

The extraordinary sex ratios in the splash pool copepod *Tigriopus californicus*

by

Travis Christopher Tai
B.Sc., University of Western Ontario, 2010

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SUPERVISORY COMMITTEE

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Supervisory Committee

Dr. Bradley R. Anholt (Department of Biology)
Supervisor

Dr. Patrick Gregory (Department of Biology)
Departmental Member

Dr. Steve Perlman (Department of Biology)
Departmental Member

ABSTRACT

Supervisory Committee

Dr. Bradley R. Anholt (Department of Biology)

Supervisor

Dr. Patrick Gregory (Department of Biology)

Departmental Member

Dr. Steve Perlman (Department of Biology)

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Fisher's adaptive sex ratio theory predicts that organisms should invest equally in sons and daughters and the sex ratio at conception should be 1:1. Hamilton's theory predicts that organisms should adjust sex ratios based on the relative strength of competition within a mating group. Testing sex ratio and sex allocation theories requires variation in sex ratio. Different sex allocation and sex allocation adjustment mechanisms can produce skewed sex ratios. I used *Tigriopus californicus*, a harpacticoid copepod with extrabinomial variation in sex ratios, to test sex ratio evolution and socially-mediated sex determination. Using artificially selected sex-biased populations, the trajectory of population sex ratios were as expected under Fisher's theory and sex ratios approached/reached 0.5 proportion males. Populations with overlapping generations had a slower rate of change towards 0.5 than populations with non-overlapping generations. I show that these data are supported by multiple different models: a mechanistic and simulation model. I tested socially-mediated sex determination using seawater conditioned with different local sex ratios of copepods. There were detectable effects found in both wild populations and isofemale lines. However, these effects may be trivial as differences were small between treatments. Sex determination in *T. californicus* is a complex mechanism, with multiple genetic and environmental components. The complex nature of sex determination in *T. californicus* and the dynamic nature of their habitat in highly ephemeral splash pools provide a possible explanation for the non-Fisherian sex ratios we see.

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CHAPTER 1 – Introduction to sex ratio theory and *Tigriopus californicus*

Introduction to sex ratio theory

Sexual reproduction is widespread and has become a topic of intense investigation among evolutionary biologists (see Otto and Lenormand [2002] review). Despite the common notion of sex benefits—i.e., increased genetic variability—mathematical models have proved that these benefits do not necessarily overcome the two-fold reduction in fitness compared with asexual reproduction and thus does not necessarily result in the evolution of sex (Otto and Lenormand, 2002). As part of this field there has been a long history of investigation into the determinants of resource allocation by parents depending on the sex of their offspring.

Sex ratio theory has been a relatively successful discipline of evolutionary biology (Bull and Charnov, 1988), and has contributed to the understanding of other general evolutionary theories (Voordouw, 2005). Sex ratio theory focuses on sex ratio as a trait and its adaptive significance. Sir Ronald Fisher (1930) was the first to introduce an explanation for the adaptive significance of equal sex ratios. He stated that the sex ratio in sexually reproducing species should be equal and parents should invest equally in each sex. If sons and daughters are equally costly to produce, the relative number of sons produced should equal the relative number of daughters produced. Fisher's idea is derived from the fact that individuals of sexually reproducing species will have exactly one mother and one father, and obtain half of their genetic make-up from either parent. As sons and daughters are equally likely to pass on their genes to the next generation, the

reproductive value of each sex must then be equal. Any deviation from this equilibrium should be countered by frequency dependent selection. If one sex is produced in excess, parents that produce the less abundant sex (as well as those individuals) will have a higher per capita genetic contribution towards subsequent generations and their genes will increase in frequency until the sex ratio is balanced. Fisher's 'balanced' sex ratio prediction does not always result in a 1:1 sex ratio of males to females. Instead, it is the resulting sex ratio produced after relative costs and benefits of either sex are taken into account. This can include costs during zygotic development (Charnov, 1982), costs of parental care, or benefits of sex-specific social behaviours that increase the lifetime reproductive success of an individual (e.g. female 'helpers' hold a greater benefit as they repay some of the costs by looking after future offspring; Emlen et al., 1986).

Evolutionary biologists have often argued whether the ubiquity of balanced sex ratios is a product of Fisher's adaptive sex ratio theory, or merely a consequence of Mendelian segregation. In organisms with sex chromosomes, a balanced sex ratio will result from independent assortment of sex chromosomes during meiosis. Mendelian segregation of sex chromosomes will produce an equal number of sons and daughters due to probability alone; organisms with sex chromosomes are constrained by Mendel's law and testing Fisher's theory is not possible (Bull et al., 1982; Bull, 1985). In order for a trait (such as primary sex ratio – PSR) to be adaptive and be subject to selection, we require genetic variation for that trait. Organisms with sex chromosomes generally lack variation in primary sex ratio (PSR), the sex ratio of offspring at conception, due to Mendelian segregation which produces sex ratios of 0.5 from probability alone. Variation in PSR is essential for testing Fisher's adaptive sex ratio theory.

In addition to variation in PSR, seven assumptions are outlined by Bull and Charnov (1988) for testing Fisher's theory. The most essential assumptions are: (1) separate sexes (Fisher, 1930), (2) biparentalism (Fisher, 1930), and (3) Mendelian segregation of alleles (Shaw, 1958; Hamilton, 1967). The first assumption is straight forward and needs no explanation of its importance in Fisher's adaptive sex ratio theory. Together, assumptions 2 and 3 create the frequency-dependent selection conditions that drive the sex ratio to 1:1, relative to the costs of sons and daughters (Voordouw, 2005). Violation of either of these assumptions usually produces extremely biased sex ratios (Bull and Charnov, 1988; Voordouw, 2005).

Fisher's adaptive sex ratio theory also assumes: (4) random mating in an infinite population (Hamilton, 1967), (5) additive offspring costs (Fisher, 1930; MacArthur, 1965), (6) absence of environmentally induced sex-specific differences (Bull, 1981), and (7) parental control (Fisher, 1930; Trivers, 1974). Violation of random mating in an infinitely large population (assumption 4) can severely influence sex ratio, such as in populations with group-structured mating (Hamilton, 1967; Voordouw, 2005). Local mate competition, where mating occurs in small groups, can affect PSR. For example, in organisms where mating occurs in small groups and males can mate multiple times (e.g., haplodiploid eusocial insects with maternally controlled fertilization), a female will maximize fecundity by producing a single son and multiple daughters (Mueller, 1991); if daughters can only mate once, the reproductive fitness of a mother increases with each daughter produced, but not with each son produced (Hamilton, 1967; Bull and Charnov, 1988). Small population sizes produce greater variation and mutations carry greater influence on the evolution of a trait. Assumption 5 requires that the production of sons

and daughters have equal fitness benefits. In species with group-structured mating, as in the example provided above, the benefit-cost ratio between sons and daughters may be imbalanced (Hamilton, 1967; Bull and Charnov, 1988). Fisher's model also assumes that environmentally induced sex-specific differences are absent in the system (assumption 6; Bull, 1981). Environmental effects on individuals can differ between the sexes and result in biased sex ratios. For example, low quality environments can affect the reproduction of daughters more than sons as daughters often require more resources to invest in reproduction (Trivers and Willard, 1973). To maximize fitness, parents should then invest in sons in low quality environments, and daughters in high quality environments. Sex ratios should be biased in favour of the sex that does better in the lower quality environments (Bull, 1981).

The final assumption of Fisher's adaptive sex ratio theory requires that the mechanism responsible for sex determination is under parental control. Examples of parental control of sex determination include: sex chromosome systems, environmental sex determination (ESD; e.g., nest selection in reptiles [Wood and Bjorndal, 2000; Kamel and Mrosovsky, 2004]), haplodiploidy (e.g., fertilization rate controlled by queens in eusocial populations (Mueller, 1991), and meiotic drive in sex chromosome systems (e.g., non-random segregation of sex chromosomes [e.g., Carvalho et al., 1998; Rutkowska and Badyaev, 2008; Blanco et al., 2002]). Fisher's model was initially exclusive to modes of parental control for sex determination, but Bulmer and Bull (1982) showed that Fisher's theory applies to zygotic control of sex determination as well. Under zygotic control, sex is ultimately determined by the zygote, not the parent (Bulmer and Bull, 1982). The rate of

evolution of sex ratio when sex determination is under zygotic control is twice that under parental control (Bulmer and Bull, 1982).

The rarity of variation in primary sex ratio has limited the number of studies that have tested Fisher's theory. Only four studies have tested Fisher's theory: in the platyfish *Xiphophorus maculatus* (Basolo, 1994), in *Drosophila mediopunctata* (Carvalho et al., 1998), in hybrids of *D. serrata* and *D. birchii* (Blows et al., 1999), and in Atlantic silversides *Menidia menidia* (Conover and Voorhees, 1990), with only one satisfying all of the model's assumptions (Carvalho et al., 1998). Chapter 2 describes these studies in further detail. Attempts at testing Fisher's theory in species with chromosomal sex determination have been mostly unsuccessful due to the lack of genetic variance for sex ratio (e.g., Falconer, 1954; Williams, 1979; Toro and Charlesworth, 1982; see Voordouw, 2005). This exemplifies the rarity with which organisms meet specific conditions in order to apply Fisher's theory. In my thesis I used *Tigriopus californicus* as a model organism to investigate sex ratio theories.

Introduction to *Tigriopus californicus*

Tigriopus californicus (Baker, 1912) is a harpacticoid copepod that has been extensively studied for its unique and complicated sex determining mechanism (e.g., Ar-Rushdi, 1958; Ar-Rushdi, 1963; Egloff, 1966; Voordouw and Anholt, 2002a; Voordouw and Anholt, 2002b; Voordouw et al., 2005b). These studies have supported a polygenic sex determining mechanism, whereby sex is determined by additive effects, both genetic and environmental, over multiple independent loci. *T. californicus* has highly variable PSRs that are extrabinomial, making them an ideal organism for testing evolutionary sex ratio theories.

T. californicus inhabits supralittoral splash pools (Dybdahl, 1995) above the high tide line along the west coast of North America, from northern Mexico to Alaska (Egloff, 1966). Extreme environmental fluctuations in wave exposure, temperature and salinity characterize their habitat (Vittor, 1971). Variable wave exposure regimes possibly drive local adaptation for life history traits affected by associated osmotic and desiccation stressors, among others (Dybdahl, 1995). *T. californicus* habitat creates ephemeral populations that interact to form metapopulations; they are intermittently connected by rainfall or high wave action (Burton and Feldman, 1981). Populations within a metapopulation have a high turnover rate—an almost complete population turnover occurs after about four successive high tides (MacKeracher, unpublished data).

The life cycle consists of five naupliar stages and six juvenile copepodid stages prior to reproductive maturity (Egloff, 1966), which under laboratory conditions (20°C) is reached in approximately 3 weeks (Voordouw and Anholt, 2002b). Throughout early development, *T. californicus* are sexually monomorphic. At copepodid stage IV individuals develop sexually dimorphic body shapes and antennae (Egloff, 1966). Sexually mature males have a tapered ‘tear-drop’ shaped body, whereas females have a less tapered body (personal observations). Further, the first antennae in females are thin, long and straight whereas males develop modified robust geniculate antennae.

Males exhibit mate guarding behaviour where they clasp immature females during copepodid stages 2-5 using their first antennae, investing up to seven days guarding any one female (Burton, 1985), thereby increasing the probability of reproductive success. Males internally inseminate the guarded female following the female terminal molt (Burton, 1985). *T. californicus* will produce non-viable egg masses until inseminated

(Burton, 1985); however, females can be successfully inseminated and produce viable egg sacs any time after the terminal molt (Burton 1985; Egloff, 1966). Females will mate only once, and produce multiple successive fertile egg sacs using stored sperm. Multiple mating events by females is either absent or rare, with any instances likely due to experimental error (Burton, 1985), indicating that sperm displacement and competition does not occur in *T. californicus* (Burton et al., 1979; Burton et al., 1981; Burton 1985). Males are able to mate multiple times, and tend to select more mature females (Burton 1985). *T. japonicus* males will 'release' a guarded immature female when exposed to water conditioned with virgin adult females (Kelly et al., 1998). Additionally, *T. japonicus* males will decrease the frequency of releases in non-virgin female conditioned water (Kelly et al., 1998). This suggests that copepods, at least in *T. japonicus*, use waterborne chemical cues to modify their behaviours. Although mate guarding behaviour increases the probability of reproductive success, the associated cost is reduced opportunities to inseminate additional females while engaged in guarding behaviour (Burton, 1985).

Individual *T. californicus* can live for more than six weeks under laboratory conditions (personal observation), and generation time in lab conditions, from the birth of a female to the hatching of her first clutch, is approximately 21 days at 20°C (Voordouw and Anholt, 2002b). Lifespan in natural populations is difficult to measure due to the ephemeral nature of metapopulations. Females from field populations can produce up to 12 clutches (Haderlie et al., 1980). Fecundity, clutch size and number in these populations are affected by temperature and salinity interactions, and food availability (Vittor, 1971). Clutch size (but not number) is positively correlated to female body size,

and development time and longevity are negatively correlated to temperature (Vittor, 1971). Temperature and salinity show some effect on sex ratio (Vittor, 1971; Voordouw and Anholt, 2002a); higher temperatures were found to be associated with an increased proportion of males in a clutch (Voordouw and Anholt, 2002a), and while salinity showed an overall effect on sex ratio, there were no clear trends (Vittor, 1971).

T. californicus populations have high migration rates between adjacent pools (Burton et al., 1979; Edmands and Harrison, 2003), with frequent turnover producing highly variable population densities throughout the year. This does not correlate with temperature, salinity, oxygen concentration or clutch size (Vittor, 1971). Storms will frequently deplete populations, and re-colonization is most likely from adjacent populations which are, for the most part, permanently populated (Vittor, 1971).

Frequent migration of individuals among adjacent populations, depletion of pools, and re-colonization events suggest high rates of among pool gene flow. Populations and even metapopulations within a site are relatively genetically homogeneous (Burton et al., 1979). The lack of genetic differentiation within a site suggests that certain mechanisms are sufficient to maintain gene flow. Such mechanisms include periodic connection by fine streams between adjacent pools (Burton and Feldman, 1981), or even ‘hitchhiking’ by clinging to shore crabs (*Pachygrapsus* sp.), which frequently move between pools within the intertidal (Egloff, 1966). However geographically distant populations—separated by geographic obstacles such as sandy beaches, large stretches of ocean, or smooth rock surfaces, with no potential for connectivity—are genetically divergent (Burton et al., 1979; Burton and Feldman, 1981) despite the high capacity for dispersal of these organisms (e.g., free swimming life stages, frequent wash-outs). Between these

distant sites, numerous studies on the genetic differentiation between populations indicate that gene flow is highly restricted (e.g., Burton et al., 1979; Burton et al., 1981; Burton and Lee, 1994).

The mechanisms responsible for variation in sex ratios in *T. californicus* have been extensively studied (e.g., Ar-Rushdi, 1958; Ar-Rushdi, 1963; Egloff, 1966; Voordouw and Anholt, 2002a; Voordouw and Anholt, 2002b; Voordouw et al., 2005b), and these studies support a polygenic sex determining mechanism. Sex determination follows a polygenic mechanism when additive effects of multiple loci determine the male or female phenotype. Three defining characteristics indicate the presence of a polygenic mechanism (Bull, 1983): 1) a large sex ratio variance among families, 2) detectable maternal and paternal effects on brood sex ratio, and 3) a sex ratio response to selection. Alternatively, a multiple factor gene with many alleles may also produce variation in observed sex ratios; however, distinguishing between the two mechanisms can be difficult. *T. californicus* was suggested to be the first example of polygenic sex determination in animals known to science (Belser, 1959), supported by studies that found no heteromorphic sex chromosomes in a cytological assay of *T. californicus* (Ar-Rushdi, 1963). Subsequent studies provide corroborative support for polygenic sex determination as the underlying mechanism. First, variation in PSR does not follow the expected binomial distribution (e.g., Egloff, 1966; Vittor, 1971; Voordouw and Anholt, 2002b), satisfying the first criterion for polygenic sex determination. Both maternal and paternal effects on offspring sex have been observed in *T. californicus* (Voordouw and Anholt, 2002b; Voordouw et al., 2005a). Further, sex ratio in *Tigriopus* responds to selection (Ar-Rushdi 1958; Alexander et al., 2014), with sex ratios reaching extreme values of <0.20

and >0.85 proportion male when selecting for female- and male-biased PSRs respectively.

Environmental factors have also been observed as having an effect on sex determination. As previously stated, higher temperatures are observed to have a masculinizing effect (Vittor, 1971; Voordouw and Anholt, 2002a). These effects are small but it can have significant implications in the evolution of sex ratios. In addition to temperature effects, salinity (Egloff, 1966) has been found to influence sex determination. Recently, observations have found an interaction between temperature and local sex ratio—the sex ratio of the surrounding population—on subsequent brood primary sex ratios in field populations (MacKeracher, unpublished data). Whether socially mediated sex determination is controlled through chemical or tactile cues has yet to be determined.

The polygenic nature of sex determination and the relatively short generation time of *T. californicus* provide us with a unique and highly tractable system to study sex determination and test evolutionary theories. This thesis will utilize *T. californicus* as a model organism for sex ratio theory. Chapter 2 focuses on testing Fisher's adaptive sex ratio theory using *T. californicus*. It presents data where artificially selected sex-biased populations were observed for sex ratio change over time. The effects of overlapping generations were also tested. A simulation model was used to compare with the empirical data. In the next chapter, I present data collected on social facilitation of sex determination, where local sex ratios were manipulated and their effects on resulting brood sex ratios were measured. The basis of Fisher's theory is applied here; individuals should maximize reproductive success and adjust the sex ratio of their offspring,

producing more of the less abundant sex in the population. Finally, in chapter 4 I conclude with an overview of the material presented and implications and contributions of my findings for general sex ratio theories.

CHAPTER 2 – Fisherian sex ratio selection in *Tigriopus californicus*

Introduction

Fisher's (1930) adaptive sex ratio theory was a landmark in the field of evolutionary biology. He stated that sex ratios in species with separate sexes are expected to be equal with a ratio of 1:1 males to females. In sexual species, males and females have an equal reproductive value as every individual has exactly one mother and one father. Sons and daughters are equal in value so parents should invest equally in both sexes. If the sex ratio is biased, frequency-dependent selection favours the less abundant sex—or individuals that produce more of the less abundant sex—as they will have a higher probability of obtaining a mate and ultimately a higher per capita genetic contribution towards subsequent generations, bringing the sex ratio back to equality (Fisher, 1930). This is the case for many species; however many exceptions exist where primary sex ratios (PSR—the sex ratio of the brood at conception) can be highly variable and skewed towards females or males within a population (e.g., Egloff, 1966; Bull and Charnov, 1989; Conover et al., 1992; Janzen, 1992; West et al., 2002).

Species that are suited to test Fisher's adaptive sex ratio theory are relatively rare in nature as there are few organisms with additive genetic variation for sex ratio. As with any trait, variation in sex ratio is essential for testing sex ratio evolution and Fisher's adaptive sex ratio theory. Heterogametic sex determination systems, as observed in most mammal and bird species, lack genetic variation in sex ratio (Bull and Charnov, 1988;

Basolo, 1994). Species that exhibit variation in sex ratio often have other sex determining mechanisms.

Some species with heterogametic sex determination have additional mechanisms that produce variation in sex ratios. For example, in certain species the heterogametic sex has epigenetic differential (non-random) segregation of sex chromosomes (e.g., Hauschteck-Jungen and Maurer, 1976; Fuge, 1994; Blanco et al., 2002; Taylor et al., 1999; Velando et al., 2002; Young and Badyaev, 2004—an extensive list can be found in Table 1 of Jaenike, 2001)—also known as sex chromosome drive (Jaenike, 2001). The non-Mendelian segregation and unequal transmission of sex chromosomes usually occurs during meiosis and can lead to biased PSRs. Sex chromosome drive is more common in males but has been reported in females (e.g., Fredga et al., 1976; Underwood and Shapiro, 1999; Fishman and Willis, 2005), especially in species with heterogametic females.

Haplodiploid species can also have sex ratio variation. Haplodiploid sex determination is common in many insect species, but is ubiquitous within the Order Hymenoptera. Sex here is determined by the ploidy of an individual. The most common and ancestral form of haplodiploidy has females developing from fertilized (diploid) eggs and males developing from unfertilized (haploid) eggs (Heimpel and de Boer, 2008). Sex ratios are often skewed in eusocial haplodiploid species as the rate of fertilization, and ultimately population sex ratio, is regulated by queens (Mueller, 1991) and workers (Verhulst et al., 2010).

Environmental sex determination (ESD) can produce non-Fisherian sex ratios. Environmental factors such as temperature (e.g., sea turtles [Raynaud and Pieau, 1985;

Mrosovsky and Provancha, 1992]) and social structure (e.g., fish [Cole and Shapiro, 1995]) can affect sex determination. PSRs in species with ESD are often skewed. For example, in sea turtles with temperature-dependent sex determination, mothers can choose a nesting site to lay her eggs (Wood and Bjorndal, 2000; Kamel and Mrosovsky, 2004). Her nest choice such as depth, will ultimately affect temperature. Atlantic silversides, *Menidia menidia*, are hypothesized to have evolved temperature-dependent sex determination to allow females to be born early in the season (colder temperatures) and grow prior to the birth of males, increasing their fecundity prior to reproduction (Conover and Voorhees, 1990). Sex ratio is initially female-biased in *M. menidia* populations. Environmentally determined traits, however, are difficult to distinguish from polygenic traits (Bull, 1983) and are not mutually exclusive. Environmentally determined traits often have a polygenic basis, and polygenic traits often have environmental components.

When sex determination is polygenic, sex is determined by additive effects over multiple, independently segregating loci. It can arise through modifications of existing sex chromosomes where a third sex chromosome is created, or from new inputs on other loci for the regulation of gonad development (Moore and Roberts, 2013). Although relatively rare in nature, polygenic sex determination has been documented in a variety of insects, invertebrates, mammals, fish, and plants (Vandeputte et al., 2007; Liew et al., 2012; Moore and Roberts, 2013). Any one of the many components—genetic or environmental—contributing to sex determination could skew population and PSRs.

In this study I used the harpacticoid copepod *Tigriopus californicus* to test Fisher's adaptive sex ratio theory. *T. californicus* have been studied for its extraordinary sex

determination mechanism and highly variable sex ratios found in nature (Egloff, 1966; Vittor, 1971; Voordouw and Anholt, 2002b). They have no sex chromosomes (Ar-Rushdi, 1963) and lack any sex-linked chromosomes (Harrison and Edmands, 2006). Many studies support the hypothesis that sex determination in *T. californicus* follows a polygenic mechanism (e.g., Ar-Rushdi, 1963; Egloff, 1966; Vittor, 1971; Voordouw and Anholt, 2002b; Voordouw et al., 2005a). Studies have supported both heritable genetic sex determination (GSD; Voordouw and Anholt, 2002b; Voordouw et al., 2005) and ESD in *T. californicus*. Environmental factors supported include: temperature (Voordouw and Anholt, 2002a), salinity (Egloff, 1966), and possibly social mediation (via local sex ratios; unpublished data). Past studies have shown that sex ratios in *T. californicus* are susceptible to selection (e.g., Ar-Rushdi, 1958; Alexander et al., 2014), making them an ideal organism to test Fisher's theory.

T. californicus can be found in splash pools above the high tideline along the eastern Pacific Ocean from northern Mexico to Alaska (Egloff, 1966). Extreme environmental conditions characterize these habitats (Vittor, 1971). Summers are characterized by high temperatures ($>25^{\circ}\text{C}$), high salinities (up to 100‰), and desiccation, while winters bring low temperatures ($<5^{\circ}\text{C}$), low salinities ($<30\text{‰}$), and potential freezing. Splash pools are usually found in slightly more wave exposed areas. Consequently, the many metapopulations found within a site are highly ephemeral (Burton and Feldman, 1981). They are maintained by high migration rates between adjacent pools and consequently high rates of gene flow (Burton et al., 1979; Edmands and Harrison, 2003; unpublished data). Frequent migration between adjacent populations within a site leaves these populations relatively genetically homogeneous, yet geographically distant

metapopulations separated by obstacles are genetically divergent (Burton et al., 1979; Burton and Feldman, 1981). Population subdivision with migration between populations within a metapopulation is known to maintain genetic variability (Whitlock, 1992).

Testing Fisher's theory is difficult as organisms that are well suited for such an experiment are rare. In fact, only four known studies have tested Fisher's adaptive sex ratio theory—in the platyfish *Xiphophorus maculatus* (Basolo, 1994), in Atlantic silversides *Menidia menidia* (Conover and Voorhees, 1990), in *Drosophila mediopunctata* (Carvalho et al., 1998), and in hybrids of *D. serrata* and *D. birchii* (Blows et al., 1999)—yet only one (Carvalho et al., 1998) satisfied all of the seven assumptions of Fisher's theory.

The seven assumptions were first outlined in Bull and Charnov (1988). The first three are arguably the most essential for testing Fisher's adaptive sex ratio theory: (1) separate sexes (Fisher, 1930), (2) biparentalism (Fisher, 1930), and (3) Mendelian segregation of alleles (Shaw, 1958; Hamilton, 1967). Assumptions 2 and 3 generate the frequency-dependent selection for Fisher's theory to hold true. Violation of either of these assumptions will often result in extremely biased sex ratios (Bull and Charnov, 1988; Voordouw, 2005). The remaining assumptions are: (4) random mating in an infinite population (Hamilton, 1967), (5) additive offspring costs (Fisher, 1930; MacArthur, 1965), (6) absence of environmentally induced sex-specific differences (Bull, 1981), and (7) parental control of sex determination (Fisher, 1930; Trivers, 1974). Assumption 4 addresses instances such as mating in populations with group structure (e.g., local mate competition), while assumption 5 refers to the disproportionate increase in fitness when producing males or females (e.g., daughters increase lifetime reproductive success but

sons do not [Mueller, 1991]). Assumption 6 requires that sexes are equally fit in a given environment. For example, sons are not worse off in a poor environment than daughters are (Charnov and Bull, 1977; Trivers and Willard, 1973).

The last assumption, parental control for sex determination, has been challenged by Bulmer and Bull (1982). Fisher's theory can also be applied to species with zygotic control of sex determination (Bulmer and Bull, 1982). Bulmer and Bull (1982) were able to show that Fisher's adaptive sex ratio theory holds true for both parental control and zygotic control of sex determination. Parental control, where the sex ratio is determined by the constitution of only one parent, include mechanisms such as sex chromosome drive, haplodiploidy, and environmental sex determination (Bulmer and Bull, 1982), whereas sex determination under zygotic control is a trait expressed by the genetic makeup of each individual offspring (Voordouw, 2005).

T. californicus satisfy all of the assumptions of Fisher's adaptive sex ratio theory—as outlined by Bull and Charnov (1988). They have separate sexes and obtain their genetic material via Mendelian segregation (Ar-Rushdi, 1963), therefore meeting the first three assumptions. Individuals within a population can be assumed to randomly mate as populations usually contain hundreds to thousands of individuals, and are known to avoid inbreeding (Palmer and Edmands, 2000). Additive and equal costs of sons and daughters have been supported by multiple studies: there is no correlation between fecundity and brood sex ratio (unpublished data); females produce egg sacs once mature whether they are mated or not (Haderlie et al., 1980); there is significant spatial and temporal variation of brood sex ratios in wild populations but the overall mean population sex ratio tends to be equal (Voordouw and Anholt, 2002a, Voordouw et al., 2005a, Voordouw et al., 2008);

and lastly, there is no observed parental care (Haderlie et al., 1980). Sex ratio distorters, such as *Wolbachia*, known to affect sex ratios in other crustaceans (Rigaud, 1997; Terry et al., 2004), have not been detected in *T. californicus* (Voordouw et al., 2008). Lastly, sex determination is not exclusively under parental control; it could additionally be under zygotic control as environmental components often affect offspring during development (e.g., temperature [Voordouw and Anholt, 2002a]).

Most models of evolving populations focus on systems with discrete generations, where an entire generation is replaced by the offspring (e.g., Trivers and Willard, 1973; Frank and Slatkin, 1990; Prügel-Bennett, 1997). Recently more realistic models with overlapping generations (OLGs) have been considered. These models tend to be more complicated but are more applicable when comparing them to empirical data. For example, theoretical models on condition-dependent sex ratios (Trivers and Willard, 1973) have been developed primarily for populations with discrete generations. The applicability of these models to populations with OLGs is limited, preventing their use in making predictions in specific populations (Schwanz et al., 2006) such as in ungulates with maternal condition-dependent sex ratios (Hewison and Gaillard, 1999; Sheldon and West, 2004; Blanchard et al., 2005). Further, the dynamics and results of some evolutionary models have shown sensitivity to OLGs (e.g., Ellner and Hairston, 1994; Ryman, 1997; Sasaki and Ellner, 1997; Rogers and Prügel-Bennet, 2000). *T. californicus* has OLGs. Individuals can live for more than six weeks, which spans more than three generations at 20° C (personal observation).

To test Fisher's theory of frequency-dependent selection on the sex ratio I made use of laboratory lines of *T. californicus* that have been selected over multiple generations for

both female- and male-biased sex ratios (Alexander et al., 2014). I assayed population brood sex ratios after artificial selection had been relaxed. I assumed that all requirements of Fisher's adaptive sex ratio theory were met, and expected frequency dependent selection to work against biased sex ratios and return PSRs back to 0.5. I further tested the role of OLGs by removing the parents every generation, or leaving older generations to overlap with subsequent ones. Selection was expected in all treatment lines, and sex ratio should return to equality. Populations with OLGs should also follow Fisher's prediction, but at a slower rate due to lower selection intensity and the genetic overlap of older generations.

Since Darwin's (1871) initial postulation for the cause of equal sex ratios, to Fisher's (1930) explanation of this ubiquitous trend, Bulmer and Bull (1982) have mathematically modelled the expected sex ratio trajectory of a population. The model predicts the population sex ratio in the next generation given the current sex ratio and heritability estimate. I fit their model to the data to estimate heritability. All treatment lines evolved towards 0.5 and OLGs evolved more slowly than discrete generations in all treatments.

Methods

Study organism and lab conditions

Populations of *Tigriopus californicus* originated from Aguilar Point, Bamfield, BC (48°51'28" N, 125°09'38" W) and Ocean Beach, San Diego, California (32°44'49" N, 117°15'16" W). Populations were obtained from selection experiments done in the lab (Alexander et al., 2014). Populations were kept separate in incubators at 20° C with a 12:12 light-dark cycle. Filtered seawater (FSW) was obtained from Bamfield Marine Science Centre (BMSC) seawater system pumped from 20 m depth in Bamfield inlet and

filtered at 0.5 μm . Populations were kept in plastic bottles in 1 litre of FSW and fed 1 mL every five days of a solution containing a ground up mix of Nutrafin[®] Spirulina flakes and TetraMin[®] tropical flakes at 0.05 grams each per 10 mL of FSW. Populations were filtered, cleaned, and replaced with new seawater every generation.

Artificial selection for sex ratio

T. californicus populations were selected for sex ratio after every generation for a minimum of 6 generations (see Alexander et al., 2014 for detailed methods). Female and male sex-biased broods were selected at <0.15 and >0.85 proportion males, respectively, to start the next generation and establish sex-biased population lines. For the last round of artificial selection, I divided each family in two—based on sex ratio—to create two replicate population lines with identical genetic backgrounds and starting sex ratios. The number of families selected for this last round of selection ranged from 22-94 families. Control lines followed the same procedure but selected families were randomly chosen from a larger haphazard sample of families to start the next generations.

Establishing discrete generations

To establish discrete generations I strained the population using 63 and 125 μm sieves. The 125 μm sieve separated adults and juveniles from nauplii, while the 63 μm sieve separated nauplii from some of the waste. Nauplii were then placed in new population bottles to start the next generation. This separation was carried out three times every generation over a period of 5-6 days to ensure sufficient nauplii to establish the next generation. Population size ranged between generations from 100 to over 1000.

Adults in populations with overlapping generations were left to breed with subsequent generations. Sampling of broods in overlapping populations occurred every 5-6 weeks, in

conjunction with when individuals were sexually mature in populations with discrete generations.

Assay of sex ratio

I measured brood sex ratios every 1-2 generations for up to 17 generations. Egg sacs from 30 gravid females were sampled from each population line and reared in individual wells of 6-well culture plates. They were fed 100 μ L of the standard food solution every 5 days or as needed. Family sex ratio was measured once individuals reached maturity and averaged for an overall population sex ratio. Sampled broods were not returned to their respective populations.

Analysis

Bulmer and Bull's model

Bulmer and Bull's (1982) model predicts sex ratio trajectory of a population where sex determination is either under parental or zygotic control. The difference between parental and zygotic control in the model is the rate at which sex ratio changes over generations; it is half the rate under parental control as the trait is only expressed by one parent, usually the mother (Bulmer and Bull, 1982; Voordouw, 2005; Figure 2-1). There is evidence for both maternal (Voordouw and Anholt, 2002b) and paternal (Voordouw et al., 2005a) inheritance, therefore sex determination in *T. californicus* is assumed to be under zygotic control in this analysis. The trajectory for estimating sex ratios over multiple generations under zygotic control of sex determination is calculated with the formula:

$$\Delta P = h_y^2 \phi^2 (1/2 - P) / P(1 - P)$$

where P denotes the sex ratio in the current generation, ΔP denotes the expected change in sex ratio between the current generation and the next, h_V^2 is the heritability estimate, and $\varphi^2/P(1 - P)$ is a probability distribution factor with values provided in Bulmer and Bull (1982). Response to selection is quicker with higher heritabilities (Figure 2-1).

The best fit to Bulmer and Bull's (1982) model was used to obtain intercept and heritability estimates. Their model assumes discrete generations and is inappropriate for populations with overlapping generations. However, heritabilities were calculated for populations with overlapping generations as they provide proxies for comparing the rates of evolution to populations with discrete generations. Estimated heritability values are assumed to be constant over time. The best fit Bulmer and Bull model was determined using non-linear least sum-of-squares (nls) regression for each one of the treatments. The intercept and heritability estimates were determined using the nls analysis with values between 0 and 1, and tested at an accuracy of 0.01. Model fits were estimated using both replicates for each treatment.

I bootstrapped the data to obtain 95% confidence intervals for the intercept and heritability estimates. The bootstrap methods resampled the data set (with replacement) and the same analysis procedures as above were used to estimate the parameters. I ran 10000 iterations. The heritability estimates were then compared with realized heritability estimates calculated from the selected lines of Alexander et al. (2014).

Bulmer and Bull's model is inappropriate for the control population lines as there is no selection for sex ratio. These lines should be stable at a sex ratio of 0.5. I used a simple

linear regression for the controls. All data were analyzed using R v2.15.2 (R Core Team, 2012).

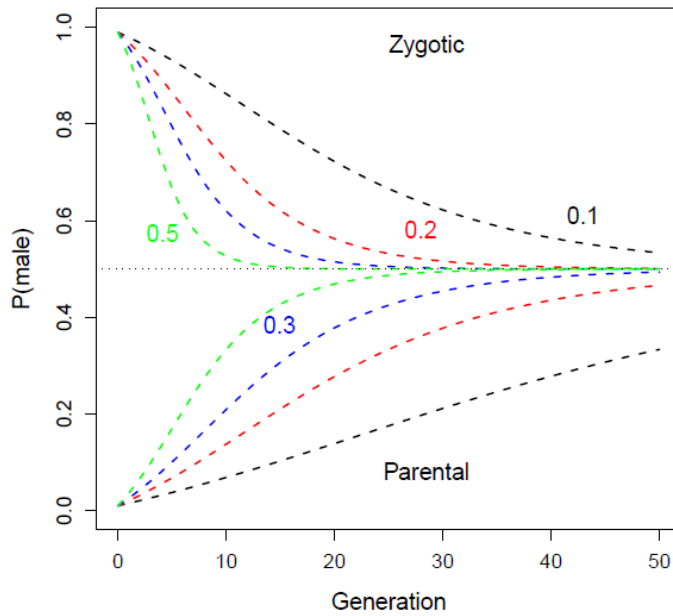


Figure 2-1. Deterministic predicted trajectories of sex ratio change over 50 generations under zygotic (top half) and parental (bottom half) control of sex determination with different heritability estimates (0.1 – black; 0.2 – red; 0.3 – blue; 0.5 – green) using Bulmer and Bull’s model (1982). Sex ratios start at 0.01 and 0.99 proportion males for zygotic and parental control of sex determination, respectively.

Generations to equilibrium

I estimated the number of generations to an equal sex ratio of 0.45 and 0.55 for female and male-biased treatments respectively. I chose these numbers as the nature of Bulmer and Bull's model has an asymptote at 0.5 and therefore never actually reaches a sex ratio of 0.5 (Figure 2-1).

Individual variance component simulation model

Background

Here I present a model that simulates Fisherian sex ratio evolution. The model is an individual variance component (IVC) model that simulated this experiment and accounted for the specifics of *T. californicus* biology and the details of the experimental procedure. Its construction is based on Roff's model from Chapter 4 in Roff (2010, *Modelling evolution: an introduction to numerical methods*). First, I included parameters that emulate the experiment: 1) carrying capacity of the population and 2) the sample size (number of broods) taken each generation. The carrying capacity was controlled by random selection of offspring/individuals to start the next generation. These parameters produced the error and variation due to experimentation.

Parameters specific to *T. californicus* biology included in the model are: 3) the lifespan of individuals, 4) the number of total mating events for each individual, 5) the number of mating events per generation for each individual, and 6) the fecundity of females. The first three covered the biology and setting of natural *T. californicus* populations where generations overlap and individuals can have multiple mating events. The lifespan of an individual was measured in generations, which affected the overlap of generations. Individuals matured after one generation and mated at random—inbreeding was not

controlled or monitored. Multiple mating events were exclusive to males, as females are known to mate only once (Burton, 1985). Males can mate with multiple females within one generation, but were limited by both time (maximum mating events per generation) and resources (maximum number of lifetime mating events). All females mated and produced a brood within a generation, unless the number of males or the number of possible mating events remaining for each male was limiting. Fecundity of *T. californicus* is known to vary greatly, ranging from 40 to over 100 (Haderlie et al., 1980); broods much smaller than 20 have also been measured in lab populations (personal observations). Fecundity was kept constant at 20 individuals per brood and females produced only one brood per generation. Lastly, general parameters of population traits in the simulation model were: 7) heritability estimate, 8) phenotypic variance and 9) the number of generations to simulate. Refer to appendix 1 for coding details of the model.

Simulations

The IVC model was used to compare with Bulmer and Bull's (1982) model fits and the experimental data. Simulations presented here held the following parameters constant: population carrying capacity at 1000, sample size at 30, and fecundity at 20. Phenotypic variance for each simulation was according to estimates from the experimental data (Table 2-3). Starting sex ratios and heritabilities for the model were obtained from those calculated using Bulmer and Bull's model (Table 2-1). Each simulation was run for 30 generations and each simulation was iterated 10 times.

Results

Male-biased treatment population lines showed the expected trajectory towards 0.5 with a negative slope, while female-biased lines showed a positive slope towards 0.5

(Figure 2-2; Table 2-1). Aguilar male-biased populations with overlapping generations did not have the expected trajectory to 0.5 and heritability was estimated to be 0 (Figure 2-2; Table 2-1). The rates of sex ratio evolution in populations with overlapping generations were all smaller in magnitude than the respective populations with discrete generations (Figure 2-2; Table 2-1).

Regression analysis on control lines did not have a significant slope ($p > 0.15$ for all regression analyses). Aguilar control lines were remained relatively stable at 0.5, but San Diego control lines remained mostly female biased (Figure 2-3). San Diego control lines with discrete generations had a slight negative slope towards being more female-biased, but this slope was non-significant ($p > 0.15$). Control lines were variable and fluctuated between generations, with no trend toward a biased sex ratio selection (Figure 2-3).

Bulmer and Bull's model

Models fit using Bulmer and Bull's (1982) equations had trajectories in the expected direction; female-biased treatments had positive slopes and male-biased treatments had negative slopes in all biased treatment lines, except Aguilar male-biased overlapping population lines (Figure 2-2; Table 2-1). These population lines also had heritability estimates greater than zero (Table 2-1). Heritability estimates were variable across treatments (Table 2-1).

The heritabilities estimated using Bulmer and Bull's (1982) model were greater in all populations with discrete generations than in the respective populations with overlapping generations (Table 2-1) as expected. This trend was consistent across locations and biased treatments, and indicates the difference in sex ratio evolution rates. This was consistent with predictions as overlapping generations slow down the rate of sex ratio

evolution due to the genetic overlap between generations. Realized heritability estimates for control lines are not informative in this case as there is no apparent selection.

Heritability estimates in Alexander et al. (2014) were less in Aguilar female-biased and San Diego male-biased populations, and greater in Aguilar male-biased and San Diego female-biased populations than those measured in this experiment (Table 2-1). There was some overlap in 95% confidence intervals for heritability estimates in Aguilar female-biased lines and San Diego male-biased lines, but the average values were still very different (Table 2-1). Aguilar male-biased and San Diego female-biased lines had much lower heritability estimates than in Alexander et al. (2014).

Generations to equilibrium

The number of generations to 0.45 or 0.55 was variable across locations, treatments, and overlapping treatments. The fewest generations to a balanced sex ratio was in Aguilar female-biased discrete generation treatments, while the greatest number of generations estimated was in Aguilar male-biased overlapping treatments (Table 2-2). The number of generations to equilibrium was a minimum two times greater in all overlapping treatments than in the discrete treatments for the respective treatment groups. This was as expected as discrete treatments had larger heritability estimates and showed a greater rate of sex ratio change (Figure 2-2; Table 2-1).

Table 2-1. Estimated intercept and heritability for each treatment combination of three variables—Location, Treatment, Overlapping generations—using Bulmer and Bull’s (1982) model. Top values represent overlapping generations treatment, while bottom italic values represent discrete treatments. Estimates and 95% lower and upper confidence limits (LCL and UCL respectively) were calculated using 10000 bootstrap iterations and model fits were determined using least sum of squares. Heritability estimates calculated here for populations with discrete generations were compared with realized heritability estimates calculated from Alexander et al. (2014).

Location	Treatment	Intercept			Heritability, h^2			Alexander† heritability, h^2		
		Model estimate	95% LCL	95% UCL	Model estimate	95% LCL	95% UCL	Model estimate	95% LCL	95% UCL
Aguilar	Female	0.22 <i>0.18</i>	0.20 <i>0.15</i>	0.25 <i>0.22</i>	0.21 <i>0.56</i>	0.16 <i>0.40</i>	0.28 <i>0.74</i>	0.26	<i>0.13</i>	<i>0.41</i>
	Male	0.76 <i>0.77</i>	0.75 <i>0.74</i>	0.78 <i>0.79</i>	0.00 <i>0.07</i>	0.00 <i>0.03</i>	0.02 <i>0.10</i>	0.36	<i>0.14</i>	<i>0.56</i>
San Diego	Female	0.17 <i>0.12</i>	0.15 <i>0.10</i>	0.19 <i>0.14</i>	0.03 <i>0.07</i>	0.02 <i>0.06</i>	0.05 <i>0.09</i>	0.58	<i>0.33</i>	<i>0.83</i>
	Male	0.74 <i>0.73</i>	0.72 <i>0.70</i>	0.77 <i>0.77</i>	0.08 <i>0.21</i>	0.05 <i>0.15</i>	0.12 <i>0.32</i>	-0.01	<i>-0.32</i>	<i>0.26</i>

†From Alexander et al. (2014)

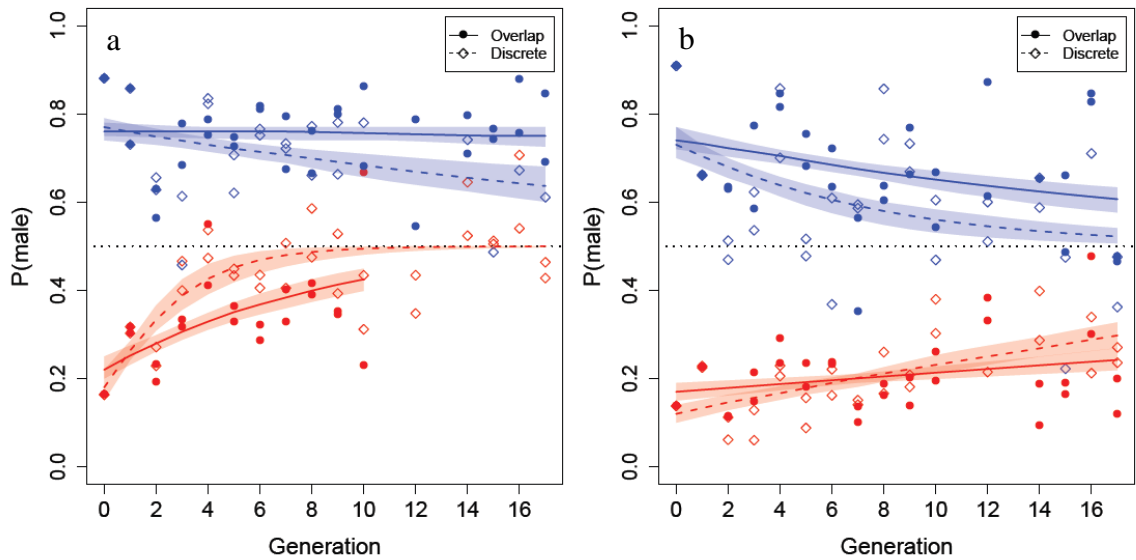


Figure 2-2. Population sex ratio (proportion males) trajectories with 95% confidence bands for up to 17 generations using Bulmer and Bull’s (1982) model to estimate heritability and an intercept (Table 2-1) using the best fitted model with least sum-of-squares. Confidence bands were generated by bootstrapping 10000 iterations. The panels shown are a) Aguilar treatment lines, and b) San Diego treatment lines. Red represents female-biased treatments, while blue represents male-biased treatments. For each combination of treatments of our three variables—Location, Biased-treatment, and Overlapping-treatment—model fits were calculated from two replicate population lines.

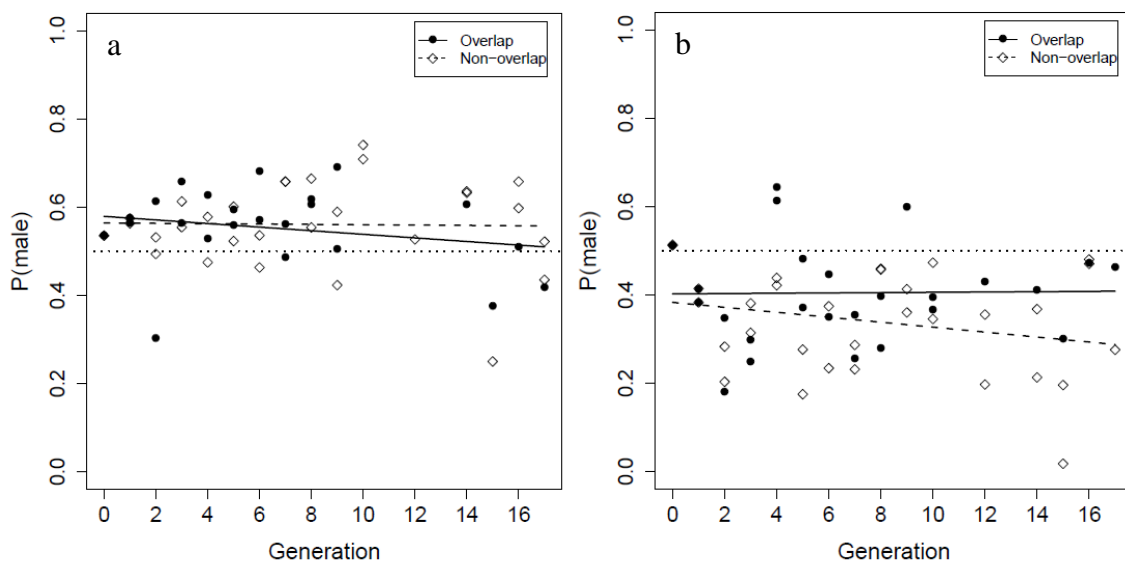


Figure 2-3. Population sex ratio linear model regressions for up to 17 generations in controls lines from a) Aguilar, and b) San Diego populations. Regressions were plotted using data from two replicate population lines but none were significant ($p > 0.15$).

Table 2-2. Predicted number of generations to an equal sex ratio with 95% lower and upper confidence limits (LCL and UCL respectively) using Bulmer and Bull's (1982) models. Top values represent overlapping generations treatment, while bottom italic values represent discrete treatments. The number of generations to a sex ratio of 0.45 was used for female-biased lines and 0.55 was used for male-biased lines.

Location	Treatment	Generations to 0.45 or 0.55 predicted by B&B†		
		Model estimate	95% LCL	95% UCL
Aguilar	Female	13	11	17
		<i>5</i>	<i>4</i>	<i>7</i>
Aguilar	Male	-	141	-
		<i>40</i>	<i>28</i>	<i>84</i>
San Diego	Female	108	67	162
		<i>49</i>	<i>39</i>	<i>60</i>
	Male	32	23	49
		<i>12</i>	<i>8</i>	<i>16</i>

†Estimates are calculated from 10000 iterations using Bulmer and Bull's (1982) model.

Individual variance component simulation model

First I simulated the model for populations with discrete generations. Lifespan, total number of mating events, and mating events per generation were set to 1. The simulations produced similar but more conservative trajectories for the rate of sex ratio evolution compared to Bulmer and Bull's model (Figure 2-4). Therefore the number of generations to an equal sex ratio of 0.5 predicted by the IVC model would be greater. The IVC model shows the possible variation and fluctuations as seen in the experimental data.

I looked at the effect of overlapping generations using the IVC by changing: lifespan to 3 generations, total number of lifetime mating events to 8, and the maximum number of mating events per generation to 3. These parameters are conservative as individuals can live well beyond 3 generations under lab conditions (personal observation). The number of mating events was estimated based on observed temporal constraints. The same heritability estimates (Table 2-1) and variance parameters (Table 2-3) calculated from populations with discrete generations were used for simulations for overlapping generations. Simulations with overlapping generations were plotted with respective simulations with discrete generations as in Figure 2-4.

There are slight but noticeable differences in the rate of sex ratio evolution between discrete and overlapping generations (Figure 2-5). Sex ratios in populations with overlapping generations change at a slower rate than those in discrete generations. These differences are not as drastic as the ones measured by Bulmer and Bull's model (Figure 2-2) from the data.

Table 2-3. Population variance used for IVC simulation models of populations with discrete generations for different treatment combinations of location and biased-treatment.

Location	Treatment	Variance
Aguilar	Female	$(0.2558)^2$
	Male	$(0.1994)^2$
San Diego	Female	$(0.1838)^2$
	Male	$(0.2745)^2$

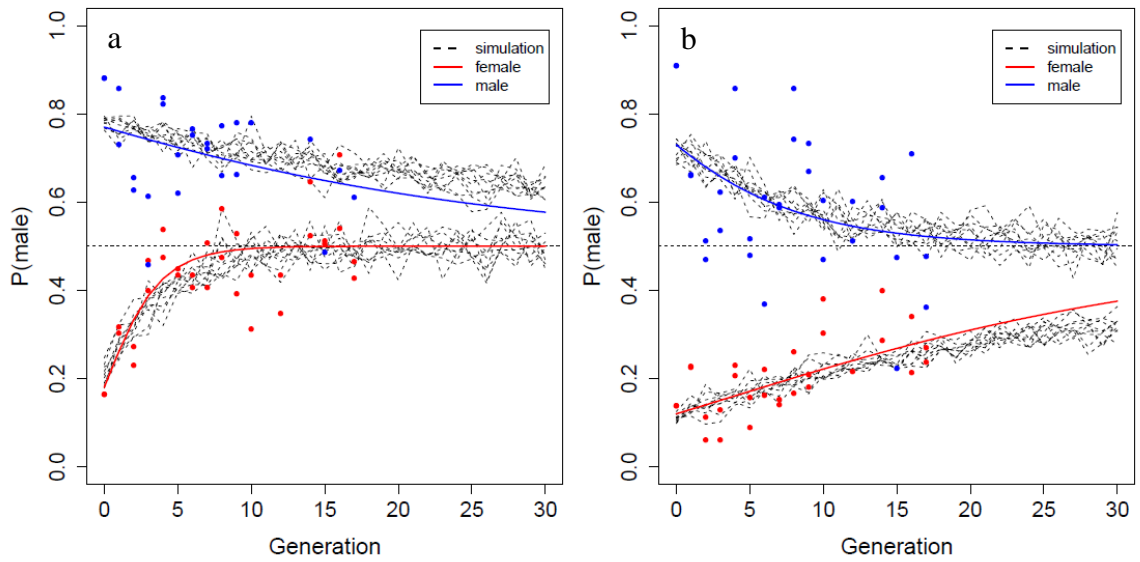


Figure 2-4. Individual variance component model plotted for a) Aguilar and b) San Diego populations with discrete generations for female- and male-biased treatments given the parameters estimated from the Bulmer and Bull model. Experimental data are plotted as points, the Bulmer and Bull model trajectories for each treatment are plotted as red and blue solid lines, and IVC model simulations are plotted as dashed black lines.

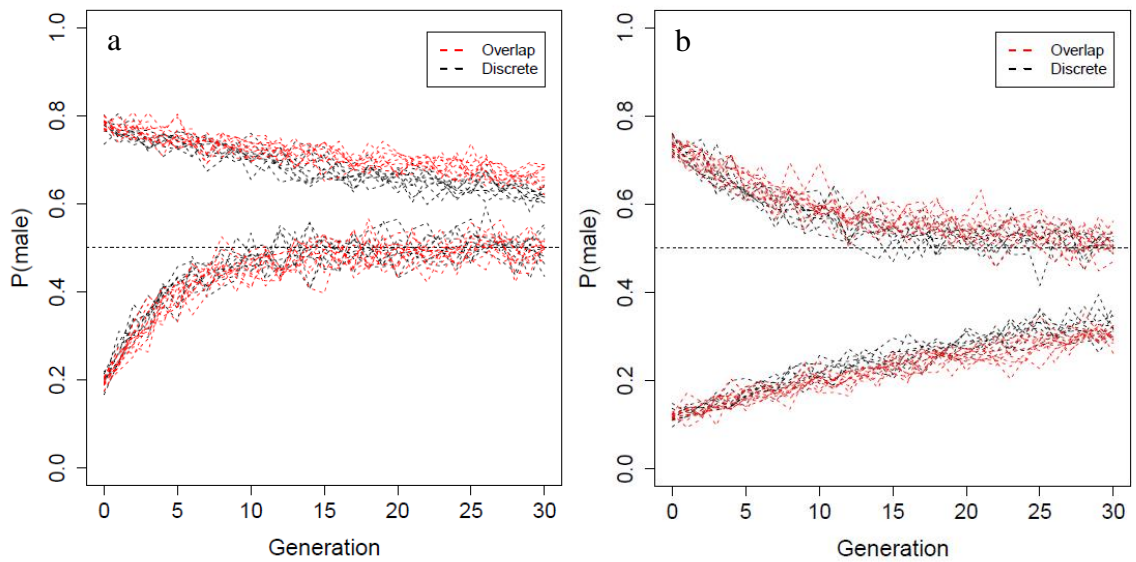


Figure 2-5. Individual variance component model plotted for a) Aguilar and b) San Diego populations with overlapping and discrete generations for female- and male-biased treatments given the parameters estimated from the Bulmer and Bull model. Simulations with overlapping generations used the same heritability and variance estimates as respective simulations with discrete generations. Parameters adjusted were: lifespan of an individual, total number of mating events per individual, and maximum number of mating events per generation.

Discussion

T. californicus treatment population lines (female- and male-biased) showed the expected trajectory towards 0.5: male-biased lines showed a negative slope while female biased lines showed a positive slope (Figure 2-2; Table 2-1). However, the heritability estimate for Aguilar male-biased overlapping populations was 0 and therefore had a slope of 0 (Figure 2-2; Table 2-1). Nonetheless, the overall data supports Fisher's (1930) adaptive sex ratio theory of frequency dependent selection in favour of a balanced sex ratio.

Heritability estimates obtained using Bulmer and Bull's (1982) model were used as proxies for the rate of Fisherian sex ratio evolution. Heritability estimates were variable across location, biased treatments, and overlapping treatments (Table 2-1). The variability in the heritability estimates indicates that the rate of change in population sex ratio towards the expected 0.5 equilibrium is different across locations and biased treatments. However, heritability estimates were consistently greater in discrete than in overlapping treatments. Discrete populations had a higher rate of change towards a balanced population sex ratio.

Heritability estimates calculated in this study were not consistent with those measured in Alexander et al. (2014; Table 2-3). The differences between heritability estimates could possibly be a result of the method of calculation (Table 2-4). In this study, I used Bulmer and Bull's (1982) model to estimate realized heritability. These estimates were calculated using population sex ratio trajectories which rely on frequency dependent selection to measure the response to selection. This provides a crude method of estimating heritability. Alexander et al. (2014) measured the response to selection to

calculate the realized heritability. The sampled broods used to calculate population sex ratio in Alexander et al. (2014) were also used to continue the next generation. Therefore the population sex ratio, the number of families, and the brood sex ratios were all known for each generation. In my study, population sex ratios were estimated from sampling from the large population every generation and other factors (e.g., number of families into the next generation) were not manipulated or controlled.

A general explanation for the differences in realized heritability estimates between populations could be attributed to the genetic divergence. Additionally, the presence of phenotypic plasticity could obscure the changes in sex ratio after artificial and frequency dependent selection, and ultimately affect the heritability estimates. These variable heritability estimates open the door to many possible explanations and suggest an intricate and complex mechanism for selection of sex determining genes.

There was high variability in the predicted number of generations to 0.5 (Table 2-4). The number of generations estimated to reach sex ratios of 0.45 and 0.55 for female- and male-biased lines respectively were different between treatments, reflective of the differences between heritability estimates.

The IVC simulation model produced similar trajectories to the ones fit using Bulmer and Bull's (1982) model (Figure 2-4). They tended to be more conservative than the Bulmer and Bull model, likely due to the fluctuations and variation within the IVC model. Further, there was a population carrying capacity with the IVC model, whereas Bulmer and Bull's model assumes an infinite population and infinite loci system. Bulmer and Bull's model did not have a variance parameter. The variance parameter in the IVC model has a large effect on the sex ratio trajectory. It was calculated from the data for

each treatment and an underestimate of variance could also explain the more conservative trajectory in the IVC model.

The IVC model simulations between discrete and overlapping generations suggest that sex ratios in populations with discrete generations change at a greater rate than overlapping generations (Figure 2-5). These differences were not as apparent in the simulations as compared to the empirical data and Bulmer and Bull's model. The IVC model is likely much more conservative. *T. californicus* have been seen to live for more than 3 generations in the lab (personal observation), and may produce more clutches than specified in the model. Further, the survival of individuals from older generations may affect the survival of the younger more current generations. There have been instances of cannibalism which would decrease the survival of younger generations in populations with overlapping generations. The IVC model does not account for this, which could explain the smaller differences between simulations of discrete and overlapping generations.

The overall results here are consistent with other studies on Fisherian sex ratio evolution (Basolo, 1994; Blows et al., 1999; Carvalho et al., 1998; Conover and Voorhees, 1990). However, the rates of change in population sex ratio, as well as the fluctuations about the expected trajectory are different. In the Atlantic silverside, *M. menidia*, it took less than ten (between 1-8) generations for sex ratio to evolve back to 0.5 (Conover and Voorhees, 1990). Hybrids of *D. serrata* and *D. birchii* produced biased sex ratios when crossed and 0.5 was reached after 16 generations (Blows et al., 1999), while in the platyfish, *X. maculatus*, sex ratio evolution was rather rapid, reaching 0.5 in 3 generations (Basolo, 1994). Conversely in *D. mediopunctata*, sex ratio evolution was

much slower, predicted to reach 0.5 in 330 generations (Carvalho et al., 1998)—only 49 generations were measured during their experiment. In comparison, the rate of sex ratio evolution in *T. californicus* populations was highly variable. Some populations were measured to reach a 0.5 sex ratio in as few as 4 generations (Table 2-4), while other populations did not reach 0.5 within the duration of this experiment (Table 2-4).

These differences in the rate of sex ratio evolution in the different studies mentioned can be attributed to the various sex determining mechanisms. In *D. mediopunctata*, the sex determination mechanism is a naturally occurring X-Y meiotic drive system with at least four autosomal suppressors (Carvalho et al., 1998). The genes are located on the autosomes and suppress meiotic drive of the X chromosome which produces variation in PSR (Carvalho et al., 1998). This genetic variation is therefore biparentally inherited. Alternatively, *X. maculatus* has a mechanism with three main factors—W, X, and Y—controlling sex determination, with only X and Y being biparentally inherited and W carried only by females (Basolo, 1994). This violates one of Fisher's assumptions (biparental inheritance), which results in a much faster rate of change back to the sex ratio equilibrium of 0.5 (Carvalho et al., 1998). Organisms with major sex determining genes such as *X. maculatus* are expected to have a greater rate of sex ratio evolution back to 0.5 (Carvalho et al., 1998). Both the *Drosophila* hybrid cross (Blows et al., 1999) and *M. menidia* (Conover and Voorhees, 1990) are also thought to also have major sex determining genes, which is supported by the relatively fast Fisherian sex ratio evolution rates.

A complex polygenic mechanism in *T. californicus* is supported by the slower and much more variable sex ratio evolution rate than species with major sex determining

genes. Similar to these other studies, my data showed fluctuations in the sex ratio trajectory within each population replicate line. Such fluctuations and overshooting 0.5 are a possible result of Mendelian segregation of sex ratio alleles during Fisherian evolution (Basolo, 1994; Voordouw, 2005). However, fluctuations are uncharacteristic of Bulmer and Bull's (1982) model. One mechanism I propose is that fluctuations could potentially be a product of natural variation in the surrounding environment, imposing other factors acting on sex determination.

In species with ESD, any environmental variation can potentially interfere and possibly even oppose Fisherian sex ratio evolution. *T. californicus* has a complex mechanism for sex determination and studies have supporting evidence for both genetic (Voordouw and Anholt, 2002b; Voordouw et al., 2005a) and environmental (e.g., Egloff, 1966; Voordouw and Anholt, 2002a) components. The fluctuations and variation in the sex ratio trajectory (Figure 2-3 and 2-4) could be explained by variation in the environmental factors, despite the experiment being carried out in incubators. Incubators used in this experiment have shown to have spatial (e.g., proximity to light source, vertical distance to the fan unit) and temporal (e.g., defrost cycle, day/night cycle) variation. These factors could affect sex determination and the sex ratio evolution in *T. californicus*.

In addition to abiotic components, evidence of socially mediated sex determination suggests that population sex ratios have a negative correlation with resulting brood sex ratios (unpublished data). This could also influence the magnitude and direction of sex ratio selection due to changes in population sex ratio.

The presence of environmental factors and its effects on sex determination have interesting implications in sex ratio evolution and genetic variance for sex ratios. It has

been shown that in a spatially variable environment, genetic variance can be maintained (Frank and Slatkin, 1990), while in a temporally variable environment with fluctuating selection, genetic variance can be maintained in populations with overlapping generations (Ellner and Hairston, 1994). Populations of *T. californicus* are faced with highly variable environmental conditions. They also have overlapping generations as individuals have been noted to live for over 6 weeks in the lab, about 3 generations. If we assume spatial and temporal variation create fluctuating selection regimes, this suggests that the genetic variance for sex ratios seen in *T. californicus* may be maintained by the spatial and temporal fluctuating selection characteristic of their habitat.

The discrete and overlapping treatments produced different heritability estimates and number of generations to 0.5. The population lines with overlapping generations had lower rates of change for population sex ratio (Figure 2-3), consistent with my hypothesis. Populations with overlapping generations allow individuals (males in *T. californicus* as females mate only once [Burton, 1985]) from previous generations to mate with younger, more current generations. This could slow down frequency dependent selection as genetic overlap can increase variation in gene frequencies (Charlesworth, 1974). As mentioned previously, overlapping generations can also help maintain genetic variation in traits subject to temporally fluctuating selection (Ellner and Hairston, 1994). Temporally fluctuating selection and maintaining genetic variance in populations with discrete generations has repeatedly been rejected (Hedrick et al., 1976; Hedrick, 1986). Each population used in this experiment was closed—no immigration or emigration occurred. However, there was both spatial and temporal environmental variation within the incubator. This, in combination with overlapping generations, could explain the

trends seen in each overlapping population line. The maintenance of genetic variance through fluctuating selection and overlapping generations could result in the observed variation and fluctuation in population sex ratios we see. Theory states that in age-structured populations with overlapping generations, ecologically induced fluctuations can cause changes in gene frequencies (Charlesworth, 1974).

Interestingly, populations with discrete generations also showed fluctuating and variable population sex ratios—again in contrast to Bulmer and Bull's (1982) predictive model. The sampling interval and generation separation in these populations with discrete generations occurred about every six weeks. At this point, I separated generations using a sieve. Generation times were determined based on the rearing time of sampled broods (for sex ratio analysis) and on the timing of sampling for subsequent generations. This allowed for individuals to mature, mate, and hatch enough broods to sustain the next generation. However, these generation times were likely overestimated. If development happened more quickly for some individuals, their first broods produced could potentially have matured to mate with their parental generations before I was able to sample and separate generations. Therefore, some of the sampled broods could have come from the mated offspring from the current parental generation. This produces some level of genetic overlap which could also be one of the causes for the fluctuations and variation in sex ratio seen in these discrete population lines. Sampling error and environmental variation could have also contributed to the fluctuations in the data.

Model comparisons

In this study I used two different models, Bulmer and Bull's (1982) model and the IVC simulation model. Fitting Bulmer and Bull's model to the data mechanistically provided

the rate of sex ratio evolution across the different treatment populations. It also showed that the rate of sex ratio evolution differed between populations with discrete and overlapping generations. The consistency of the IVC simulation model with Bulmer and Bull's model provided further support for the data. It also confirmed the fluctuations and variability due to the experimental procedure.

The use of a mechanistic and simulation model makes this study more robust, supporting Fisher's (1930) adaptive sex ratio theory in *T. californicus*. It indicates the complexity of sex ratio theory and the exceptionally complex sex determination system in *T. californicus*. Further it highlights the importance of using simulation models that can reproduce trends and the variation seen in the experimental data.

Conclusion

This experiment augments the existing few empirical studies that support Fisher's sex ratio evolution theory. It provides support for the theoretical models on polygenic sex determination and Fisher's sex ratio theory. Lastly, it highlights the importance of considering overlapping generations when comparing theoretical models with empirical studies. The variability in population sex ratio trajectories depicts the complex nature of empirical studies on Fisher's sex ratio theory, and the complex sex determination mechanism in *T. californicus*.

CHAPTER 3 – Socially mediated sex determination

Introduction

Sexually-reproducing organisms depend on finding a mate for fitness. The sex of an individual and the relative sex ratio in the population determines the probability of reproductive success. Given this there is potential for individuals to increase fitness by modifying sex and sex ratios such that the probability of obtaining a mate increases. Sex and sex ratio adjustment have been of great importance to sex ratio theory and is one of the successes of modern evolutionary biology (Moore et al., 2002).

Sex and sex ratio adjustment in response to the population sex ratio can be under parental or zygotic control. This distinction is synonymous to the definitions of parental or zygotic control of sex determination, as outlined in Bulmer and Bull (1982) and Voordouw and Anholt (2002b). Sex allocation adjustment occurs when the parents control the sex ratio of their offspring in response to environmental cues. Alternatively, zygotes may respond to environmental cues during development and sex determination. To avoid confusion throughout this paper I define sex adjustment in response to population sex ratios under parental control as “socially mediated sex allocation”, and under zygotic control as “socially mediated sex determination”. The two mechanisms together will be referred to as “socially mediated sex ratio adjustment”.

Fisher’s (1930) adaptive sex ratio theory implies that individuals that produce more of the rare sex are favoured (further explained in: Shaw and Mohler, 1953; Bodmer and Edwards, 1960; Kolman, 1960). In populations with biased sex ratios, the less abundant sex will have a higher per capita genetic contribution towards subsequent generations and

parents producing more of the less abundant sex will have a higher fitness (Fisher, 1930). His theory predicts the evolution of sex ratios, but these principles can also apply to socially mediated sex ratio adjustment. Individuals able to adjust sex or sex ratios in response to the population sex ratio may increase their fitness by producing more of the less abundant sex. To test this hypothesis, we require an organism with sex ratio variation.

Clutch sex ratios in populations of *Tigriopus californicus* are highly variable and exhibit extrabinomial variation (Egloff, 1966; Vittor, 1971; Voordouw and Anholt, 2002b). *Tigriopus californicus* is a harpacticoid copepod found in splash pools above the high tideline along the eastern Pacific Ocean coastline from northern Mexico to Alaska (Egloff, 1966). Their habitats are characterized by extreme conditions including: temperature and salinity fluctuations, wave exposure, and desiccation (Vittor, 1971). Metapopulations (made up of splash pool subpopulations on the same rocky outcrop) are highly ephemeral (Burton and Feldman, 1981) and are frequently washed out by the high tide. Populations within a site are relatively genetically homozygous, but geographically distant metapopulations are genetically divergent (Burton et al., 1979; Burton and Feldman, 1981). Average clutch sex ratios are often not 1:1, inconsistent with Fisher's (1930) adaptive sex ratio theory which states that natural selection will always favour sex ratios such that parents invest equal resources in sons and daughters. Sex-determination in *T. californicus* is hypothesized to be polygenic—determined by additive effects of multiple loci on several chromosomes. *T. californicus* has heritable genetic variation for clutch sex-ratio (Voordouw and Anholt 2002b; Voordouw et al. 2005a) and there is evidence to suggest that temperature (Egloff, 1966; Voordouw and Anholt 2002a), and

salinity (Egloff, 1966) influence sex determination. *T. californicus* has highly variable sex ratios and the polygenic nature of sex determination in this species could explain the lack of a balanced Fisherian sex ratio. Therefore, *T. californicus* provide us with a unique and highly tractable system in which we can manageably test socially mediated sex ratio adjustment under Fisher's theory.

Responses to social environmental cues have been documented in a wide range of copepod species. Copepods in general show a preference towards conspecifics, suggesting an ability to physically or chemically detect differences between species (Lonsdale et al., 1998). Further, some copepods follow pheromone trails left by prospective mates (e.g., Frey et al., 1998; Weissburg et al., 1998; Bagøien and Kjørboe, 2005; Yen et al., 2011). Within the genus *Tigriopus*, mate recognition proteins have been identified and can explain mate preferences and changes in behaviour with changes in population makeup (Ting and Snell, 2003). Studies with *T. japonicus* found that seawater chemically-conditioned with different combinations of males, virgin females, and non-virgin females altered male mate-guarding behaviours (Kelly et al., 1998; Ting and Snell, 2003). Additionally, *T. japonicus* was found to delay the hatching of broods in response to higher population densities (Kahan et al., 1988), suggesting a mechanism to detect the surrounding social environment. These observations in *T. japonicus*, a sister species to *T. californicus*, suggest *T. californicus* is likely to have an ability to detect changes in social environment.

Currently, there are no documented instances of socially mediated sex ratio adjustment such as the one described here for *T. californicus* but other mechanisms that are similar in function exist. In sequentially hermaphroditic fish species with hierarchical group

structure, the timing of sex-change is influenced by the social status of individuals within the group (e.g., Cole and Shapiro, 1995; Hobbs et al., 2004; Liu and Sadovy, 2004). Certain shrimp species have also shown responses in sex-change and age at maturity to social cues (Tziouveli and Smith, 2009). In some invertebrate species with separate sexes, individuals are able to control offspring sex in response to the environment (West et al., 2005). This is extremely common in the Order Hymenoptera. For example, fig wasps are able to adjust the sex ratio of their brood according to the level of inbreeding within the mating group (Herre, 1985). Related females laying eggs in the same fig fruit produce more female-biased broods; fitness is increased because males are related to each other. Where a larger number of unrelated females are laying eggs in the same fig, a more equal brood sex ratio is favoured. The competition within a mating group is known as local mate competition (LMC) and fig wasps alter sex ratios of their brood in response to the relative strength of LMC. Alternatively, vertebrates have limited ability for sex allocation adjustment given the constraints of chromosomal sex determination and Mendelian segregation of the sex chromosome (e.g., Seychelles warblers, *Acrocephalus sechellensis*, are one of few vertebrate species with the ability for sex allocation adjustment [Komdeur, 1996]); invertebrates more frequently can alter sex allocation in response to environmental conditions (West et al., 2005).

In this study I investigate the effects of social environment on sex adjustment in wild and lab-reared isofemale lines of *T. californicus*. The role of socially mediated sex ratio adjustment in *T. californicus* has only recently been considered. Preliminary field evidence shows that clutch sex ratios were negatively correlated with local sex ratios, suggesting the social environment may affect sex allocation/determination

(MacKeracher, unpublished data). I test the occurrence of socially mediated sex ratio adjustment in this study without addressing whether it is under parental (sex allocation) or zygotic (sex determination) control. Studies of socially mediated sex ratio adjustment are rare and have never been reported, to my knowledge, in crustaceans. If such a mechanism exists, then I expect brood sex ratios to be negatively correlated with population sex ratios.

Methods

Study organisms and lab conditions

The effect of population sex ratios on the sex ratio of developing egg masses was tested in both wild out-bred and lab-reared isofemale lines of *T. californicus*. Wild populations were collected from the Ross Islets (48°52'25.88"N, 125° 9'40.75"W) in Barkley Sound, British Columbia. Subpopulations within the site were collected and pooled; previous studies have shown that frequent mixing occurs among pools on a rocky outcrop, resulting in little genetic diversity among pools (Edmands and Harrison, 2003). These populations were acclimatized to lab conditions for one week prior to experimentation. Individuals captured from the wild were kept in 1 litre of filtered seawater in a 20 °C incubator with a 12:12 hour light:dark cycle. Filtered seawater (FSW) was obtained from Bamfield Marine Science Centre (BMSC) seawater system pumped from 20 m depth in Bamfield inlet and filtered at 0.5 microns before use. Populations were fed 1000 µL of a solution containing a mix of ground Nutrafin® Spirulina flakes and ground TetraMin® tropical flakes at 0.05 grams each per 10 mL of FSW.

Sex adjustment experiment

To assess the role of social environment on sex adjustment, gravid females from wild populations with ripe egg sacs were haphazardly selected and randomly assigned to one of four conditioned-water treatments: all non-virgin female (F), all males (M), 50:50 pairs of males with virgin females (P; mate-guarding male with virgin female), and a control with plain FSW and no copepods (C). Egg sacs (clutches) were removed from the urosome and placed in a single well of a 6-well culture plate filled with 10 mL of conditioned FSW. The females were then placed in the adjacent well of the culture plate in the same respective conditioned treatment water. Up to three clutches were removed from each female to assess individual variation. Therefore, the first broods sampled from each female was subject to the treatment conditions isolated from the mother, while subsequent broods were subject to treatment conditions while attached to the mother. Subsequent broods were removed from females once they had become red in colour (indicating ripeness) and placed in a new well. Occasionally egg sacs hatched before they were removed from the female. In such instances, the females were transferred to a new well and the clutch was allowed to mature in the well in which it had hatched because females will cannibalise their offspring (Lazzaretto et al., 1990; personal observation). I have never detected sex-biased mortality of offspring (Voordouw et al., 2002a). Broods were fed 75 μ L of the standard food (see above) every five days as needed and reared in treatment conditions until sexual maturity. At maturity individuals in a clutch were anaesthetized using $MgCl_2$ solution and individuals were identified as male or female under a dissecting microscope. For each female (n=30 per treatment), sex ratio was calculated as the proportion male offspring produced summing, across all clutches.

Set-up of conditioned-water treatments

Conditioned water used for treatments (all female F, all male M, and 50:50 male-female P) of social environment were prepared by holding wild-collected copepods at a density of 200 individuals per 200 mL for three days. Chemically conditioned water was used as previous research suggests that *Tigriopus* have the ability to detect chemical cues (Kelly et al., 1998; Ting and Snell, 2003). Individuals used to condition seawater were fed 200 μ L of food solution (as above). After three days, copepods were strained from the water and removed. Three days was chosen to minimize buildup of wastes, and accumulation of deceased copepods, newly born individuals, and any individuals that may have matured or changed from their virgin state.

Sex adjustment in isofemale lines

To further explore the potential for social mediation of sex adjustment, I conducted a second experiment using isofemale lines, originating from San Diego, California, created to reduce genetic variation in the sex ratio within a line. Isofemale lines were created by imposing strong inbreeding (full-sib crosses) for 9 generations, after which 3 gravid females were selected and placed in a bottle. Offspring and subsequent generations from these females were allowed to breed at will and persist as a population. Isofemale lines were kept in the lab at room temperature, averaging 20 °C, in natural light conditions (approximately 14:10 hour light:dark cycle).

I used seven different isofemale lines. Populations 1, 2, and 3 are male-biased, populations 5, 6 and 7 are female-biased, and population 4 is slightly male-biased, but shows a more balanced sex ratio during inbreeding crosses. For each isofemale line, five

individuals were randomly assigned to each of the four water treatments; all other details are identical to those described above for the wild population.

Analysis

All data were analyzed using R v2.15.2 (R Core Team, 2012). The proportion of males produced by each female as a function of treatment was analyzed as a generalized linear model (glm) with quasibinomial errors due to overdispersed data. I used an F-test on the residual deviance for model selection to compare the original and simplified models.

Families with fewer than four individuals in the clutch were removed from data analysis as such small family sizes cannot provide accurate estimates of brood sex ratio.

Similarly, analysis for the isofemale lines used the same glm, but with ‘population’ included as an additional fixed factor. Data were also overdispersed and quasibinomial errors were specified. Where treatments differed, I performed a post-hoc analysis to contrast the different treatment levels using the package ‘phia’ (De Rosario-Martinez, 2013) in R v2.15.2 (R Core Team, 2012).

Brood number was tested as a factor in both experiments but had no effect on sex ratio.

Results

Sex adjustment in wild populations

Brood sex ratios were highly variable within treatments. The sex ratio of broods developed in male conditioned water was slightly female biased (back-transformed mean (\pm SE), 0.4137 (+0.0464/-0.0449) from model 1.1), but 95% confidence intervals still overlapped 0.5 and the control mean (Figure 3-1). All other treatments overlapped 0.5 and the control treatment. There was no evidence of a treatment effect; including

treatment in the model did not significantly improve model fit over that of the null model (Table 3-1; Δ deviance= 19.532, Δ df=3, $F=1.077$, $P=0.3622$).

Sex adjustment in isofemale lines

Looking at the treatment effects only, the sex ratio of the broods being produced tended to oppose that of treatment sex ratio (Figure 3-2), however only the male-biased treatment was significantly different from the control. The back-transformed overall mean (\pm SE) sex ratios for control, female, male and paired treatment groups from model 2.3 were 0.501 (± 0.046), 0.481 ($+0.049/-0.048$), 0.392 ($+0.047/-0.045$), and 0.464 ($+0.051/-0.050$) proportion males respectively (Figure 3-2).

Treatment effects were highly variable among populations (Figure 3-3). The expected trend in female treatments was only seen in population 3; however the 95% confidence interval still overlapped the control (Figure 3-3). In female treatments, clutch sex ratios in populations 4, 5 and 7 did not differ from their respective control groups, while in populations 1, 2, and 6, clutch sex ratios were opposite to what was expected (female biased). In male treatments, clutch sex ratios in populations 2, 4, 5, and 6 showed trends in the expected direction (female biased), while populations 1, 3, and 7 did not differ from the controls (Figure 3-3). Paired treatments tended to show the same pattern as female treatments.

Model 2.2, which includes the population and treatment effects with no interaction term, was the best fit to the data (Table 3-2). The absence of an interaction term suggests that there are overall population differences, but the treatment effects within populations were consistent. In the post hoc analysis on Model 2.2 (Table 3-2) comparing the different levels of a treatment—with population considered—I found that male treatments

were significantly different from the control group, but not from either the female or the paired treatments (Figure 3-2).

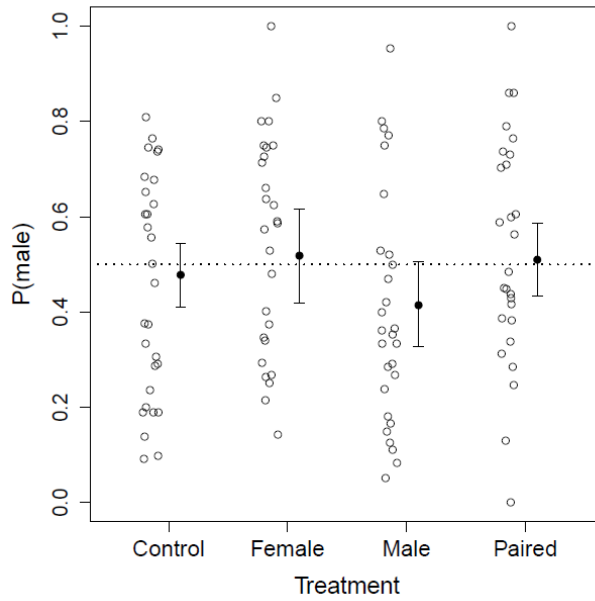


Figure 3-1. Mean (\pm 95% C.I.) brood sex ratios (proportion male) in four treatments of conditioned seawater in wild populations of *T. californicus* (indicated with ●). The control treatment was plain seawater, the female treatment had seawater conditioned with 100% non-virgin females, the male treatment was conditioned 100% males, and the paired treatment was conditioned with a 50/50 mix of males to virgin females. Raw data are indicated with open circles (○).

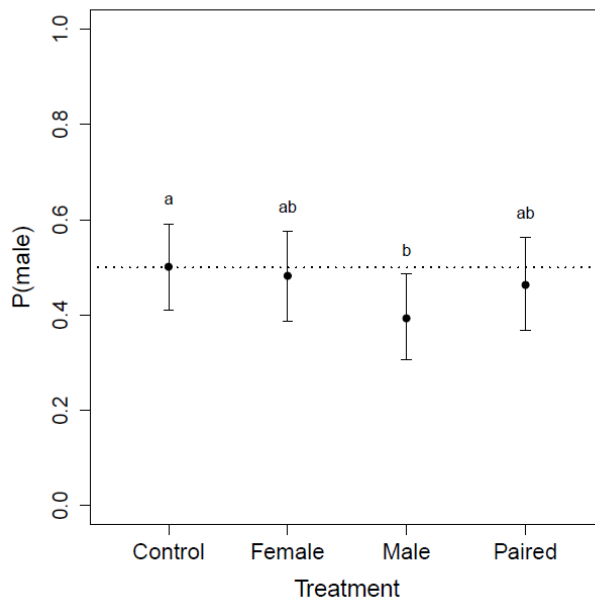


Figure 3-2. Mean (\pm 95% CI) proportion of males for each treatment with population removed as a fixed effect in isofemale lines (Model 3 in Table 3-2). Letters indicate significant differences in contrasts between the different treatment levels.

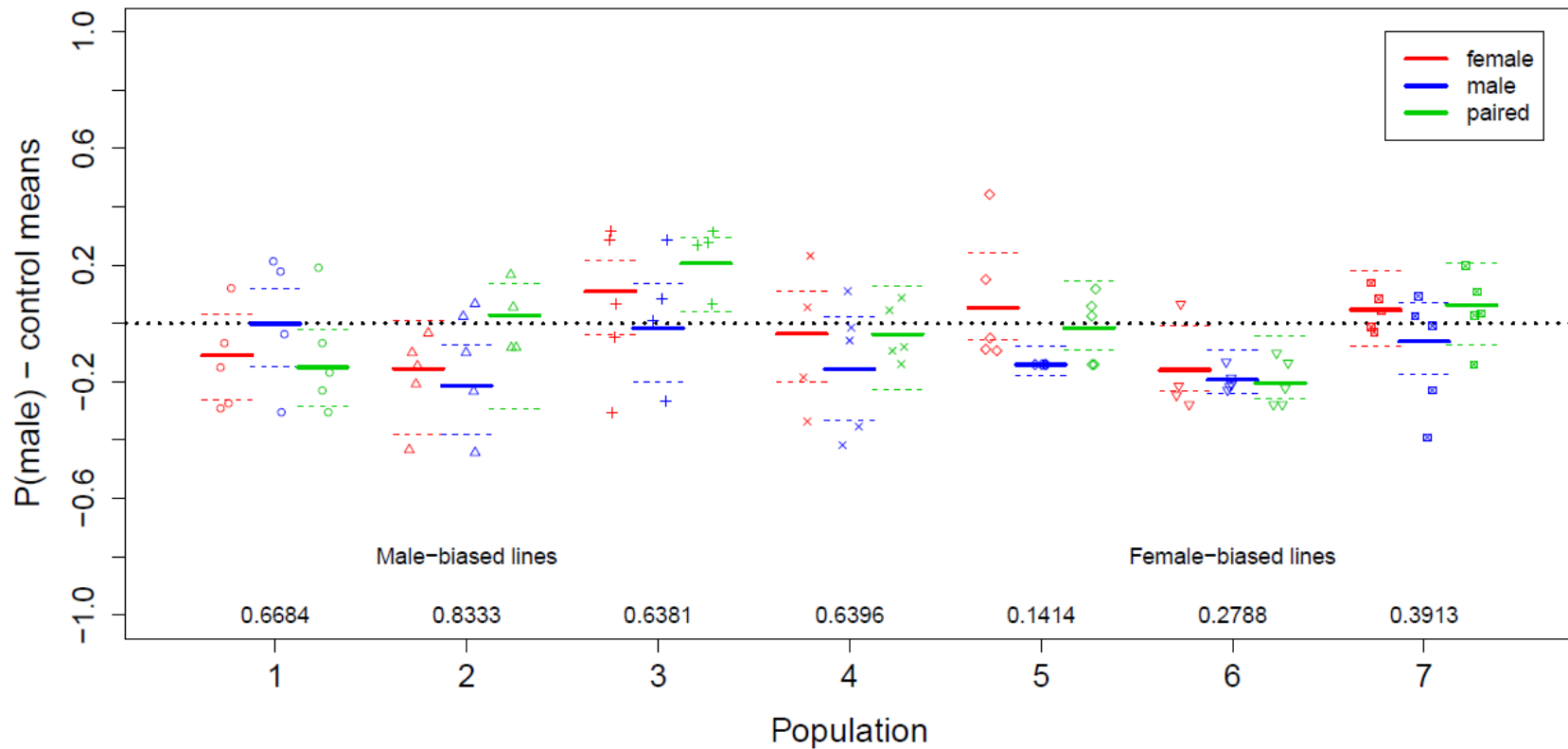


Figure 3-3. Deviations of brood sex ratios raised in conditioned water relative to the control means from respective isofemale lines. Control treatment mean values from each population are written along the bottom of the x-axis. Legend and colours represent treatment groups, and coloured horizontal lines represent the means (\pm 95% CI) for each treatment group relative to the control mean from respective populations. The control treatment was plain seawater, the female treatment had seawater conditioned with 100% non-virgin females, the male treatment was 100% males, and the paired treatment was a 50/50 mix of males to virgin females.

Table 3-1. Analysis of deviance table for the effects of local sex ratio treatments (100% male, 100% non-virgin female, 50:50 male:virgin female) on subsequent clutch sex ratios in wild populations of *T. californicus* using a generalized linear model with quasibinomial errors to account for overdispersion.

Model	Formula	Residual df	Residual deviance	Δdf	ΔDeviance	F	P[>F]
1.1	$y = 1 + treatment$	107	699.6	3	19.5	1.077	0.3622
1.2	$y = 1$	110	719.1	-	-	-	-

Table 3-2. Analysis of deviance tables and paired model F-test comparisons for the effects of isofemale lines (with known sex ratio bias) and local sex ratio treatments (100% male, 100% non-virgin female, 50:50 male:virgin female) on subsequent clutch sex ratios in *T. californicus* using a generalized linear model with quasibinomial errors to account for overdispersion.

Model	Formula	Residual df	Residual deviance	Δ df	Δ Deviance	F	P[>F]
2.1	$y = 1 + \text{population}$ * <i>treatment</i>	104	342.0	18	81.03	1.457	0.1215
2.2	$y = 1 + \text{population}$ + <i>treatment</i>	122	423.0	-	-	-	-
2.2	$y = 1 + \text{population}$ + <i>treatment</i>	122	423.03	6	716.1	36.65	<0.001
2.3	$y = 1 + \text{treatment}$	128	1139	-	-	-	-
2.2	$y = 1 + \text{population}$ + <i>treatment</i>	122	423.0	3	26.26	2.688	0.0494 3
2.4	$y = 1 + \text{population}$	125	449.3	-	-	-	-
2.3	$y = 1 + \text{treatment}$	128	1139	3	22.89	1.009	0.3913
2.5	$y = 1$	131	1162	-	-	-	-
2.4	$y = 1 + \text{population}$	125	449.3	6	712.7	34.65	<0.001
2.5	$y = 1$	131	1162	-	-	-	-

Discussion

Local sex ratios have a small, yet detectable effect on subsequent brood sex ratios in isofemale lines but not in wild populations. Isofemale lines allowed me to detect small sex ratio adjustments in the different treatments as expected. The reduced genetic variance in the isofemale lines was enough to make treatment a significant factor in the analysis. The data suggest that socially mediated sex ratio adjustment may exist in *T. californicus*, but that the effects produced in this study were small relative to individual variation in clutch sex ratio. The negative effect of treatment sex ratios on brood sex ratios (e.g., more female-biased broods were produced in the male-conditioned water treatment) in isofemale lines, suggests that socially mediated sex ratio adjustment is consistent with Fisher's (1930) theory, but further testing would better support such inferences. Paired treatments tending to show the same pattern as female treatments suggests that the presence of females in the population may have a greater socially mediated sex ratio adjustment effect than males.

While the inclusion of treatment effects was supported in the isofemale lines (Table 3-2), they were variable across different populations (Figure 3-3). Lack of greater overall treatment effect sizes seen in the isofemale experiment could be attributed to the small sample sizes. Isofemale lines were often small and not as densely populated as wild populations. I was only able to sample 20 females from each isofemale line, 5 for each treatment. In contrast, 30 females for each treatment were used from wild populations. Increasing the sample size in isofemale lines could refine my results; either increasing or diminishing any treatment effects currently detected.

The treatment effects of socially mediated sex ratio adjustment detected in *T. californicus* appear to be triggered by waterborne chemical cues. There was no direct contact of females with the inducing animals. Species of *Tigriopus* are known to use chemical cues to alter mate-guarding behaviours (Kelly et al., 1998; Ting and Snell, 2003). In *T. japonicus*, water conditioned with virgin females resulted in an increase in the number of ‘releases’ by a mate-guarding male and decreased in water conditioned with non-virgin females (Kelly et al., 1998). This suggests that when there is an abundance of females males search for the most developed virgin female (Kelly et al., 1998). In freshwater species of copepods, pheromone trails left by conspecifics are used to locate prospective mates (Weissburg et al., 1998). However, responses may increase with the inclusion of physical cues. In addition to the chemical diffusible pheromones mentioned, *T. japonicus* appear to also use physical cues when altering their mate-guarding behaviour (Kelly et al., 1998). In the presence of both physical and chemical cues, *T. californicus* males release less developed females and choose more developed females when available (Burton, 1985). In other species, social mediation is primarily triggered by visual cues (e.g., Shapiro, 1981; Ross et al., 1983; Shapiro, 1983; Cole and Shapiro, 1995; reviewed in Devlin and Nagahama, 2002), but possibly waterborne pheromonal or chemical cues as well (e.g., Cole and Shapiro, 1995; reviewed in Devlin and Nagahama, 2002). The exact mechanism for information transfer has yet to be identified in *T. californicus* and including all possible cues in future experiments may better define sex ratio adjustment effects.

While I did not determine whether socially mediated sex ratio adjustment is under parental or zygotic control, the experimental design suggests it could possibly be under

zygotic control. The first broods sampled were exposed to treatment conditions only while separated from the mother; subsequent broods were exposed to treatment conditions during development attached to the mother. There was no effect of brood number on the brood sex ratio and results were consistent across broods. Despite these differences between broods, treatment effects were still detected (Table 3-2; Figure 3-2 and 3-3). Maternal control of socially mediated sex determination has been documented in haplodiploid species (e.g., maternal control of fertilization rates [Werren, 1980a]), but to our knowledge this would be the first organism to support zygotic control of socially mediated sex determination in response to local sex ratios.

Producing the less abundant sex in response to the overall sex ratio as a mechanism I have proposed for *T. californicus* is not known to occur in any other species. However, there are other similar mechanisms that affect sex determination, sex allocation, and sex change in response to social environmental cues. For example, socially mediated sex allocation appears to be most common in species with local mate competition regimes (e.g., Taylor and Bulmer, 1980; Izraylevich and Gerson, 1996; Fellowes et al., 1999; King, 2002; Foitzik et al., 2010; Hu et al., 2010; Pickup and Barrett, 2013). Local mate competition (LMC) is an important aspect in reproductive success. Segregated mating groups—most often geographically isolated from other groups—can vary in density and relative sex ratios. This can affect the strength of competition between reproductive individuals and the level of inbreeding (Hamilton, 1967; Hardy, 1994; Hu et al., 2010; Janicke et al., 2013). In some species, sex allocation adjustment mechanisms exist to alter brood sex ratios and increase reproductive success. This phenomenon is most notably studied in Hymenopteran species (e.g., Werren, 1980a; Molbo and Parker, 1996; Hardy et

al., 2000). For example, in a few species of wasp, such as *Nasonia vitripennis*, mothers are known to adjust offspring sex ratio in response to the strength of LMC in mating groups (Herre, 1985). *N. vitripennis* is a parasitic wasp that lays eggs in pupae of cyclorrhaphous flies. If the eggs within a pupa host come from a single foundress, female biased broods are produced, but increasing the number of foundresses shifts the sex ratio towards 0.5 (Werren, 1980b; Werren, 1983).

Similarly in the simultaneously hermaphroditic flatworm, *M. lignano*, the initial investment in the proportion of males produced is positively correlated to mating group size (Janicke et al., 2013). This correlation diminishes once a threshold mating group size is reached and sex ratio stabilizes, as predicted by theory (Hamilton, 1967) and consistent with other LMC examples. Although the mechanism proposed for *T. californicus* is different, it follows the same principles as offspring sex ratio changes in response to the strength of LMC and the relative sex ratios and might represent an example of convergent evolution for socially mediated sex ratio adjustment.

Similarly, sex change has been observed to be triggered by social cues and changes in the surrounding environment. Socially mediated sex change has been documented in certain species of fish (e.g., Cole and Shapiro, 1995; Hobbs et al., 2004; Liu and Sadovy, 2004). These species operate as sequential hermaphrodites, where individuals start as one sex and develop into the opposite sex triggered by changes in group structure. For example, clown fish form mating groups and adhere to a dominance hierarchy system (Hobbs et al., 2004). The most aggressive, and often largest, individual will be the only reproducing female in the group. The next largest individual in the hierarchy will be the only reproducing male, and the remaining individuals will be comprised of juvenile

males. Maturation or sex-change of individuals next in the hierarchy is triggered by the death or departure of the reproducing female or male (Hobbs et al., 2004). This mechanism has evolved independently multiple times in species with very different origins. Again, this mechanism is very different to the mechanism proposed for *T. californicus*, but provides further evidence for the diverse existence of the effects of social mediation on sex-determining components.

The capacity and sensitivity of socially mediated sex ratio adjustment in *T. californicus* is unknown and confounding variables—including population density, turnover rate, and structure—could affect the underlying social mediation mechanism. Altering brood sex ratios in response to the local sex ratio may in fact be of trivial importance if population sex ratios frequently change, especially with the ephemeral populations of *T. californicus*. Metapopulations of *T. californicus* are highly variable with respect to size, density, sex ratio, and age structure. Populations are known to have high turnover rates (unpublished data) which would lead to a “false” sense of increased reproductive success as local sex ratios are dynamic and unstable. The local sex ratio when socially mediated sex adjustment occurs could be very different to when individuals are sexually mature and actively looking for a mate. In summary, three factors could affect socially mediated sex ratio adjustment and the potential benefit to reproductive success in *T. californicus*: 1) the overall sex ratio across all connected metapopulations, 2) the sex ratio at the time when socially mediated sex determination occurs, and 3) the sex ratio at the time when individuals are sexually mature and ready to mate. The presence of socially mediated sex ratio adjustment and these three factors could have implications for sex ratio evolution in *T. californicus*.

Socially mediated sex adjustment in *T. californicus* could reduce the rate of sex ratio evolution and help maintain phenotypic variation for sex ratios. *T. californicus* have brood and population sex ratios that are often not 1:1 as predicted by Fisher (1930). They have a complex sex determining mechanism including genetic (Voordouw and Anholt, 2002b; Voordouw et al., 2005a) and environmental (Egloff, 1966; Voordouw and Anholt, 2002a) components. Changing environmental conditions, such as temperature and local sex ratios, can produce fluctuating selection regimes. Fluctuating selection can maintain genetic variance in populations with overlapping generations (Ellner and Hairston, 1994)—as in *T. californicus*. Therefore, if local sex ratios are constantly changing then selection for brood sex ratios under social mediation will also be variable. This fluctuating selection could contribute to the maintenance of genetic variation for sex ratios in *T. californicus* and explain the lack of a 1:1 sex ratio.

Establishing sex determination mechanisms and the process in which they operate can help us better understand population dynamics, which can be applied to species with similar sex determining mechanisms. Socially mediated sex adjustment can allow individuals to potentially maximize their reproductive success. Utilizing these results, we can further develop our knowledge in the complex evolutionary field of sex ratio theory and sex allocation theory.

CHAPTER 4 – Conclusion

My thesis highlights the complex sex determination mechanism in *Tigriopus californicus*, while making a significant contribution to better understanding the mechanism. The results consistently produce extrabinomial sex ratios (chapter 2 & 3) which further support the hypothesis for polygenic sex determination. Past studies have shown genetic heritability for brood sex ratios (e.g., Voordouw and Anholt, 2002b; Voordouw et al., 2005a), and further support is provided by the artificially selected sex-biased populations (from Alexander et al., 2014) established to test Fisher's adaptive sex ratio theory (Chapter 2). Artificial selection of these population lines showed a consistent trend to become more sex-biased over time.

This thesis contributes to sex ratio theory by providing support of Fisher's (1930) adaptive sex ratio theory. Models developed by Bulmer and Bull (1982) show the expected sex ratio trajectory under different heritability estimates. Realized heritability estimates calculated in Alexander et al. (2014) on the same populations used in this experiment (chapter 2) were all relatively low, which support the data obtained. Most populations showed a relatively slow trajectory back to the expected 0.5 sex ratio, indicative of a low heritability. These low heritability estimates correspond to the predictions for the number of generations to 0.5 using Bulmer and Bull's model.

Fisher's (1930) adaptive sex ratio theory is one of the most fundamental theories developed in evolutionary biology. Natural populations of *T. californicus* do not conform to the predictions of Fisher's theory—a balanced sex ratio of 0.5. Instead, *T. californicus* shows extrabinomial variation in PSR. This variation in PSR and variation for the

numerous factors affecting sex determination can possibly be maintained by the dynamic nature of their habitat, which would lead to non-Fisherian sex ratios. The overall fitted trajectories and sex ratio evolution of population sex ratio presented in chapter 2 support Fisher's theory of frequency dependent selection for the less abundant sex. However, the fluctuations of the sex ratio trajectory emphasize the variation in *T. californicus* sex ratios. More generations of experimentation are needed to reach 0.5 according to model predictions. If possible, this experiment could benefit from a more controlled environment to minimize any confounding effects (e.g., environment) on sex determination.

Overlapping generations (OLGs) have also shown to be of great importance and need to be considered in relevant systems in which they exist. Theoretical models often disregard overlapping generations and instead assume discrete populations. However, the outcome of models can drastically change with the inclusion of OLGs. OLGs are a pervasive life history trait. This creates a genetic overlap and can alter genetic variation (Charlesworth, 1974). Overlapping generations have shown to increase the rate of genetic drift (Rogers and Prügel-Bennett, 2000) which can affect sex ratio evolution. Sex ratio models often assume discrete generations. Predictions of sex ratio trajectory when testing Fisher's adaptive sex ratio could overestimate the rate at which sex ratio changes. Overlapping generations need to be accounted for especially when estimating parameters in which accuracy is of importance, such as in conservation applications.

One major contribution of this thesis to evolutionary theories is the importance of balancing empirical support and the use of models. Bulmer and Bull's (1982) mechanistic model was fit to my data, providing valuable predictions for sex ratio evolution (chapter

2). A simulation model was then developed to specifically accommodate *T. californicus*. Modelling sex ratio evolution with simulations that emulate the experimental design provides valuable support for the data and Bulmer and Bull's model. Simulation models strengthen the existing support of statistical models and justify fitting the models and parameters to the data. The two models were consistent with each other. These techniques can be easily applied with little effort, and is especially important to consider in studies on complex systems. They also provide a basis for predictions beyond the scope of the experiment.

In chapter 3, I found that brood sex ratios show slight effects in chemically treated water with different local sex ratios. These effects were very small when testing wild populations, but more clear in isofemale lines. Environmental sex determination in *T. californicus* has been linked to many other factors (i.e., temperature [Vittor, 1971; Voordouw and Anholt, 2002a], salinity [Egloff, 1966]) in addition to my results. These findings produce a complex scenario for sex determination in *T. californicus*. The supralittoral habitat of *T. californicus* is characterized by a highly variable environment, including large changes in abiotic factors such as temperature, salinity, desiccation rates, wave exposure, and wash-outs of splash pools (Dybdahl, 1995). The interaction between this dynamic environment and factors that influence sex ratio makes predicting sex ratios in response to the relative environmental components complicated and maybe even impossible. Future studies could aim to better define the underlying mechanisms involved in sex determination.

Any sex determination effects detected in the social mediation experiment were attributed to chemical cues—a consequence of the experimental design. The detection of

chemical cues and pheromones has been noted in sister species of *T. californicus* (e.g., Kahan et al., 1988; Kelly et al., 1998; Ting and Snell, 2003) and copepods in general (e.g., Lonsdale et al., 1998, Yen et al., 2011). The minor effects detected in my experiment could be attributed to waterborne chemical cues not having enough of an influence on sex allocation. The physical presence of individuals that comprise the local sex ratio could provide better resolution of any effects, a direction for additional studies. The ability to detect chemical cues could have also been confounded by contaminated treatment water. During the water-conditioning period, some copepods died while some females hatched broods. This could confound and dilute any effects on sex allocation. Instead, physical cues or more definitive chemical treatment conditions could enhance any effects of social mediation on sex determination. Sample size in isofemale lines were also limited by the population size and number of gravid females within each population line. Ideally we would have more than 5 replicate females assigned to each treatment within an isofemale line.

To my knowledge, chapter 3 provides the first evidence of this type of social mediation of sex determination in crustaceans. Most instances of socially mediated sex determination/change occur in response to local mate competition, local resource competition, or local resource enhancement (extensive review in West et al., 2005), as well to group structure in sequential hermaphrodites (e.g., Cole and Shapiro, 1995; Hobbs et al., 2004; Liu and Sadovy, 2004). Although similar to sex allocation in LMC, this study distinguishes from other studies as it shows socially mediated sex determination in response to the absolute local sex ratios.

Future studies on sex determination mechanisms in *T. californicus* should better refine any effects through genetic analysis. Genetic markers are available for *T. californicus* (Foley et al., 2011) and can provide the tools necessary to use QTL analysis and identify sex determining genes. This could help us determine the presence and the relative effects each component has on sex determination. Identifying sex determining mechanisms in *T. californicus* provides valuable information when testing Fisher's (1930) adaptive sex ratio theory—the focus of chapter 2.

In conclusion, this is the first study to use a polygenic system to support Fisher's adaptive sex ratio theory to my knowledge. Socially mediated sex determination in response to local sex ratios is also the first of its type to be reported. This thesis presents the importance of including specific life histories—such as overlapping generations—when testing fundamental theories. I used different modelling techniques to support data, which strengthens any inferences made. My thesis makes valuable contributions to sex ratio theory and sex allocation theory.

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APPENDIX A

R-code for IVC model

```
#####
#
#
# Fisherian sex-ratio selection
# Simulation model of fisherian experiment_v9
# by Travis Tai
#
#
#####
#####
# Modelling sex-ratio trajectories
#
# Packages required:
#   lestat
#   boot
#
#####
rm(list=ls())
library(lestat)
library(boot)

#####
#
#
# ASSUMPTIONS OF MODEL:
# -all females have equal fecundity (success is equal)
# -all females will mate unless limited by males or number of possible male matings
# -males can mate more than once (if specified)
# -males can mate more than once within a generation (if specified)
# -males can only mate multiple times within generation if (#females > #males)
# -females produce clutches til death, one per generation
# -the end of a generation/start of a new generation occurs AFTER the MATING function
# -selection of individuals for next generation is limited by carrying capacity
# -older individuals have higher probability of surviving (unless at max lifespan)
# -two contributors to overlapping generations:
#   females producing multiple broods
#   males mating with subsequent generations
#
# Sources of variation within the model
# -binomial probability of sex
# -generating offspring genotype with rnorm
# -sampling for sex ratio
# -carrying capacity random selection
#
#####
#
# IMPORTANT NOTES ON VARIABLES OF DATA FRAME
#
# Genotype: represents binomial probability of becoming male/female
# Phenotype: 0=female; 1=male; 2=average genotype (represent mated females [for overlapping gens])
# Life: indicates number of generations lived thus far
# Mated: number of mating events for individual
#         -for females: 0=no; 1=yes
#         -for males: 0=no; 1:n where n is MaxMated
#
#####
#####
#
# FUNCTIONS
#
#####
##### Generate population function #####
GENERATE<-function(X){ ## X = initial population N
  ##### generate genotypes for N individuals using normal distribution
  ## confined by 0 and 1
  G<-matrix(0,10000,2) ## matrix to store generated geno/phenotypes
  G[,1]<-rnorm(10000,mean=mu,sd=0.002) ## create a distribution of genotypes to draw from with
  mean mu
  G[G[,1]>1,]<-1 ## change the probabilities to be confined to 0,1
  G[G[,1]<0,]<-0

  G[,2]<-apply(G,1,function(x){ ## assign sex based on binomial probability
    rbinom(n=1,size=1,prob=x[1])
  })
  m<-G[G[,2]==1,] ## separate males
```

```

f<-G[G[,2]==0,] ## separate females
## sample male and female genotypes with sex ratio=mu
Y<-rbind(m[sample(1:nrow(m),
                size=X*mu,replace=T),],
        f[sample(1:nrow(f),
                size=X*(1-mu),replace=T),])

##### creating the initial data frame #####
colnames(Y)<-c("Geno","Pheno")
ID<-vector(mode="numeric",length=nrow(Y)) ## create individual IDs
Life<-vector(mode="numeric",length=nrow(Y)) ## column for storing how many generation and
individual has lived for
Mated<-vector(mode="numeric",length=nrow(Y)) ## column for how many times an individual has mated
Y<-data.frame(ID,Y,Life,Mated)
Y$ID<-as.numeric(rownames(Y)) ## enter in unique IDs using row names of dataframe

return(Y)
}

##### Mate pairing function #####
MATING<-function(X){ ## X = male and female genotypes (from GENERATE function)
  GM<-X[which(X$Pheno==1 & X$Mated<MaxMate),] ## separate reproductive males (can't be at max mated)
  GF<-X[which(X$Pheno==0),] ## separate females
  Fecundity<-Fe ## fecundity

  ##### create a dummy data frame to replicate males for multiple matings within generation #####
  RepVect<-ifelse((MaxMate-GM$Mated) > MatePerGen, MatePerGen, (MaxMate-GM$Mated))
  DummyMVect<-rep(1:nrow(GM),times=(RepVect))

  ##### Randomly pair all competing reproductive individuals #####
  ## index
  MSamp<-sample(DummyMVect,size=ifelse(length(DummyMVect)>nrow(GF),nrow(GF),length(DummyMVect)))
  FSamp<-sample(1:nrow(GF),size=ifelse(length(DummyMVect)>nrow(GF),nrow(GF),length(DummyMVect)))
  ## successfully mated males and females
  MGenoSuc<-GM[MSamp,]
  FGenoSuc<-GF[FSamp,]

  ##### create dataframe for average genotypes #####
  ## these average genotypes will persist and represent "females" as females
  ## produce multiple broods from the same sperm
  AveGeno<-c(MGenoSuc$Geno+FGenoSuc$Geno)/2 ## average genotype of each pair
  Pheno<-rep(2,length(AveGeno)) ## create a phenotype column; assign values as
  2

  Life<-vector(mode="numeric",length=length(AveGeno)) ## create a generation count
  Mated<-rep(1,length(AveGeno)) ## created a mated column
  ID<-c((max(X$ID)+1):(max(X$ID)+length(AveGeno))) ## create ID column
  Geno<-AveGeno ## must rename this vector so that it matches
  for rbind
  AveGeno.dat<-data.frame(ID,Geno,Pheno,Life,Mated)

  ##### remove female genotypes that have mated; they are represented by the AveGeno now #####
  X<-X[!X$ID%in%FGenoSuc$ID,]

  ##### indicated that individual has mated, adds one for each mating #####
  mated<-aggregate(MGenoSuc$Pheno,by=list(MGenoSuc$ID),length) ## aggregate # matings
  within 1 gen
  X$Mated[X$ID%in%mated$Group.1]<-X$Mated[X$ID%in%mated$Group.1]+mated$x ## add to the data frame

  Z<-rbind(X,AveGeno.dat) ## combine individual and average geno dataframe
  Z$Life<-Z$Life+1 ## add one 'generation' to life for each mating

  return(Z)
}

##### Sample function #####
## randomly sample from population of mated individuals
## This replicates the experimental procedure used in the actual experiment
## produces experimental/sampling error
SAMPLE<-function(X){
  Fecundity<-Fe
  Ave<-X[which(X$Pheno==2),] ## isolate average geno of mated pairs from data frame
  Y<-Ave[sample(1:nrow(Ave),size=ifelse(nrow(Ave)<SampleSize,nrow(Ave), ## sample paired individual
  from
  SampleSize)),]

  TempFam<-matrix(0,Fecundity*nrow(Y),2) ## store each individual for every family
  TempFam[,1]<-apply(Y,1,function(x){
    OffGeno<-rnorm(Fecundity,mean=x[2],sd=SDa) ## create individual genotypes variation=SDa^2
    OffGeno[OffGeno>1]<-1 ## confine to 1
    OffGeno[OffGeno<0]<-0 ## confine to 0
    return(OffGeno)
  })
  TempFam[,2]<-apply(TempFam,1,function(x){ ## assign sex based on binomial probability
    rbinom(n=1,size=1,prob=x[1])
  })

  ##### OPTIONAL #####
  ## output for more details:
  ## family sex ratio, variance, etc.

```

```

#Output<-as.data.frame(matrix(0,nrow(Y),3))    ## output for clutch samples
#index<-rep(1:SampleSize,each=Fecundity)

#Output[,1]<-aggregate(TempFam[,1],
#                      by=list(index),mean)[2]    ## mean family genotype
#Output[,2]<-aggregate(TempFam[,2],by=list(index),sum)[2]    ## number of males in family
#Output[,3]<-Fecundity-Output[,2]    ## number of females in family
##### END #####

meansexratio<-sum(TempFam[,2])/nrow(TempFam)    ## mean family sex ratio of sample (balanced)

return(meansexratio)
}

##### offspring function #####
## create offspring geno/phenotypes from parental genotypes
OFFSPRING<-function(X){
  Fecundity<-Fe
  Ave<-X[which(X$Pheno==2),]    ## average geno of mated pairs
  Geno<-matrix(0,nrow(Ave)*Fecundity,1)    ## matrix to store offspring genotypes

  Geno[,1]<-apply(Ave,1,function(x){    ## for each average geno, generate offspring geno
    OffGeno<-rnorm(Fecundity,mean=x[2],sd=SDa)
    OffGeno[OffGeno>1]<-1    ## confine to 1
    OffGeno[OffGeno<0]<-0    ## confine to 0
    return(OffGeno)
  })
  Pheno<-as.vector(apply(Geno,1,function(x){    ## binomial probability of being male given
    genotype
    rbinom(1,1,prob=x[1])
  }))

  Geno<-Geno[,1]
  ID<-(max(X$ID)+1):(max(X$ID)+length(Geno))
  Life<-rep(0,times=length(Geno))    ## generation count
  Mated<-vector(mode="numeric",length=length(Geno))
  temp<-data.frame(ID,Geno,Pheno,Life,Mated)
  Y<-rbind(X,temp)

  return(Y)
}

## from this point we have three items in the datasets:
## 1) adult individuals (males + non-mated female [if any]),
## 2) average genotypes (mated females),
## 3) newly produced offspring.

##### Next generation function #####
## sample individuals that survive to the next generation
## determined by carrying capacity
NEXTGEN<-function(X){
  surv.prob<-vector(mode="numeric",length=nrow(X))    ## vector for surviving probabilities

  ## remove individuals at lifespan max
  X<-X[X$Life<lifespan,]

  ##### Optional #####
  ## probability of surviving into the next generation; CAN SET TO EQUAL
  surv.prob<-X$Life+1    ## add one to surviving probability

  ## sample from data frame given the probabilities calculated above
  SurvVect<-sample(1:nrow(X),size=ifelse(nrow(X)>=K,K,nrow(X)),prob=surv.prob)
  X<-X[SurvVect,]

  return(X)
}

##### Temporal function 2 #####
## measures average brood sex-ratio of population from a sample over given number of generations
TIME2<-function(G){
  Pop<-GENERATE(N)    ## X number of generations
  propmale<-vector(length=(G+1))    ## generate a starting population
  propmale<-vector(length=(G+1))    ## mean proportion male from sampling

  for(i in 1:(G+1)){    ## loop for maxGen
    ## running the functions created and storing the mean of the broods sampled
    Z<-MATING(Pop)    ## matching/mating males and females
    propmale[i]<-SAMPLE(Z)    ## obtaining samples for sex ratio estimate (experiment)
    offs<-OFFSPRING(Z)    ## create offspring and combine with parent dataframe
    Pop<-NEXTGEN(offs)    ## sample individuals for next generation
  }
  generation<-0:(G)    ## start at generation zero to match with data
  data<-data.frame(generation,propmale)

  return(data)
}

#####
#

```

```

# END FUNCTIONS
#
#####
#####
##### Main program #####
set.seed(100)    ## set seed for random number generator

N<-1000        ## start population size

## Simulation ##
MaxGen<-50     ## number of generations
Iterate<-10    ## number of iterations to run
SampleSize<-30 ## sample size

## Traits ##
h2<-0.2        ## heritability estimate
Vp<-(0.22)^2   ## phenotypic variance-----from data, the mean SD is 0.182 (underestimated cause
of Gen=0)
Fe<-20         ## fecundity
mu<-0.1        ## initial trait mean genetic value; starting sexratio

## Life histories ##
lifespan<-1    ## potential lifespan of individuals; **measured in generations (must be >0)**
K<-1000        ## carrying capacity
MaxMate<-1     ## maximum number of matings for males in entire life
MatePerGen<-1  ## maximum number of matings for males in one gen

Va<-Vp*h2      ## additive genetic variance
SDa<-sqrt(Va)  ## SD of Va

##### Run 1 iteration #####
#TIME2(MaxGen)

##### Graphing the output #####
## run and plot multiple iterations
graphics.off()
x<-0:MaxGen
windows(width=10, height=10)
plot(x, type="n",
      xlab="Generation", ylab="P(male)",
      cex.lab=1.3, cex.axis=1.2,
      xlim=c(0,50), ylim=c(0,1))
abline(h=0.5, lty=2, lwd=0.8)

for(i in 1:Iterate){
  dummy<-TIME2(MaxGen)
  lines(propmale~generation, data=dummy, lty=3, lwd= 0.3, col="blue")
}

#####

```