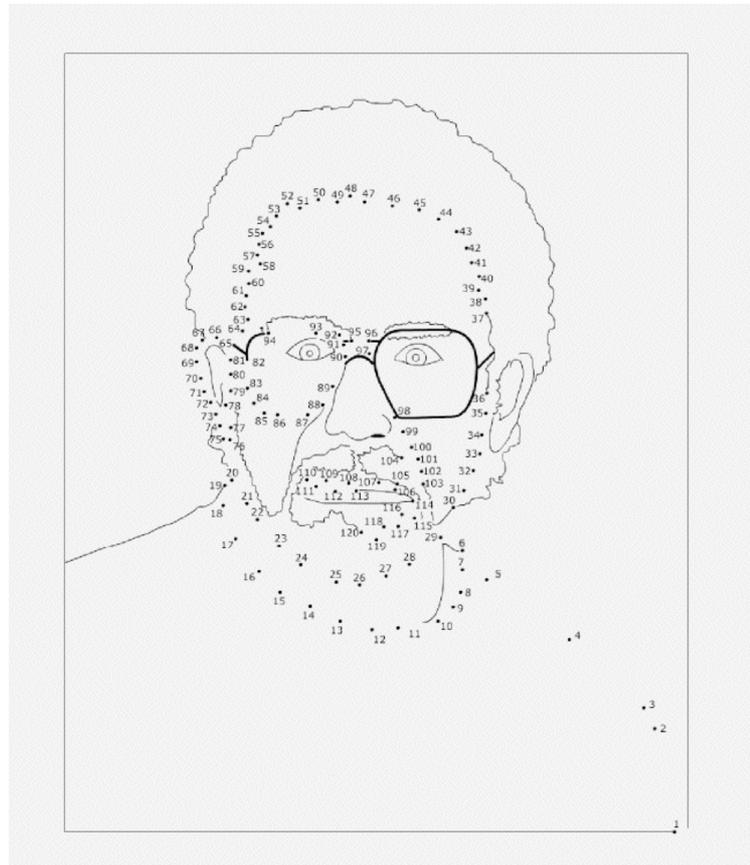# The Evolutionary Ecology of Sex Change

by

David John Allsop

Submitted for the Degree of Doctor of Philosophy

The University of Edinburgh 2003

Contents

List of Figures

List of Tables

Declaration

I declare that this thesis has been composed by myself and is entirely my own work, except for the collaborative inputs mentioned below:

Chapter Three. The 'Sex Change Theory' section was written by Stuart West.

Chapter Six. The simulations that are the basis for Figure 6-1 were performed by Andy Gardner.

List of papers arising from this thesis

Copies of papers followed by an asterisk * are included in the thesis appendix.

- ♣ Allsop, D. J. and West, S. A. 2003. Changing sex at the same relative body size. *Nature*. **425**, 783 –784.*

- ♣ Allsop, D. J. and West, S. A. 2003. Constant relative size and age at sex change for sequentially hermaphroditic fish. *Journal of Evolutionary Biology*. **16**, 5: 921 – 925.*

- ♣ Allsop, D. J. and West, S. A. 2004. Sex ratio evolution for sex changing animals. *Evolution*. In press.

- ♣ Allsop, D. J. and West, S. A. 2004. Sex allocation in the sex changing goby *Coryphopterus personatus* on atoll fringing reefs. *Evolutionary Ecology Research.* In press.

- ♣ Allsop, D. J. 2003. Trans-sexual fish from dimensionless space. *Planet Earth* (NERC newsletter article): **Autumn**, 30 - 31.*

Acknowledgements

First I would like to thank my supervisor, Stuart West, for being so flexible and allowing me to pursue the things that I find interesting, whilst always providing a solid guide rail to help constrain my wild cogitations to something (relatively) meaningful. My learning curve has been exponential in Stu's company and it has been a genuine pleasure to work with him. However, I still feel that my Mother Earth pie was worthy of the "I Baked Off and Won" T-shirt, and as such the gauntlet is still down for a rematch!

I thank Dave Shuker, who has given critical and far-reaching advice at every stage of the production of this thesis. Many thanks to Andy Gardner for coming to my aid with his mathematical wizardry when the pressure was on. Thanks to the staff at the University of Belize field station on Calabash Cay for their logistical support in the field, and to the Wildlife Conservation Society on Glovers Atoll for sending a boat to rescue me from the clutches of the mad woman. I thank Camillo Morra and the rest of the Sale group for their contagious enthusiasm and limitless ingenuity. Thanks to James Humphreys for being there at 25 meters below sea level when I ran out of air.

ICAPB has been a total research experience and I thank all of the staff members for creating such an exciting and stimulating atmosphere in which to work. Many researchers from around the world have advised or generously donated data-sets, I thank them all for their unselfish behaviour. Their names are listed in the acknowledgements of the published papers in the appendix to this thesis. In particular thanks to Eric Charnov for his support and encouragement.

Thanks also to the friends and colleagues who kept me sane or joined me in escaping sanity (more of the latter, I must say!) over the last three years: Dan Halligan, Tim Sands, Dan Gaffney, Tom Little, Stu West, Ashleigh Griffin, Zoe Hodgson, Penny Haddrill, Stu Killick, Alex Hayward, Ali Duncan (and her harem – which includes Dan), Aleta Graham, my flatmates Chris, Matias, Ross, Julia and Bingo, and my brothers Nick and Paul. Thanks to Carla Burns for her unnerving ability to distract me in the most critical stages of the PhD.

Finally thanks to the Natural Environmental Research Council (NERC) for funding me through this work, and the Davis Expedition Fund, Edinburgh University for their contribution to my fieldwork costs.

And God said, Let the earth bring forth grass, the herb yielding seed.....

Genesis, 1:11

And God said, Behold, I have given you every herb bearing seed, which is upon the face of all the earth...

Genesis, 1:29

My oven is on high, when I roast the quail
Tell bill clinton to go and inhale
Exhale, now you felt the funk of the power
Now feel the effects...

(Cypress Hill - Black Sunday).

Abstract

In this thesis I investigate the evolutionary causes and consequences of the sex change life history strategy.

I test *a priori* predictions regarding the social and ecological context in which selection favours sex change and/or the production of alternative mating strategies with a field study and laboratory experiment using populations of the marine teleost fish *Coryphopterus personatus*. The experimental data demonstrates that *C. personatus* retains the ability to facultatively switch its gender in response to local social conditions, but demographic field observations indicate that the fish may use this trait relatively rarely on atoll fringing reefs. I discuss these observations in the context of the distribution of essential ecological resources, the emergent mating system, and the resulting impact of the mating system on selection for sex change.

I examine the evolution of the sex ratio in sex changing organisms. I analyse sex ratio data for 121 species of sex changing animals using both standard cross species techniques and formal comparative methods. The data supports the theories that (a) the sex ratio should be biased towards the first sex, and (b) the magnitude of the sex ratio bias should be more for protogynous species than for protandrous species. I found support amongst the vertebrates for the theory that the sex ratio bias should be less extreme for species in which a proportion of the 'second' sex mature directly from the juvenile phase (early maturers), but this support does not extend to the whole sex change phylogeny, although the reason my be a lack of appropriate data.

I draw upon the statistical techniques of dimensionless analysis in order to test the prediction that sex change should occur at the same *relative* age and body size for populations or species that share similar relationships between crucial life history parameters: k/M, $\alpha \cdot M$ and δ. These are the relative growth rates k (the Bertalanffy coefficient), the adult instantaneous mortality rate M, the age at first breeding α, and the coefficient in the equation relating male fertility to size δ (where male fertility is proportional to L^δ, and L is size). My analyses reveal startling empirical support for the theory across 77 species of sex changing animals from five phyla.

Chapter 1. General Introduction

The aim of evolutionary biology is to explain the origin of organic diversity. To do this requires that we understand the pattern and mechanism of adaptation by natural selection. Adaptation is the development of physical and/or behavioural characteristics (the phenotype) that allow organisms to survive and reproduce in their habitats. Consequently adaptation is responsible for the myriad diversity of behaviours and body-forms that we see when we look out at the living world. This thesis investigates the evolutionary ecology of natural sex change - an adaptation that has evolved to allow certain organisms to optimise their Darwinian fitness through facultative resource allocation to male and female function in a single adult lifespan. In order to perform this investigation I use a combination of field observations, experimental methods, and comparative techniques. In each case the theoretical grounding is either spelled out or developed.

In this introduction I first give a brief overview of the work presented in the subsequent chapters. I then go on to give a general review of sex allocation theory with specific emphasis on the sex change life history, and I finish with a synopsis of the study of allometry with specific emphasis on dimensionless life history theory as applied to sex change.

The main data chapters open with an intra-specific experiment and field study of the factors selecting for sex change and the production of alternative male reproductive tactics in populations of a coral reef fish, the Masked Goby – *Coryphopterus personatus* on atoll fringing reefs in the Caribbean (Chapter 2). Specifically I investigate the link between the distribution of essential ecological resources and the mating system (Emlen and Oring 1977), and the subsequent effects of the mating system on the sex change decisions (Warner 1984).

In Chapter 3, I investigate the consequences of facultative sex change on the overall population sex ratio using a comparative approach looking at sex ratios for 121 species. Specifically, I test the predictions that (1) the sex ratio should be biased

towards the 'first sex' (Charnov 1982a; Frank and Swingland 1988; Charnov and Bull 1989a; Charnov 1993); (2) the sex ratio bias should be less extreme in species where a proportion of the 'second' sex mature directly from the juvenile stage (Charnov 1989), and (3) the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species (Charnov 1982a).

In Chapter 4, I compile a comparative dataset for sex changing fish to test the prediction of (Charnov and Skuladottir 2000) that sex change should take place at an invariant relative size and age. This prediction arises from the realisation that certain key aspects of the sex change life history have an invariant relationship with body size/age, and that those invariant relationships are also present in the Evolutionary Stable Strategy (ESS) calculations for optimal timing of sex change (Charnov 1979b; Charnov 1982a).

In Chapter 5, I extend the fish dataset from Chapter 4 to incorporate the whole phylogeny of sex changing organisms, spanning the chordata, echinodermata, arthropoda, mollusca and annelida. I investigate the extent to which the relative size at sex change can be explained by Charnov's invariant rule, taxonomy and various life history parameters.

Finally, in Chapter 6, I conclude with a brief review of the preceding chapters and a look at areas in which future research would be useful. In particular I highlight the need for the further development of theory to hang meaning onto the empirical findings of sex change invariance reported in Chapters 4 & 5, and pose some suggestions as to how this might be achieved.

1.1. Sex allocation

Any anisogamous organism has to face a number of reproductive decisions in its lifetime that will impact upon its fitness in some way. Such decisions include when to first reproduce, how many times to reproduce in a lifetime, who to reproduce with, how many offspring to produce, and what proportion should be male. The way in

which an organism allocates resources to male and female function (sex allocation) in their offspring can have a direct and large effect upon their genetic fitness. Due to its direct impact on fitness, selection may favour the ability to control sex allocation. Indeed, many examples of such control exist in the literature, notably using haplodiploid hymenoptera as a model system (see Charnov et al. 1981; Charnov 1982a; West et al. 2000a). However, recent research is unearthing evidence of such control in many other taxa with genetic sex determination, including vertebrates (West and Sheldon 2002).

1.1.1. Fishers Principle of equal investment

Sex allocation theory was founded on the basis of R. A. Fisher's (Fisher 1930) account of Darwin's (Darwin 1871) observations on the sex ratio, which were formalised mathematically by Carl Düsing in the late 19th century (Edwards 2000). Fisher noted that each offspring of a diploid dioecious pair receives half of its genetic complement from its mother and half from its father (under autosomal inheritance). In other words, any such organism is derived from only one mother and one father. This fact sets the scene for the evolution of frequency dependent selection on the sex ratio: if you consider a situation where there is an excess of males in a population, males would on average have access to less than one mate, so females would have higher fitness. Thus, natural selection favours mothers that produce an excess of female offspring (the rarer sex). The argument is mirrored for a sex ratio biased in the opposite direction. The resultant ESS sex ratio (Maynard Smith 1978) is the commonly observed ratio of unity, or 0.5.

1.1.2. Unequal sex ratios

Although Fisher's principle of equal investment forms the foundation stone for all studies of the sex ratio, equal investment is not always the best sex allocation strategy. This is because the principle of equal investment assumes that fitness-returns from male and female offspring are equal. This assumption is commonly violated in nature because the way that fitness is distributed amongst the sexes can be

affected by many factors. These can be summarised into two broad categories. First, sex biased interactions between relatives (Hamilton 1967), as is observed when : (a) relatives interact cooperatively to increase the beneficial resources available to offspring/siblings (Local Resource Enhancement: Trivers and Willard 1973; Schwarz 1988), (b) relatives compete for limited resources (Local Resource Competition: Hamilton 1967; Clark 1978) or (c) sons compete to mate with their sisters (Local Mate Competition: Hamilton 1967). Second, when environmental conditions affect the sexes differently, where offspring sex is determined either by: (a) a key environmental trigger which tells the developing embryo which sex will do best in the given environmental conditions (Environmental Sex Determination: Charnov and Bull 1977), such as temperature dependence in some reptiles (Bull 1980), or (b) a facultative decision made by the mother based upon her perception of which sex will give greater fitness returns given the current environment (Trivers and Willard 1973). Here 'environment' can be in an ecological sense, (e.g. resource availability), or in a condition-dependent sense (e.g. mother's maternal condition or social rank in a group) (West et al. 2002a).

1.1.3. Sex Change Theory

Relative to the majority of animals, which reproduce as gonochores (with separate sexes), many reproduce as hermaphrodites (Charnov 1982a; Policansky 1982; Allsop and West 2004a). Hermaphrodites can be either simultaneous (having mature gametes of both sexes present in the same individual at the same time), or sequential (able to completely reorganise their gonad- and genderspecific behaviours at some point in their life history). If sequential hermaphroditism (sex change) occurs from male to female, the condition is termed *protandrous* sex change, and if sex change occurs from female to male, the condition is known as *protogynous* sex change.

Theory predicts that natural selection will favour genes coding for sex change in circumstances where male and female reproductive values are closely related to size or age. This relationship is different for each sex (Figure 1-1. and Warner et al. 1975; Warner 1988b). The direction of sex change (protandrous or protogynous) is

determined by the relative fitness-returns over the course of a lifetime for the two sexes. If male fitness increases at a slower rate than female fitness when young or small, and accelerates above that of the females later on in life when larger sizes or older ages are reached, sex change will be protogynous. The reverse holds for protandrous sex change. The evolution of such a sex allocation mechanism enables the organisms to maximise their genetic contribution to the next generation by being the best sex for the prevailing circumstances.
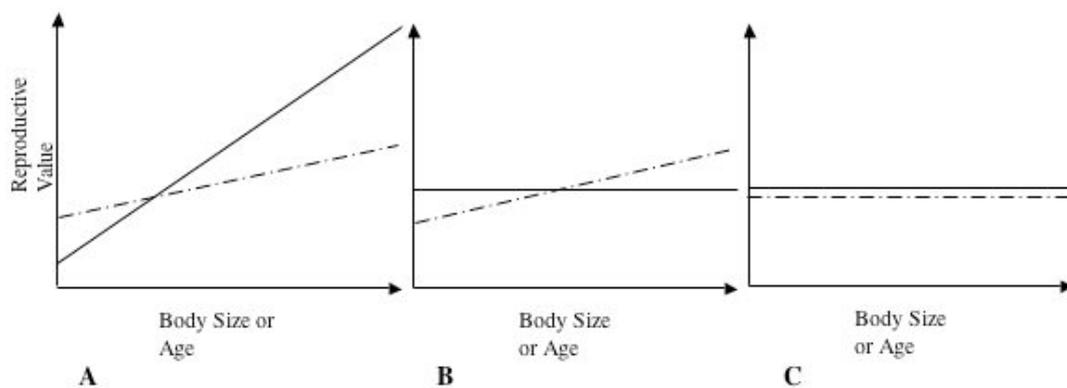
Figure 1-1. The size advantage model for sex change.
Solid lines represent male reproductive value, dashed lines female reproductive value. A: Protogynous sex change (female to male); B: Protandrous sex change (male to female); C: Gonochorism (separate sexes). Figure adapted from Warner (1984).

Which circumstances would create a situation where male and female reproductive values are so different over a lifetime as to select for the evolution of sex change? Here we must look to the mating system that a species or population adopts. In nearly all instances of protogynous (female to male) sex change studied, the mating system is some form of polygynous dominance hierarchy, with larger males holding either permanent or temporary harems, or at least defending resources which attract females (Ross 1990). Such a mating system creates a marked skew in mating success in the population, with the largest or oldest males monopolising access to the females. As female reproductive success is probably limited by egg production, and small (or young) males are competitively excluded from mating, it is easy to see how

11

the reproductive values of the two sexes are so different over the course of a lifetime (Figure 1-1 A). A similar argument follows for protandrous species (Figure 1-1 B), although here the sex differential in fitness occurs due to mating being random with respect to males. Yet female fitness increases with size (or age), as fecundity increases approximately to the cube power of body size (Charnov 1982a). If there is no difference in reproductive value between the sexes across the course of a lifetime, selection favours separate sexes with no sex change (Figure 1-1 C). These ideas are elaborated on and illustrated with examples in Chapter 3.

Variation in the mating system occurs both intra- and inter-specifically (Emlen and Oring 1977), and this can cause variation in the level of mating skew in the population (Clutton-Brock and Vincent 1991). Consequently there is great intra- and inter-specific diversity in the selective forces that shape the timing of sex change, and indeed the actual occurrence of sex change (Warner 1984). The external manifestation of these diverse selective forces is a broad range in the size at sex change both within and across species. The timing of sex change is often linked to changes in relative condition or social group membership (Robertson 1972; Warner et al. 1975; Fricke and Fricke 1977; Warner and Robertson 1978; Warner and Swearer 1991; Munday et al. 1998), suggesting that the sex change strategy has evolved to be plastic so as to enable an animal to optimise its fitness in any given social context (see Chapter 2).

1.2. The Comparative Approach

Whilst the diversity of sex change strategies and the variation therein is spectacular, this does not preclude the possibility of the existence of broad scale general principles governing the phenomenon. The search for such universal principles in biology is often undertaken by looking for general patterns that exist across many species. The comparative approach to studying biology has been in use at least since Darwin began his investigations into the natural world. Recently however, the technique has been formalised using explicit evolutionary and statistical models that enable cross-species studies to remove the effects of evolutionary relatedness when

investigating adaptive patterns and processes (Harvey and Pagel 1991). The details of these advances applied to sex change are covered in Chapters 3, 4 and 5.

1.2.1. Allometry

Allometry is the study of biological scaling relationships and is concerned with the way in which certain aspects of organismic biology change with changes in body size. The quantitative study of such phenomena has a distinguished history, receiving close attention from scholars such as D'Arcy Thompson (Thompson 1959) and Julian Huxley (Huxley 1993). Allometric analysis can be conducted at the level of the individual, since organisms change size throughout life. However, by far the best-studied scale for researching allometric relationships is at the cross-species level, since this allows for the formulation and testing of theory across many orders of magnitude of variation in body size. A well-known example of such an approach is the scaling of the mammalian metabolic rate as the 3/4 power of body mass (Kleiber 1932). This allometry has been shown to hold across animals as diverse in body size as mice and elephants. The scaling of metabolic processes with body size is a particularly important example to highlight, as a recent general model designed to explain the meaning of these scaling rules in biology highlights the fundamental nature of the conversion of external products into energy (metabolism) for all living things (West et al. 1997).

Allometric equations take the form

$$Y = Y_0 M^b$$

where Y is some dependent variable, Y_0 is a normalisation constant, M is an independent variable (usually body mass), and b is the scaling exponent. These biological scaling relationships are called *allometric* because the exponent, b, differs from unity. If $b = 1$, the relationship is said to be invariant to changes in body size (see *dimensionless invariants* section below) and is termed *isometric*. West et al's (West et al. 1997) model highlights the fact that the presence of isometries are a

universal principle in biological scaling, and occur in conjunction with an integrated set of allometries that scale to the 3/4 power exponent of body mass.

1.2.2. Dimensionless analysis

A particular example of isometry is in the use of dimensionless numbers to categorise and classify life histories. Dimensionless analysis can be employed when mathematical functions are constructed between quantities with the same dimensions, such as the age at maturity and maximum lifespan. Here both quantities have the common dimension of time, and the rules of dimensional analysis (Stephens and Dunbar 1992) say that we can eliminate the common unit of time. This analytical technique has a rich tradition in the mathematical sciences (such as engineering and physics) and offers many potential benefits for evolutionary ecology. Amongst its benefits are: (a) the reduction of analytical complexity of models by reducing the number of variables, (b) they express the nature of the *relationship* between variables, and (c) in removing the common dimension, they enable measured variables to be compared over transitions of many orders of magnitude, since the unit-less numbers have absolute meaning from case to case.

1.2.3. Dimensionless analysis in life history evolution

In the field of life history evolution, the seminal application of the dimensionless approach was to the analysis of fisheries data, which attempted to summarise the relationships between growth, mortality and maturation (Beverton and Holt 1959). Since then the gauntlet has been picked up by Eric Charnov and co-workers, who have expanded the use of the dimensionless approach into many aspects of evolutionary ecology, including sex allocation, ageing, alternative life histories and determinate/indeterminate growth (Charnov and Berrigan 1991; Stephens and Dunbar 1992; Charnov 1993; Mangel 1996).

Charnov's general approach for the dimensionless classification of life histories employs optimisation models for aspects of the life history, and then searches for

support for these models in the literature across taxa. This optimisation approach uses the simplifying assumptions of (a) stationary population size, (b) density-dependent juvenile mortality and (c) age-independent adult mortality - to output the evolutionary equilibrium values for the appropriate life history characteristics in the face of trade-offs when maximising fitness. An important point here is that the resulting dimensionless invariants, which can be used to highlight patterns across diverse taxa, are statistical – to say something is invariant does not imply that all values are identical, other than statistically, as with all analysis of biological data.

The study of Life History Invariants (Charnov 1993) is much broader than the study of allometry, which restricts itself to the impact of body size on adaptive traits. With Life History Invariants, a given life history characteristic of plants and animals remains invariant across major transformations, such as those inherent in age, body size (within a species or phylogeny), population size (total or density), geography and time itself. A well-known example is the invariant relationship between age at maturity and lifespan. Here we see that the ratio of age at maturity and lifespan remains the same within major taxonomic groups (e.g. birds, mammals, reptiles, fish):
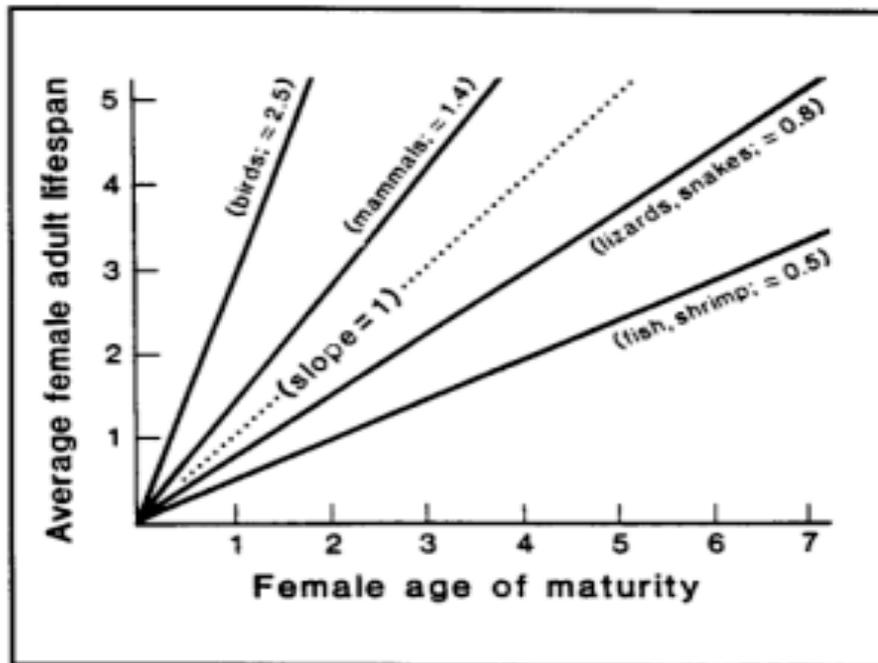
Figure 1-2. Average adult lifespan (E) versus age at maturity (a) for several taxa.
The number on each line refers to estimated slope (E/a) and is approximately a constant within groups, while differing greatly between groups (from Charnov 1993).

This invariance approach can well be applied to the study of sex allocation and reproductive strategy. To give an example, consider Figure 1-3. Here Shapiro (Shapiro 1979) demonstrates how the breeding sex ratio of groups of the sex changing reef fish *Pseudoanthias squamipinnis* remains invariant over changes in the population body size distribution. Figure 1-3 shows the sex/size distribution of two breeding populations of the same species collected from different habitats. The body sizes of males and females are different at the two locations, possibly reflecting environmental differences in habitat quality. Despite this transition in body sizes, the breeding sex ratio remains the same for the two groups.
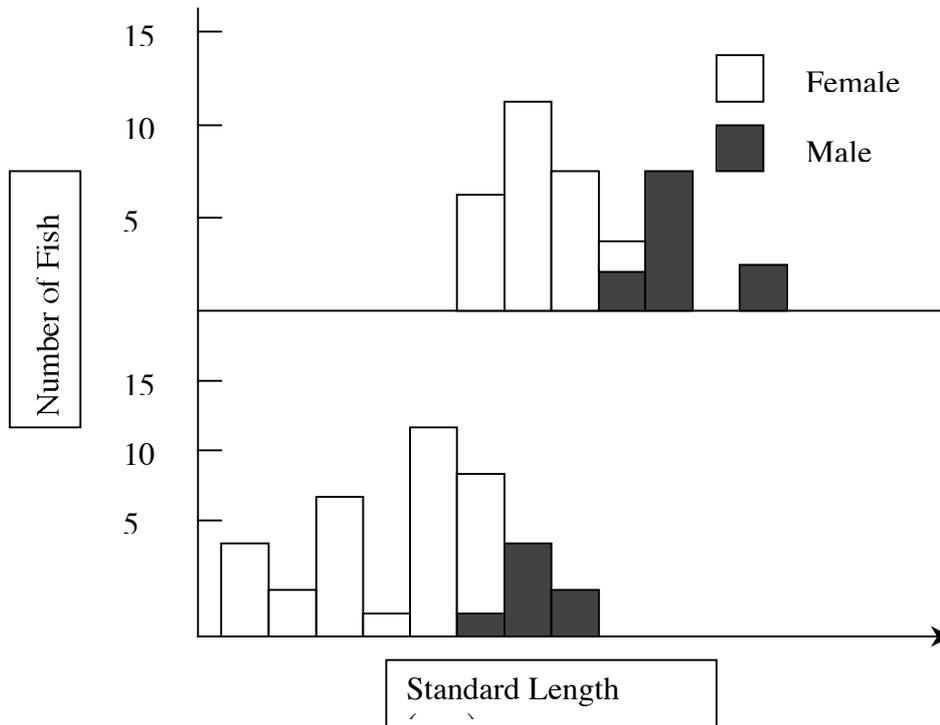
Figure 1-3. Adult body size distribution for male and female *Pseudoanthias squamipinnis.* Populations sampled from two locations (800m apart) on the same Aldabra Island Reef (from Shapiro 1979).

A major advantage of using life history theory to predict invariants is that invariants can make predictions based on variables that are relatively easy to measure. In particular, invariants often do not depend upon hard to measure relationships such as specific fitness functions or lifetime reproductive success.

1.2.4. Dimensionless invariants for sex change

Building on the dimensionless approach, Charnov and Skulladottir (2000) rephrased the classical ESS analysis of sex change (Warner et al. 1975; Leigh et al. 1976.) in terms of dimensionless numbers. This analysis predicted, under certain conditions, three dimensionless life history numbers for the equilibrium size and age at sex change. Specifically, their model predicted invariance in: (a) the *relative* age at sex

17

change, (b) the relative size at sex change, and (c) the breeding sex ratio. They provided empirical support for one of these predictions by demonstrating an invariant *relative* size at sex change across 21 separate breeding populations of the northern shrimp *Pandalus borealis* from four distinctly different growth regimes. I elaborate on the theoretical background and provide an empirical test of these predictions in Chapters 4 and 5 of this thesis. In addition, I discuss possible future developments to the theory in Chapter 6

Chapter 2. Sex allocation in the sex changing marine goby *Coryphopterus personatus* on atoll fringing reefs.

2.1. Abstract

Sex allocation theory applied to sex changing animals predicts that the amount of sex change and the proportion of individuals that mature early as the second sex depend upon the mating system of the species or population in question. In turn, theory suggests that the mating system is governed by the size and distribution of resources critical to reproduction, and by population density. Here I investigate the social and ecological factors that govern the amount of selection for sex change and the production of alternative male strategies in a protogynous (female first) goby, *Coryphopterus personatus* on atoll fringing reefs in Belize. I found that: (1) increasing population density leads to an increase in the proportion of early maturing males on leeward facing reefs, as predicted, but not on windward reefs; (2) contrary to predictions, the proportion of early maturing males was higher on continuously distributed coral gardens than on isolated patches of reef in windward locations, with no difference in leeward locations; and (3) the proportion of early maturing males can be used as a predictor of the population sex ratio, with less biased sex ratios occurring with a higher proportion of early maturing males, as predicted by theory. I discuss these conflicting results in terms of the differences between windward and leeward reefs that might lead to differening selective regimes acting in these locations.

2.2. Introduction

Many animals and plants have evolved the ability to change sex at some point during their lives (Warner 1975; Charnov 1982a; Policansky 1982; Allsop and West 2003a, b, 2004a). Sex change is favoured when males and females differ in their capacity to produce offspring over the course of a lifetime (Warner et al. 1975; Leigh et al. 1976.; Charnov 1982a; Warner 1988b). Specifically, selection favours individuals that mature as the sex whose reproductive value increases more slowly with age (first sex), and then change to the other sex (second sex) when older (Ghiselin 1969; Charnov 1982a; Warner 1988a, b).

In some sex changing species there are a proportion of individuals who mature early as the second sex, here termed early maturers or EMs. EMs can arise either directly from the juvenile stage, having never passed through the 'first sex' (Fennessy and Sadovy 2002), or they can arise by *pre-maturational sex change* (de Girolamo et al., 1999). Here we treat both developmental routes as being functionally equivalent for the purposes of our investigation and lump both under the umbrella term EMs. These EMs often employ alternative mating strategies, such as sneaking or group spawning (Robertson and Choat 1974; Robertson and Warner 1978; Warner and Robertson 1978; Warner and Hoffman 1980a, b; Charnov 1982a). Their production depends upon the magnitude of the difference between the male and female reproductive value curves, and the abundance of EMs in a population can be used as a measure of the amount of sex change that is taking place. Sex change is at a maximum when EMs are absent and at a minimum when EMs form 50% of the population (Warner 1984).

The adaptive benefit of sex change and the production of early maturers depends to a large extent on the structure of the mating system (Warner 1984), which is largely determined by the distribution of ecological resources required by a species (Emlen and Oring 1977). Emlen and Oring (1977) argued that the distribution of resources (patchy or continuous) determines the ability of one sex to monopolise access to

potential mates. Such mate monopolisation will act to set the level of variance in mating success in a population, which dictates the intensity of inter- and intra-sexual selection, and thus the form of mating system (Clutton-Brock and Vincent 1991).

In protogyny (sex change from female to male) the differences between male and female reproductive value curves are invariably associated with polygynous dominance hierarchies (Warner 1984; Ross 1990). For example, in the cleaner wrasse *Labroides dimidiatus*, males have total harem control, as females feed at predictable and limited 'cleaning stations' that large males can readily monopolise (Robertson 1972; Robertson and Hoffman 1977). Small males are unable to gain any matings, so all fish are born female. Towards the other end of the selective spectrum, in the blue-head wrasse *Thalassoma bifasciatum*, large males are often unable to completely control the preferred spawning sites that females are attracted to. Small males are able therefore to gain some reproduction in this case (by adopting alternative mating strategies), so their production is selected for (Warner and Hoffman 1980a).

Interacting with the effects of resource distribution (patchy or continuous), population density has been also shown to influence the potential for mate monopolisation in sex changing reef fish (Warner 1984). Early work on Caribbean labrids demonstrated that the proportion of EMs increased with increasing population density across several species (Warner and Robertson 1978). More detailed field studies using the bluehead wrasse, *Thalassoma bifasciatum*, showed that males are capable of defending females in small but not in large populations. Population size and density increased with increasing reef size, and so selection for sex change was highest on small reefs with low population densities (Warner and Hoffman 1980a, b). In contrast, the pacific rainbow wrasse, *T. lucasanum*, has dramatically higher population densities than the bluehead wrasse, and accordingly very little sex change takes place in this species, to the extent that it appears gonochoristic, with a sex ratio of 0.5 (Warner 1982).

Studies of non-labrid fish have highlighted similar effects of resource size or distribution shaping the mating system and subsequent selection for sex change. For example, numerous species within the damselfish genus *Dascyllus* have been investigated from the perspective of the potential for mate monopolisation (Fricke 1980; Shpigel and Fishelson 1986; Godwin 1995). The general pattern is that small patchily distributed habitats are more easily defended and hence lead to high reproductive skew and polygynous mating systems, favouring more sex change. In contrast, larger or more continuously distributed habitats, with more potential for the formation of large groups or for migration between groups, result in more promiscuous mating systems with little or no skew, less sex change and more equal sex ratios.

Here I test these ideas in a field study of the Masked goby, *Coryphopterus personatus* on atoll fringing reefs in the Caribbean. Prior to conducting field observations, I performed a laboratory experiment to demonstrate that Belizean populations are capable of changing sex, and to test the effects of the social environment on the sex change decision. I then carried out field observations to test the predictions that: (1) Increasing local population density makes it more difficult to defend females or resources critical to females, leading to a reduction in the amount of sex change and an increase in the proportion of early maturing males. (2) Females on isolated patches of reef are easier to defend than females on continuous coral garden habitats, leading to more sex change and fewer early maturing males on isolated patches of reef. (3) The difference in energetic environment between windward and leeward reef locations (Stoddart 1962) leads to differences in the selection pressures for sex change - specifically the higher turbulence on windward reefs restricts inter-group movement, leading to easier mate monopolisation, higher selection for sex change, and fewer EM-males (EMMs) than on more protected leeward reefs.

Further, I take the opportunity to test a final prediction that the population sex ratio should show less extreme biases in partially sex changing populations, where some individuals mature directly into the second sex (Charnov 1989; Allsop and West

2004a). Previous empirical work attempting to test this prediction has found quantitative support for the theory at a cross species level when using the presence or absence of EMMs as a discreet binomial predictor, but failed to find any relationship between sex ratio and the proportion of EMMs when using the proportion of EMMs as a continuous predictor across species (Allsop and West 2004a).

2.3. Materials and methods

2.3.1. Study Sites

The Eastern Coast of Belize, Central America, is home to the second longest barrier reef system in the world, extending from the Mexican Border to the Gulf of Honduras. To the East of the Barrier Reef system, formed from the fault-controlled Yucatan continental block, lie three of the Caribbean's coral atolls, Turneffe, Glovers and Lighthouse (Figure 2-1). The present study focuses on Turneffe and Glovers Atolls. Both atolls are orientated roughly N – S, and are surrounded on both Windward and Leeward sides by fringing reef. Windward sides generally receive high wave energy, and as a consequence have developed segmented reefs of spectacular high relief, often consisting of spur and groove topography (Stoddart 1962). Leeward sides, on the other hand, are generally more protected, receiving considerably less wave energy. As a consequence, leeward reefs tend to be of lower relief, forming a more continuous distribution of 'coral gardens'.

2.3.2. Sample Collections & Basic Reef Ecology

I sub-sampled assemblages of the marine goby, *Coryphopterus personatus*, from fore-reef habitats on the fringing reefs around Glovers Atoll (16°42'00"N to 16°55'00"N; 87°53'00"W to 87°41'00"W) and Turneffe Atoll (17°09'00"N to 17°38' 00"N; 87°44'30"W to 87°57'30"W). I sampled locations randomly from around the leeward and windward sides of both atolls at a depth of 15 – 30 metres using SCUBA and the anaesthetic Quinaldine Sulphate, administered in aqueous solution from a squeezy bottle. We were able to capture a large sub-sample of the individual

populations using a single cloud of the anaesthetic, due to the small size of the

animals (maximum body size 34mm).
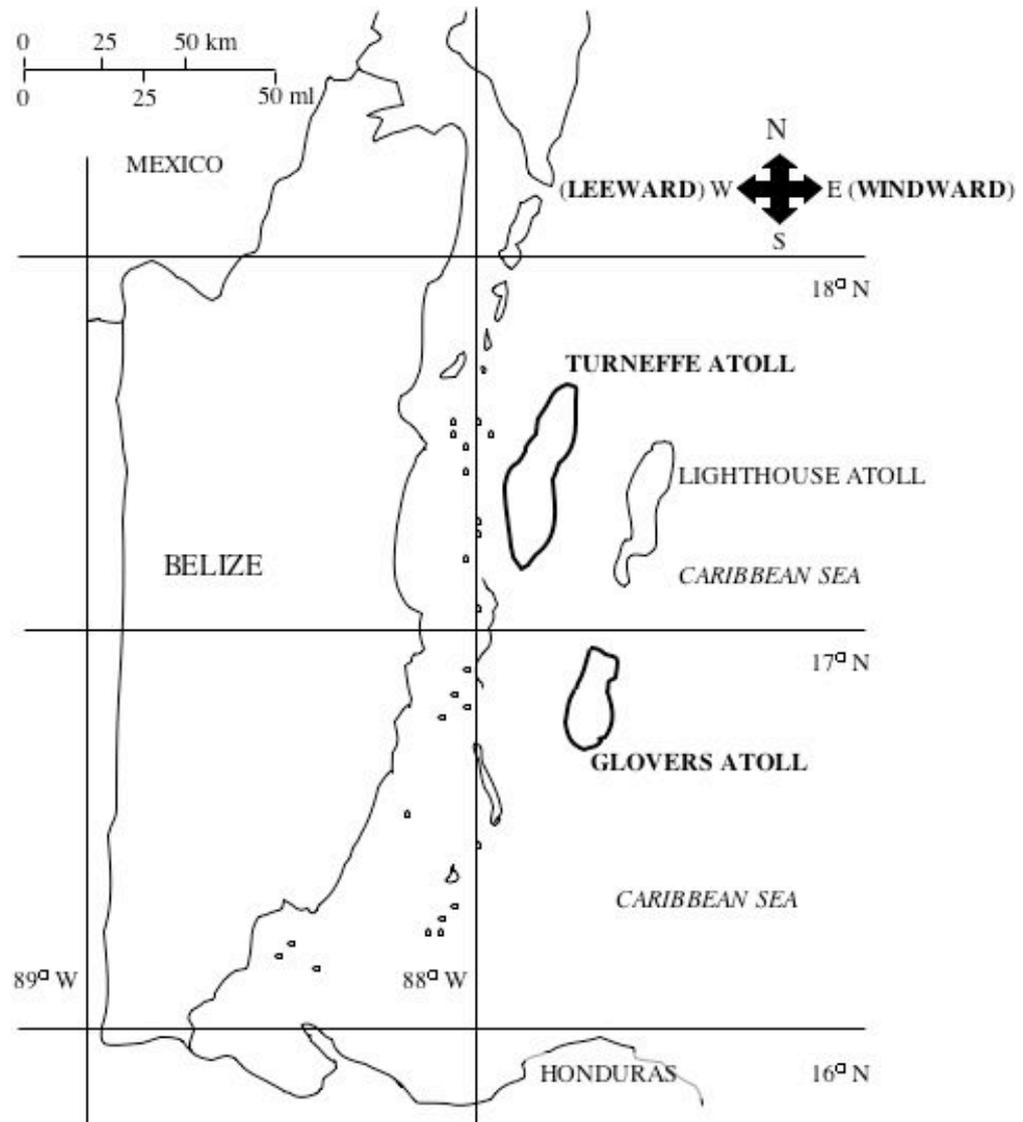
Figure 2-1. Map of study area. The Belize Barrier Reef is demarked by the string of small islands (running N – S) to the west of the atolls. Study atolls are outlined in bold.

I collected basic ecological data for each individual population in order to assess the influence of ecology on the sex change decisions of *C. personatus*. I made measurements of the surface dimensions of the section of reef from which the population was taken and converted these measurements to surface area. I then used these surface area scores to calculate the population density for each group, as number of fish per m^2. In addition, I further classified each capture site as Isolated or Continuous by subjective interpretation, depending on whether the section of reef on which the fish lived was a single, prominent topographic entity (such as a bluff, bolder or turret), or an area of reef with little or no topography (such as low lying coral gardens).

Following capture, I transported fish to the shore whereupon populations were processed for sex/size distribution data. I sexed fish using the external appearance of the genital papillae, which is short and rounded for females and long and pointed for males (Cole and Robertson 1988), and maximum length was recorded as the distance in mm from the tip of the snout to the distal tip of the caudal fin, using callipers accurate to 0.1mm.

2.3.3. Social Control of Sex Change Experiment

I performed the sex change experiment at the University of Belize marine field station, on Calabash Caye, Turneffe Atoll. I made separate collections of fish from the nearby fringing reefs in front of the field station using the same methodology as above. Following capture, I sexed and measured fish before placing them into experimental groups of known sex and size distribution in aquaria in the flow through sea-water laboratory. In order to test for an effect of social environment (sex/size ratio) on sex change, I carried out the following experiment. I established a control 'inhibition only' treatment consisting of a single large male and five smaller females, in which I expect the dominant effects of the larger male to inhibit sex change in the smaller females. The experimental 'inhibition/stimulation' treatment consisted of one larger male and ten smaller females, in which I am testing for a stimulatory effect of small females above that of the males inhibitory effects. I maintained fish for the duration of the experiment (20 days) with a constant supply of fresh, aerated sea water, and fed them *ad libitum* on a diet of ground fish flakes.

2.3.4. Estimating Life History Variables for Natural Populations

As there is no difference between the gonads of sex changed males and males that mature directly from the juvenile phase in this species (Cole and Robertson 1988), we were unable to directly estimate the proportion of early maturing males (EMMs) using gonadal histology. Instead I estimated the proportion of EMMs for each population separately using a logistic regression procedure, with sex ratio (proportion male) as the response variable and body size as the predictor variable. This procedure outputs a roughly S-shaped curve, with a higher proportion of females in the smallest size categories and a higher proportion of males in the largest size categories.

I then used the logistic regression parameters to calculate the sex ratio (proportion male) at the 5th percentile of the population body size distribution using the following equation:

$$\text{Sex ratio (proportion male)} \ = \ \frac{e^{(a+bx)}}{1+e^{(a+bx)}}$$

where a is the intercept of the logistic regression, b is the slope, and x is the body size at the 5th percentile. I chose to use the sex ratio at the 5th percentile because this is sufficiently close to the lower end of the population size distribution to ensure that the males present were early maturing males and not the product of sex change, and yet sufficiently far in from the smallest animal so as to minimise the noise inherent in measuring and sexing these smallest size groupings.

2.3.5. Statistical analysis

Proportion data, such as the proportion of early maturers, are bound between 0 and 1, and usually characterised by non-normal error variances and unequal sample sizes. The most powerful way to allow for this is to assume a binomial error structure and a logit link function in a Generalised Linear Model (GLM: Crawley 1993; Wilson and Hardy 2002). The results of the sex change experiment are analysed using this procedure, and the analysis of the prediction of less extreme sex ratio bias in partial sex changers is performed using a combination of angular transformation and GLM with binomial error structure. However, I was unable to use the GLM approach for analysis of the effects of socio-ecology on the proportion of EMMs because I estimated the proportion of EMMs using the logistic regression (see above). Thus, I did not have access to the population size information required for this approach. Consequently I used the next best method of arcsine square root transforming our proportion EM data, and performing standard analysis assuming normal errors. In order to investigate the factors affecting habitat preferences and the proportion of EMMs, I constructed general linear models including all main effects and higher-

level interactions. I then carried out model simplification with standard methodology, through stepwise deletion (Crawley 1993).

2.4. Results

I sampled a total of 49 populations of *Coryphopterus personatus* from depths ranging from 10 m to 25 m, with a mean capture depth of 17.8 m (± 95% C.I: 16.7 m - 18.9 m). Populations ranged in size from 28 individuals up to 302, with a mean population size of 93.2 (± 95% C.I: 77.2 - 109.4). Collections were split equally between the two atolls, with 24 populations collected from Glovers Atoll and 25 from Turneffe Atoll. Sex ratios (proportion male) ranged from 0.11 to 0.50, with an average of 0.33 (± 95% C.I: 0.3 – 0.36).

My estimate of the proportion of early maturing males ranged from 0.11 to 0.79 with an average of 0.44 (± 95% C.I: 0.38 – 0.49). There was a high incidence of overlap between male and female size distributions in all populations studied (Mean proportion overlap = 0.78 ± 95% C.I: 0.72 – 0.84, range = 0.33 – 1; Figure 2-2), and the proportion of females in the upper fifth of the population size distributions was generally high (Mean proportion female in upper 5th of size distribution = 0.5 ± 95% C.I: 0.4 – 0.6, range = 0 - 1).
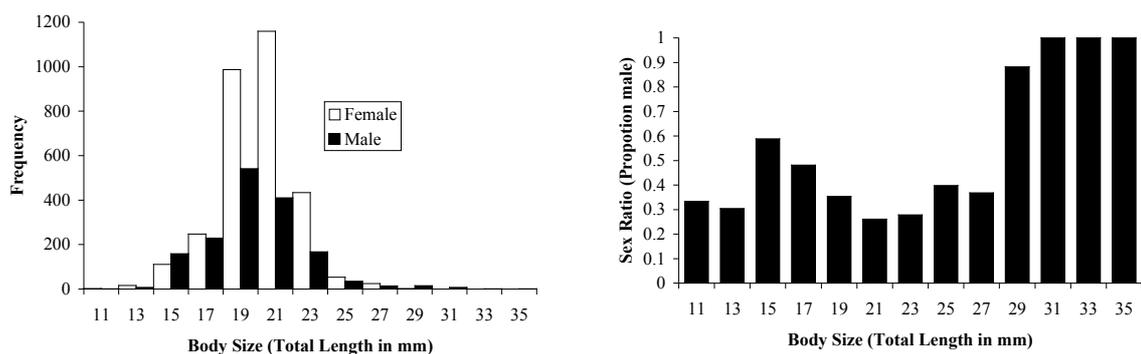
Figure 2-2. Sex-size distribution for whole study population of *C. personatus* collected on atoll fringing reefs in Belize. Left of figure = frequency distribution, Right of figure = proportion male. Note the large overlap in size distribution between the two sexes.

2.4.1. Social Control of Sex Change Experiment

I observed sex change taking place in six out of ten of the 'inhibition/stimulation' experimental replicates and no instances of sex change in the 'inhibition only' control populations. This difference between experimental and control treatments was highly significant using a GLM with binomial error structure and a logit link function ($F_{1,\,17} = 9.00$, $p = 0.008$; Figure 3). The experiment demonstrates that *C. personatus* on atoll fringing reefs in Belize does have the ability to change sex, and indicates that the proximal mechanism controlling sex change includes a stimulatory effect of the presence of smaller females, and/or an inhibitory effect of larger males.

Four of the populations in which sex change occurred fitted the predictions of the size-advantage hypothesis (Warner 1988b), with the largest female changing sex. One of those populations had multiple sex changes occurring, with the second largest female changing sex also. In the remaining two sex changing populations it was the second largest female who changed sex. Overall, there was a significant tendency for the females that changed sex to be larger then the average sized non sex-changing female in the group (Mean difference = 3.3, $t_5 = 2.7$, $p = 0.05$).
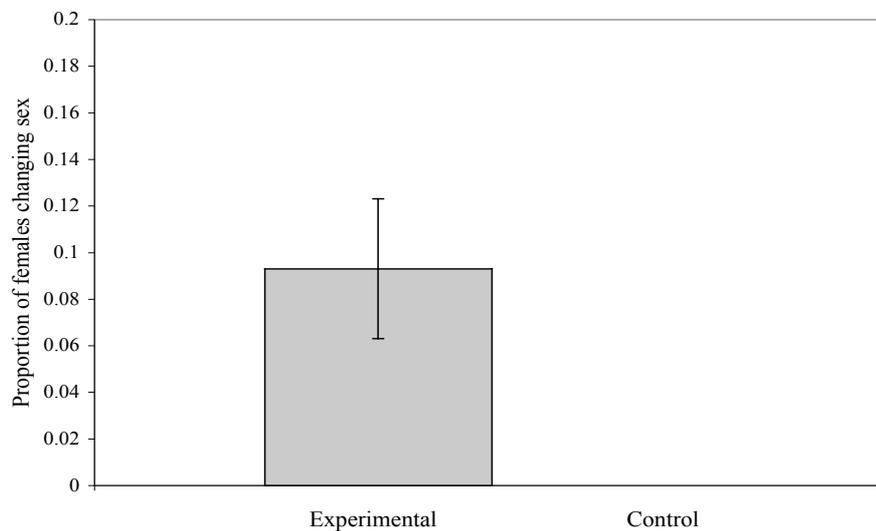
Figure 2-3. Sex change is mediated by social environment (sex ratio) in a laboratory experiment. The proportion of females changing sex was significantly higher in the experimental treatment (one male, ten females) than in the control treatment (one male, five females). Standard Error bar is shown for the experimental treatment; no sex change occurred in the control treatment.

2.4.2. Basic ecology and population density

Considering both atolls together, reef areas from which captures took place ranged in size from 2m^2 to 70 m^2 with mean reef size of 21 m^2 (± 95% C.I: 17 – 25 m^2, n = 49). Of the 49 populations sampled, 25 were taken from continuous coral cover habitats and 24 from topographically isolated patches of reef. On the windward sides of the atolls, where wave energy is highest, 61% of the habitats sampled were topographically isolated patches of reef, and on the leeward sides of the atolls 88% of habitats sampled were continuous coral cover with low lying, indistinct topography.

Population size increases with increasing reef area (OLS Regression: Population size = 53.18 + 1.93 * Reef Area, significance of slope: t = 3.68, p < 0.01, r^2 = 0.22, n = 49), but population density decreases with increasing reef size in a quadratic fashion (Significance of quadratic term: $F_{1, 46}$ = 23.99, p < 0.01; best fit predictor of population density is given by: log(Population density) = 1.28 - 0.004*Reef Area + 0.0005*Reef Area^2 (±95% CI of the linear (α) and quadratic (β) terms: α ± 0.001, β ±0.0002). There was no significant difference between population density on isolated and continuously distributed reefs (Isolated: Mean = 8.12 fish per m^2 (±95% C.I: 2.18), n = 24; Continuous: Mean = 4.08 fish per m^2 (±95% C.I: 1.01), n = 25; $F_{1, 47}$ = 2.10, p > 0.1). There was however a significant difference in population density between windward and leeward reefs, with higher population densities on windward sides of the study site (Windward mean population density = 7.7 fish per m^2 (±95% C.I: ±1.7), n = 33; Leeward mean population density = 2.8 fish per m^2 (±95% C.I: 0.4), n = 16; $F_{1, 47}$ = 19.09, p < 0.0001).

2.4.3. Ecological factors and sex change

I investigated the effects of population density, habitat distribution (isolated or continuous), and windward or leeward reef location on the amount of sex change and the production of EMMs using a Generalised Linear Model with stepwise deletion of non-significant terms. Our minimal model included significant interactions between: (1) population density and windward or leeward location ($F_{1,44} = 5.17$, $p < 0.05$; Figure 2-4), and (2) habitat distribution (isolated or continuous reefs) and windward or leeward reef location ($F_{1,44} = 6.3$, $p < 0.05$; Figure 2-5).

The significant interaction between population density and windward or leeward reef location (Figure 2-4) arises because the proportion of EMMs increases with increasing population density on low energy leeward reefs (slope = 0.98, ±95% C.I: 0.07, t = 14, p = 0.01, $r^2 = 0.35$), but there is no such relationship on higher energy, windward reefs (slope = -0.16, ±95% C.I: 0.22, t = 0.73, p > 0.1, $r^2 = 0.06$). The significant interaction between windward or leeward locations and habitat distribution arises because there are more EMMs on isolated reefs than on continuous reefs in windward locations ($F_{1, 32} = 5.06$, $p < 0.05$; Figure 2-5), but no significant difference on leeward locations ($F_{1, 15} = 0.43$, $p > 0.1$), with the trend actually being in the opposite direction.
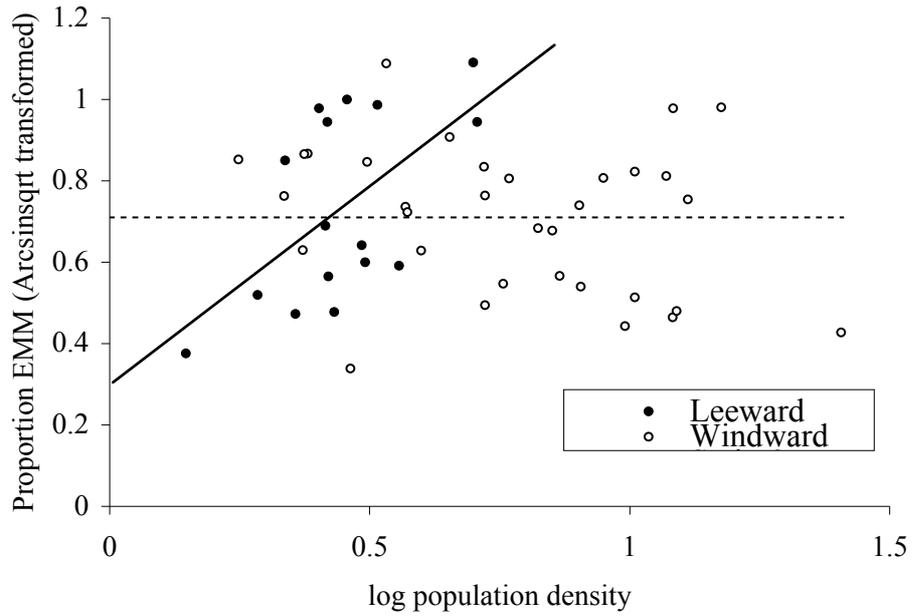
Figure 2-4. The correlation between population density and the proportion of early maturing males (EMMs), on windward and leeward reefs.
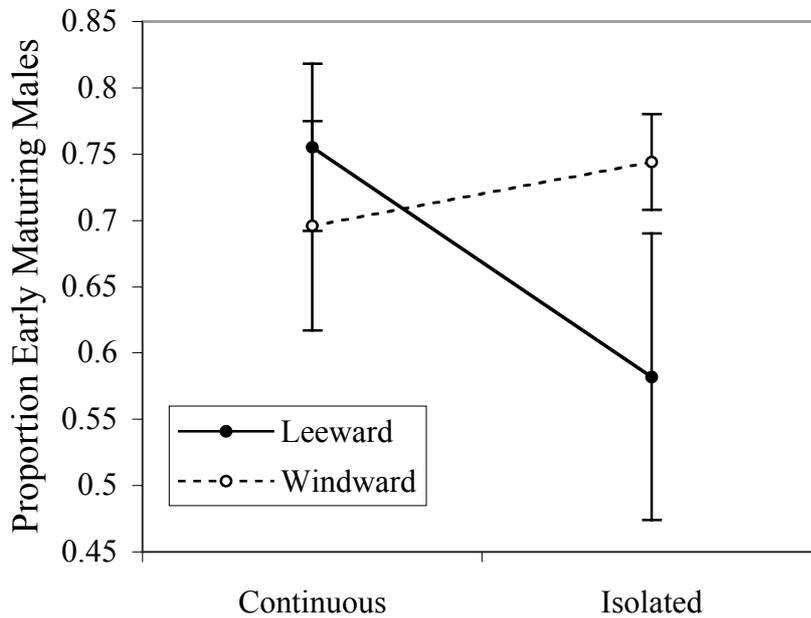
Figure 2-5. Isolated reefs have fewer early maturing males (EMMs) than continuous coral gardens on leeward locations, but not windward locations.

2.4.4. Sex ratio bias and the proportion of EMMs

I tested whether the sex ratio bias was less for populations with a greater proportion of EMMs by angular transformation of the proportion EMM (see Statistical Analysis in Methods section), and using that transformed data as a predictor of the population sex ratio as defined using a GLM with a binomial error structure and a logit link function. The analysis demonstrates that indeed there is a trend for a less bias sex ratio as the proportion of EMMs increases across populations (Intercept = -1.5 ± 95% C.I: 0.4, Slope = 1.3 ± 95% C.I: 0.5, r^2 = 0.29; $F_{1, 47}$ = 18.8, p < 0.0001).

2.5. Discussion

I have carried out a laboratory experiment, and observational field collections from 49 populations on the masked goby, *Coryphopterus personatus* from atoll fringing reefs in Belize. The results show that: (1) *C. personatus* from this population is capable of facultative sex change, and that this sex change process is consistent with a proximal control mechanism involving the net balance of an inhibitory effect of larger males and a stimulatory effect of smaller females (Figure 2-3), (2) The proportion of early maturing males (EMMs) is positively correlated with population density in leeward environments, as predicted, but this relationship does not hold in windward locations (Figure 2-4). (3) The proportion of EMMs is higher on isolated patches of reef than on continuous coral gardens in windward environments, contrary to predictions (Figure 2-5), and (4) The proportion of EMMs is positively correlated with the population sex ratio, with higher proportions of EMMs being associated with less extreme sex ratio biases, as predicted by theory (Charnov 1989; Allsop and West 2004a).

Overall, the results give mixed support to the predictions I set out to test. Whilst I do find support for the idea that increasing population density leads to an increase in the proportion of EMMs, possibly because higher population densities destabilise the potential for mate monopolisation and thus selection for sex change (Warner 1982, 1984), the result only holds on the leeward sides of the atolls (Figure 2-4). Why

might the effects of population density break down in windward environments? One possibility might be that animals find it more difficult to migrate between groups in high-energy turbulent environments such as those found on windward reefs, especially small-bodied animals such as *C. personatus*. Restricting inter-group movement in this way would encourage mate monopolisation and select for higher levels of sex change and fewer EMMs, in an analogous way to the restriction of movement in patchily distributed habitats (Fricke and Fricke 1977; Godwin 1995). However, the data also highlights the fact that population densities on windward sides of the atolls are at least double those in the leeward environments. If increasing population density in any way destabilises group structure leading to difficulty in harem control, then the observation of higher population densities on windward sides would predict less sex change to occur here and more EMMs to be produced. Population density may therefore always be so high on windward sides that variation in population density has negligible effect on selection for EMMs (and sex change).

My finding of a higher proportion EMMs on isolated patches of reef than on continuous coral gardens is contrary to my original prediction. Aside from the possible effects of the energetic environment in windward locations, it may be that windward and leeward reefs differ in other characteristics that might influence the timing of life history events. For instance, it is possible that food supply varies between these sites, which would have an effect on the growth rates and thus the timing of life history decisions for the animals living in the respective environments (Kerrigan 1994). Alternatively, there may be variability in population recruitment rates between leeward and windward reefs caused by physical differences in the local ocean currents (Sammarco and Andrews 1988; Wolanski and Hamner 1988; Swearer et al. 1999), which would alter the population demographics and potentially lead to differences in selective pressures in the two environments. Whilst I do find support for the prediction that sex change should be more frequent on isolated reefs than on continuous reefs when I look at the leeward side of the study site, the low sample size for isolated reefs in this area means that we can make no inference about these results. More data on isolated, leeward reefs would be required to see if this trend is real.

My finding of less extreme sex ratio bias in populations with a larger proportion of EMMs supports the idea that the presence of EMMs lowers the average reproductive value of males in the population (EMMs plus sex changed males), leading to a lower ratio of females to males being needed to equalise the genetic contribution of males and females to the next generation (Allsop and West 2004a).

As a general observation, my data reveals that there is a high proportion of EMMs present in all populations captured (average = 0.44 ±95% C.I: 0.06), a large overlap in the male and female size distributions (Mean proportion overlap = 0.78 ±95% C.I: 0.06; Figure 2-2) and a relatively high proportion of females in the upper 5th of the population size distributions (Mean proportion of females in upper 5th of size distribution = 0.5 ±95% C.I: 0.1). When taken together these findings could indicate that there is very little sex change taking place in these natural populations of *C. personatus*. Yet, the experiment demonstrates that these animals are indeed capable of changing sex. Why then do we see such low levels of sex change taking place? One potentially important factor is that *C. personatus* exhibits male parental care, where males guard the eggs in their nest until hatching (Cole and Robertson 1988). This could reduce the males potential reproductive rate (Clutton-Brock 1991; Clutton-Brock and Vincent 1991; Kokko and Jennions 2003), and hence reduce selection for sex change (Warner and Lejeune 1985).

Another possible explanation for these patterns in the data may be that there is a high risk of sperm competition for these fish in the atoll fringing reef environments of the present study. Certainly, the very presence of the high numbers of small males in the lower size categories would indicate the opportunity for high levels of sperm competition to take place, through sneak, streak or group spawning tactics (Gross 1982, 1991). Such high risks of sperm competition can act to reduce the reproductive value (potential future gains in reproduction) of following the classical size advantage route, and changing sex when old and/or large (Munoz and Warner 2003). Thus, it is also possible that the large overlaps in sex-size distributions observed across populations are the product of small females changing sex rather than the

largest females. Such a strategy could be selected for if there are opportunities for engaging in sperm competition and using 'small male interference reproductive strategies' (Munoz and Warner 2003). However it should also be mentioned that my method for estimating the proportion of EMMs (using the logistic regression) and my measures of sexual size dimorphism (proportion overlap in size distribution of the sexes) may be sensitive to other factors that can affect the degree of sexual size dimorphism, such as the effects that variation in the population density can have on intra-sexual competition (and thus male size) and on levels of food competition between the sexes, leading to sex specific selection on body size to reduce dietary overlap (Stamps et al. 1997).

Considering all potential factors that might disrupt a large male's reproductive value begs the question 'Why bother changing sex at all?'. One explanation may be that large males are able to care for many egg clutches at the same time, as is observed in many demersal spawning fish species (Williams 1975; Blumer 1979; Clutton-Brock and Vincent 1991; Reynolds and Jones 1999; Kokko and Jennions 2003). Thus, if a female's reproductive potential is limited by her rate of egg production, but parental males can care for many females' eggs, the detrimental impact of paternal care on the male's reproductive value may be ameliorated considerably. Alternatively, sex change can be selected for in smaller females in order to exploit any opportunity to become involved in sperm competition, as discussed above, and not selected for in larger females because of the risk of sperm competition reducing the value of being a large male. It may be that the variation that we observe in the proportion of EMMs, in the percentage of sex-size overlap and in the proportion of large females in the populations in this study represent points along a continuum of the various forces selecting for sex change.

Chapter 3. Sex ratio evolution in sex changing animals.

3.1. Abstract

Sex allocation theory is often able to make clear predictions about when individuals should facultatively adjust their offspring sex ratio (proportion male) in response to local conditions, but not about the consequences for the overall population sex ratio. A notable exception to this is in sex changing organisms, where theory predicts that: (1) organisms should have a sex ratio biased towards the 'first' sex; (2) the bias should be less extreme in partially sex changing organisms, where a proportion of the 'second' sex mature directly from the juvenile stage, and (3) the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species. I tested these predictions with a comparative study using data from 121 sex changing animal species spanning 5 phyla, covering fish, arthropods, echinoderms, molluscs, and annelid worms. I found support for the first and third predictions across all species. The second prediction was supported within the protogynous species (mainly fish), but not the protandrous species (mainly invertebrates).

3.2. Introduction

Sex allocation theory describes how organisms should divide their resources between male and female reproduction (Charnov 1982a). Some of the most striking successes of sex allocation theory have been in explaining cases in which individuals facultatively adjust their offspring sex ratios (proportion male) in response to local conditions (Charnov 1982a; Hardy 2002; West et al. 2002a), as originally suggested by Trivers and Willard (1973). For example, numerous parasitic wasps have been shown to lay male eggs on relatively small hosts and female eggs on large hosts, presumably because female offspring gain a greater fitness benefit from extra resources and larger body size (West and Sheldon 2002). In contrast, when such facultative sex ratio adjustment occurs, sex allocation theory has been much less successful in predicting and explaining variation in the overall population or breeding sex ratio (West et al. 2002a). The reason for this is that the population sex ratio is often predicted to depend upon biological details that are rarely known, such as the details of male and female life histories, and whether other behaviours such as clutch size are also facultatively adjusted (Frank 1987; Frank 1990; Pen and Weissing 2000, 2002; West and Sheldon 2002).

Here I consider a case in which it is possible to make clear predictions for the overall population sex ratio. I am concerned with species in which sex change occurs, also termed sex reversal or sequential hermaphroditism. This has been documented in a variety of fish, invertebrates and plants (Charnov 1982a; Policansky 1982; Allsop and West 2003a). Sex allocation theory suggests that sex change is favoured when: (a) the reproductive value of an individual varies with age or size, and (b) the relationship is different for males and females. In this case natural selection favours individuals who mature as the sex whose reproductive value increases more slowly with age (first sex), and then change to the other sex (second sex) when older (Ghiselin 1969; Leigh et al. 1976.; Charnov 1982a; Warner 1988a, b).

Sex allocation theory is able to make three predictions for the overall breeding sex ratio in species where sex change occurs (See section 3.2.1). First, the sex ratio

should be biased towards the sex that individuals mature as, termed the first sex (Charnov 1982a; Frank and Swingland 1988; Charnov and Bull 1989a; Charnov 1993). Second, the sex ratio is predicted to show less extreme biases in partially sex changing species where some individuals mature as the second sex (Charnov 1989). Third, the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species (Charnov 1982). Previous work testing these predictions has been largely anecdotal, and relied primarily upon only a few species from a limited number of taxa, such as a comparison of female first (protogynous) fish with male first (protandrous) shrimps (Charnov 1982a; Charnov and Bull 1989a; Charnov 1993). Comparisons between limited numbers of taxa can be a problem because other phylogenetically conserved differences can lead to misleading correlations (Harvey and Pagel 1991). Closely related species tend to share many characters through common descent rather than independent evolution, and so differences in the sex ratios of fish and shrimps, for example, could be explained by other factors that also differ between them.

Here I carry out a quantitative test of these three predictions for the population sex ratios of sex changing species. I expand upon previous work in two ways. First, I use a taxonomically comprehensive data set with 121 animal species, spanning a wide range of taxa, including fish, crustaceans, molluscs, echinoderms and annelid worms. Second, I carry out the first phylogenetically based comparative tests of the theoretical predictions. I construct a 'super-tree' of relationships for sex changing animals combining information from a wide variety of published sources that use both taxonomic and phylogenetic information. I map the direction of sex change and presence of early maturers onto this tree, which allows me to test the predictions of sex allocation theory using the comparative methodology of independent contrasts (Felsenstein 1985). Before describing my work, I explain the theoretical predictions that I am testing.

3.2.1. Sex change theory

Firstly, why is the sex ratio predicted to be biased towards the sex that individuals mature as first (Charnov 1982a; Frank and Swingland 1988; Charnov and Bull 1989a; Charnov 1993; Charnov and Skuladottir 2000)? This can be explained following Charnov (1993). Consider the case of a protogynous diploid species, where individuals mature as females and then change sex to males when older (bigger). In this case the relative fitness of males increases faster with age than it does for females. Males and females must make an equal genetic contribution to the next generation, because all offspring have two parents. Consequently, it must be true that

$$N_m W_m = N_f W_f \qquad\qquad (1)$$

where N_m and N_f are the number of mature males and females, and W_m and W_f represent the reproductive value (fitness) of a male and a female. Given that the reproductive value of a male at the point of sex change will be equal to that of a female, and that male reproductive value increases faster with age, it follows that $W_m > W_f$, because any individual that has become a male must have a higher fitness than individuals that are still female. Consequently, for equation 1 to hold it also follows that $N_m < N_f$. This means that there will be more females than males, and hence a female-biased sex ratio. The converse prediction for protandrous (male first) species can equally be made, showing that a male-biased sex ratio is predicted.

Second, why should the sex ratio be less biased if 'early maturers' of the second sex are present (Charnov 1989)? Consider a protogynous population (changes sex from female to male) with a proportion of males arising directly from the juvenile state without prior transition through the female phase (termed 'early maturers' or 'pure males'). The males in the population will therefore be divided between early

maturing males and those matured as females, but then changed sex to male, termed terminal phase males.

On average, terminal phase males will have a higher reproductive value than early maturing males, mating with more females per unit time. This must be the case because sex change is only stable if male reproductive value increases substantially with age (Ghiselin 1969; Charnov 1982a; Warner 1988a, b). As early maturing males have a lower reproductive value than terminal phase males, the presence of early maturing males lowers the average reproductive value of males (W_m). Consequently, a lower ratio of N_f / N_m will be required to satisfy equation 1, leading to a less female-biased sex ratio. The converse prediction for protandrous (male first) species can equally be made, with the presence of early maturing females leading to a less male-biased sex ratio.

Third, why should protogynous (female first) species have more biased sex ratios than protandrous (male first) species (Charnov 1982a). In invertebrates and fish, female fecundity almost always increases rapidly with size, often following a cubed relationship (Charnov 1982a; Charnov 1993). This means that differences in the direction of sex change are determined primarily by variation in how male fitness varies with size: in protogynous species male fitness must increase even more rapidly with size, whereas in protandrous species male fitness increases little or nothing with larger body size (Charnov 1982a; Warner 1984). Consequently, if we assume that the fitness of individuals at the size of sex change is 1.0, then in protandrous species the average W_m will be approximately or just less than 1.0, and the average W_f will be much greater than 1.0. In contrast, with protogynous species, the average W_m will be much greater than 1.0 and the average W_f will be much lower than 1.0. This leads to a greater difference between W_m and W_f in protogynous species, and so according to equation 1, a more biased sex ratio.

3.3. Methods

3.3.1. Data collection

I gathered sex ratio data for sex changing animals by: (1) performing ISI Web of Science database searches using keywords "sex change" + "sex ratio"; (2) searching in key reviews on the topic (Reinboth 1975; Robertson and Warner 1978; Warner and Robertson 1978; Policansky 1982; Charnov and Bull 1989a); (3) searching citations in all papers found; (4) directly from the field, for the marine goby *Coryphopterus personatus* as part of a larger study (see Chapter 2); (4) utilising data from previous comparative studies on life history variables in sex changing organisms (Allsop and West 2003a, b, 2004a). In some studies authors determined sex by macroscopic observation of the genitalia or secondary sexual characters (Cole 1983; Abe and Fukuhara 1996). In others, sex was determined by histological examination of the gonads (Gillanders 1995; Brule et al. 1999). In all cases, I have only included data for the sexually mature members of any populations sampled, and so the analysis does not include juveniles, as assumed by theory.

For the purposes of investigating the impact of mixed populations (sex changers and 'early maturers' together) on the sex ratio, I carried out the analysis in two ways. I first followed (Charnov 1989) by categorising a species as a pure sex changer (not mixed) if the proportion of early maturing males or females (animals of the 'second' sex in the initial phase) was less than 2%. However, the proportion of primary individuals has been quantified in only a limited number of species, mainly protogynous fish. Consequently, to examine this question more generally, I also carried out separate analyses, assigning mixed or pure sex changing status based upon the authors' description of the organisms' life history – specifically, whether or not the presence of any early maturing individuals had been noted. For example, Pollock's study on the protandrous yellowfin bream, *Acanthopagrus australis* (Pollock 1985), states that "..most juveniles become functional males by the age of two years but a small proportion of juveniles develop directly into functional females

(primary females)." Whilst Pollock goes on to suggest the possible developmental origin of these small females, he does not at any point quantify their proportion in the population. In cases such as this we assign the species as being mixed (having early maturers of the second sex), for our second analysis. Alternatively, if a study presents sex/size frequency data, and highlights that there is no overlap between the large males (females) and small females (males), such as is discussed in (Lowry and Stoddart 1986) for populations of the amphipod *Acontiostoma marionis*, I assigned such species as being pure sex changers (having no early maturers). Whilst it is certainly possible that other populations of these species might show plasticity in the production of early maturers in different locations, I believe the assignment of mixed or pure status using these criteria for the populations under study here is appropriate for the current analysis.

We excluded data for the anemone fish (Amphiprioninae) from all analyses, as the sex ratio has been argued to be extrinsically constrained to 0.5. This is because the size and spatial distribution of their host anemones necessitates the formation of monogamous pairs (Fricke and Fricke 1977). This is analogous to the situation in apicomplexan (protozoa) parasites, where syzygy leads to a form of monogamy and selection for an unbiased sex ratio even with high levels of inbreeding (West et al. 2000b; West et al. 2002b).

3.3.2. Phylogenetic relationships

I obtained relationships within the invertebrates from the following sources: (i) the Crustaceans from Brook et al. (1994) and Tsai et al. (1999); (ii) the Echinoderms from Sewell (1994); (iii) relationships amongst the other invertebrate taxa from the Tree of Life web project (http://tolweb.org/tree/phylogeny.html), as I was unable to locate recent published records of relationships. These invertebrate phylogenies are based primarily upon morphological characters.

For the phylogenetic relationships within fish, I was able to use recently published information in most cases. Specifically, relationships for the: (i) Pomacentridae were

inferred from Godwin (1995), based upon morphology and biogeography; (ii) Lethrinidae were obtained from Lo Galbo et al.(2002), who generated a molecular phylogeny using the cytochrome b gene; (iii) Scaridae relations were taken from Streelman et al. (2002), who used nuclear and mitochondrial DNA genes; (iv) Labridae were obtained from Hanel et al. (2002), and Westneat et al. (pers. comm. 2002), based upon morphological characters as well as mitochondrial and nuclear DNA; (v) Sparidae relations were inferred from Hanel and Sturmbauer (2000), Orrell et al. (2002), Desdevises et al. (2002), and from Hanel (pers.comm. 2003). In cases where published information was not available, and for higher-level relationships, we used Nelson (1994).

Whilst the tree of relationships is constructed from a combination of taxonomic and phylogenetic information, and hence suffers from the problem of housing paraphyletic groups (Starck 1998), such as invertebrates, crustacea and fish, it is the first attempt we know of to comprehensively investigate the evolutionary pathways of the sex change life history strategy. As such, it allows the best current effort to investigate the evolution of the sex ratio in sex changers whilst controlling for the confounding effects of evolutionary relatedness.

3.3.3. Statistical analyses

I analysed the data using two methods: assuming species were independent data points (SI), and independent contrasts (IC). The pros and cons of different comparative methods have been much debated, and a recent review focused on sex allocation is provided by Mayhew and Pen (2002). I analysed the data with two methods because: (1) the theoretical predictions are in some cases for specific values of the sex ratio (i.e. > or < 0.5) – this can only be assessed with SI, as IC test for correlations and relative differences, not specific values (see West et al. (2000b) for further discussion on this point); (2) this is necessary to show how differences with previous conclusions (Charnov 1989; Charnov and Bull 1989a) arise due to either my expanded data set or methods of analysis; (3) I wish to test how robust our results are to different forms of analysis; (4) differences and similarities between the

conclusions drawn from these different analyses can be very informative (reviewed by Mayhew and Pen 2002) - for example, they can show the extent of phylogenetic effects (Pagel 1993), and whilst IC examines evolutionary correlations, SI examines trends in extant characters that are the product of these evolutionary correlations (Mayhew and Pen 2002). More generally, studies of the sex ratio have provided some of the best examples of the adaptive process in action, often with staggering fits between theoretical predictions and empirical data (West and Herre 2002), and considerable inroads have been made towards defining the problem in a phylogenetic context (Herre et al. 2001; Mayhew and Pen 2002). However, the present study is the first such study to address the evolution of the sex ratio in sex changing organisms using a phylogenetically based comparative method.

I first analysed the data assuming that species were independent data points. I am interested in the sex ratio, defined as the proportion of individuals that are male. Proportion data such as sex ratio usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems, I first analysed the data with a generalized linear model analysis of deviance, assuming binomial errors, and a logit link function (Crawley 1993; Wilson and Hardy 2002). However, the data were highly overdispersed, with the residual deviance being 174 - 252 times the residual degrees of freedom (this ratio is the heterogeneity factor), suggesting that a binomial error structure was not appropriate. Consequently, I arcsine transformed the sex ratio data, confirmed that the error variance was normally distributed using the Kolmogorov-Smirnov test for normality ($\chi^2 = 1.34$, p > 0.1), and carried out the analysis using standard ANOVA and ANCOVA. All mean sex ratio results presented are back transformed. I tested the extent of sex ratio deviation from 0.5 by calculating a 'mean magnitude of deviation' (MMD) for each species (i.e. for protandrous species, MMD = sex ratio – 0.5; for protogynous species, MMD = 0.5 – sex ratio). The sex ratio deviation data fitted the assumption of normal error distribution without the need for any further transformation (Kolmogorov-Smirnov test for normality: $\chi^2 = 2.4$, p > 0.1). I investigated the effects of pure and mixed populations on the sex ratio in three ways: performing ANOVA using pure/mixed

status assigned using the two methods above as a dichotomous predictor, and by treating the proportion of 'early maturers' as a continuous predictor.

I also analysed the data using a phylogenetically based comparative method. There are a variety of different statistical methods for removing the effects of evolutionary relatedness from cross-species analyses. These include the phylogenetic generalised least squares method (Grafen 1989), the method of independent contrasts (Felsenstein 1985), the phylogenetic eigenvector regression (Diniz-Filho et al. 1998) and the autoregressive method (Cheverud and Dow 1985). Whilst the debate over which method is the best to use is still active (Rohlf 2001), I chose to use the most commonly applied technique, the method of independent contrasts (Felsenstein 1985), as implemented in the CAIC statistical package (Purvis and Rambaut 1995). Independent contrasts (ICs) are derived by calculating the difference in the response and the explanatory variables across pairs of species, or higher nodes that share a common ancestor. The CAIC package uses the method of Pagel (1992) for continuous variables and Burt (1989) for dichotomous variables. I tested for significance in the dichotomous analyses using a one-sample t-test, comparing the mean of the standardised contrasts to 0. In all cases, unless otherwise stated, the data fitted the assumption of normality (Mayhew & Pen 2002). I assigned branch lengths using both the Grafen (1989) and Pagel (1992) methods – in all cases these gave the same result, and so I have only reported analyses using the Pagel method. The species relationships that we used for the independent contrasts analysis are given in Figure 3-1.

3.4. Results and Discussion

My dataset represents 196 separate populations from 121 species spanning 26 families from 5 phyla (Annelida, Mollusca, Arthropoda, Echinodermata and Chordata; see Figure 3-1. and Table 1). Broadly speaking, I find protogynous (female to male) sex change within the vertebrates, and protandrous sex change in the invertebrate taxa. Notable exceptions to these generalities are as follows. Within the vertebrates (which are all fish) the families Sparidae (Porgies) and Pomacentridae

(Damselfishes) have both protandry and protogyny; the sole representative from the family Polynemidae (Threadfins) is protandrous; the Gobiidae (Gobies) and Cirrhitidae (Hawkfishes) (no members of the Cirrhitidae family are represented in the current analysis) are now known to have species capable of switching back and forth between the sexes (both ways sex change); and the Clupeidae (Herring, Sardines etc.) are protandrous. Within the invertebrates the Peracaridan crustaceans (containing the isopods, amphipods and tanaidaceans) have both protogynous and protandrous species; within the order Isopoda (woodlice and relatives) there are both protandrous and protogynous species; and the sex changers in the crustacean order Tanaidacea are all protogynous. Where information on the presence or absence of early maturers was available, there appeared to be no clear pattern for its distribution throughout the taxonomic groups, with both mixed and pure populations occurring in all major phyla except for the Mollusca, which appear to have no recorded instances of early maturing individuals of the second sex.
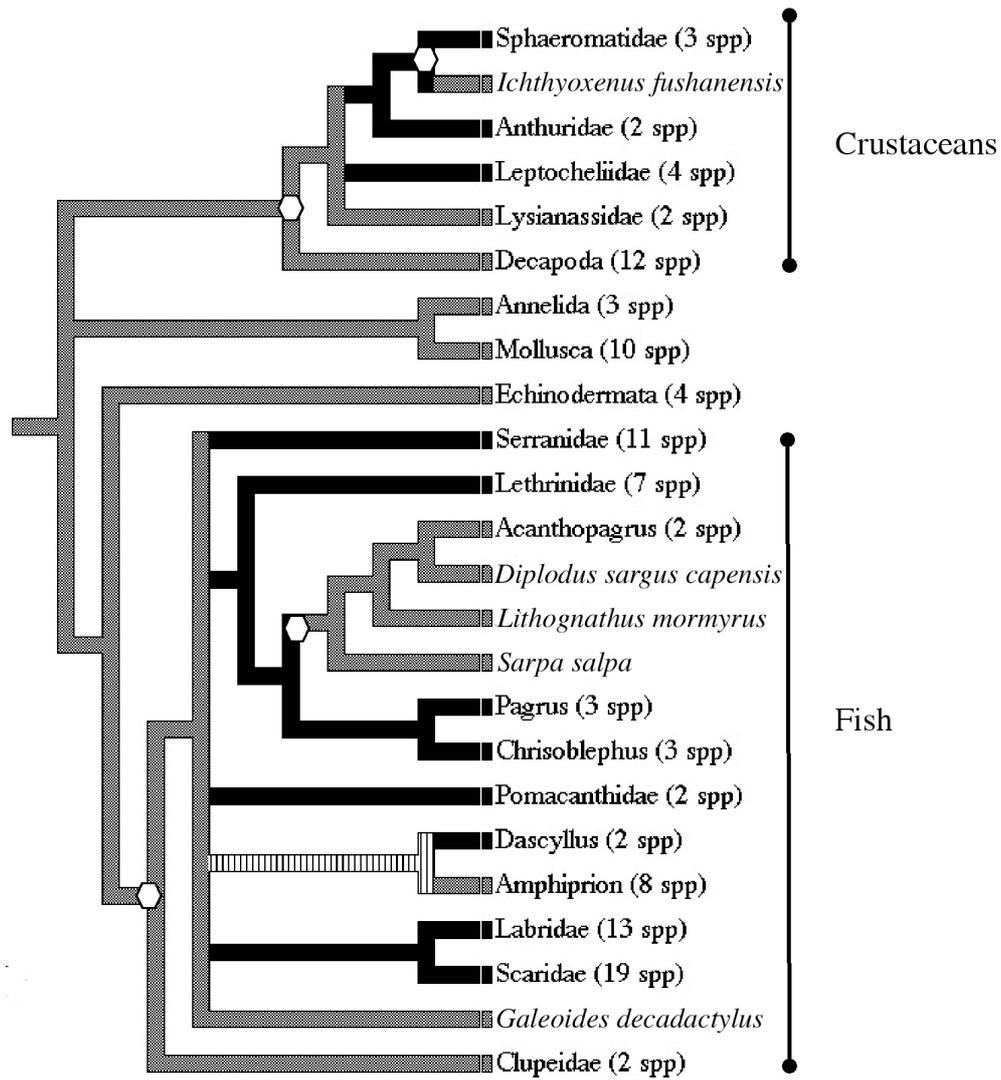
Figure 3-1. A composite phylogeny for sex changing animals.
Black = female first (protogynous), Grey = male first (protandrous), Vertical lines = equivocal.
Branch lengths do not represent evolutionary time. Independent Contrasts for the direction of sex
change are marked on as white hexagons.

3.4.1. Direction of sex change and the sex ratio

I first tested the prediction that the sex ratio should be biased towards the first sex (Charnov 1982a; Frank and Swingland 1988; Charnov and Bull 1989a; Charnov 1993). All our analyses supported this prediction. Assuming species as independent data points: (1) the sex ratio of protogynous (female first) animals is significantly female biased (one sample t-test comparing sex ratio to 0.5: $t = 4.1$, d.f. $= 72$, $p < 0.001$), with a mean of 0.32 (SE $= 0.02$, n $= 73$); (2) the sex ratio of protandrous (male first) species were significantly male biased ($t = 10.9$, d.f. $= 39$, $p < 0.001$), with a mean of 0.57 (SE $= 0.03$, n $= 40$; Figure 3-2). This difference between groups is highly significant, with the sex ratio of protogynous species being more female biased than that of protandrous species ($F_{1, 111} = 59.4$, $p < 0.001$). Overall, 96% of protogynous species had a female biased sex ratio and 70% of protandrous species had a male biased sex ratio (Figure 3-2).

Protogynous Species

Protandrous Species
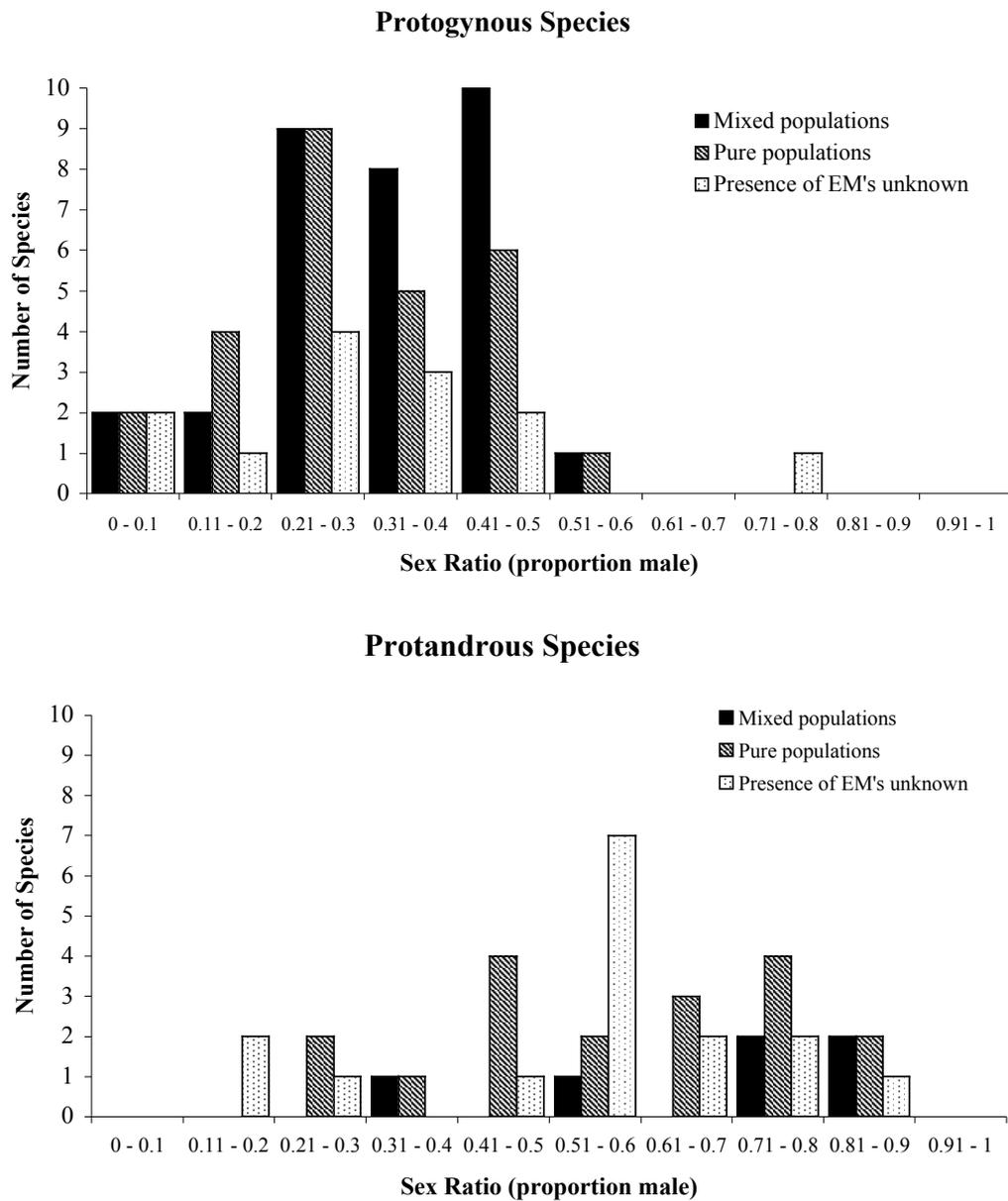
Figure 3-2. Frequency distribution of population sex ratios for protogynous and protandrous sex changers.
Histograms are split between pure sex changing species (with no early maturers of the second sex), mixed sex changing species (with a proportion 'early maturers' of the second sex) and species for which the presence or absence of 'early maturers' is unknown. (Figure excludes 8 species of anemone fish – see methods for details)

This result was confirmed by a comparative analysis with independent contrasts. In this case the hypothesis under test is that the sex ratio of protogynous taxa should be more female biased than the sex ratio of protandrous sister taxa. The phylogeny of sex changing animals reduced to four independent contrasts in which the direction of

sex change differs between sister taxa (Figure 3-1). As predicted, in all cases the species that change sex from female to male were more female biased than the sister taxa that changed from male to female (Figure 3-3). This difference was statistically significant, with the mean of the standardised contrasts being below 0 ($t = -4.3$, d.f. = 3, $p < 0.05$, n = 4 Independent Contrasts (ICs)).
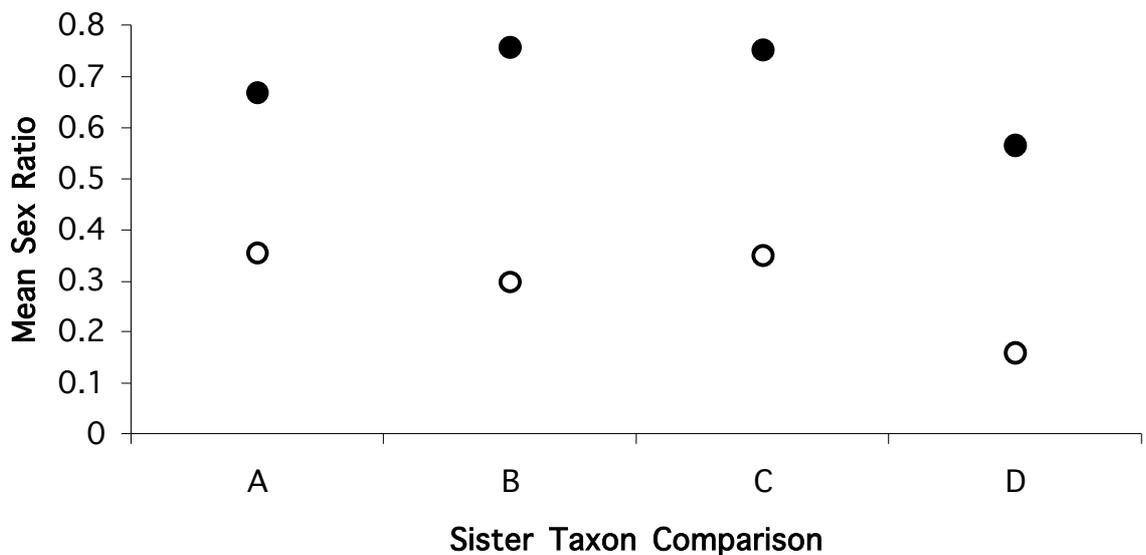
Figure 3-3. Independent contrasts for mean sex ratios of sex changing taxa.
Showing protandrous (male first; filled circles) and protogynous (female first; unfilled circles) animals separately. All protogynous animals have a sex ratio below that of the protandrous animals, as predicted by theory. Letters along the X axis refer to the taxanomic groups being contrasted in our formal comparative analysis: A = Sparidae fish (5 protandrous species, 6 protogynous species), B = Flabelliferan Isopods (1 protandrous, 3 protogynous), C = Teleost fish (other than those in the families Sparidae and Pomacentridae, for whom lower level contrasts are computed; 3 protandrous, 56 protogynous), D = Peracaridan crustaceans (other than those included in the lower level contrast described above in letter C; 2 protandrous species, 6 protogynous species)**.**

3.4.2. Early maturers and the sex ratio

I then tested the prediction that the sex ratio should be less biased in partially sex changing species where a proportion of individuals mature directly into the second sex (Charnov 1989). I found support for this in the protogynous (female first) species, but not in the protandrous (male first) species (Figures 3-2 & 3-4).
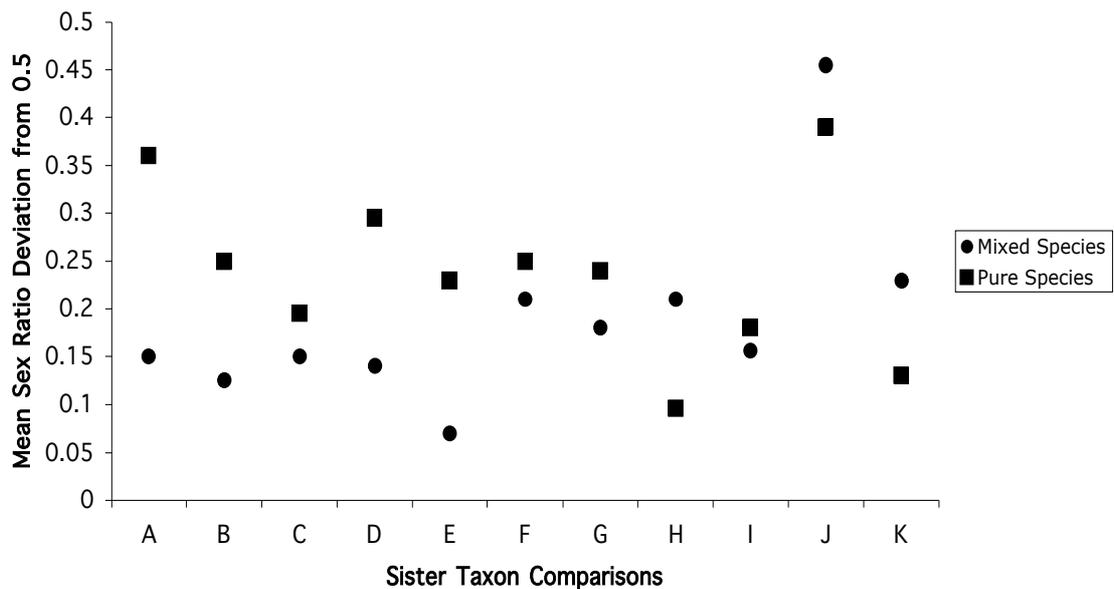
Figure 3-4. Independent contrasts for the extent of sex ratio bias from 0.5 in all protogynous species (vertebrates and invertebrates).
Showing mixed species (those with Early Maturers of the second sex present; circles) against pure species (those with no Early Maturers present; squares). There is a significant trend for pure sex changing species to have a larger sex ratio deviation from 0.5 than species with early maturers. Letters along the X axis refer to the taxanomic groups being contrasted in our formal comparative analysis: A = Sub-branch of the Labridae fish family containing Semicossyphus pulcher and Bodianus rufus (one mixed taxa, one pure taxa), B = Sub-branch of the Labridae fish family containing genus Halichoerus and species Labroides dimidiatus (one mixed taxa, one pure taxa), C = Sub-branch of the Scaridae fish family containing the genus Scarus (ten mixed taxa, two pure taxa), D = Sub-branch of the Scaridae fish family containing the genus Sparisoma (five mixed taxa, two pure taxa), E = Sub-branch of the Serranidae fish family (multiple node) containing the genus Epinephelus (one mixed taxa, one pure taxa), F = Sub-branch of the Serranidae fish family (multiple node) containing the genus Epinephelus (one mixed taxa, one pure taxa), G = Sub-branch of the Serranidae fish family containing Pseudoanthias squamipinnis and Plectropomus leopardus (one mixed taxa, one pure taxa), H = The fish family Lethrinidae (one mixed taxa, eight pure taxa), I = the fish family Gobiidae (one mixed taxa, one pure taxa), J = the Peracaridan crustaceans Hargeria rapax and Leptochelia forresti and Leptochelia dubia (two mixed taxa, one pure taxa), K = root of the tree (one mixed taxa, one pure taxa).

Considering protogynous fish, sex ratios were less female biased in species with early maturing males. This result held when using species as data points (Using 2% rule: Species with early maturers mean magnitude of deviation from a sex ratio of 0.5 (EM MMD) = 0.12, SE = 0.02, n = 20; Pure sex changers mean magnitude of deviation (Pure MMD) = 0.22, SE = 0.03, n = 20; $F_{1, 38}$ = 6.27, p < 0.05; Relaxing 2% rule: EM MMD = 0.14, SE = 0.02, n = 30; Pure MMD = 0.21, SE = 0.03, n = 26; $F_{1, 54}$ = 4.4, p < 0.05), or when using IC's (Using 2% rule: only 3 contrasts, and so no test; Relaxing 2% rule: t = 2.9, d.f. = 8, p < 0.05, n = 9 ICs). In the fish species where the proportion of 'early maturers' had been quantified, I also tested whether the variation in the proportion of 'early maturers' could be used as a continuous predictor of the sex ratio. In this case there was no significant correlation between the sex ratio and the proportion of early maturing males, irrespective of whether species ($F_{1, 41}$ = 0.68, p > 0.1, r^2 = 0.02, n = 43) or independent contrasts ($F_{1, 19}$ = 0.64, p > 0.1, r^2 = 0.03, n = 19 ICs) were used as data points.

Examining all protogynous species (vertebrates and invertebrates together), there was no significant trend for species with early maturers to have a less biased sex ratio when data were analysed with species as data points (2% rule relaxed: EM MMD = 0.16, SE = 0.02, n = 33; Pure MMD = 0.21, SE = 0.03, n = 27; $F_{1, 58}$ = 0.08, p > 0.1), but there was a significant effect with the method of independent contrasts (t = 2.3, d.f. = 10, p < 0.05, n = 11 ICs; Figure 3-4). This difference of results between the IC and the SI methods of analysis reflects the fact that sister taxa that differ in the presence or absence of early maturers do have significant differences in the magnitude of sex ratio bias, but these differences are hidden in a simple SI analysis because there is so much variation in the magnitude of sex ratio bias when all taxonomic groups in the dataset are considered together. This provides a clear example of exactly the kind of problem that the method of IC can deal with (Harvey & Pagel 1991). To my knowledge, this is the first time that such a discrepancy has been found in comparative work on sex allocation, as previous analyses have usually found qualitatively and quantitatively identical results with IC and SI analyses (Herre et al. 2001; Mayhew and Pen 2002).

Examining the protandrous species, there was no significant trend for species with early maturers to have a less biased sex ratio, when using species as data points (2% rule relaxed: EM MMD = 0.17, SE = 0.08, n = 6; Pure MMD = 0.08, SE = 0.04, n = 18; $F_{1, 22}$ = 0.88, p > 0.1). Indeed the method of independent contrasts even hints at the trend being reversed in protandrous animals, with mixed species having more biased sex ratios (t = 4.8, d.f. = 2, p < 0.05, n = 3 ICs), although this is based upon only three IC's.

Consequently, my results support Charnov's (1989) prediction that species with early maturers should have less biased sex ratios when considering protogynous species (mainly fish), but not when considering protandrous species (mainly invertebrates). Furthermore, whilst protogynous species show a difference between species with and without early maturers, there was no significant effect of the proportion of early maturers. Possible explanations for these differences fall into three broad categories. First, the sex ratio is predicted to depend upon a number of factors (Charnov 1982a; Frank and Swingland 1988; Charnov and Bull 1989a; Frank 1998; Charnov and Skuladottir 2000), and so the effect of proportion of early maturers may be confounded by other variables. Analysing the data with independent contrasts can get around this problem (Harvey & Pagel 1991; Mayhew & Pen 2002), as my analyses have shown, but in other cases limited phylogenetic resolution severely limited statistical power (e.g. n = 3 ICs in some analyses). It would be extremely useful to create more resolved phylogenies for these species, as well as quantifying the proportion of early maturers in more species, and other factors that may influence the sex ratio. Second, the biology of the protandrous species may differ in important ways from the assumptions of theory. This seems particularly possible with the invertebrates, which have been relatively less well studied. For example: (a) in the Tanaid crustacean *Leptochelia dubia*, males are born with no mouthparts and thus suffer much higher mortality rates than females (Highsmith 1983), in contrast to the constant mortality assumption of theory (Charnov 1993); (b) in the flesh burrowing parasitic isopod *Ichthyoxenus fushanensis,* which inhabits a membranous cavity in the body wall of the freshwater fish *Varicorhinus bacbatulus* (Tsai et al. 1999), resource limitation may lead to a monogamous lifestyle which could constrain the

sex ratio to be 0.5 as with anemone fish (see methods section). Third, there could be some sampling bias with the data collection, possibly due to factors such as the small size of one sex, or relatively extreme habitats. This possibility is illustrated by the fact that it has been possible to quantify the occurrence of early maturing individuals only rarely in some species (especially invertebrates), compared with others (some fish).

3.4.3. Sex ratio bias: protogynous versus protandrous species

Finally, I tested Charnov's (1982) prediction that the sex ratio should be more biased in protogynous (male first) than in protandrous (female first) species. I found some, but not complete, support for this prediction. When considering species as data points, the mean magnitude of the deviation from 0.5 was significantly greater for protogynous species (MMD = 0.19, SE = 0.02, n = 73), than it was for protandrous species (MMD = 0.07, SE = 0.03, n = 40) ($F_{1, 111}$ = 13.1, p < 0.001). However, when using the method of independent contrasts, I did not find statistical support for this result (t = 2.13, d.f. = 3, p > 0.1, n = 4 ICs), although our power to test this prediction is limited due to having only four independent contrasts. However the data are in the predicted direction, with three out of four ICs above 0. Clearly, increased phylogenetic resolution and targeted data collection would be extremely useful for expanding the number of IC's that could be used in this analysis, and hence determining if the same result will be obtained as with SI analyses.
In particular, it would be extremely useful to construct molecular phylogenies of the invertebrate species, and to increase resolution within the Teleost fish order Perciformes, which is polyphyletic with poor phylogenetic resolution of the suborders (Lauder and Liem 1983; Asoh and Kasuya 2002)

3.5. Conclusions

I have analysed data on the adult sex ratio in 121 sex changing animal species from five phyla, covering fish, crustaceans, echinoderms, molluscs, and annelid worms (Figure 3-1). I have found support for the theoretical predictions that: (1) the sex ratio is biased towards the sex that individuals first reach reproductive maturity as

(first sex) (Figures 3-2 & 3-3); (2) the sex ratio is less biased in species where there are some individuals who mature early as the second sex, when examining protogynous (female first) species (Figures 3-2 & 3-4); (3) protogynous species show a greater deviation from a sex ratio of equality than protandrous (male first) species (Figure 3-2). In contrast, I did not find support for the prediction that the sex ratio should be less biased in species where there are some individuals who mature early as the second sex, when examining protandrous species. Possible explanations for this discrepancy with theory include multiple factors influencing the sex ratio, theory not matching biology and sampling bias. Furthermore, it suggests that there may be important differences between vertebrate and invertebrate species. Future progress in this area requires increasing phylogenetic resolution, quantification of further factors that may influence the sex ratio, and in particular more detailed species specific studies of protandrous invertebrates.

3.6. Appendix

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
1.VERTEBRATES (Phylum Chordata) Class: Osteichthyes (Bony fish) Subclass: Actinopterygii Division: Teleostei Order:Clupeiformes Family: Clupeidae					
Tenualosa macrura	U**	PD	0.32	1	(Blaber et al. 1999)
Tenualosa toil	U**	PD	0.74	2	(Blaber et al. 1996)
Order Perciformes Family Gobiidae					
Coryphopterus glaucofraenum	U*	PG	0.31	2	(Cole and Shapiro 1992)
Coryphopterus nicholsi	U*	PG	0.38	1	(Cole 1983)
Coryphopterus personatus	U*	PG	0.34	1	(Allsop and West 2004b)
Gobiosoma multifasciatum	U**	PG	0.32	1	(Robertson and Justines 1982)
Family Labridae					
Achoerodus viridis	P	PG	0.05	2	(Gillanders 1995)
Bodianus rufus	P	PG	0.12	1	(Warner and Robertson 1978)
Clepticus parrae	P	PG	0.24	1	(Warner and Robertson 1978)
Coris julis	U	PG	0.28	1	(Lejeune 1987)
Halichoeres bivittatus	M	PG	0.46	1	(Warner and Robertson 1978)
Halichoeres garnoti	M	PG	0.26	1	(Warner and Robertson 1978)
Halichoeres maculippina	M	PG	0.29	1	(Warner and Robertson 1978)
Halichoeres pictus	M	PG	0.24	1	(Warner and Robertson 1978)
Halichoeres poeyi	M	PG	0.42	1	(Warner and Robertson 1978)
Labroides dimidiatus	P	PG	0.25	1	(Robertson and Choat 1974)

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Semicossyphus pulcher	U*	PG	0.35	5	(Warner 1974; Cowen 1990)
Thalassoma bifasciatum	M	PG	0.46	1	(Warner and Robertson 1978)
Thalassoma lunare	M	PG	0.49	1	(Robertson and Choat 1974)
Family Lethrinidae					
Lethrinus choerynchus	P	PG	0.46	1	(Young and Martin 1982)
Lethrinus fraenatus	P	PG	0.24	1	(Young and Martin 1982)
Lethrinus lentjan	U**	PG	0.41	3	(Young and Martin 1982; Grandcourt 2002)
Lethrinus mahsena	U*	PG	0.29	1	(Grandcourt 2002)
Lethrinus nebulosus	P	PG	0.46	1	(Young and Martin 1982)
Lethrinus nematacanthus	P	PG	0.46	1	(Young and Martin 1982)
Lethrinus variegates	P	PG	0.46	1	(Young and Martin 1982)
Family Pomacanthidae					
Centropyge interruptus	U	PG	0.28	1	(Moyer and Nakazono 1978a)
Genicanthus semifasciatus	U**	PG	0.37	1	(Shen and Liu 1976)
Family Pomacentridae					
Amphiprion akallopisos	U	PD	0.50	1	(Fricke 1979)
Amphiprion bicinctus	U	PD	0.50	1	(Fricke 1979)
Amphiprion clarkii	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Amphiprion frenatus	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Amphiprion oscellaris	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Amphiprion perideraion	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Amphiprion polymnus	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Amphiprion sandrasinos	U	PD	0.50	1	(Moyer and Nakazono 1978b)
Dasyllus aruanus	U	PG	0.48	5	(Godwin 1995; Cole 2002)
Dasyllus flavicaudis	U	PG	0.41	3	{Godwin, 1995 #41

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Family Scaridae					
Chlorurus sordidus	M	PG	0.48	1	(Choat and Robertson 1975)
Cryptotomus roseus	M	PG	0.23	1	(Robertson and Warner 1978)
Scarus dubius	M	PG	0.43	1	(Choat and Robertson 1975)
Scarus festivus	M	PG	0.46	1	(Choat and Robertson 1975)
Scarus frenatus	M	PG	0.33	1	(Choat and Robertson 1975)
Scarus ghobban	U*	PG	0.22	1	(Grandcourt 2002)
Scarus globiceps	M	PG	0.46	1	(Choat and Robertson 1975)
Scarus iserti	M	PG	0.18	1	(Robertson and Warner 1978)
Scarus niger	P	PG	0.38	1	(Choat and Robertson 1975)
Scarus psittacus◆	M	PG	0.35	2	(Choat and Robertson 1975)
Scarus rivulatus	M	PG	0.42	1	(Choat and Robertson 1975)
Scarus rubroviolaceus	U*	PG	0.17	1	(Grandcourt 2002)
Scarus vetula	P	PG	0.23	1	(Robertson and Warner 1978)
Sparisoma atomarium	P	PG	0.15	1	(Robertson and Warner 1978)
Sparisoma aurofrenatum	P	PG	0.26	1	(Robertson and Warner 1978)
Sparisoma chrysopterum	M	PG	0.37	1	(Robertson and Warner 1978)
Sparisoma radians	M	PG	0.49	1	(Robertson and Warner 1978)
Sparisoma rubripinne	M	PG	0.42	1	(Robertson and Warner 1978)
Sparisoma viride	M	PG	0.29	1	(Robertson and Warner 1978)
Family Serranidae					
Epinephelus aeneus	U**	PG	0.39	1	(Brusle and Brusle 1975)
Epinephelus andersoni	U*	PG	0.57	1	(Fennessy and Sadovy 2002)

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Epinephelus chlorostigma	U*	PG	0.29	1	(Grandcourt 2002)
Epinephelus guaza	U**	PG	0.34	1	(Brusle and Brusle 1975)
Epinephelus marginatus	P	PG	0.21	1	(Marino et al. 2001)
Epinephelus morio	P	PG	0.16	1	(Brule et al. 1999)
Epinephelus rivulatus	U**	PG	0.21	6	(Mackie 2000)
Mycteroperca bonaci	P	PG	0.06	1	(Crabtree and Bullock 1998)
Plectropomus leopardus	U*	PG	0.32	2	(Ferreira and Russ 1995)
Pseudoanthius squamipinnis	P	PG	0.26	3	(Suzuki et al. 1978; Shapiro 1981b)
Sacura margaritacea	U	PG	0.34	1	(Okada 1965)
Family Sparidae					
Acanthopagrus australis	U*	PD	0.76	1	(Pollock 1985)
Acanthopagrus berda	U	PD	0.52	2	(Tobin et al. 1997)
Chrysoblephus cristiceps	U	PG	0.16	5	(Buxton 1993)
Chrysoblephus laticeps	U	PG	0.38	1	(Penrith 1972)
Chrysoblephus puniceus	U	PG	0.28	1	(Garrat 1986)
Diplodus sargus capensis	U*	PD	0.34	1	(Mann and Buxton 1998)
Lithognathus mormyrus	U	PD	0.56	2	(Kraljevic et al. 1995; Lorenzo et al. 2002)
Pagrus auriga	P	PG	0.21	1	(Alekseev 1983)
Pagrus ehrenbergi	P	PG	0.51	1	(Alekseev 1983)
Pagrus pagrus	P	PG	0.42	2	(Alekseev 1983)
Sarpa salpa	U	PD	0.66	2	(van der Walt and Mann 1998; Villamil et al. 2002)
Family Polynemidae					
Galeoides decadactylus	M	PD	0.82	1	(Longhurst 1965)
2. ECHINODERMS (Phylum Echinodermata)					
Class Asteroidea (Starfish)					
Order Spinulosida Family Asterinidae					
Asterina gibbosa	U	PD	0.54	1	(Bacci 1951)
Patiriella exigua	U	PD	0.11	1	(Byrne 1992)
Class Holothuroidea (Sea cucumbers)					
Order Apodida Family Synaptidae					
Leptosynapta clarki	M	PD	0.83	4	(Sewell 1994)

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Class Ophiuridea (Brittle-stars)					
Order Ophiurida Family Ophiacanthidae					
Ophiacantha bidentata	U	PD	0.30	1	(Tyler and Gage 1982)
3. INVERTEBRATES					
a. Phylum Mollusca					
Class Gastropoda (Slugs, snails & limpets)					
Order Neotaenioglossa Family Aglossa					
Balcis shaplandi	U	PD	0.49	1	(Morton 1979)
Mucronalia fulvescens	U	PD	0.54	1	(Morton 1979)
Subclass Prosobranchia Superorder Caenogastropoda					
Order Neogastropoda Family Coralliophilidae					
Coralliophila violacea	U**	PD	0.65	1	(Soong and Chen 1991)
Order Neotaenioglossa Family Calyptraeidae					
Crepidula adunca	U**	PD	0.63	1	(Collin 2000)
Crepidula convexa	U**	PD	0.49	7	(Hoagland 1978)
Crepidula fornicata	U**	PD	0.62	8	(Hoagland 1978; Collin 1995)
Crepidula lingulata	U**	PD	0.49	1	(Collin 2000)
Crepidula norrisiarium	N	PD	0.59	1	(Warner et al. 1996)
Order Archaeogastropoda Family Acmaeidae					
Lottia gigantean	U**	PD	0.73	3	(Wright 1989)
Order Patellogastropoda Family Patellidae					
Patella vulgata	U	PD	0.75	1	(Charnov and Bull 1989a)
b. Phylum Crustacea					
Class Malacostraca Subclass Eucarida					
Order Decapoda Infraorder Caridea Family Atyidae					
Paratya curvirostris	U	PD	0.81	1	(Carpenter 1978)
Family Hippolytidae					
Thor manningi	U**	PD	0.73	1	(Bauer 1986)
Family Pandalidae					

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Pandalopsis dispar	U**	PD	0.75	4	(Butler 1964)
Pandalus borealis	U**	PD	0.42	1	(Butler 1964)
Pandalus danae	U*	PD	0.53	1	(Butler 1964)
Pandalus goniurus	U	PD	0.14	2	(Butler 1964)
Pandalus hypsinotus	U*	PD	0.71	2	(Butler 1964)
Pandalus jordani	U**	PD	0.59	2	(Butler 1964)
Pandalus montagui tridens	U**	PD	0.29	1	(Butler 1964)
Pandalus platyceros	U**	PD	0.89	3	(Butler 1964)
Pandalus stenolepis	U**	PD	0.26	1	(Butler 1964)
Infraorder Anomura Family Hippidae					
Emerita analoga	U	PD	0.53	1	(Barnes and Wenner 1968)
Subclass Peracarida					
Order Isopoda Family Anthuridae					
Cyathura carinata	U	PG	0.01	1	(Legrand and Juchault 1963)
Cyathura profunda	U	PG	0.78	1	(Kensley 1982)
Family Sphaeromatidae					
Gnorimosphaeroma naktongense	U	PG	0.22	1	(Abe and Fukuhara 1996)
Gnorimosphaeroma oregonense	U*	PG	0.27	1	(Brook et al. 1994)
Paraleptosphaeroma glynni	U	PG	0.34	1	(Buss and Iverson 1981)
Order Amphipoda Family Lysianassidae					
Acontiostoma marionis	U**	PD	0.46	1	(Lowry and Stoddart 1986)
Stomacontion pungapunga	U	PD	0.52	1	(Lowry and Stoddart 1986)
Order Tanaidacea Leptocheliidae					
Hargeria (=Leptochelia) rapax	U*	PG	0.06	1	(Modlin and Harris 1989)
Heterotanais oerstedi	U	PG	0.08	1	(Jazdzewski 1969)tsai
Ichthyoxenus fushanensis	U	PD	0.51	1	(Tsai et al. 1999)
Leptochelia dubia	U*	PG	0.03	4	(Highsmith 1983; Stoner 1986)
Leptochelia forresti	U**	PG	0.11	3	(Stoner 1986)
c. Phylum Annelida					
Class Polychaeta					

SPECIES	Mixed/ Pure pops	Direction of Sex Change	Sex ratio	# Populations Averaged	Reference
Order Phyllodocida Family Syllidae					
Syllis amica	U	PD	0.67	2	(Durchon 1975)
Syllis prolifera	U**	PD	0.84	2	(Durchon 1975)
Syllis vittata	U	PD	0.73	5	(Durchon 1975)

Table 1. Sex ratio and breeding system data for sex changers.
Mixed/Pure Populations column: M=Mixed, P = Pure, U = Unknown (based on Charnov 1989, 2% rule for quantifying the proportion of early maturers of the 'second' sex). If an author stated that a species was mixed, but did not quantify it, we have placed an asterix by the Unknown (U) designation, and if the author stated the species was a pure sex changer, but did not quantify it, we have placed two asterix by the Unknown (U) designation. Direction of Sex Change column: PD=Protandrous (female first), PG=Protogynous (male first).♦Note: also provide data for *Scarus venosus* but is a synonym of *Scarus psittacus* (as is their *Scarus forster*).

Chapter 4. Constant relative age and size at sex change for sequentially hermaphroditic fish

4.1. Abstract

A general problem in evolutionary biology is that quantitative tests of theory usually require a detailed knowledge of the underlying trade-offs, which can be very hard to measure. Consequently, tests of theory are often constrained to be qualitative and not quantitative. A solution to this problem can arise when life histories are viewed in a dimensionless way. Recently, dimensionless theory has been developed to predict the size and age at which individuals should change sex. This theory predicts that the size at sex change / maximum size (L_{50}/L_{max}), and the age at sex change / age at first breeding (τ/α) should both be invariant. I found support for these two predictions across 52 species of fish. Fish change sex when they are 80% of their maximum body size, and 2.5 times their age at sexual maturity. This invariant result holds despite a 60 and 25 fold difference across species in maximum size and age at sex change. These results suggest that, despite ignoring many biological complexities, relatively simple evolutionary theory is able to explain quantitatively at what point sex change occurs across fish species. Furthermore, my results suggest some very broad generalities in how male fitness varies with size and age across fish species with different mating systems.

4.2. Introduction

Sex change occurs in a variety of fish, invertebrates and plants (Charnov 1982a; Policansky 1982; Allsop and West 2004a: also termed sex reversal or sequential hermaphroditism). Sex allocation theory provides an explanation for sex change, in terms of which species should do it, and in what direction it should be (i.e. male to female or vice versa: Warner et al. 1975; Charnov 1982a). The general idea is that sex change is favoured when: (a) the reproductive value of an individual is closely related to their age or size, and (b) the relationship is different for the sexes. In this case, selection favours genes that cause an individual to first be the sex whose reproductive value increases more slowly with age, and then change to the other sex at a later stage. Although this theory has been quite successful, tests of at what point sex change should occur, and the consequences for population sex ratios, have generally been qualitative and not quantitative (Charnov 1982a; Policansky 1982; Charnov and Bull 1989b). The reason for this is that quantitative tests require reliable estimates of the underlying trade-offs, which can be difficult to obtain (Charnov 1982a; Frank and Swingland 1988).

Charnov & Skuladottir (2000) have recently shown that a solution to this problem lies in dimensionless life history theory (Charnov 1993). They developed an optimisation model to predict at what point sex change should occur, and then expressed the predictions dependent upon several dimensionless quantities - k/M, $\alpha \cdot M$ and δ. These parameters represent the relative growth rates (k, the Bertalanffy coefficient), the adult instantaneous mortality rate (M), the age at first breeding (α), and the coefficient in the equation relating male fertility to size (δ; where male fertility is proportional to L^{δ}, and L is size). Charnov & Skuladottir showed that populations/species with the same values of these dimensionless quantities are predicted to have the same: (1) size at sex change / maximum size (L_{50}/L_{max}); (2) age at sex change / age at first breeding (τ/α); (3) breeding sex ratio, defined as the proportion of breeders that are male. Several comparative studies have suggested that k/M and $\alpha \cdot M$ are invariant within taxa (reviewed by Charnov 1993).

Consequently, Charnov & Skuladottir's three predictions will hold whenever δ is similarly invariant. Importantly, the predictions can be tested quantitatively without a detailed knowledge of the relationship between size and male fertility (δ) – all that is required is that the relationship is approximately the same shape across the populations studied (e.g. squared or cubed). They provided support for their first prediction, by showing that across populations of a single shrimp species, the size at sex change / maximum size was invariant (Skuladottir and Petursson 1999; Charnov and Skuladottir 2000).

Here I test the first two of Charnov & Skuladottir's invariance predictions in a comparative study across 52 sex changing fish species. The extent to which these predictions are expected to hold across species depends upon variation in δ, the coefficient in the equation relating male size to fertility. We know that δ will vary between species in which sex change occurs from male to female (protogynous) to those in which it is female to male (protandry). The reason for this is that the exponent in the female fitness function is approximately 3 for fish (Charnov, 1993), and so for sex change to be favoured, $\delta<3$ for protandrous species and $\delta>3$ for protogynous species. However, δ may also vary within species that change sex in the same direction, due to factors such as variation in the mating system, or the occurrence of individuals that mature early into the second sex (diandry). For example, in systems where mating opportunities can be monopolised by a few large males, such as harems and leks, one might expect relatively large values of δ compared to species with more open mating systems, such as in non-territorial schooling species or those where large aggregations are formed (Coleman et al. 1996). Consequently, I also test the extent to which the relationships predicted by Charnov & Skuladottir (2000) are influenced by the direction of sex change, mating system and presence of diandry.

4.3. Materials and methods

4.3.1. Data collection

I collected data on the sex-size frequency distribution and the ages at sex change and maturity for 61 populations from 52 different species of sex changing fish (See Table 2 in the appendix to this chapter). Importantly, there is sufficient data to ensure at least one or two representative species for most of the major taxonomic groups containing sex changers (Figure 4-1). We obtained these data from the literature, and by donation from currently active projects in fisheries management. I located relevant studies by: (1) searching the ISI Web of Science database, using the key words "sex change" + "fish" + "population" + "size"; (2) searching citations in all papers found as well as reviews on the topic; (3) directly contacting researchers. The original authors collected their data by a variety of methods, including the use of traps, hook and line fishing, anaesthetics nets, and trawls. Further details on the methodology used in different studies can be found in the relevant articles (see references to Table 2).

FAMILY

FIRST SEX

Serranidae
- *Epinephelus_marginatus*
- *Epinephelus_morio*
- *Epinephelus_rivulatus*
- *Epinephelus_chlorostigma*
- *Plectropomus_leopardus*
- *Mycteroperca_bonaci*
- *Pseudoanthias_squamipinnis*

Lethrinidae
- *Lethrinus_lentjan*
- *Lethrinus_mahsena*
- *Lethrinus_choerorynchus*
- *Lethrinus_fraenatus*
- *Lethrinus_nebulosus*
- *Lethrinus_nematacanthus*
- *Lethrinus_variegatus*

F

Sparidae
- *Sarpa_salpa*
- *Lithognathus_mormyrus*
- *Acanthopagrus_berda*
- *Chrysoblephus_puniceus*

M

Gobiidae
- *Coryphopterus_nicholsi*
- *Gobiosoma_multifasciatum*

F

Pomacanthidae
- *Centropyge_interruptus*
- *Genicanthus_semifasciatus*

Pomacentridae
- *Dascyllus_flavicaudus*
- *Amphiprion_akallopisos*
- *Amphiprion_frenatus*
- *Amphiprion_oscellaris*
- *Amphiprion_perideraion*
- *Amphiprion_polymnus*
- *Amphiprion_sandrasinos*

M

Labridae
- *Semicossyphus_pulcher*
- *Achoerodus_viridis*
- *Bodianus_rufus*
- *Clepticus_parrae*
- *Thalassoma_bifasciatum*
- *Thalassoma_lunare*
- *Halichoerus_bivatattus*
- *Halichoerus_pictus*
- *Halichoerus_garnotti*
- *Halichoerus_poeyi*
- *Halichoerus_maculippina*
- *Labroides_dimidiatus*

F

Scaridae
- *Scarus_iserti*
- *Scarus_vetula*
- *Scarus_ghobban*
- *Scarus_rubroviolaceus*
- *Cryptotomus_roseus*
- *Sparisoma_rubripinne*
- *Sparisoma_atomarium*
- *Sparisoma_aurofrenatum*

Synbranchidae
- *Synbranchus_marmoratus*

Clupeidae
- *Tenualosa_toli*
- *Tenualosa_macrura*

M

Figure 4-1. A composite phylogeny for sex changing fish.

My criteria for inclusion of a species in the study were that: (i) it should be a unidirectional sex changer, and (ii) if it is diandric (having a proportion of animals maturing directly or early into the second sex – which for convenience we shall term 'Early Maturers' or EM's), the dataset should either distinguish between the EM's and the true sex changers, or the population should have a minimal amount of EM's

68

(i.e. sex change is the predominant strategy – e.g. Warner and Hoffman 1980a). These criteria were necessary to fit the assumptions of the theoretical model being tested (Charnov & Skulladotir, 2000), and to allow the required data to be calculated. The model does not make predictions for simultaneous hermaphrodites with sequential allocation patterns (bi-directional sex changers - Kobayashi and Suzuki 1992; Sunobe and Nakazono 1993; St Mary 1997; Munday et al. 1998; St Mary 2000; Munday 2002; Munday and Molony 2002) and so I was unable to include them. The only exception to this is that I have included data for the cleaner wrasse *Labroides dimidiatus* despite the fact that it has recently been demonstrated to have the ability of bi-directional sex change (Kuwamura et al. 2002). I include it on the basis that its normal mode of sequential hermaphroditism is unidirectional, and it would appear that it is rare and difficult for it to go the other way. I would add that this appears to be usual for the reported cases of bi-directional sex change, and that it may well be that many more instances of its occurrence will become apparent with further studies.

The size at sex change (L_{50}= size at which 50% are the second sex (male for protogynous fish, female for protandrous fish)) was calculated for each population using the logistic regression of the proportion of second sex breeders against the population body size distribution. Previous work investigating variation in size at sex change within a species has used the size of the smallest second sex individual as the "size at sex change" (Shapiro 1981a). I chose to use the sigmoidal logistic regression curve, consistent with the method used to develop the theory that I am testing, on the basis that it provides a more statistically rigorous and quantitatively consistent estimation of the population average size at sex change. Several populations were extremely clear outliers, with an L_{50} well out of the range of the natural size distributions – for example, populations of the Lethrinid *Lethrinus lentjan* from the Seychelles (Grandcourt 2002) have overlapping sex size distributions, which I interpret to mean that very little sex change is occurring. In contrast, populations of the same species from the North West shelf of Australia (Young and Martin 1982), show clear evidence of sex change occurring. These 'non sex-changing' populations are not suitable for testing Charnov & Skulladotir's predictions, and so were

eliminated from my analysis (although this variation across populations is obviously very interesting from the perspective of why sex change is favoured).

The maximum size (L_{max}) was recorded for each population. Testing the age invariant predictions required knowledge of the age at maturity (α) and the age at sex change (τ). Fish age is calculated by rearing fish from egg, or more commonly by extracting the otoliths (ear bones) and counting the daily or annual rings therein (Jones 1992). I was able to find adequate age data for 15 species of sex changing fish. I was also able to obtain information on the type of mating system for five of those species.

Data on the breeding sex ratio would require detailed information on the breeding condition of all fish in the population, which ultimately can only be obtained through histological analysis of the gonads of all fish. Hence I was not able to collect this data, and so could not test Charnov & Skuladottir's third invariant prediction.

I classified mating systems on the basis of the potential for mate monopolisation, where information was available. In instances where there is good opportunity for permanent mate monopolisation (e.g. resource or female defence polygyny), I grouped them under the umbrella term Haremic (H). If mate monopolisation occurs temporarily (at a certain time each day for instance) we term these species lekking (L). Monogamous species are termed so (M) and more open mating systems (with potentially less opportunity for mate monopolisation) are termed aggregation spawners (A). The mating system is unknown for many species (U).

4.3.2. Statistical analyses

I tested whether the relative size (L_{50}/L_{max}) and age (τ/α) at sex change are invariant. If these relationships are invariant then a log-log regression of the numerator against the denominator, would give a slope of 1.0. For example, a regression of ln(L_{50}) against ln (L_{max}) would give a slope of 1.0. I carried out these regressions in three ways. First, I carried out regressions using each sample as an

independent data points. Second, I averaged multiple samples from the same species, and carried out a regression using species as independent data points.

Third, I carried out an independent contrasts analysis across species. Species may not be independent data points because they are phylogenetically related (Felsenstein 1985). This leads to problems if a trait is evolutionary conserved or highly correlated with an unknown phylogenetically inert third variable. A formal method to control for this is to carry out an analysis with independent contrasts. Independent contrasts are derived by calculating the difference in the response and explanatory variables across pairs of species or higher nodes that share a common ancestor (Felsenstein 1985; Harvey and Pagel 1991). These contrasts can then be analysed by a regression through the origin; the expected value of the slope through the origin equals the true relationship between the variables in the absence of phylogenetic effects (Pagel 1993). I carried out such an analysis with the CAIC statistical package (Purvis and Rambaut 1995), assuming equal branch lengths.

The phylogeny for the species that I examined was constructed based upon molecular and morphological phylogenies (Figure 4-1). Westneat et al., pers. comm. provided detailed information for the Labridae family, which they obtained using traditional morphological techniques as well as molecular analysis of both mitochondrial and nuclear DNA. Relationships amongst the Sparidae were taken from De la Herran et al. (2001) and Day (2002) who used centromeric EcoRI and subtelomeric DraI satellite DNA families and traditional morphological techniques. Lethrinid relations were obtained from Lo Galbo et al. (2002) who used cytochrome b gene sequence variation, and Scarid relations were taken from Streelman et al. (2002) using nearly 2 kb of nuclear and mitochondrial DNA sequence. We obtained higher order relationships and that of any groups for which we could not readily locate information from Nelson (1994).

I carried out regression analyses using two methods. The use of standard Ordinary Least Squares (OLS) regression is based upon the assumption that the x variable is fixed or controlled by the observer (i.e. no error in the x term). This is the most

commonly used method of estimating the functional relationship between biological variables, and yet its assumptions are often violated, leading to a biased slope estimate. Although I may often be more confident of my estimates of the x variables in our analyses, they are clearly subject to measurement error. A possible solution to this problem is to carry out a Major Axis (MA) regression. MA (or Model II) regression is used when there may be no causal structure between the y and the x variable and they are both measured with equal error. The Major Axis produces a line that minimises the sum of the squared deviations perpendicular to itself. In order to examine any affect of error assumptions, we repeated all analyses using both OLS and MA regression (Gemmill et al. 1999), using SYSTAT 10.2.

4.4. Results

The species presented here span a size range from 27 mm maximum length for the Caribbean goby *Gobiosoma multifasciatum* to 1500 mm maximum length for the Western Atlantic serranid *Mycteroperca bonaci*. The fish range in age at sex change from 9 months for the tropical shad *Tenulosa macrura* to 18 years for the wrasse *Achoerodus viridis*. The analysis contains 52 species representing three orders within the class Actinopterigii, and ten families (see Figure 4-1). Of the 52 species, eleven are protandric (change sex from male to female) (see Table 2 and Figure 4-1). The remaining 41 species are protogynous (change sex from female to male), and of those, ten species are definitely known to have early maturing fish (EM's) of the second sex.

4.4.1. Size invariant

The relationship between L_{50} and L_{max} shows a slope not significantly different from 1 (Figure 4-2). This suggests that the ratio of L_{50}/L_{max} is invariant across species, and that fish are changing sex at a constant proportion of their maximum size. This result holds when the data are analysed with populations as data points (OLS: intercept = -0.22 (± 95% C.I). ±0.24, slope = 0.99 ±0.04, $r^2 = 0.971$, n= 61; Major Axis: intercept = -0.29 ±0.24, slope = 1 ±0.05), species as data points (OLS: intercept = -

0.25 $\pm$ 0.28, slope = 0.99 $\pm$ 0.28, r^2 = 0.967, n=52; Major Axis: intercept = -0.33 $\pm$ 0.28, slope = 1 $\pm$ 0.06), or the method of independent contrasts (OLS: slope = 0.98 $\pm$ 0.098, r^2 =0.982, n=25; Major Axis: slope = 0.98 $\pm$ 0.08). Figure 4-2 shows the OLS regression with species as independent data points. The average value across species of L_{50}/L_{max} was 0.79.

Figure 4-2. A logarithmic plot of the size at sex change (L50) versus maximum size (Lmax) for 52 species of sex changing fish.
Species are as independent data points). The slope of the relationship (0.99) is not significantly different from 1.0 (95% C.I. $\pm$ 0.28; r^2=0.967), suggesting that fish change sex at a constant proportion (0.79) of their maximum size. Filled symbols are for protandrous fish (change sex from male to female), open symbols for protogynous fish (change sex from female to male). The presence or absence of Early Maturing fish of the second sex is shown by the shape of the symbols. A circle represents Diandric species (with EMs present), a triangle represents Monandric species (EMs absent) and a diamond represents fish for which I am unsure of the status for EMs.

When comparing across species, the relationship between L_{50} and L_{max} is not influenced significantly by: (1) the mating system, comparing species that are monogamous, lekking, form harems, or aggregations (ANCOVA, Intercept: $F_{3,25}=0.63$, n.s. ; Slope: $F_{3,25}=0.55$, n.s.); or (2) comparing species in which the first sex is male (protandry) or female (protogyny) (ANCOVA, Intercept $F_{1,48}=3.27$, n.s.; Slope $F_{1,48}=3.03$, n.s.), although these values are close to significance. However, when I looked at the effects of the presence of early maturing individuals of the second sex (i.e. whether a species is monandric or diandric) I did see a significant difference in both the slope and the intercept (ANCOVA, Intercept: $F_{1,46}=5.2$, $p < 0.05$; Slope: $F_{1,46}=4.5$, $p < 0.05$, $r^2=0.971$). However, the separate regression models are still showing a positive relationship between L_{50} and L_{max}, and the slopes of the respective relationships are still not significantly different from 1 (monandrics: Intercept: -0.12 ± 0.28, Slope: 0.98 ± 0.046, N= 40, Diandrics: Intercept: -1.3 ± 1.3, Slope: 1.2 ± 0.25, N= 10). Adding in the presence of Early Maturers as an explanatory variable increases r^2 by 0.004, from 0.967 to 0.971.

4.4.2. Age invariant

Similarly the relationship between τ/α (age at sex change / age at maturity) shows a slope not significantly different from 1 (Figure 4-3). This suggests that the ratio of τ/α (relative age of sex change) is invariant across species, and that fish are changing sex at a constant multiplier of their age at maturity. This result holds when the data are analysed with populations as data points (OLS: intercept = 0.66 ($\pm 95\%$ C.I). ± 0.27), slope = 1.12 ± 0.21, $r^2 = 0.872$, n=21; Major Axis: intercept = 0.56 ± 0.28, slope = 1.22 ± 0.23), species as data points (OLS: intercept = 0.69 ± 0.3, slope = 1.17 ± 0.25, $r^2 = 0.885$, n= 15; Major Axis: intercept = 0.59 ± 0.33, slope = 1.26 ± 0.27) or independent contrasts (OLS: slope = 0.99 ± 0.48, $r^2 = 0.753$, n= 11; Major Axis: slope = 1.3 ± 0.69). Figure 4-3 shows the OLS regression with species as independent data points. The average value across species of τ/α was 2.5.
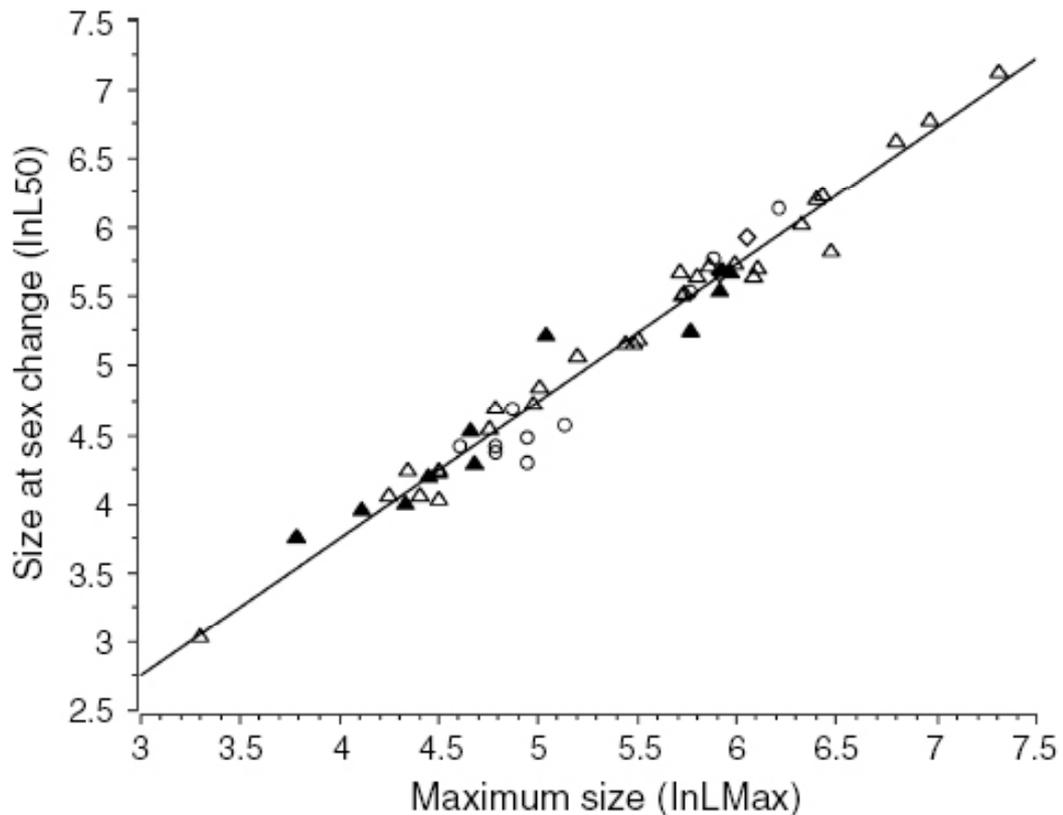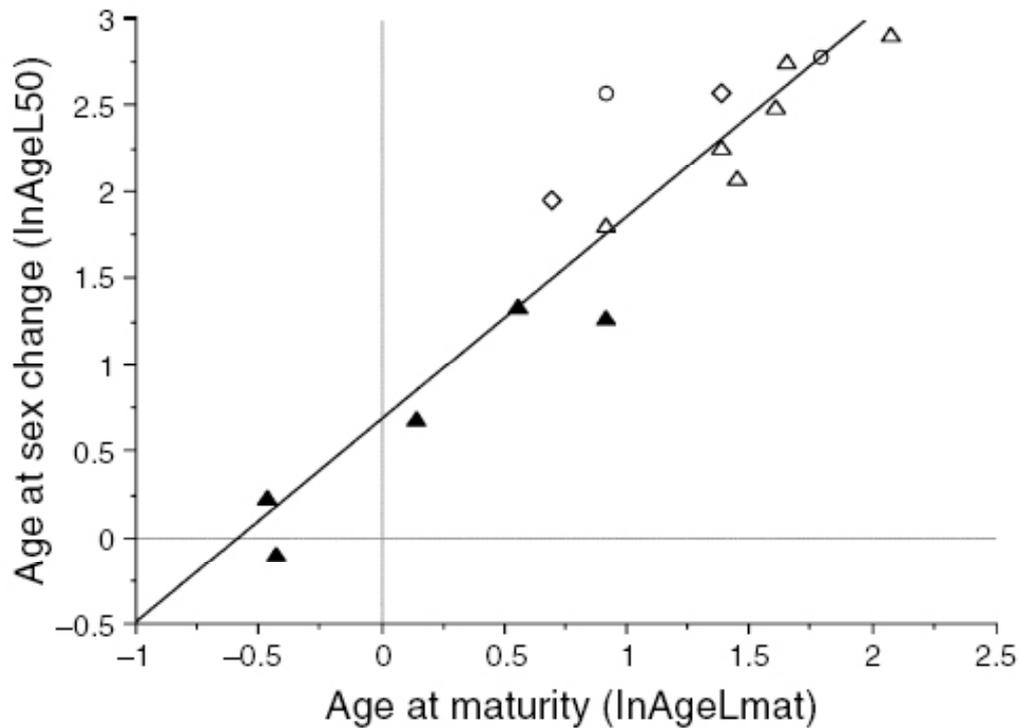
Figure 4-3. A logarithmic plot of the age at sex change (τ) versus the age at maturity (α) for 15 species of sex changing fish.
Species are as independent data points. The slope of the relationship (1.17) is not significantly different from 1.0 (95% C.I. $\pm$ 0.25; $r^2 = 0.885$), suggesting that fish change sex at a constant ratio (2.5) to their age at maturity. Filled symbols are for protandrous fish (change sex from male to female), open symbols for protogynous fish (change sex from female to male). The presence or absence of Early Maturing fish of the second sex is shown by the shape of the symbols. A circle represents Diandric species (with EM's present), a triangle represents Monandric species (EMs absent) and a diamond represents fish for which I are unsure of the status for EMs.

As with the size invariant analysis, when comparing across species, the relationship between τ and α is significantly influenced by the occurrence of Early Maturers (ANCOVA, Intercept: $F_{1,9} = 14.5$, $p < 0.01$; Slope: $F_{1,9}=7.8$, $p < 0.05$, n=13, $r^2=0.943$). However this analysis is limited as there are only two samples for diandric species within the age data. My dataset on mating systems for fish where I have age information was also too small to make meaningful inference (N=5), although a non significant ANCOVA is suggestive that the type of mating system has no effect on the relationship between τ and α. Comparing species in which the first sex is male (protandry) or female (protogyny), there was a significant difference on the intercept

(ANCOVA, $F_{1,11}$=10.2, p < 0.01), but not the slope (ANCOVA, $F_{1,11}$=1.35, n.s.) of the relationship between τ and α (n=15, r^2=0.940). This means that fish are changing sex at a constant ratio to their age at maturity, but that this proportion differs between protandrous and protogynous species – protogynous species change sex at a higher ratio of their age at maturity. Adding in 'first sex' as an explanatory variable increased r^2 by 0.055, from 0.885 to 0.940.

4.5. Discussion

4.5.1. Sex change invariants in fish

I have demonstrated two life history invariants for sex change across several fish species. First, fish change sex at a constant proportion of their maximum size - the size at sex change divided by the maximum size (L_{50}/L_{max}) is 0.79. Second, fish change sex at a constant ratio to their age at maturity - the age at sex change divided by the age at maturity (τ/α) is 2.5. Given the differences across species in life history and method of data collection, the degree of invariance observed is striking, especially for the size at sex change invariant, where r^2=0.967. More noise is observed with the age at sex change invariant, where r^2=0.885, but this could be explained by the relative imprecision involved in estimating fish age (see materials and methods section and Bell (2001). Of course, it is not surprising that individuals of larger fish species change sex when bigger and older. What is surprising is that when viewed dimensionlessly, they change sex at the same relative size and age.

Charnov & Skulladottir's (2000) model predicts these two invariants if δ, the exponent in the male fitness function is invariant. Although the two invariants hold very well across all species examined, as shown by the r^2 values, the data also suggest that slightly different invariants hold: (i) for the size at sex change depending upon whether early maturers of the second sex occur (diandry); (ii) for the age at sex change invariant dependent upon the occurrence of diandry and the direction of sex change (male or female first). These effects could be expected since they are factors that may be expected to influence δ. However, their biological significance is not

clear because: (i) they lead to a very small increase in the amount of variance explained (e.g. r^2 for size at sex change increases from 0.967 to 0.971), that is statistically significant because r^2 is extremely high and consequently the error (residual) variance is so low; (ii) they often rely on analyses with subsets of the data where we have the relevant information, and a small number of data points. Indeed, the most striking point is that these factors have very little explanatory power for the size and age of sex change. This suggests that the value of δ: (a) is approximately invariant across fish species that change sex in the same direction; (b) for protandrous and protogynous species is such that it favours sex change at the same relative size and age. We are currently extending theory in order to address this, and to use the size at sex change data to indirectly estimate δ.

My results suggest that Charnov & Skuladottir's (2000) model can quantitatively explain when sex change occurs across fish species. This is particularly remarkable because the model ignores many biological details in which there is variation, such as the mating system (from harems, leks and monandry to large temporary spawning aggregations), maximum size and age, the presence of early maturing individuals of the second sex, and the cues and mechanisms involved in determining when sex change occurs. Instead, their model approximates all sex changing species by a single life history, with fitness being related to size raised to the power δ in males, and size raised to the power 3 in females. The fact that the invariant occurs across fish species therefore suggests that their approximation captures the important points of the underlying biology extremely well for fish. To give a specific example, the cues/mechanisms involved in sex change have been the subject of much debate (Warner et al. 1975; Shapiro 1979; Shapiro and Lubbock 1980; Shapiro and Boulon 1982; Charnov 1986; Warner 1988a, b; Lutnesky 1996; St Mary 1997; Munday 2002). Charnov & Skuladottir's (2000) model assumes that fitness depends primarily upon size (age), and so sex change occurs when individuals reach a certain size (age). However, it has been argued that in some species fitness depends upon the social environment, and that sex change occurs in response to the local ratio of females to males (Shapiro and Lubbock 1980). Our empirical support for Charnov & Skuladottir's (2000) model suggests that assuming a fixed relationship between body

size and fitness provides a very good approximation irrespective of the underlying mechanism controlling when sex change occurs.

How do my results compare with previous research in this area? To my knowledge, this is the first comparative study across species on size and age at sex change. Previous empirical work has focused on either: (i) determining why and when sex change takes place in specific species (Shapiro 1981a; Cole 1983; Hoffman et al. 1985; Lejeune 1987; Warner and Swearer 1991; Gillanders 1995); (ii) descriptive work on whether, in what direction, and when sex change takes place for several species (Choat and Robertson 1975; Robertson and Warner 1978; Warner and Robertson 1978; Kobayashi and Suzuki 1992), or (iii) descriptive physiology of the gonads of several sex changing species (Cole 1988, 1990; Cole et al. 1994). To an extent this has been because theoretical work prior to Charnov & Skulladotir (2000) has been phrased in terms of relatively hard-to-measure life history parameters, and so has been less useful for making testable quantitative predictions across species (Warner et al. 1975; Leigh et al. 1976.; Charnov 1979a; Charnov and Bull 1989b). Although, there have been some related notable studies across populations of the same species, on the amount of individuals that mature early as the second sex (Charnov 1979b; Charnov 1982a; Charnov and Andersson 1989; Charnov and Hannah 2002).

4.5.2. Future directions

As with other discoveries of dimensionless life history invariants, my results pose numerous questions, and suggest a number of future directions (Charnov, 1993). (1) Are Charnov & Skulladottir's (2000) invariant predictions model dependent, or can they be made with other approaches / assumptions? (2) Do the invariant rules that I have demonstrated across fish also hold within species (Charnov and Skuladottir 2000)? This seems quite likely given that we would expect less variation in δ between populations of the same species then between species. (3) Do the invariant rules hold in other taxa where sex change occurs? If so, do they lie on the same slope as the fish? Differences within or between taxa would reflect fundamental differences in trade-offs linking growth, reproductive success and mortality, which in turn might be reflections of general differences in energetic/growth schedules (Charnov, 1993). (4) Is the breeding sex ratio invariant, within or across species? This invariant has yet to be tested because breeding sex ratios are difficult to estimate. (5) Is δ really invariant across species? If so, does this reflect some underlying fundamental constraint associated with sex change? (6) Several other areas of sex allocation, such as condition dependent sex ratio adjustment and environmental sex determination rely on the same underlying theory as sex change (Charnov 1982a; Frank and Swingland 1988). Can similar invariant predictions can be made for these areas?

4.6. Conclusions

My results demonstrate the novel insights that may be gained with a dimensionless approach to evolutionary theory. A general problem in evolutionary biology is that quantitative tests of theory require a detailed knowledge of the underlying trade-offs, which can be very hard to measure (Stearns 1992). The advantage of the invariant predictions that I have tested here are that they allow quantitative tests without a detailed knowledge of the underlying trade-off function – in this case, how male fertility changes with size or age. Within the field of sex change, it is no coincidence that the previously most striking quantitative success, predicting the proportion of

individuals that mature as the second sex (Charnov, 1982), relied on theory that similarly finessed away this problem. Another advantage of this approach is that it may provide a useful indirect approach to understanding the more intractable breeding systems, such as the massive breeding aggregations observed in many of the commercially important grouper species (Shapiro et al. 1993). To date the processes involved in sex ratio regulation of these large aggregations have remained elusive, despite its possible importance for stock management (Tupper 1999). If, as the theory suggests, the invariants demonstrate that the males of all of these species share similarly shaped trade off curves linking size to reproductive value (δ), then one might reasonably assume that the unknown mating systems will turn out to approximate some form of mate monopolisation (for protogynous species, sensu Robertson and Choat 1974; Robertson and Warner 1978; Warner and Robertson 1978; Warner 1988a).

4.7. Appendix

Species	Study location	Mating system	First sex	EMs *	L_{50} (in mm)	L_{Max} (in mm)	Age at Sex Change (in years)	Age at Maturity (in years)
Acanthopagrus berda[1]	Australia	U	M	N	191	310	1.95	1.15
Achoerodus viridis[26]	Australia	U	F	N	526	620	18	8
Amphiprion akallopisos[33]	Indian Ocean	M	M	N	72	108		
Amphiprion frenatus[34]	Japan	M	M	N	66	85		
Amphiprion oscellaris[34]	Japan	M	M	N	40	44		
Amphiprion perideraion[34]	Japan	M	M	N	54	76		
Amphiprion polymnus[34]	Japan	M	M	N	92	106		
Amphiprion sandrasinos[34]	Japan	M	M	N	51	61		
Bodianus rufus[5]	Caribbean Panama	H	F	N	172.8	230		
Centropyge interruptus[40]	Japan	H	F	N	127	150		
Chrysoblephus puniceus[37]	SE Coast Africa	U	F	N	377	450		
Clepticus parrae[6]	Caribbean Panama	L	F	N	157.8	180		
Coryphopterus nicholsi[38]	BC, Canada	H	F	N	69	90		
Cryptotomus roseus[7]	Caribbean Panama	L	F	N	57.5	70		
Dascyllus flavicaudus[42]	Hawaii	H	F	N	68	77		
Epinephelus chlorostigma[28]	Seychelles	A	F	Y	464	500	13	2.5
Epinephelus marginatus[8]	Mediterranean Sea	A	F	N	880	1050	12	5
Epinephelus morio[9]	Gulf of Mexico	U	F	N	755.5	895	9.5	4
Epinephelus rivulatus[10]	Australia	A	F	N	756	895		
Genicanthus semifasciatus[41]	Taiwan	H	F	N	93	116		
Gobiosoma multifasciatum[39]	Caribbean Panama	U	F	N	20	27		

Species	Study location	Mating system	First sex	EMs *	L_{50} (in mm)	L_{Max} (in mm)	Age at Sex Change (in years)	Age at Maturity (in years)
Halichoeres bivittatus[11]	Caribbean Panama	L	F	Y	302	350		
Halichoeres garnoti[12]	Caribbean Panama	L	F	N	73	140		
Halichoeres maculippina[13]	Caribbean Panama	L	F	Y	109	120		
Halichoeres pictus[14]	Caribbean Panama	L	F	Y	79	120		
Halichoeres poeyi[15]	Caribbean Panama	L	F	Y	83	100		
Labroides dimidiatus[16]	Australia	H	F	N	88	140		
Lethrinus choerorynchus[36]	NW Australia	U	F	N	281	331		
Lethrinus fraenatus[36]	NW Australia	U	F	N	246	305		
Lethrinus lentjan[35, 36]	Seychelles, NW Australia	U	F	N	308	398		
Lethrinus mahsena[29]	Seychelles	U	F	Y	320	360	16	6
Lethrinus nebulosus[36]	NW Australia	U	F	N	408	562		
Lethrinus nematacanthus[36]	NW Australia	U	F	N	180	245		
Lethrinus variegatus[36]	NW Australia	U	F	N	291	304		
Lithognathus mormyrus[4]	Canary Islands, E. Adriatic	U	M	N	252.5	370	3.5	2.5
Mycteroperca bonaci[27]	Florida	A	F	N	1233	1500	15.5	5.2
Plectropomus leopardus[17]	Australia	L	F	N	321	360	16.00	6.00
Pseudoanthius squammipinis[18,43]	Red Sea, Indian Ocean, Japan	H	F	N	50	62.5		
Sarpa salpa[2]	South Africa, Canary Islands	U	M	N	250.5	375	3.75	1.75
Scarus ghobban[30]	Seychelles	U	F	U	290	370	7	2
Scarus iserti[19]	Caribbean Panama	H	F	Y	96	170		
Scarus rubroviolaceus[31]	Seychelles	U	F	U	374	425	13	4
Scarus vetula[20]	Caribbean Panama	H	F	Y	252	320		

Species	Study location	Mating system	First sex	EMs *	L_{50} (in mm)	L_{Max} (in mm)	Age at Sex Change (in years)	Age at Maturity (in years)
Semicossyphus pulcher[21]	California	L	F	N	301	440	7.9	4.25
Sparisoma atomarium[22]	Caribbean Panama	H	F	N	55	90		
Sparisoma aurofrenatum[23]	Caribbean Panama	H	F	N	175	240		
Sparisoma rubripinne[24]	Caribbean Panama	L	F	N	250	310		
Synbranchus marmoratus[46]	Pearl Island Pacific Panama	U	F	N	340	650		
Tenualosa macrura[47]	Sumatra	U	M	N	190	320	0.9	0.65
Tenualosa toli [3]	Sarawak, Malaysia	U	M	N	110	130	1.24	0.63
Thalassoma bifasciatum[32]	Caribbean Panama	L	F	Y	83	120		
Thalassoma lunare[25]	Australia	L	F	Y	110	130		

Table 2. Fish species used to test for sex change invariance.
Mating system has been simplified to either: Unknown (U) if there is no or ambiguous information available; Haremic (H) if mate monopolisation is permanent; Lek (L) if monopolisation is temporary for mating; Aggregation (A) if the species is known to form large breeding aggregations periodically (mostly the groupers); and Monogamous (M) for monogamy. The exact nature of the mating system is often unknown for most species, as it can vary greatly from location to location (Robertson 1981). The column headed 'First Sex' refers to direction of sex change (protogyny = female to male (F); protandry = male to female (M)). *The EMs column ('Early Maturers') refers to whether all fish are born into one sex (N), or whether both sexes are produced to start with, and a certain fraction of the 'first sex' goes on to change later (early maturers present, Y). The size and age at sex change were calculated as shown in section 2 of this paper. References to the table: (1) Tobin, et al., 1997 (2) van der Walt & Mann, 1998; Villamil et al., 2002 (3) Blaber et al., 1996 (4) Kraljevic et al., 1995; Lorenzo et al., 2002 (5, 6 & 11 –15, 32) Warner & Robertson, 1978 (7, 19 & 20, 22-24) Robertson & Warner, 1978 (8) Marino et al., 2001 (9) Brule et al., 1999 (10) Mackie, 2000 (16 & 25) Robertson & Choat, 1974 (17) Ferreira, 1995 (18) Shapiro, 1981 (21) Cowen, 1990 (26) Gillanders, 1995 (27) Crabtree & Bullock, 1998 (28-31, 35). Grandcourt, 2002. (33) Fricke, 1979 (34) Moyer & Nakazono, 1978b (36) Young and Martin, 1982 (37) Garrat, 1986 (38) Cole, 1983 (39) Robertson & Justines, 1982 (40) Moyer & Nakazono, 1978a (41) Shen & Liu, 1976 (42) Godwin, 1995 (43) Suzuki et al., 1978 (46) Liem, 1968 (47) Blaber et al., 1999.

Chapter 5. Dimensionless invariants for the timing of gender switch in sex changing animals

5.1.   Abstract

A common approach in evolutionary biology is the use of optimality models. These are used to determine what value of a particular trait maximises Darwinian fitness in response to trade-offs and constraints. Although this approach has been very successful, it is generally only applied qualitatively. The reason for this is that quantitative predictions depend upon the exact forms of the underlying trade-offs and constraints, which are hard to determine. Recent work has suggested that viewing aspects of life history theory in a dimensionless way can sometimes offer a solution to this problem.

Here I use the dimensionless approach to examine the size of sex change across 77 species from a range of animal taxa, including fish, echinoderms, crustaceans and molluscs. I found that the relative size at sex change was surprisingly invariant across species, independent of many biological details, including variation in maximum body size, which ranged from 2 mm for the crustacean *Thor maningi* to 1500 mm for the fish *Mycteroperca bonaci*.

Although this invariant relationship has been predicted within species, the degree to which it holds across such a broad range of taxonomic groups is astounding - 98% of the variation in size at sex change across all animals can be explained by the simple rule that individuals change sex when they reach 72% of their maximum size. This suggests a fundamental similarity across all sex changing animals in the underlying trade-offs and fitness functions associated with sex change.

5.2. Introduction

Sex change occurs in a variety of animals, including fish, echinoderms, crustaceans, molluscs and polychaete worms (Ghiselin 1969; Charnov 1982a; Policansky 1982). Evolutionary theory suggests that sex change is favoured when: (i) the reproductive success (fitness) of an individual varies with their age or size, and (ii) the relationship differs between the sexes. In this case, natural selection favours a strategy where individuals start as the sex whose fitness increases more slowly with age, and then change to the other sex at a later stage (Charnov 1982a; Warner 1988b). Although this idea is well established, quantitative tests of sex change theory have been hampered by the requirement for reliable estimates of the underlying trade-offs, such as the relationship between age and fitness for the two sexes.

Charnov & Skulladottir (2000) have recently developed an optimisation model to predict when sex change should occur, and shown that their predictions can be given in terms of several dimensionless quantities. Specifically, their results depend upon (1) k/M (the quotient of the relative growth rate (the Bertalanffy coefficient k) and the adult instantaneous mortality rate); (2) $\alpha \cdot M$ (the product of the age at first breeding and the adult instantaneous mortality rate); and (3) δ (the coefficient in the equation relating male fertility to size, where male fertility is proportional to L^{δ}, and L is size). Charnov & Skuladottir showed that populations/species with the same values of these dimensionless quantities are predicted to have the same: (a) size at sex change / maximum size (L_{50}/L_{max}); (b) age at sex change / age at first breeding (τ/α); (c) breeding sex ratio, defined as the proportion of breeders that are male. Several studies have suggested that k/M and $\alpha \cdot M$ are invariant within taxa (Charnov 1993). Consequently, Charnov & Skuladottir's three predictions will hold whenever δ is invariant.

The use and novelty of Charnov & Skulladottir's predictions are that they can be tested quantitatively across populations or species, without a detailed knowledge of the relationship between size and male fertility (δ). All that is required is that the

relationship is approximately the same shape across populations studied. Charnov &
Skulladotir suggested that this is likely to be the case within species, and consistent
with this, they found that the relative size at sex change was invariant across
populations of the shrimp *Pandalus borealis*. This invariant would also be extended
to hold across species if they had the same δ, which at the very least might be
expected between closely related species with similar mating systems. Consistent
with this, I have previously found that the invariant holds across sex changing fish
(Allsop and West 2003b).

5.3. Methodology

5.3.1. Data collection

Building on a previous study investigating the existence of life history invariants in
fish (Allsop and West 2003b), I expanded my dataset to incorporate the known
diversity of sex changing organisms. I located relevant studies by: (1) searching the
ISI Web of Science database, using the key words "sex change" + "population" +
"size" NOT "fish"; (2) searching citations in all papers found as well as reviews on
the topic; (3) directly contacting researchers. I collected data on: (a) the size at sex
change, and (b) the maximum body size achieved in a population (defined as the
Total Length from the most distal part of the caudal fin to the tip of the snout). The
size at sex change (L_{50}= size at which 50% are the second sex (male for protogynous
animals, female for protandrous animals)) was calculated for each population using
the logistic regression of the proportion of second sex breeders against the population
body size distribution. I used the sigmoidal logistic regression curve as it is
consistent with the method used to develop the theory that we are testing, on the
basis that it provides a rigorous and quantitatively consistent estimation of the
population average size at sex change (Allsop and West 2003b). My criteria for
inclusion of a species in the study were as described in Chapter 4 (see Allsop and
West 2003b).

5.3.2. Statistical analyses

I tested whether the relative size at sex change (L_{50}/L_{max}) was invariant across all populations and species studied, using a log-log regression of L_{50} against L_{max}. If the data fit an invariant life history rule for governing the size at which to change sex, such a regression would give a slope of 1.0. I carried out these regressions in two ways: (1) using species as independent data points, and (2) an independent contrasts analysis across species employing a phylogeny for sex changing animals. The phylogeny we used is presented in Chapter 3 (Allsop and West 2004a). Species may not be independent data points because they are phylogenetically related (Felsenstein 1985), and thus may share derived traits through a variety of processes, including phylogenetic niche conservatism, phylogenetic time lags and through similar adaptive responses (Harvey and Pagel 1991). This leads to problems if a trait is evolutionarily conserved or highly correlated with an unknown phylogenetically inert third variable. A formal method to control for this is to carry out an analysis with independent contrasts. Independent contrasts are derived by calculating the difference in the response and explanatory variables across pairs of species or higher nodes that share a common ancestor (Felsenstein 1985; Harvey and Pagel 1991). These contrasts can then be analysed by a regression through the origin; the expected value of the slope through the origin equals the true relationship between the variables in the absence of the effects of evolutionary relatedness (Pagel 1993). I carried out such an analysis with the CAIC statistical package (Purvis and Rambaut 1995), assuming equal branch lengths.

I carried out regression analyses using two methods. The use of standard Ordinary Least Squares (OLS) regression is based upon the assumption that the *x* variable is fixed or controlled by the observer (i.e. no error in the *x* term). This is the most commonly used method of estimating the functional relationship between biological variables, and yet its assumptions are often violated, leading to a biased slope estimate. Although I may often be more confident of our estimates of the *x* variables in my analyses, they are clearly subject to measurement error. A possible solution to this problem is to carry out a Major Axis (MA) regression (Gemmill et al. 1999).

MA (or Model II) regression is used when there may be no causal structure between the y and the x variable and they are both measured with equal error. The Major Axis produces a line that minimises the sum of the squared deviations perpendicular to itself. In order to examine any affect of error assumptions, we repeated all analyses using both OLS and MA regression (Gemmill et al. 1999), using SYSTAT 10.2.

5.4. Results and Discussion

Here, I test the degree of invariance in the relative size at sex change across all sex changing animals (Figure 5-1). We collected data on 77 species of fish, echinoderms, crustaceans and molluscs (see supplementary information). If the relative size at sex change is invariant then a plot of log(L_{50}) against log(L_{max}) would give a slope of 1.0. I first analysed the data using the formal comparative method of independent contrasts (Felsenstein 1985; Harvey and Pagel 1991), based upon a composite phylogeny of sex changing animals (n=38 independent contrasts). This analysis gave a slope of 0.97 $\pm$ (95%CI) 0.05, which was not significantly different from 1.0 (t_{37}=1.2, p>0.1; Figure 5-1). The amount of variance in size at sex change explained by this regression was 96%, and dropped only 5% to 91% when we forced the regression to have a slope of 1.0. I then analysed our data using species as independent data points. This analysis gave a slope of 1.05 $\pm$ (95%CI) 0.03, which was significantly higher than 1.0 (t_{75}=3.3, p<0.01; Figure 5-2). However, this difference reflects the extremely low residual/error variance and is of negligible biological importance – forcing the regression to have a slope of 1.0 caused the % of the variation in size at sex change explained to drop only 1.8% to 96.7%. Consequently, 95-97% of the variation in mean size at sex change across species can be explained by the simple rule that individuals change sex when they reach 72% (95%CI: 67 – 77%) of their maximum size.
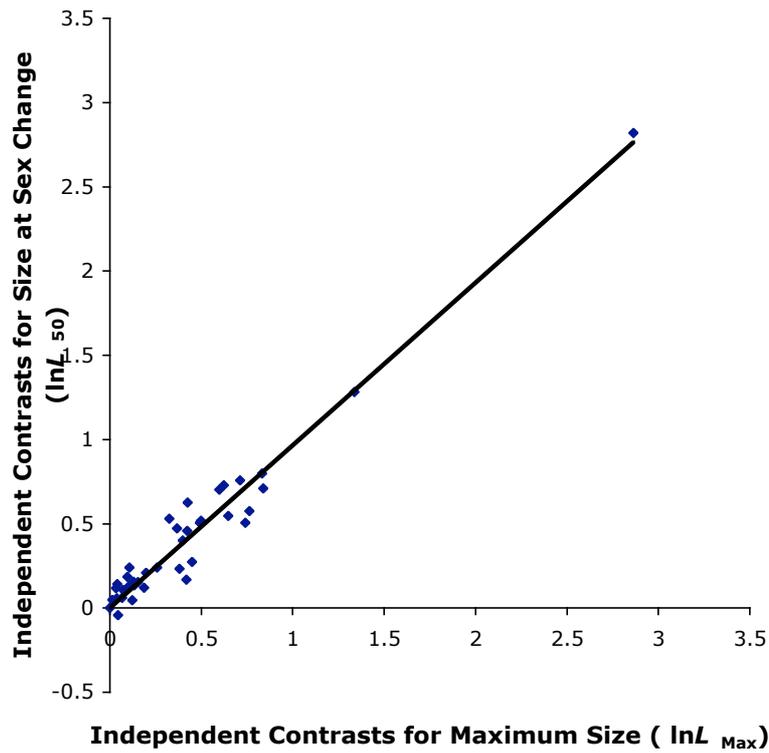
Figure 5-1. Log-Log plot of Independent Contrasts for L_{50} vs L_{max} for sex changing organisms. Demonstrating that all sex changers make their gender transition at a constant proportion of their maximum body size, irrespective of evolutionary history. The slope is fixed at 1 and is driven through the origin as required for the analysis of independent contrasts ($r^2 = 0.91$, n = 38 independent contrasts). The OLS slope is $0.97 \pm$ (95%CI) 0.05 ($r^2 = 0.96$).
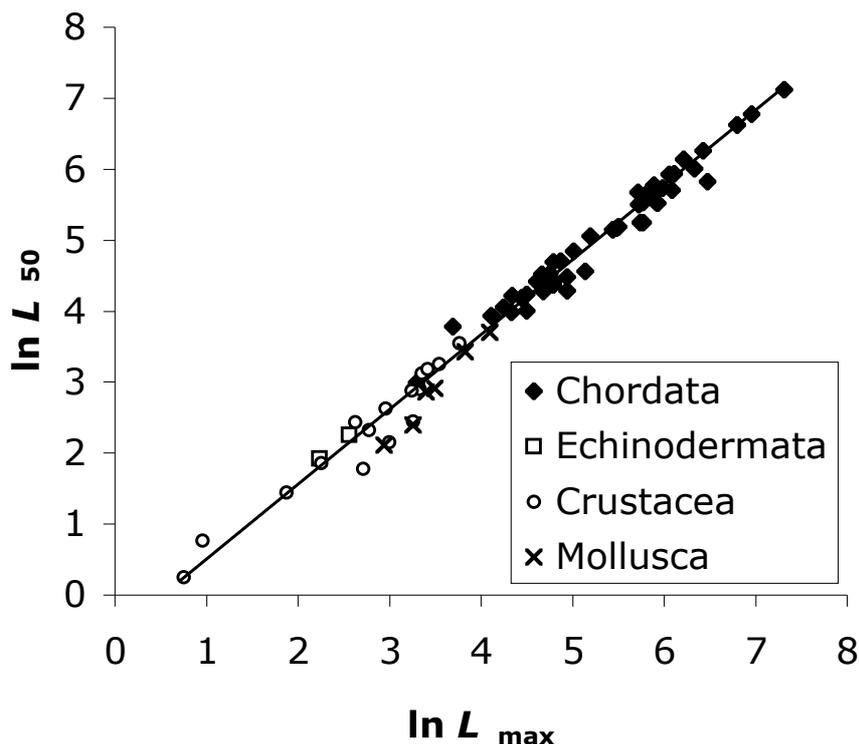
Figure 5-2. Log-Log plot of L_{50} vs L_{max} for 77 species of sex changing animals with species as independent data points.
Data are split by taxa: = Echinodermata, o = Crustacea, ◆ = Chordata, × = Mollusca. The regression has a slope fixed at 1 giving an intercept of $-0.3 \pm$ (95%CI) 0.05, ($r^2 = 0.97$, $n = 77$ species). The OLS slope is $1.05 \pm$ (95%CI) 0.03, and the intercept is $-0.5 \pm$ (95%CI) 0.07 ($r^2 = 0.98$). The relative size at sex change (L_{50}/L_{max}) is 0.72, implying that individuals change sex when they reach 72% of their maximum size.

The amount of variation that can be explained across species in the mean size at sex change is not increased by taking into account life history variables or taxonomic differences. I tested whether possibly important life history variables (Leigh et al. 1976.; Charnov 1982a; Warner 1988b; Charnov 1993; Allsop and West 2003b) and taxonomic groupings could significantly improve the relationship between the average size at sex change ($\log(L_{50})$) and maximum size ($\log(L_{max})$). Considering possibly important life history variables (Leigh et al. 1976.; Charnov 1982a; Warner 1988b; Charnov 1993; Allsop and West 2003b), there was no significant effect of: (a) whether the direction of sex change was from male to female or female to male (Intercept: $F_{1,73} = 1.4$, $p > 0.1$; Slope: $F_{1,73} = 0.3$, $p > 0.1$; $n=77$ species), or (b) the presence of individuals who mature early as the second sex (termed 'early maturers';

Intercept: $F_{1,64} = 0.02$; $p > 0.1$, Slope: $F_{1,64} = 0.02$, $p > 0.1$; $n=68$ species where the presence or absence of early maturers has been identified). Considering taxonomy, there was no significant difference between the groupings of Chordata, Crustacea, Echinodermata and Mollusca (Intercept: $F_{3,\,69} = 2.7$, $p>0.05$, Slope: $F_{3,\,69} = 1.9$, $p>0.1$).

It is not surprising that there is a positive relationship across species between the size at sex change and the maximum size. This simply reflects the fact that larger species change sex at a larger size. What is astounding is that the relative size at sex change is so similar (invariant) in sex changing animal species, regardless of the direction of sex change or the presence of early maturing individuals. Overall, 95-97% of the variation in the average size at sex change across all animals can be explained by assuming that animals change sex when they reach 72% ± (95%CI: $67 - 77\%$) of their maximum size. Invariance in the relative size at sex change was predicted by Charnov & Skulladottir's (Charnov and Skuladottir 2000) model. However, it had not been predicted to hold to such an extent across species. The fact that it does has several implications and begs a number of further questions. In particular, it suggests a fundamental similarity across animals in the underlying trade-offs and fitness functions associated with sex change, despite considerable variation in mating system, cues / mechanisms involved in sex change, and numerous other life history details.

The prediction of invariance in relative size at sex change arises when k/M (the quotient of the relative growth rate and the adult instantaneous mortality rate), $\alpha \cdot M$ (the product of the age at first maturity and the adult instantaneous mortality rate) and δ (the exponent relating male fertility to body size) are invariant. Whilst $\alpha \cdot M$ is invariant across the taxa examined here, this does not appear to be the case with k/M (Beverton 1963; Charnov 1993). This suggest that either: (a) being a sex changer correlates with certain invariant values of k/M and δ (for a given direction of sex change); (b) in sex changers the values of k/M and δ are correlated such that their effects on the optimal relative time at sex change cancel each other out, or (c) δ is invariant and variation in k/M across taxa has negligible influence on the optimal

relative time at sex change. The lack of a difference in relative size at sex change between protandrous (male first) and protogynous (female first) species is particularly intriguing because we know that δ varies between them. The relationship between size and fitness for females of the species that we have examined is likely to be cubed (i.e. fitness $\propto$ body size^3) (Charnov 1993). In order for sex change to be favoured, fitness must increase with body size more slowly in the first sex, and so δ must be <3 in protandrous species, and >3 in protogynous species. This suggests that the values of δ for protandrous and protogynous species are such that they give such similar relative sizes at sex change. All of these points can only be resolved by detailed empirical studies on a number of species from the different taxa, and further theoretical work.

How do my results fit in with the findings of other studies? Early work on fish life history invariants demonstrated that L_{mat}/L_{max} (size at maturity/maximum size) is approximately invariant within certain taxonomic groups (Beverton and Holt 1959; Beverton 1963; Mitani 1970; Charnov 1993). Various explanations for the L_{mat}/L_{max} invariant have been proposed (Iles 1974; Jones 1976; Pauly 1984), and analogous to the sex change invariant, it can be predicted if the underlying trade-offs for growth, maturation and mortality hold the same shape across populations or species (Charnov 1993). Pauly (1981, 1984) has suggested that a possible mechanistic explanation for this in female fish is the constraint imposed by oxygen limitation in larger fish, due to gill size and oxygen consumption scaling with size at different rates. An analogous argument could be applied to our observation of invariance in the relative size at sex change, as all the species we have examined are aquatic. However, this would not necessarily predict the same size fitness relationship in males (i.e. invariant δ), as male reproductive success will not always be linearly correlated to the amount of gametes that can be produced.

5.5. Conclusions

The dimensionless approach to evolutionary and optimality theory is still in its infancy. My data demonstrate the usefulness of this approach, and in particular its ability to make quantitative predictions and provide novel insights that will stimulate further work. In addition, my data show the specific use of applying this approach to the field of sex allocation. This is particularly exciting because the problem of when to change sex is formally equivalent to numerous other problems in sex allocation where there is condition dependent sex ratio adjustment or environmental dependent sex determination (Trivers and Willard 1973; Charnov 1982a; Frank 1998; West and Sheldon 2002). These include sex ratio adjustment in response to parental condition (e.g. maternal rank in ungulates, or male attractiveness in birds), resources (e.g. host size in parasitoid wasps or nematodes) or the environment (e.g. incubation temperature in reptiles). It will be extremely interesting to see in which of these areas sex ratio invariants can also be predicted or found.

Chapter 6. General Discussion

Detailed discussions of the findings for each of the central data chapters are provided within those chapters. Here, I present a brief review of the main findings of the thesis and highlight where to take this work in the future.

6.1. Selection for sex change and alternative reproductive strategies

In Chapter 2, I test theories of the way in which the mating system can be shaped by the underlying distribution of ecological resources (Emlen and Oring,1977). I also investigate the consequences of the mating system for reproductive skew and the selective forces shaping the evolution of sex change and alternative reproductive strategies (Warner 1984). Field and experimental data for populations of the coral inhabiting goby fish *Coryphopterus personatus* provided some support for the theories being tested, but not total support. In particular, there was conflict between the data collected on windward and leeward sides of the atolls. These differences may be the result of some factor of key importance to the life history decisions of the animals, such as food availability, predation risk or the effects of wave energy on the possibility for migration between groups. Alternatively, the conflicting results may reflect an incompatibility between the predictions to be tested and the study system chosen to test those predictions.

The choice of a species for which it was not possible to determine the sexual history of the second sex (i.e. whether males were born so or originated from females by sex change) using histology, meant that I was unable to accurately quantify the level of selection for sex change. Instead, I had to rely on a statistical estimate of the proportion of Early Maturing Males (EMMs) using the logistic regression of sex ratio on body size. Whilst I defend this technique, because it provides a consistent measure across populations that yield the correct *relative* comparisons for the study, undoubtedly the findings of the research would have been much more robust given a species for which it is possible to accurately quantify the proportion of EMMs.

The general observation that there appears to be either very little sex change taking place, or sex change taking place in the smaller size classes, has large implications for the appropriateness of the study organism and study location for testing the predictions. In particular, the fact that there appeared to be a high proportion of early maturing males in all populations studied meant that there was actually very little variation in selection for sex change on which to perform exploratory analysis. The ideal situation for addressing the predictions would have greater variation in the amount of sex change taking place.

In all, the study was productive in giving more insight into the selective forces in operation for sex change in *C. personatus*, but it seems that both the choice of species and site were not suitable for addressing the impact of resource distribution on selection for sex change.

6.2. Sex ratio evolution for sex changers

In Chapter 3, I find empirical support for the predictions that (1) sex changing organisms should have a sex ratio biased towards the 'first' sex; and (2) the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species. Whilst finding support amongst the vertebrate taxa for the third prediction that sex ratio bias should be less extreme in partially sex changing organisms, global support for this prediction was not detected in analysis across the whole sex change phylogeny. In particular, the analysis highlights some difference between the predominantly invertebrate protogynous species and the predominantly invertebrate protandrous species. Certainly, any future attempt at trying to apply a more rigorous test to this final aspect of the predictions would benefit greatly from both an improved phylogenetic resolution within the invertebrates, and from more detailed quantitative species-specific studies of the adaptive value of sex change for species that produce early maturing individuals of the second sex.

One of the major failings of Chapter 3 is that there simply is not sufficient data in the literature to fully address all of the predictions. The vast majority of studies attempting to describe and quantify the adaptive value of sex change have taken place within the protogynous (female to male) fish. This may be due to the fact that protogyny appears to be the most common form of sex change in nature. However, it may be that protogyny appears to be most common due to the fact that most of the sex-changing reef fish are protogynous, and researchers have had a natural tendency to favour working in the reef environment for its ease of access. I do not doubt the immense value that studying protogynous reef fish has brought to our understanding of the selective forces favouring sex change, and indeed to our understanding of wider issues in evolutionary biology. However, here I am addressing the future of research into the evolution of hermaphrodite sexuality, and I believe that this future lies in the encouragement of studies into the more difficult protandrous systems about which we actually know very little in an adaptive context (although see Charnov et al. 1978; Charnov 1979b). The protandrous fish tend to be found in more hostile, inaccessible environments than coral reefs, such as turbulent and silty estuaries. The majority of protandrous species, however, are invertebrates, such as the crustaceans and the molluscs. It is here that future basic research should be focused if we are to ever fully grasp the selective forces leading to the evolution of the spectrum of hermaphrodite sexuality.

6.3. Dimensionless Invariants for sex change

In Chapters 4 and 5, I collated data from the sex change literature in order to test Charnov's prediction that the *relative* size and age at sex change should be invariant across species. This is predicted for species that share similar relationships between crucial life history parameters: k/M, $\alpha \cdot M$ and δ, (which are the relative growth rates k, the Bertalanffy coefficient, the adult instantaneous mortality rate (M), the age at first breeding (α), and the coefficient in the equation relating male fertility to size δ, where male fertility is proportional to L^{δ}, and L is size). The analyses revealed striking empirical support for the predictions, with $91 - 97\%$ of the variation in size at sex change across all species being explained by the simple rule that species

change sex when they reach 72% of their maximum body size. The findings are made all the more surprising when the amount of variation in breeding systems is considered, with both protogynous and protandrous species in the dataset, and with species both with and without alternative reproductive strategies (early maturers) present.

These finding have, however, received criticism from some quarters (Buston et al. 2004), because of the possibility that the average size at sex change may be constrained to give the observed patterns, since sex change has to occur at some point between the size at maturity and the maximum size. In support of their criticism, Buston et al. invoke an *ad hoc* null model in which they generate values for the size at sex change randomly given a size at maturity of 50% of the maximum body size. However, they have made the mistake of generating a 'non-null' model - by setting the size at maturity to 50% of the maximum body size, their simulations are constrained to give the same output as the empirical findings reported in Chapters 4 & 5. Specifically, Buston et al.'s 'null model' assumes an invariant relative size at maturity, which follows from two of the dimensionless invariants assumed by Charnov's model – $\alpha \cdot M$ and k/M. If these are invariant then their product $\alpha \cdot k$ is invariant, and so the relative size at maturity ($L_{mat}/L_{max} = 1 - \exp(-\alpha \cdot k)$) is also an invariant. These are the crucial invariants for Charnov's model, so we would expect their null model to produce an invariant relative size at sex change, hence fitting the data.

If a true null model is constructed, where the size at maturity is generated randomly between 0 and 100% of the maximum body size, the resulting output differs considerably from the empirical findings, and the difference between the observed data and the null model are highly significant (Figure 6.1).
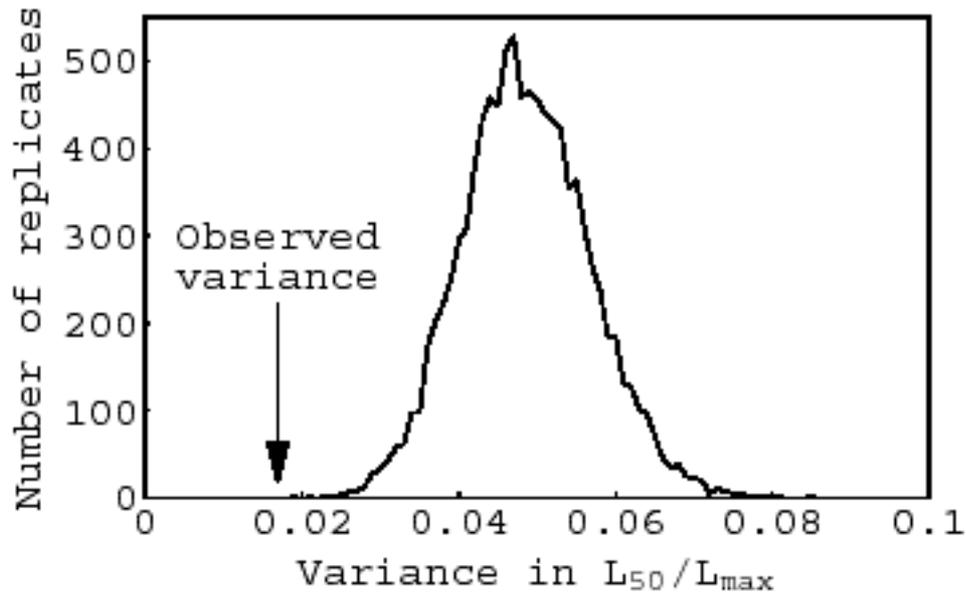
Figure 6-1. A true null model.
The results from 10,000 replicates of the more appropriate null model ($L_{mat}/L_{max} \sim U[0,1]$, $L_{50}/L_{max} \sim U[L_{mat}/L_{max},1]$). In each replicate an independent L_{50} is generated for each of the 77 species given its L_{max}, as above. Shown is the resulting distribution for the variance in L_{50}/L_{max}. The arrow indicates the value obtained from the real data, with zero out of 10,000 replicates giving the observed variance or lower. This suggests the invariant result holds to $P < 0.0001$. Similar results are obtained if analyses are focused on the r^2 value of a log-log plot of L_{50} versus L_{max} with the slope forced to be unity, where 102/10,000 replicates equalled or bettered the observed r^2 (estimated $P = 0.01$). Simulation carried out by Andy Gardner.

However, rather than questioning whether the observed ecological patterns arose by chance, I would like to look to the future, and discuss how we can move forward in our understanding of the observed phenomena. As with classical studies in allometry, the discovery of empirical patterns is interesting, but without a rigorous theoretical framework, we can only go so far in our interpretation of the meaning of the findings (West et al. 1997). Thus, it would be extremely worthwhile to carry out analytical simulations based on Charnov's model (Charnov and Skuladottir 2000), to test how variation in k/M, $\alpha \cdot M$ and δ (the underlying invariants in Charnovs model) influences the predicted relationship between size at sex change and maximum size, and hence the possible relative size at sex change invariant. Specifically, it may prove fruitful to: (a) use the existing information on k/M, $\alpha \cdot M$ and the relative size at sex change to make estimates for δ (the exponent linking male size to fitness); (b) estimate how variation in k/M, $\alpha \cdot M$ and δ influences the extent and nature of the relative size at sex change invariant that would be expected; (c)

estimate the amount of variation in k/M, $\alpha \cdot M$ and δ that is consistent with the empirical data. This work is currently being investigated in collaboration with Andy Gardner, Eric Charnov and Stuart West, and preliminary results indicate that the prediction of an invariant size at sex change relies primarily upon invariance in k/M and $\alpha \cdot M$, with variation in δ having little effect.

6.4. Concluding remarks

Many of the great leaps forward in the study of evolution have come from incorporating techniques and practices from other disciplines, such as the adoption of game theory from the field of economics (Maynard Smith, 1982). The search for dimensionless generalities across vastly different systems is a normal pastime for physicists and engineers and yet is anathema to many biologists, who have developed and tested theories based on the study of variation. My hope is that the use of both philosophical perspectives in this thesis to investigate the evolution of sex change presents a convincing argument for the potential value of studying biology at both levels. We need to intensify our efforts to study the variation that is the driving force of evolutionary change, but must also keep an eye on the general patterns that emerge from this data, as it is here where the greatest potential lies for novel insights into the assembly rules for life.

Bibliography

Abe, M., and H. Fukuhara. 1996. Protogynous Hermaphroditism in the Brackish and Freshwater Isopod, *Gnorimosphaeroma naktongense* (Crustacea: Isopoda, Sphaeromatidae). *Zool. Sci.* 13:325 - 329.

Alekseev, F. E. 1983. Hermaphroditism in Porgies (Perciformes, Sparidae). II. Sexual Structure of the Populations, Mechanism of its Formation and Evolution in Scups, *Pagrus pagrus, P. orphus, P. ehrenbergi,* and *P. auriga. Journal of Ichthyology* 23:61-73.

Allsop, D. J., and S. A. West. 2003a. Changing Sex at the Same Relative Body Size. *Nature* 425:783 - 784.

---. 2003b. Constant relative age and size at sex change in sequentially hermaphroditic fish. *J. Evol. Biol.* 16:921-929.

---. 2004a. Sex Ratio Evolution in Sex Changing Animals. *Evolution.* In press

---. 2004b. Sex allocation in the sex changing marine goby *Coryphopterus personatus* on atoll fringing reefs. *Evolutionary Ecology Research.* In press.

Asoh, K., and M. Kasuya. 2002. Gonadal development and mode of sexuality in a coral-reef damselfish, *Dascyllus trimaculatus. Journal of Zoology* 256:301-309.

Bacci, G. 1951. On two sexual races of *Asterina gibbosa* (Penn.). *Experientia* 15:31-33.

Barnes, N. B., and A. M. Wenner. 1968. Seasonal variation in the sand crab *Emerita analoga* (Decapoda, Hippidae) in the Santa Barbara area of California. *Limnol. Oceanogr.* 13:465-476.

Bauer, R. T. 1986. Sex change and life history pattern in the shrimp *Thor manningi* (Decapoda: Caridea): A novel case of partial protandric hermaphroditism. *Biol. Bull.* 170:11-31.

Bell, M. A. 2001. Fish do not lie about their age ......but they might lose count. *TREE* 16:599-600.

Beverton, R. J. H. 1963. Maturation, growth and mortality of clepeid and engraulid stocks in relation to fishing. *Rapp. P. -V. Reun. Cons. Int. Explor. Mer.* 154:44-67.

Beverton, R. J. H., and S. J. Holt. 1959. A review of the lifespans and mortality rates of fish in nature and the relation to growth and other physiological characteristics. Pp. 142 - 177. The lifespan of animals. Churchill, London.

Blaber, S. J. M., D. A. Milton, J. Pang, P. Wong, O. BoonTeck, L. Nyigo, and D. Lubim. 1996. The life history of the tropical shad *Tenualosa toli* from Sarawak: First evidence of protandry in the Clupeiformes? *Environ. Biol. Fishes* 46:225-242.

Blaber, S. J. M., D. T. Brewer, D. A. Milton, G. S. Merta, D. Efizon, G. Fry, and T. van der Velde. 1999. The life history of the protandrous tropical shad *Tenualosa macrura* (Alosinae: Clupeidae): Fishery Implications. *Estuarine, Coastal and Shelf Science* 49:689-701.

Blumer, L. S. 1979. Male parental care in bony fishes. *Q. Rev. Biol.* 54:149 - 161.

Brook, H. J., T. A. Rawlings, and R. W. Davies. 1994. Protogynous Sex-Change in the Intertidal Isopod *Gnorimosphaeroma oregonense* (Crustacea, Isopoda). *Biol. Bull.* 187:99-111.

Brule, T., C. Deniel, T. Colas-Marrufo, and M. Sanchez-Crespo. 1999. Red grouper reproduction in the southern Gulf of Mexico. *Trans. Am. Fish. Soc.* 128:385-402.

Brusle, J., and S. Brusle. 1975. Ovarian and testicular intersexuality in two protogynous Mediterranean groupers, *Epinephelus aeneus* and *Epinephelus guaza*. Pp. 222-227 *in* R. Reinboth, ed. Intersexuality in the animal kingdom. Springer-Verlag, Berlin.

Bull, J. J. 1980. Sex determination in reptiles. *Q. Rev. Biol.* 55:3 - 21.

Burt, A. 1989. Comparative methods using phylogenetically independent contrasts. *Oxford Surveys in Evolutionary Biology* 6:33-53.

Buss, L. W., and E. W. Iverson. 1981. A new Genus and Species of Sphaeromatidae (Crustacea: Isopoda) with experiments and observations on its reproductive biology, Interspecific Interactions and Colour Polymorphisms. *Postilla* 184:1-23.

Buston, P., P. L. Munday, and R. R. Warner. 2004. Understanding sex change. *Nature*. Submitted.

Butler, T. H. 1964. Growth, Reproduction, and Distribution of Pandalid Shrimps in British Columbia. *Journal of the Fisheries Research Board of Canada* 21:1403-1452.

Buxton, C. D. 1993. Life-History Changes in Exploited Reef Fishes on the East-Coast of South-Africa. *Environ. Biol. Fishes* 36:47-63.

Byrne, M. 1992. Reproduction of sympatric populations of *Patiriella gunnii, P. calcar* and *P. exigua* in New South Wales, asterinid seastars with direct development. *Mar. Biol.* 114:297-316.

Carpenter, A. 1978. Protandry in the freshwater shrimp, *Paratya curvirostris* (Heller, 1862) (Decapoda: Atyidae), with a Review of the Phenomenon and its significance in the Decapoda. *Journal of the Royal Society of New Zealand* 3:343-358.

Charnov, E. L. 1979a. The genetical evolution of patterns of sexuality: Darwinian fitness. *Am. Nat.* 113:465-480.

---. 1979b. Natural Selection and Sex Change in Pandalid Shrimp: test of a Life-History theory. *The American Naturalist* 113:715-734.

---. 1982a. The Theory of Sex Allocation. Princeton University Press, Princeton.

---. 1982b. Alternative Life-Histories in Protogynous Fishes: A General Evolutionary Theory. *Mar. Ecol.-Prog. Ser.* 9:305-307.

---. 1986. Size Advantage May Not Always Favor Sex Change. *J. Theor. Biol.* 119:283-285.

---. 1989. Evolution of the Breeding Sex-Ratio under Partial Sex Change. *Evolution* 43:1559-1561.

---. 1993. Life History Invariants. Oxford University Press, Oxford.

Charnov, E. L., and J. Bull. 1977. When is sex environmentally determined? *Nature* 266:828-830.

Charnov, E. L., and J. J. Bull. 1989a. Non-fisherian sex ratios with sex change and environmental sex determination. *Nature* 338:148-150.

Charnov, E. L., and P. J. Andersson. 1989. Sex Change and Population Fluctuations in Pandalid Shrimp. *The American Naturalist* 134:824-827.

Charnov, E. L., and J. J. Bull. 1989b. The primary sex-ratio under environmental sex determination. *Journal of Theoretical Biology* 139:431-436.

Charnov, E. L., and D. Berrigan. 1991. Dimensionless numbers and the assembly rules for life histories. *0962-8436* 332:41-48.

Charnov, E. L., and U. Skuladottir. 2000. Dimensionless invariants for the optimal size (age) of sex change. *Evolutionary Ecology Research* 2:1067-1071.

Charnov, E. L., and R. W. Hannah. 2002. Shrimp adjust their sex ratio to fluctuating age distributions. *Evolutionary Ecology Research* 4:239-246.

Charnov, E. L., D. Gotshall, and J. Robinson. 1978. Sex ratio: Adaptive response to Population Fluctuations in Pandalid Shrimp. *Science* 200:204-205.

Charnov, E. L., R. L. Los-den Hartogh, W. T. Jones, and J. Van den Assem. 1981. Sex ratio evolution in a variable environment. *Nature* 289:27-33.

Cheverud, J. M., and M. M. Dow. 1985. An autocorrelation analysis of genetic variation due to lineal fission in social groups of Rhesus Macaques. *Am. J. Physic. Anthropol.* 67:113 - 121.

Choat, J. H., and D. R. Robertson. 1975. Protogynous hermaphroditism in fishes of the family Scaridae. Pp. 263-283 *in* R. Reinboth, ed. Intersexuality in the animal kingdom. Springer-Verlag, Berlin.

Clark, A. B. 1978. Sex ratios and local resource competition in a Prosimian primate. *Science* 201:163 - 165.

Clutton-Brock, T. H. 1991. The Evolution of Parental Care. Princeton University Press, Princeton.

Clutton-Brock, T. H., and A. C. J. Vincent. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature* 351:58 - 60.

Cole, K. 1983. Protogynous hermaphroditism in a temperate zone territorial marine goby. *Copeia* 3:809-812.

Cole, K., and D. R. R. Robertson. 1988. Protogyny in the Caribbean reef goby, *Coryphopterus personatus*: gonad ontogeny and social influences on sex change. *Bull Marine Sciences.* 42:317-333.

Cole, K. S. 1988. Predicting the Potential for Sex-Change on the Basis of Ovarian Structure in Gobiid Fishes. *Copeia*:1082-1086.

---. 2002. Gonad morphology, sexual development, and colony composition in the obligate coral-dwelling damselfish *Dascyllus aruanus*. *Mar. Biol.* 140:151-163.

Cole, K. S., and D. Y. Shapiro. 1992. Gonadal Structure and Population Characteristics of the Protogynous Goby *Coryphopterus glaucofraenum*. *Mar. Biol.* 113:1-9.

Cole, K. S., D. R. Robertson, and A. A. Cedeno. 1994. Does Gonad Structure Reflect Sexual Pattern in All Gobiid Fishes. *Environ. Biol. Fishes* 41:301-309.

Cole, K. S., and Shapiro, D. Y. 1990. Gonad structure and hermaphroditism in the Gobiid genus *Coryphopterus* (Teleostei: Gobiidae). *Copeia* 4:996-1003.

Coleman, F. C., C. C. Koenig, and L. A. Collins. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. *Environ. Biol. Fishes* 47:129-141.

Collin, R. 1995. Sex, size, and position: A test of models predicting size at sex change in the protandrous gastropod *Crepidula fornicata*. *The American Naturalist* 146:815-831.

---. 2000. Sex change, reproduction, and development of *Crepidula adunca* and *Crepidula lingulata* (Gastropoda : Calyptraeidae). *Veliger* 43:24-33.

Cowen, R. K. 1990. Sex-Change and Life-History Patterns of the Labrid, *Semicossyphus pulcher*, across an environmental gradient. *Copeia*:787-795.

Crabtree, R. E., and L. H. Bullock. 1998. Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters. *Fish. Bull.* 96:735-753.

Crawley, M. 1993. GLIM for Ecologists. Blackwell Scientific, Oxford.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. Murray, London.

Day, J. J. 2002. Phylogenetic relationships of the Sparidae (Teleostei : Percoidei) and implications for convergent trophic evolution. *Biological Journal of the Linnean Society* 76:269-301.

de Girolamo, M., Scaggiante, M. and Rasotto, M. B. 1999. Social organization and sexual pattern in the Mediterranean parrotfish *Sparisoma cretense* (Teleostei : Scaridae). *Mar. Biol.* **135**:353-360.

De la Herran, R., C. R. Rejon, M. R. Rejon, and M. A. Garridos-Ramos. 2001. The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. *Heredity* 87:691-697.

Desdevises, Y., S. Morand, O. Jousson, and P. Legendre. 2002. Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): The study of a complex host-parasite system. *Evolution* 56:2459-2471.

Diniz-Filho, J. A. F., C. E. R. de Sant' Ana, and L. M. Bini. 1998. An eigenvector method for estimating phylogenetic inertia. *Evolution* 52:1247 - 1262.

Durchon, M. 1975. Sex reversal in the *Syllinae* (Polychaeta: Annelida). Pp. 41-47 *in* R. Reinboth, ed. Intersexuality in the Animal Kingdom. Springer-Verlag, Berlin.

Edwards, A. W. F. 2000. Carl Dusing (1884) on *The Regulation of the Sex-Ratio*. *Theoretical Population Biology* 58:255-257.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215-223.

Felsenstein, J. 1985. Phylogenies and the Comparative Method. *American Naturalist* 125:1-15.

Fennessy, S. T., and Y. Sadovy. 2002. Reproductive biology of a diandric protogynous hermaphrodite, the serranid *Epinephelus andersoni*. *Mar. Freshw. Res.* 53:147-158.

Ferreira, B. P., and G. R. Russ. 1995. Population-Structure of the Leopard Coralgrouper, *Plectropomus leopardus*, on Fished and Unfished Reefs Off Townsville, Central Great-Barrier-Reef, Australia. *Fish. Bull.* 93:629-642.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University press, Oxford.

Frank, S. A. 1987. Individual and population sex allocation patterns. *Theoretical Population Biology* 31:47-74.

---. 1990. Sex allocation theory for birds and mammals. *Annual Reviews of Ecology and Systematics* 21:13-55.

---. 1998. Foundations of Social Evolution. Princeton University Press, Princeton.

Frank, S. A., and I. R. Swingland. 1988. Sex-Ratio under Conditional Sex Expression. *Journal of Theoretical Biology* 135:415-418.

Fricke, H., and S. Fricke. 1977. Monogamy and sex change by aggressive dominance in coral reef fish. *Nature* 266:830-832.

Fricke, H., W. 1979. Mating System, Resource Defense and Sex Change in the Anemonefish *Amphiprion akallopisos*. *Z. Tierpsychol.* 50:313-326.

Fricke, H. W. 1980. Control of different mating systems in a coral reef fish by one environmental factor. *Anim. Behav.* 28:561-569.

Garrat, P. A. 1986. Protogynous hermaphroditism in the slinger, *Chrysoblephus puniceus* (Gilchrist & Thompson, 1908) (Teleostei: Sparidae). *J. Fish Biol.* 28:297-306.

Gemmill, A. W., A. Skorping, and A. F. Read. 1999. Optimal timing of first reproduction in parasitic nematodes. *J. Evol. Biol.* 12:1148-1156.

Ghiselin, M. T. 1969. The Evolution of hermaphroditism amongst animals. *Quart. Rev. Bio.* 44:189-208.

Gillanders, B. M. 1995. Reproductive biology of the protogynous hermaphrodite *Achoerodus viridis* (Labridae) from south-eastern Australia. *Mar. Freshw. Res.* 46:999-1008.

Godwin, J. 1995. Phylogenetic and habitat influences on mating system structure in the humbug damselfishes (Dascyllus, Pomacentridae). *Bull. Mar. Sci.* 57:637-652.

Grafen, A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society of London (Series B)* 326:119-157.

Grandcourt, E. M. 2002. Demographic characteristics of a selection of exploited reef fish from the Seychelles: preliminary study. *Mar. Freshw. Res.* 53:123-130.

Gross, M. R. 1982. Sneakers, satellites and parentals: polymorphic mating strategies in North American sunfishes. *Z. Tierpsychol.* 60:1-26.

---. 1991. Evolution of alternative reproductive strategies: frequency-dependent sexual selection in male bluegill sunfish. *Phil. Trans. R. Soc. Lond. B.* 332:59-66.

Hamilton, W. D. 1967. Extraordinary sex ratios. *Science* 156:477-488.

Hanel, R., and C. Sturmbauer. 2000. Multiple recurrent evolution of trophic types in northeastern Atlantic and Mediterranean seabreams (Sparidae, Percoidei). *J. Mol. Evol.* 50:276-283.

Hanel, R., M. W. Westneat, and C. Sturmbauer. 2002. Phylogenetic Relationships, Evolution of Broodcare Behaviour, and Geographic Speciation in the Wrasse Tribe Labrini. *J. Mol. Evol.* 55:776-789.

Hardy, I. C. W. 2002. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Harvey, P. H., and M. D. Pagel. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press

Herre, E. A., C. A. Machado, and S. A. West. 2001. Selective Regime and Fig Wasp Sex Ratios: Towards Sorting Rigor from Pseudo-Rigor in Tests of Adaptation. Pp. 191-218 *in* S. Orzack and E. Sober, eds. Adaptionism and Optimality. Cambridge University Press, Cambridge.

Highsmith, R. C. 1983. Sex Reversal and Fighting Behavior: Coevolved Phenomena in a Tanaid Crustacean. *Ecology* 64:719-726.

Hoagland, K. E. 1978. Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia* 17:365-391.

Hoffman, S. G., M. P. Schildhauer, and R. R. Warner. 1985. The cost of changing sex and the ontogeny of males under contest competition for mates. Evolution. *Evolution* 39:915-22.

Huxley, J. 1993. Problem of Relative Growth. John Hopkins University Press, Baltimore.

Iles, T. D. 1974. The tactics and strategy of growth in fishes. Pp. 331-345 *in* F. R. Harden Jones, ed. Sea fisheries research. Elek Science, London.

Jazdzewski, K. 1969. Biology of two hermaphroditic crustacea, *Cyathura carinata* (Kroyer) (*Isopoda*) and *Heterotanais oerstedi* (Kroyer) (*Tanaidacea*) in waters of the Polish Baltic Sea. *Zoologica Poloniae* 19:5-25.

Jones, C. M. 1992. Development and Application of the Otolith Increment Technique. Pp. 1-11 *in* D. K. Stevenson and S. E. Campana, eds. Otolith microstructure examination and analysis. Canadian Spec. Publ. Fish. Aquat. Sci., Ottawa.

Jones, R. 1976. Growth of fishes. Pp. 251 - 279 *in* D. H. Cushing and J. J. Walsh, eds. The Ecology of the Seas. Blackwell Scientific Publishing.

Kensley, B. 1982. Deep water Atlantic Anthuridea (Crustacea: Isopoda). *Smithsonian Contrib. Zool.* 346:1-51.

Kerrigan, B. A. 1994. Postsettlement Growth and Body-Composition in Relation to Food Availability in a Juvenile Tropical Reef Fish. *Mar. Ecol.-Prog. Ser.* 111:7-15.

Kleiber, M. 1932. Body Size and Metabolism. *Hilgardia* 6:315 - 353.

Kobayashi, K., and K. Suzuki. 1992. Hermaphroditism and sexual function in *Cirrhitichthys aureus* and other Japenese Hawfishes (Cirrhitidae: Teleostei). *Japan. J. Ichthyology.* 38

Kokko, H., and M. Jennions. 2003. It takes two to tango. *TREE* 18:103 - 104.

Kraljevic, M., J. Dulcic, A. Pallaoro, P. Cetinic, and J. Jugdujakovic. 1995. Sexual-Maturation, Age and Growth of Striped Sea Bream, *Lithognathus mormyrus* L, on the Eastern Coast of the Adriatic Sea. *J. Appl. Ichthyol.-Z. Angew. Ichthyol.* 11:1-8.

Kuwamura, T., N. Tanaka, Y. Nakashima, K. Karino, and Y. Sakai. 2002. Reversed sex-change in the protogynous reef fish Labroides dimidiatus. *Ethology* 108:443-450.

Lauder, G. V., and K. F. Liem. 1983. The evolution and inter-relationships of the actinopterygian fishes. *Bull. Mus. comp. Zool.* 150:95 - 197.

Legrand, J.-J., and P. Juchault. 1963. Mise en evidense d'un hermaphrodisme protogynique fonctionnel chez l'Isopode Anthuride Cyathura carinata (Kroyer) et etude du mecanisme de l'inversion sexuelle. *C. R. Acad. Sci. Paris.* 256:2931-2933.

Leigh, E. G., E. L. Charnov, and R. R. Warner. 1976. Sex ratio, sex change and natural selection. *Proc. Natl. Acad. Sci.* 73:3655-3660.

Lejeune, P. 1987. The effect of local stock density on social behavior and sex change in the Mediterranean labrid *Coris julis*. *Environmental Biology of Fisheries* 18:135-141.

Lo Galbo, A. M., K. E. Carpenter, and D. L. Reed. 2002. Evolution of trophic types in emperor fishes (Lethrinus, Lethrinidae, Percoidei) based on cytochrome b gene sequence variation. *J. Mol. Evol.* 54:754-762.

Longhurst, A. R. 1965. The Biology of West African Polynemid Fishes. *J. Cons. perm. int. Explor. Mer.* 30:58 - 74.

Lorenzo, J. M., J. G. Pajuelo, M. Mendez-Villamil, J. Coca, and A. G. Ramos. 2002. Age, growth, reproduction and mortality of the striped seabream, *Lithognathus mormyrus* (Pisces, Sparidae), off the Canary Islands (Central-east Atlantic). *J. Appl. Ichthyol.* 18:204-209.

Lowry, J. K., and D. R. Stoddart. 1986. Protandrous hermaphrodites among the Lysianassoid amphipods. *J. Crustacean Biology* 6:742-748.

Lutnesky, M. M. F. 1996. Density-Dependent protogynous sex-change in territorial haremic fishes - models and evidence. *Behav. Ecol.* 5:375-383.

Mackie, M. 2000. Reproductive biology of the halfmoon grouper, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. *Environ. Biol. Fishes* 57:363-376.

Mangel, M. 1996. Life History Invariants, age at maturity and the ferox trout. *Evol. Ecol.* 10:249-263.

Mann, B. Q., and C. D. Buxton. 1998. The reproductive biology of *Diplodus sargus capensis* and *D cervinus hottentotus* (Sparidae) off the south-east Cape coast, South Africa. *Cybium* 22:31-47.

Marino, G., E. Azzurro, A. Massari, M. G. Finoia, and A. Mandich. 2001. Reproduction in the dusky grouper from the southern Mediterranean. *J. Fish Biol.* 58:909-927.

Mayhew, P. J., and I. Pen. 2002. Comparative analysis of sex ratios. Pp. 132-156 *in* I. C. W. Hardy, ed. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Maynard Smith, J. 1978. Optimization theory in evolution. *Annual Reviews of Ecology and Systematics* 9:31-56.

---. 1982. Evolution and the Theory of Games. Cambridge University Press, Cambridge.

Mitani, F. 1970. A comparative study on growth patterns of marine fishes. *Bull. Jap. Soc. scient. Fish* 36:258-265.

Modlin, R. F., and P. A. Harris. 1989. Observations on the Natural History and experiments on the reproductive strategy of *Hargeria rapax* (Tanaidacea). *Journal of Crustacean Biology* 9:578-586.

Morton, B. 1979. The population dynamics and expression of sexuality in *Balcis shaplandi* and *Mucronalia fulvescens* (Mollusca: Gastropoda: Aglossa) parasitic upon *Archaster typicus* (Echinodermata: Asteroidea). *Malacologia* 18:327-346.

Moyer, J. T., and A. Nakazono. 1978a. Population structure, Reproductive Behavior and Protogynous Hermaphroditism in the Angelfish *Centropyge interruptus*, at Miyake-jima, Japan. *Japan. J. Ichthyology.* 25:25-38.

---. 1978b. Protandrous hermaphroditism in six species of the Anemonefish Genus Amphiprion in Japan. *Japan. J. Ichthyology.* 25:101-106.

Munday, P. L. 2002. Bi-directional sex change: testing the growth-rate advantage model. *Behav. Ecol. Sociobiol.* 52:247-254.

Munday, P. L., and B. W. Molony. 2002. The energetic cost of protogynous versus protandrous sex change in the bi-directional sex-changing fish *Gobiodon histrio*. *Mar. Biol.* 141:1011-1017.

Munday, P. L., M. J. Caley, and G. P. Jones. 1998. Bi-directional sex change in a coral-dwelling goby. *Behav. Ecol. Sociobiol.* 43:371-377.

Munoz, R. C., and R. R. Warner. 2003. A New Version of the Size-Advantage Hypothesis for Sex Change: Incorporating Sperm Competition and Size-Fecundity Skew. *The American Naturalist* 161:749 - 761.

Nelson, J. S. 1994. Fishes of the world. Wiley, New York ; Chichester.

Okada, Y. K. 1965. Sex reversal in the serranid fish *Sacura margaritacea*. *Jap. Acad., Proc.* 41:741-745.

Orrell, T. M., K. E. Carpenter, J. A. Musick, and J. E. Graves. 2002. Phylogenetic and Biogeographic Analysis of the Sparidae (Perciformes: Percoidei) from Cytochrome *b* Sequences. *Copeia* 3:618-631.

Pagel, M. D. 1992. A method for the analysis of comparative data. *Journal of Theoretical Biology* 156:431-442.

---. 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. *J. Theor. Biol.* 164:191-205.

Pauly, D. 1981. The relationship between gill surface area and growth performance in fish: a generalisation of von Bertalanffy's theory of growth. *Ber. dt. Wiss. Kommn Meeresforsch* 23:366-370.

---. 1984. A mechanism for the juvenile-to-adult transition in fishes. *J. Con. int. Explor. Mer* 41:280-284.

Pen, I., and F. J. Weissing. 2000. Sex ratio optimization with helpers at the nest. *Proc. Roy. Soc. Lond. B* 267:539-544.

---. 2002. Optimal sex allocation: steps towards a mechanistic theory. Pp. 26-45 *in* I. C. W. Hardy, ed. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Penrith, M. J. 1972. Sex reversal in the sparid fish *Chrysoblephus laticeps. Koedoe* 15:135-139.

Policansky, D. 1982. Sex Change in Plants and Animals. *Annu. Rev. Ecol. Syst.* 13:471-495.

Pollock, B. R. 1985. The reproductive cycle of yellowfin bream, *Acanthopagrus australis* (Gunther), with particular reference to protandrous sex inversion. *J. Fish Biol.* 26:301-311.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Computer Appl. Biosciences* 11:247-251.

Reinboth, R. 1975. Intersexuality in the Animal Kingdom. Springer-Verlag, Berlin.

Reynolds, J. D., and J. C. Jones. 1999. Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschististus microps*). *Behav. Ecol.* 10:149 - 154.

Robertson, D. R. 1972. Social control of sex reversal in a coral reef fish. *Science.* 177:1007-1009.

Robertson, D. R., and J. H. Choat. 1974. Protogynous hermaphroditism and social systems in Labrid fish. *Proc. Second. Intnl. Coral Reef Symposium 1.* 1:217-225.

Robertson, D. R., and S. G. Hoffman. 1977. The roles of female mate choice and predation in the mating systems of some tropical labroid fishes. *Z. Tierpsychol.* 45:298-320.

Robertson, D. R., and R. R. Warner. 1978. Sexual patterns in the labroid fishes of the western Carribbean, II: The Parrotfishes (Scaridae). *Smithsonian Contrib. Zool.* 255:1-26.

Robertson, D. R., and G. Justines. 1982. Protogynous hermaphroditism and gonochorism in four Caribbean reef gobies. *Environ. Biol. Fishes* 7:137-142.

Rohlf, F. J. 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* 55:2143 - 2160.

Ross, R. M. 1990. The Evolution of Sex-Change Mechanisms in Fishes. *Environ. Biol. Fishes* 29:81-93.

Sammarco, P. W., and J. C. Andrews. 1988. Localised dispersal and recruitment in Great Barrier Reef corals: the helix experiment. *Science* 239:1422 - 1424.

Schwarz, M. P. 1988. Local resource enhancement and sex ratios in a primitively social bee. *Nature* 331:346-348.

Sewell, M. A. 1994. Small Size, Brooding, and Protandry in the Apodid Sea Cucumber *Leptosynapta clarki*. *Biol. Bull.* 187:112-123.

Shapiro, D. Y. 1979. Social behaviour, group structure and sex reversal in hermaphroditic fish. *Adv. Study. Behav.* 10:43-102.

---. 1981a. Size, maturation and the social control of sex reversal in the coral reef fish Anthias squamipinnis (Peters). *Journal of Zoology* 193:105-128.

---. 1981b. Size, Maturation and the Social-Control of Sex Reversal in the Coral-Reef Fish Anthias-Squamipinnis. *Journal of Zoology* 193:105-128.

Shapiro, D. Y., and R. Lubbock. 1980. Group sex ratio and sex reversal. *J. Theor. Biol.* 82:411-426.

Shapiro, D. Y., and R. H. Boulon. 1982. The Influence of Females on the Initiation of Female-to-Male Sex Change in a Coral-Reef Fish. *Horm. Behav.* 16:66-75.

Shapiro, D. Y., Y. Sadovy, and M. A. McGehee. 1993. Size, composition, and spatial structure of the annual spawning aggregation of the red hind, *Epinephelus guttatus* (Pisces, Serranidae). *Copeia* 2:399-406.

Shen, S.-C., and C. H. Liu. 1976. Ecological and morphological study of the fish fauna from the waters around Taiwan and its adjacent islands. *Acta Oceanographica Taiwanica* 6:140-150.

Shpigel, M., and L. Fishelson. 1986. Ecology and sociobiology of coexistence in two species of *Dascyllus* (Pomacentridae, Teleostei). *Bull. Inst. Ocean Fish. (Egypt)* 9

Skuladottir, U., and G. Petursson. 1999. Defining populations of northern shrimp, *Pandalus borealis* (Kroyer 1938), in Icelandic waters using maximum length and maturity ogive of females. *Rit Fiskideildar* 16:247-262.

Soong, K., and J. L. Chen. 1991. Population-Structure and Sex-Change in the Coral-Inhabiting Snail *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Mar. Biol.* 111:81-86.

St Mary, C. M. 1997. Sequential patterns of sex allocation in simultaneous hermaphrodites: do we need models that specifically incorporate this complexity? *The American Naturalist* 150:73-97.

---. 2000. Sex allocation in Lythrypnus (Gobiidae): variations on a hermaphroditic theme. *Environ. Biol. Fishes* 58:321-333.

Stamps, J. A., J. B. Losos, and R. M. Andrews. 1997. A comparative study of population density and sexual size dimorphism in Lizards. *American Naturalist* 149:64 - 90.

Starck, J. M. 1998. Non-independence of data in biological comparisons. A critical appraisal of current concepts, assumptions, and solutions. *Theory in Biosciences* 117:109-138.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Stephens, D. W., and S. R. Dunbar. 1992. Dimensional analysis in behavioural ecology. *Behav. Ecol.* 4:172-183.

Stoddart, D. R. 1962. Three Caribbean atolls: Turneffe Islands, Lighthouse Reef, and Glovers Reef, British Honduras. *Atoll research bulletin* 87:1-128.

Stoner, A. W. 1986. Cohabitation on algal habitat islands by two hermaphroditic Tanaidacea (Crustacea: Peracarida). *Journal of Crustacean Biology* 6:719-728.

Streelman, J. T., M. Alfaro, M. W. Westneat, D. R. Bellwood, and S. A. Karl. 2002. Evolutionary history of the parrotfishes: Biogeography, ecomorphology, and comparative diversity. *Evolution* 56:961-971.

Sunobe, T., and A. Nakazono. 1993. Sex-Change in Both Directions by Alteration of Social-Dominance in Trimma-Okinawae (Pisces, Gobiidae). *Ethology* 94:339-345.

Suzuki, K., K. Kobayashi, S. Hioki, and T. Sakamoto. 1978. Ecological Studies of the Anthiine Fish, *Franzia squamipinnis*, in Suruga Bay, Japan. *Japan. J. Ichthyology.* 25:124-140.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral reef fish. *Nature* 402:799 - 802.

Thompson, D. 1959. On Growth and Form. Cambridge University Press

Tobin, A. J., M. J. Sheaves, and B. W. Molony. 1997. Evidence of protandrous hermaphroditism in the tropical sparid *Acanthopagrus berda*. *J. Fish Biol.* 50:22-33.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92.

Tsai, M. L., J. J. Li, and C. F. Dai. 1999. Why selection favors protandrous sex change for the parasitic isopod, *Ichthyoxenus fushanensis* (Isopoda : Cymothoidae). *Evol. Ecol.* 13:327-338.

Tupper, M. 1999. A brief review of grouper reproductive biology and implications for management of the Gulf of Mexico gag grouper fishery. Pp. 12 pp. Southeastern Fisheries Association, Inc., Tallahassee, FL.

Tyler, P. A., and J. D. Gage. 1982. The reproductive biology of *Ophiacantha bidentata* (Echinodermata: Ophiuroidea) from the Rockall trough. *J. mar. biol. Ass. UK* 62:45-55.

van der Walt, B. A., and B. Q. Mann. 1998. Aspects of the reproductive biology of *Sarpa salpa* (Pisces : Sparidae) off the east coast of South Africa. *South Afr. J. Zool.* 33:241-248.

Villamil, M. M., J. M. Lorenzo, J. G. Pajuelo, A. G. Ramos, and J. Coca. 2002. Aspects of the life history of the salema, *Sarpa salpa* (Pices, Sparidae), off the Canarian Archipelago (central-east Atlantic). *Environ. Biol. Fishes* 63:183-192.

Warner, R. R. 1974. The reproductive biology of the protogynous hermaphrodite *Pimelometopon pulchrum* (Pisces: Labridae). *Fish. Bull.* 73:262-283.

---. 1975. The adaptive significance of sequential hermaphroditism in animals. *Amer. Natur.* 109:61-82.

---. 1982. Mating systems, sex change and sexual demography in the rainbow wrasse, *Thalassoma lucasanum. Copeia* 3:653-661.

---. 1984. Mating behavior and Hermaphroditism in Coral Reef Fishes. *American Scientist* 72:128-136.

---. 1988a. Sex Change in Fishes - Hypotheses, Evidence, and Objections. *Environ. Biol. Fishes* 22:81-90.

---. 1988b. Sex Change and the Size-Advantage Model. *Trends in Ecology & Evolution* 3:133-136.

Warner, R. R., and D. R. Robertson. 1978. Sexual patterns in the labroid fishes of the western Carribbean, I: The wrasses (Labridae). *Smithsonian Contrib. Zool.* 254:1-27.

Warner, R. R., and S. G. Hoffman. 1980a. Local population size as a determinant of mating system and sexual composition in two tropical marine fishes (Thalassoma spp.). *Evolution* 34:508-518.

---. 1980b. Population density and the economics of territorial defense in a coral reef fish. *Ecology* 61:772-780.

Warner, R. R., and P. Lejeune. 1985. Sex change limited by paternal care: a test using four Mediterranean labrid fishes, genus Symphodus. *Mar. Biol.* 87:89-99.

Warner, R. R., and S. E. Swearer. 1991. Social-Control of Sex-Change in the Bluehead Wrasse, Thalassoma-Bifasciatum (Pisces, Labridae). *Biol. Bull.* 181:199-204.

Warner, R. R., D. R. Robertson, and E. G. Leigh. 1975. Sex change and Sexual Selection: The reproductive biology of a labrid fish is used to illuminate a theory of sex change. *Science.* 190:633-638.

Warner, R. R., D. L. Fitch, and J. D. Standish. 1996. Social control of sex change in the shelf limpet, *Crepidula norrisiarum*: Size-specific responses to local group composition. *J. Exp. Mar. Biol. Ecol.* 204:155-167.

West, G. B., J. H. Brown, and B. J. Enquist. 1997. A general model for the origin of allometric scaling laws in Biology. *Science* 276:122 -126.

West, S. A., and B. C. Sheldon. 2002. Constraints in the evolution of sex ratio adjustment. *Science* 295:1685-1688.

West, S. A., and E. A. Herre. 2002. Using sex ratios: why bother? Pp. 399-413 *in* I. C. W. Hardy, ed. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

West, S. A., E. A. Herre, and B. C. Sheldon. 2000a. The benefits of allocating sex. *Science.* 290:288-290.

West, S. A., T. G. Smith, and A. F. Read. 2000b. Sex allocation and population structure in Apicomplexan (Protozoa) parasites. *Proc. R. Soc. London Ser. B* 267:257-263.

West, S. A., S. E. Reece, and B. C. Sheldon. 2002a. Sex Ratios. *Heredity* 88:117 - 124.

West, S. A., T. G. Smith, S. Nee, and A. F. Read. 2002b. Fertility insurance and the sex ratios of malaria and related hemospororin blood parasites. *J. Parasitol.* 88:258-263.

Williams, G. C. 1975. Sex and Evolution. Princeton University Press, Princeton.

Wilson, K., and I. C. W. Hardy. 2002. Statistical analysis of sex ratios: an introduction. Pp. 48-92 *in* I. C. W. Hardy, ed. Sex Ratios: Concepts and Research Methods. Cambridge University Press, Cambridge.

Wolanski, E., and W. Hamner. 1988. Topographically controlled fronts in the ocean, and their influence on the distribution of organisms. *Science* 241:177 - 181.

Wright, W. G. 1989. Intraspecific Density Mediates Sex-Change in the Territorial Patellacean Limpet *Lottia gigantea*. *Mar. Biol.* 100:353-364.

Young, P. C., and R. B. Martin. 1982. Evidence for protogynous hermaphroditism in some lethrinid fishes. *J. Fish Biol.* 21:475-484.

Appendix – published papers