and latitudinal variation in growth patterns and adult body size of Swedish moose (*Alces alces*). *Oecologia* 102: 433-442

- Sargent RC, Taylor PD, Gross MR (1987) Parental care and the evolution of egg size in fishes. *American Naturalist* 129: 32-46
- Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* 24: 35-68
- Schluter D (1993) Adaptive radiation in sticklebacks: Size, shape and habitat use efficiency. *Ecology* 74: 699-709
- Schluter D (2001) Ecology and the origin of species. *Trends in Ecology and Evolution* 16: 372-380
- Schluter D, McPhail JD (1992) Ecological character displacement and speciation in sticklebacks. *American Naturalist* 140: 85-108
- Scholander PF, Flagg W, Walters V, Irving L (1953) Climatic adaptation in arctic and tropical poikilotherms. *Physiological Zoology* 26: 67-92
- Schultz ET, Conover DO (1997) Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). *Oecologia* 109: 516-529
- Schultz ET, Conover DO, Ehtisham A (1998) The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1149-1157
- Schultz ET, Reynolds KE, Conover DO (1996) Countergradient variation in growth among newly hatched *Fundulus heteroclitus*: geographic differences revealed by common-environment experiments. *Functional Ecology* 10: 366-374
- Sebens KP (1987) The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics* 18: 371-407
- Shepherd JG, Cushing DH (1980) A mechanism for density-dependent survival of larval fish as the basis of a stock-recruitment relationship. *Journal du Conseil* 40: 67-75
- Shine R, Charnov EL (1992) Patterns of survival, growth, and maturation in snakes and lizards. *American Naturalist* 139: 1257-1269
- Shulman MJ (1998) What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? *Australian Journal of Ecology* 23: 216-225
- Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. *Evolution* 49: 897
- Sibly R, Calow P (1983) An integrated approach to life-cycle evolution using selective landscapes. *Journal of Theoretical Biology* 102: 527-547
- Sibly RM, Atkinson D (1994) How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* 8: 486-493
- Sibly RM, Calow P (1986) *Physiological Ecology of Animals: an Evolutionary Approach*. Blackwell Scientific Publications, Oxford
- Sibly RM, Calow P, Nichols N (1985) Are patterns of growth adaptive? *Journal of Theoretical Biology* 112: 553-574
- Simpson SD, Meekan MG, McCauley RD, Jeffs A (2004) Attraction of settlement-stage coral reef fishes to reef noise. *Marine Ecology Progress Series* 276: 263-268
- Sinervo B (1990) The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44: 279-294
- Sinervo B, Adolph SC (1989) Thermal sensitivity of growth rate in hatchling *Sceloporus* lizards: environmental, behavioural and genetic aspects. *Oecologia* 78: 411-419

- Sinervo B, Adolph SC (1994) Growth plasticity and thermal opportunity in *Sceloporus* lizards. *Ecology* 75: 776-790
- Sinervo B, Doughty P (1996) Interactive effects of offspring size and timing of reproduction on offspring reproduction: Experimental, maternal, and quantitative genetic aspects. *Evolution* 50: 1314-1327
- Sirois P, Dodson JJ (2000) Critical periods and growth-dependent survival of larvae of an estuarine fish, the rainbow smelt *Osmerus mordax*. *Marine Ecology Progress Series* 203: 233-245
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *American Naturalist* 108: 499-506
- Smoker WW (1986) Variability of embryo developmental rate, fry growth, and disease susceptibility in hatchery stocks of chum salmon. *Aquaculture* 57: 219-226
- Solemald P (1967) The effect of salinity on buoyancy, size and development of flounder eggs. *Sarsia* 29: 431-442
- Somero GN, Childress JJ (1980) A violation of the metabolism size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-sized fish. *Physiological Zoology* 53: 322-337
- Sponaugle S, Cowen RK, Shanks A, Morgan SG, Leis JM, Pineda J, Boehlert GW, Kingsford MJ, Lindeman KC, Grimes C, Munro JL (2002) Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. *Bulletin of Marine Science* 70: 341-375
- Srinivasan M, Jones GP, Caley JM (1999) Experimental evaluation of the roles of habitat selection and interspecific competition in determining patterns of host use by two anemonefishes. *Marine Ecology Progress Series* 196: 283-292
- Stearns SC (1977) The evolution of life history traits: a critique of the theory and a review of the data. *Annual Review of Ecology and Systematics* 8: 145-171
- Stearns SC (1980) A new view of life-history evolution. *Oikos* 35: 266-281
- Stearns SC (1989) The evolutionary significance of phenotypic plasticity. *BioScience* 39: 436-446
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, Oxford, UK
- Stearns SC, Koella J (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40: 893-913
- Stobutzki I, Bellwood DR (1997) Sustained swimming abilities of the late pelagic stages of coral reef fishes. *Marine Ecology Progress Series* 149: 35-41
- Storfer A (1996) Quantitative genetics: a promising approach for assessment of genetic variation in endangered species. *Trends in Ecology and Evolution* 11: 343-348
- Strathmann RR, Hughes TP, Kuris AM, Lindeman KC, Morgan SG, Pandolfi JM, Warner RR (2002) Evolution of local recruitment and its consequences for marine populations. *Bulletin of Marine Science* 70: 377-396
- Sultan SE (1987) Evolutionary implications of phenotypic plasticity in plants. *Journal of Evolutionary Biology* 21: 127-178
- Summerfelt RC, Hall GE (1987) *Age and Growth of Fish*. Iowa State University Press, Ames
- Sutherland WJ, Grafen A, Harvey PH (1986) Life history correlations and demography. *Nature* 320: 88
- Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. *Nature* 402: 799-802

- Sweatman HPA (1988) Field evidence that settling coral reef fish larvae detect resident fishes using dissolved chemical cues. *Journal of Experimental Marine Biology and Ecology* 124: 163-174
- Sweeney BW, Vannote RL (1984) Influence of food quality and temperature on life history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. *Freshwater Biology* 14: 621-630
- Tamate T, Maekawa K (2000) Interpopulation variation in reproductive traits of female masu salmon, *Oncorhynchus masou*. *Oikos* 90: 209-218
- Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* 98: 185-207
- Taylor EB, McPhail JD (1985) Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Canadian Journal of Fisheries and Aquatic Sciences* 42: 2029-2033
- Taylor MS, Hellberg ME (2003) Genetic evidence for local recruitment of pelagic larvae in a Caribbean reef fish. *Science* 299: 107-109
- Temple GK, Johnston IA (1998) Testing hypotheses concerning the phenotypic plasticity of escape performance in fish of the family Cottidae. *Journal of Experimental Biology* 201: 317-331
- Thompson JD (1991) Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution* 6: 246-249
- Thorpe JE, Miles MS, Keay DS (1984) Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. *Aquaculture* 43: 289-305
- Thresher RE (1984) *Reproduction in reef fishes*. T.F.H. Publications Inc., Neptune City, N.J.
- Thresher RE, Brothers EB (1989) Evidence of intra- and inter-oceanic regional differences in the early life history of reef-associated fishes. *Marine Ecology Progress Series* 57: 187-205
- Thresher RE, Colin PL, Bell LJ (1989) Planktonic duration, distribution and population structure of western and central pacific damselfishes (Pomacentridae). *Copeia* 2: 420-434
- Tolimieri N, Haine O, Jeffs A, McCauley R, Montgomery J (2004) Directional orientation of pomacentrid larvae to ambient reef sound. *Coral Reefs* 23: 184-191
- Valente LMP, Rocha E, Gomes EFS, Silva MW, Oliveira MH, Monteiro RAF, Fauconneau B (1999) Growth dynamics of white and red muscle fibers in fast- and slow-growing strains of rainbow trout. *Journal of Fish Biology* 55: 675-691
- Van Berkum FH (1988) Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *American Naturalist* 132: 327-343
- Van der Have TM, De Jong G (1996) Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology* 183: 329-340
- Van Tienderen PH (1991) Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* 45: 1317-1331
- Van Voorhies WA (1996) Bergmann size clines: a simple explanation for their occurrence in ectotherms. *Evolution* 50: 1259-1264
- Vernberg FJ (1984) Fiddler crabs: ecosystems-organisms-molecules. *American Zoologist* 24: 293-304
- Via S (1987) Genetic constraints on the evolution of phenotypic plasticity. In: Loeschcke V (ed) *Genetic constraints on adaptive evolution*. Springer Verlag, Berlin, pp 46-71

- Via S (1993) Adaptive phenotypic plasticity: target or by-product of selection in a variable environment. *American Naturalist* 142: 352-365
- Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution* 10: 212-217
- Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39: 505-522
- Videler JJ (1993) *Fish swimming*. Chapman & Hall, London
- Vigliola L, Meekan MG (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia* 131: 89-94
- Walker JA (1997) Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological Journal of the Linnean Society* 61: 3-50
- Walker SPW, McCormick MI (2004) Otolith-check formation and accelerated growth associated with sex change in an annual protogynous tropical fish. *Marine Ecology Progress Series* 266: 201-212
- Ware DM (1975) Relation between egg size, growth and natural mortality of larval fish. *Journal of the Fisheries Research Board of Canada* 32: 2503-2512
- Warner RR (1988) Sex change in fishes: hypotheses, evidence, and objections. *Environmental Biology of Fishes* 22: 81-90
- Warner RR (1997) Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. *Coral Reefs* 16: 115-120
- Warner RR, Robertson DR (1978) Sexual patterns in the labroid fishes of the western Caribbean. 1. The wrasses (Labridae). *Smithsonian Contributions to Zoology*: 1-27
- Webb PW (1984) Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist* 24: 107-120
- Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). *Marine Biology* 101: 557-567
- Whitlock MC (1996) The red queen beats the jack-of-all-trades: The limitations on the evolution of phenotypic plasticity and niche breadth. *American Naturalist* 148 S: 65-77
- Williams AJ, Davies CR, Mapstone BD, Russ GR (2003) Scales of spatial variation in demography of a large coral-reef fish - an exception to the typical model? *Fishery Bulletin* 101: 673-683
- Williams DM, Wolanski E, Andrews JC (1984) Transport mechanisms and the potential movement of planktonic larvae in the central region of the Great Barrier Reef. *Coral Reefs* 3: 229-236
- Wilson DT, Meekan MG (2002) Growth-related advantages for survival to the point of replenishment in the coral reef fish *Stegastes partitus* (Pomacentridae). *Marine Ecology Progress Series* 231: 247-260
- Winemiller KO, Rose KA (1993) Why do most fish produce so many tiny offspring? *American Naturalist* 142: 585-603
- Winkler DW, Wallin K (1987) Offspring size and number: a life-history model linking effort per offspring and total effort. *American Naturalist* 129: 708-720
- Wootton RJ (1998) *Ecology of teleost fishes*. Kluwer Academic Publishers, Dordrecht
- Yamahira K, Conover DO (2002) Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? *Ecology* 83: 1252-1262

- Yamamoto S, Morita K, Goto A (1999) Geographic variations in life-history characteristics of white-spotted charr (*Salvelinus leucomaenis*). Canadian Journal of Zoology 77: 871-878
- Yampolsky LY, Scheiner SM (1996) Why larger offspring at lower temperatures? A demographic approach. American Naturalist 147: 86-100
- Zar JH (1999) Biostatistical Analysis. Prentice Hall, Upper Saddle River, N.J.