Presentation Title: “An Introduction to Environmental Sex Determination from the Perspective of Behavioral Ecology”

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GSD versus ESD Sex Determination

With **Genetic Sex Determination (GSD**) the sex of the offspring is determined at fertilization by chromosomal inheritance by the presence or absence of a sex-determining gene. This is commonly seen in mammals (XX/XY) and birds (ZW/ZZ). With GSD there is no plasticity in sex phenotype across the range of viable developmental temperatures.

On the other hand, for **Environmental Sex Determination (ESD)** offspring can develop as males or as females depending on the developmental environment. Such that plasticity in sex phenotype is observed across the range of viable developmental temperatures. ESD is common in some fish and reptiles, with many turtle species, most crocodilians, and some lizards (Gekkota, Agamidae, Scincidae) displaying this pattern of sex determination.

Types of Environmental Sex Determination

ESD occurs in some seasonally reproductive species as a response to some sort of environmental cue (such as temperature, light, water) that provides reliable information about the growing/breeding season. This cue can be under selection to act as a phenotypic switch point for male/female development. There are two requirements for the environmental cue that is tracked by the species: (1) the environmental cue affects reproductive success differently for each sex, and (2) the cue which is tracked is a good indicator of growth time before the breeding season.

(1) **Temperature Sex Determination (TSD):** Sex ratios are a function of incubation temperature, with temperature acting directly on a gene or on transcription factors causing the differential development of one sex or the other. Sex determination for an individual depends on its incubation temperature relative to any pivotal/threshold temperatures. TSD *I* & TSD *II* are two patterns of sex determination distinguished by the number of pivotal temperatures/threshold points (TSD I has one pivotal temperature versus TSD II has two). (**Figure 1.1)**

- **Pivotal/threshold temperature:** Defined as the incubation temperature that produces a balanced sex ratio in the offspring, such that incubation temperatures that deviate from the pivotal threshold produced skewed sex ratios in the offspring.

(2) **Ph-Dependent Sex Determination**: some cichlid fish (*Apistogramma*, *Pelvicachromis* spp., *Xiphophorus*.)

(3) **Water or precipitation Sex Determination**

(4) **Photoperiod (day length) Sex Determination**: in brackish water shrimp *Gammarus diebeni*

The proximate mechanism of ESD are the environmental conditions that influence the expression of male or female gonad developing genes (such as hormones, temperature-induced conformational changes to enzymes, and the level of hormone receptor expression in gonads). For example, the hormone estrogen leads to the feminization of the gonads and the expression of female developing genes, also temperatures can control the expression of the aromatase gene.

Types of Temperature Sex Determination

First, we need to go over three important terms for understanding how TSD evolves: thermosensitive period, pivotal temperature, and the transitional range of temperature. The **thermosensitive period** is the specific window of embryonic development where temperature determines male or female gonadal development. Typically, sex is determined in the first third of development. The **pivotal temperature** is the incubation temperature that when held constant produces offspring in a 1:1 sex ratio. The **transitional range (TR)** or **transitional range of temperature (TRT)** is the range of temperatures that if held constant would produce a mixture of male and female offspring. Some species can have wide, narrow, or intermediate TRT's. Populations can experience no variation in TRT or have a great deal of individual variation.

TSD *I* - Single pivotal/threshold temperature & single range of transitional temperatures, incubation temperatures either above or below this temperature determines the sex of the offspring and incubation at this temperature produces a 1:1 sex ratio. There are two patterns of type 1 TSD that are seen in nature: MF and FM

- **TSD *Ia*/MF**: Incubating below the pivotal temperature produces males and incubating above the pivotal temperature produces females. Ex: Some turtles and fish
- **TSD *Ib*/FM**: Incubating below the pivotal temperature produces females and incubating above the pivotal temperature produces males. Ex: Atlantic Silverside fish (*Menidia menidia*)

TSD *II* - Two pivotal/threshold temperatures and two ranges of transitional temperature, such that incubation between the two pivotal temperatures produces one sex and incubation both below and above this temperature produces the other sex. There are two patterns of type 2 TSD: FMF and MFM.

- **TSD *IIa*/Female-male-female (FMF):** Incubating between the pivotal temperatures produces males, whereas incubation both below and above the pivotal temperatures produces females. The 1:1 sex ratio is achieved at the pivotal temperatures. Ex: Lizards, some turtles, crocodilians
- **TSD *IIb*/Male-female-male (MFM):** Incubating between the pivotal temperatures produces females, whereas incubation both below and above the pivotal temperatures produces males. Ex: in Flatfishes (Paralichthys) the genetic expression of the **cytochrome P450 aromatase gene*** is inhibited beyond the pivotal temperatures by temperature either acting directly on this gene or on transcription factors to promote male development by preventing aromatase from converting androgens to estrogens. *Note: **cytochrome P450 aromatase gene** encodes an enzyme (aromatase) that converts androgens to estrogen.* In flatfish, the highest ratio of females is produced at intermediate temperatures (between the pivotal temperatures), and offspring are male-biased at extreme temperatures.

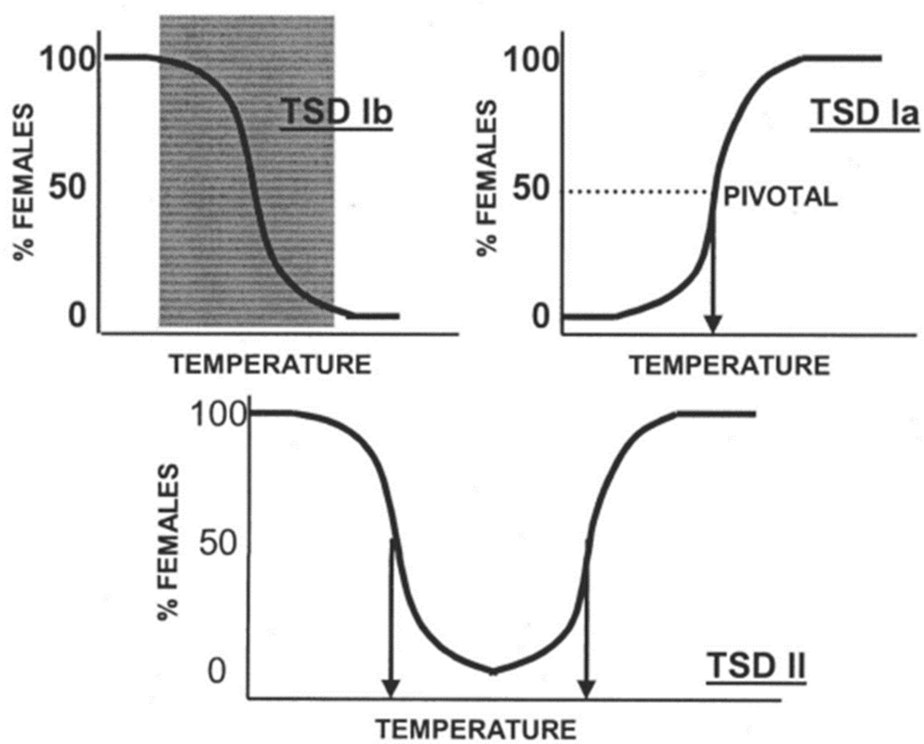

Figure 1.1: Shaded region represents the transitional range of temperatures that produce both sexes, pivotal temperatures (the point at which offspring are produced in a 1:1 sex ratio) are represented by arrows. Note that these graphs show the population reactive norm to incubation temperatures but that the individual reactive norm may be different. **(**Credit: Valenzuela, N., and Lance, V. 2004. Temperature-Dependent Sex Determination in Vertebrates. *Smithsonian Institution.*).

Example of TSD in Nature: Female-Male Pattern in the Atlantic Silverside Fish (Menidia menidia)

The Atlantic Silverfish (*Menidia menidia*) is a great example of TSD being documented in the wild, this fish has a widespread distribution ranging from Nova Scotia to Florida and reproduces from May to July. In most of the populations the mechanism of sex determination is TSD *Ib* (Female-Male pattern), with females produced at lower temperatures and males produced from higher incubation temperatures. By comparing populations of silverfish from different latitudes (Figure 2.3) it became apparent that their sex ratio response to temperature depends on the population being studied (considerable variation in the population reactive norm). Low latitude populations which experienced longer breeding and growing seasons demonstrated the greatest change to sex ratios in response to changes in the incubation period. Whereas the higher latitude populations with shorter breeding and growing seasons showed a diminished change in sex ratios in response to changes in the incubation temperature. For example, the most northern population of Nova Scotia is the only Atlantic Silverfish population that demonstrates GSD instead of TSD, in this population the breeding season is the shortest and only lasts 1 month. This demonstrates that the mechanism of sex determination can be widely variable over the range of a species with a large distribution. the potential for interpopulation variation in sex determination is one population with GSD instead of TSD, this is the Nova Scotia population with a breeding season that only lasts 1 month.

Overall, in most populations of Atlantic Silverfish the proportion of female offspring decreases with increasing temperatures. Such that eggs laid at the beginning of spring under cooler temperatures develop as females, and eggs laid in the warmer summer months develop as males. The net result is that female offspring are larger than male by the breeding season, and this size difference provides an adaptive benefit because the larger size provides more of a reproductive advantage to the fitness of females than to males, because larger sized females can produce more eggs than smaller females. Additionally, experiments with captive populations of Atlantic Silversides revealed that there is individual variation in their sensitivity to temperature and sex ratio response to environmental temperatures, such that over generations when raised in extremely hot or cold temperatures that the sex ratio is brought back to a balance 1:1 (this demonstrates that TSD can be a rapid evolutionary response).

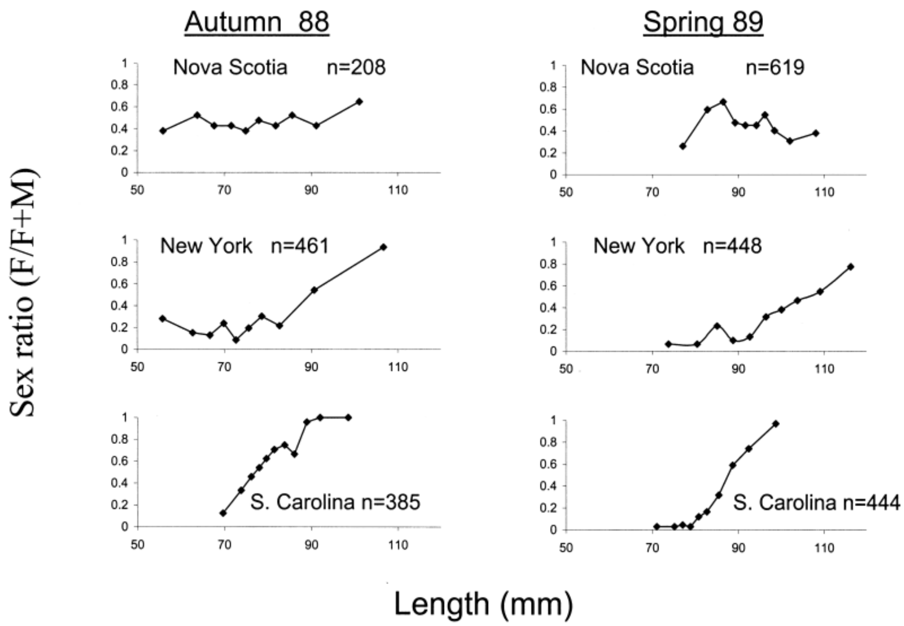

Figure 2.3 Differences in sex ratio as a function of body length among latitudinal populations of silversides that differ in the level of TSD. Samples of the 1988-year class were collected in autumn at the end of the growing season and in spring during the breeding season. The sex ratio within each quantile of the size distribution is plotted. In South Carolina (SC) and New York (NY), where the change in sex ratio with temperature is high and moderate, respectively, the smaller fish are nearly all males and the larger fish are nearly all females. In Nova Scotia (NS), however, where thermal effects on sex ratio are nil, the sex ratio does not change systematically with size (D. O. Conover, unpubl. data).

Figure 2.3 from: Valenzuela, N., and Lance, V. 2004. Temperature-Dependent Sex Determination in Vertebrates. *Smithsonian Institution.*

The takeaway from Figure 2.3 with the silversides is that geographic variation in temperature sex determination evolved in response to length of the growing season. High latitude populations such as Nova Scotia with shorter growing seasons do not exhibit TSD, because there would be no differential sex advantage because the growing season is so short that males and female offspring will be the same size by next breeding season. This is in contrast to low latitude populations in NY and SC with longer growing seasons that exhibit TSD, because in these populations there is greater adaptive benefit (increased fitness) for females to be born at the beginning of the season, because then females are significantly larger than males by the next breeding season and able to produce more offspring. In conclusion, the adaptive benefit of having females born first through TSD provides increased fitness for Atlantic Silverside fish populations <u>in certain environments</u>, because then they have more time to grow before the next breeding season and larger females have greater reproductive success, whereas there is no benefit for males to be larger than females.

Genotype x Environmental Sex Determination Interaction and the Minimum Threshold Model

In some cases, the environment can mask or override genotype, causing sex reversal in reptiles, amphibians, and fish with GSD. For example, in Tilapia (which were originally believed to be XX/XY species) the XX genotypes develop as females and the XY and YY genotypes develop as males at low temperatures, but when the temperatures are high then the XX genotype is sex-reversed and develop as the male phenotype whereas the YY genotype will develop as the female phenotype.

This type of sex determination is called the **Minimum Threshold Model**, which incorporates sex dosage and TSD temperature thresholds into one model and hypothesizes that sex determination is affected by genotype and environmental interaction. Under this model the sex determining signals are thermosensitive, such that the signal produced from the genotype x temperature interaction leads to female or male gonadal development. The component of genetic influence is variable, with sex ratios and temperature sensitivity differing by population and family. Thus, some individuals do not always produce 100% of one sex because some genotypes are not as sensitive to temperature. The sex ratio and temperature response are maintained by frequency dependent selection.

A **MDF (male determining factor)** is a factor that if genetic expression is maintained above a certain threshold during the developmental window, then facilitates male gonadal development. Otherwise, if the expression of the MDF falls below the threshold then the offspring develops as a female. In many species with G x E sex determination the incubation temperature interacts with the threshold for male development, such that depending on the temperature a lower or higher level of MDF expression is needed to facilitate male gonadal development.

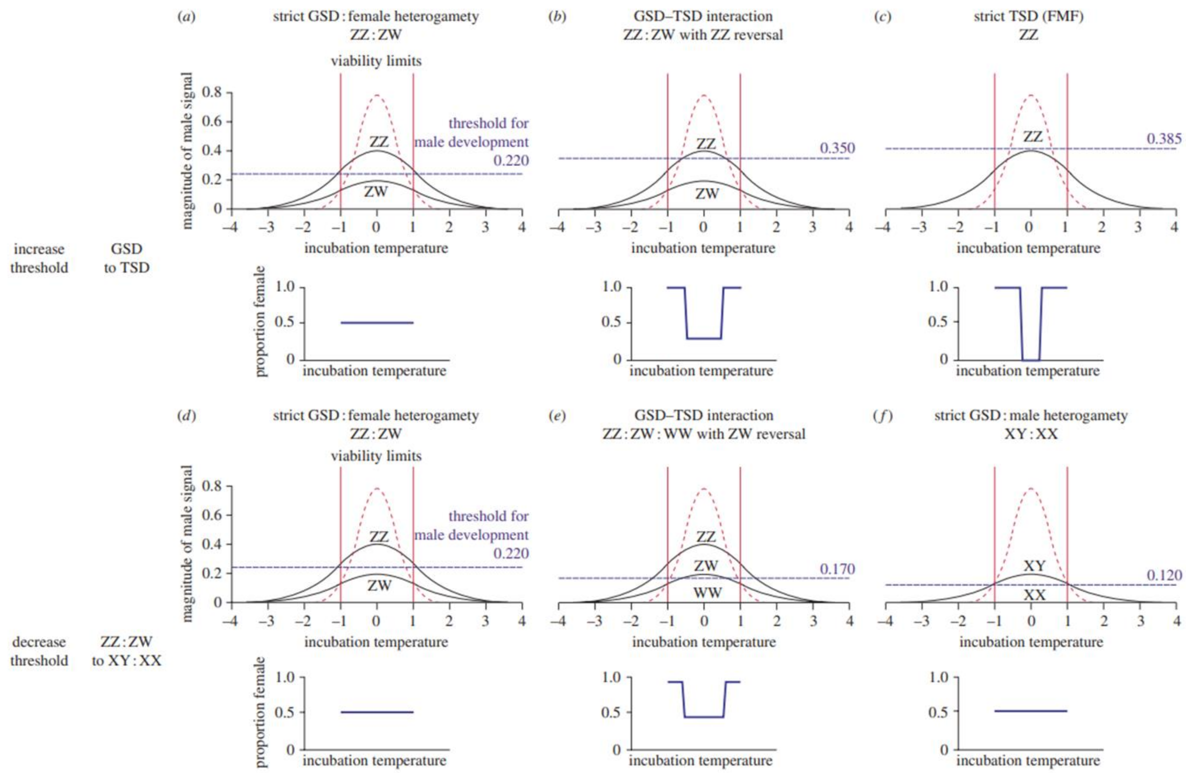

Figure 1. Transitions between sex-determining mechanisms caused by shifts in the sex-determining threshold. Black curves, magnitude of the regulatory signal for male development (arbitrarily scaled); dashed blue line, threshold value for male development; dashed red line, nest site distribution; vertical red lines, upper T_H and lower T_L thermal limits for embryo viability; solid blue line, population thermal reaction norm for sex ratio. (*a–c*) Effect of increasing the threshold for male development; (*d–f*) the effect of decreasing the threshold.

Figure 1: The Minimum Threshold Model hypothesizes that sex determination is affected by both the genotype of the individual and the interaction of this genotype with the environment. This model predicts that there is a minimum threshold for which a male determining signal will result in male gonadal development. (Credit: Quinn, A., Sarre, S., Ezaz, T., Graves, J., & Georges, A. 2011. Evolutionary transitions between mechanisms of sex determination in vertebrates. Biology Letters, 7, 443-448.)

G x E sex determining systems require that the species has poorly differentiated sex chromosomes (meaning that the sex chromosomes can recombine with each other). The interaction of genetic and environmental sex determining factors can explain why TSD is commonly seen in species with poorly differentiated sex chromosomes (such as reptiles and fish), but not in birds and mammals with highly differentiated sex chromosomes. TSD can evolve from GSD if three conditions are met: (1) there is temperature sensitivity in the genetic sex determination, (2) there is selective pressure for different levels of temperature sensitivity, and (3) the sex chromosomes are not highly differentiated (instead male and female sex chromosomes are roughly equal in size and genetic identity).

Ultimate Explanations and the Differential Fitness Hypothesis (Charnov and Bull 1977)

The **Differential Fitness Hypothesis** proposed by Charnov and Bull in 1977 treats ESD as a form of phenotypic plasticity and states that temperature-sex determination allows for the matching of sex with the environmental conditions that maximize individual fitness. So, in this way, ESD can be viewed as an adaptation for improving sex-specific fitness of offspring, where offspring develop into the sex that would have the highest reproductive fitness in that environment (differential reproductive success). This hypothesis predicts that TSD correlates with sex-specific traits that determine individual fitness, which inhibits the evolution of genetic sex determining systems. This hypothesis requires that the species breeds in a heterogenous environment where some environmental patches provide a greater benefit to the reproductive fitness of one sex over the other. So, variable or heterogenous environments would favor the evolution of ESD, whereas stable homogeneous environments would favor the evolution of GSD.

To elaborate, imagine that certain environmental conditions during incubation affect the fitness of males and females differently by affecting other aspects of their phenotype such as size, color, growth, locomotive ability, survival, fecundity. There are plenty of resources that show that incubation temperature affects many aspects of phenotype, so its very well likely that temperature could control survival or reproductive fitness. For example, in Leopard Geckos the gonadal sex and incubation temperature both affect the growth rate of developing geckos, adult body size, metabolic capacity, sex steroids, aggression levels, and sexual behavior. High incubation temperatures favor female offspring and low temperatures favor male offspring. Temperature has a sex-specific effect on adult sexual and agonistic behavior, with males from female-biased temperatures having more pronounced sexual behavior than males produced from male-biased temperatures. It is hypothesized that temperature modulates either aromatase activity or estrogen production (where offspring develop as females above a minimum estrogen level threshold).

This hypothesis assumes that male and female offspring are equally costly to produce, because if one sex was more energetically costly to produce then there would be selection for a biased sex ratio to produce the less costly sex. However, this mode of reproduction is a stable strategy as long as there is equal investment in male and female offspring, but not necessarily an equal number of both sexes. A skewed sex ratio could still be stable if it costs more to produce one sex, because maternal influence can maintain the persistent of TSD over generations if the females can select nest sites to match maternal investment. The selective pressures and exact mechanism that leads to TSD depends on life history of species and the benefit, because in order for this behavior to be an evolutionarily stable strategy the species must produce multiple clutches of offspring each year, and there needs to seasonal variation in environmental temperature. Otherwise, without exposure to a range of temperatures above and below the threshold/pivotal point then only one sex would be produced, and without both sexes occurring within a population the species would eventually become extinct. Examples where TSD might provide a differential fitness advantage to male and female offspring in different environments:

(1) Differential Dispersal: When the dispersal rates of males and females are different then the evolutionary stable strategy is to overproduce the dispersing-sex (usually males) in poor habitats and to underproduce the dispersing sex in high quality habitats.

(2) Differential Mortality: If males and female offspring suffer different morality rates at incubation temperatures then TSD allows for the production of the best fit offspring at extreme temperatures.

(3) Differential Body Size Advantage: Sexual selection for seasonal sex ratio shifts.

Examples for the Differential Fitness Hypothesis & Sexual Selection for Seasonal Sex Ratio Shifts: Australian Agamids

There is sexual selection for TSD seasonal sex ratio shifts when one sex would benefit more from hatching earlier or later during a reproductive season. The most common explanation is that an earlier hatching time has the advantage of providing an individual with more growth time before the cold season. The benefit to females of larger size is increased fecundity and the male benefit of larger size is increased combat and territory defense.

Image: Fighting between male Jacky Dragons (From Robbie Fishing)

In Australian Agamids reproduction is seasonal, with vitellogenesis and mating occurring from August to November, and then egg laying happens from November to February. They are multi-clutching species so they will lay multiple clutches of eggs during the reproductive season, with each clutch containing 2 to 41 eggs. The earliest hatchlings emerge in the early Summer and will have 4 months of growth before cold season versus only a few weeks for hatchlings from late summer. The early hatchlings will be able to reproduce next spring, but later hatchlings need to wait an extra year. For example, the Jacky dragon is an Australian lizard with MF TSD and a polygamous mating system, males hatch earlier in breeding season at colder incubation temperatures, which gives them a longer growth period than the females which hatch months later in the breeding season. So, by the next mating seasons the males have a considerably larger body size than the females, and there is strong selection for large body size in males because then they can defend territories with more females. Thus, TSD in this example is a case of differential increase in fitness as a result of seasonal hatching order, because in this species it is more advantageous for males to hatch first than it is for females to hatch first.

Sex-biased seasonal hatching orders are only seen in multi-clutching species, but more is commonly seen in species with annual life cycles versus bi-annual life cycles, because in an annual life cycle there is usually intense sexual selection for the individual to be ready for breeding by the following season. Furthermore, in order for seasonal shifts in sex ratio to be an evolutionary stable strategy, then the life history strategy of males and females must differ seasonally, and generations of a population need to overlap. The sex that will benefit more from a larger body size depends on the life history of the species, Female-Male TSD is favored when a larger body size provides females with a differential increase in fitness by increasing her fecundity (such as in species where the reproductive success is controlled by maternal body condition). On the other hand, Male-Female TSD is adaptive in species with male body size sexual dimorphism and when males engage in territorial combat (such as in species that exhibit a resource-defense or female-defense polygynous mating system).

Evidence for the Differential Fitness Hypothesis and Sexual Selection for Sex-Biased Hatching Orders: Brackish Water Shrimp

The Brackish Water Shrimp (*Gammarus diebeni*) is a widespread species of crustacean found in the temperate waters of the North Atlantic Ocean, they exhibit temperature-dependent and photoperiod-dependent ESD. The sex ratio is further distorted by infection by a microsporidian parasite that feminizes the host and turns some males into large females. This species reproduces with positive size-assortative mating, meaning that large male’s mate with large females. The pairing success depends on the ability of the male to carry the female around (mate guarding), because during this time the male will stop eating and lose weight, which is what results in selection for there to be a significant positive relationship between male and female body size. Female reproductive success if constrained by the availability of suitable males, because a female cannot successfully breed with a male that is smaller than her because he will be unable to perform courtship behaviors such as mate guarding.

Image Credit: Hatcher, M., Dunn, A., & McCabe, J. 1997. Size and pairing success in *Gammarus diebeni*: can females be too big? *Animal Behavior*, 54, 1301-1308.

This sexual dimorphism is maintained by ESD, with males being produced at the beginning of the breeding season during longer photoperiods and females produced at the end of the breeding period. This means that males have a longer growing period than females do by the next breeding season, and the result of the males hatching earlier is that they are larger than the females by the time they breed. Body size also affects female fecundity; intermediate-sized females produce less eggs than large-sized females, but the intermediate-sized females still had higher reproductive success than the larger females because they were more successful in finding an adequately large male. Thus, there is selection for males to hatch first, because they have a greater advantage from body size (larger increase in reproductive success) than females do (Figure 10.11(d)).

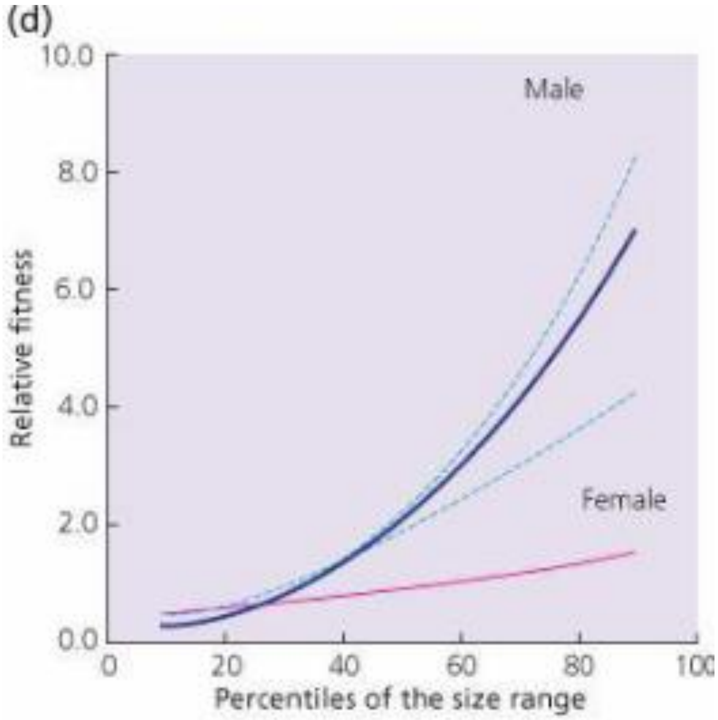

Figure 10.11 (d): Relative fitness of male Brackish Water Shrimp increases more rapidly with size than females.

The reproductive success for a population of Brackish Water Shrimp depends on encounter and pairing of same sized male and female, large females within the population are at disadvantage to smaller females, because large males are a small proportion of the overall total male population so there are not enough large males to carry all of the very large females (Figure 1 below). An experiment "Size and pairing success in *Gammarus diebeni*: can females be too big?" in 1997 by Hatcher, Dunn, and McCabe looked for evidence that ESD in the Brackish Water Shrimp is explained by sexual selection for larger male body size.

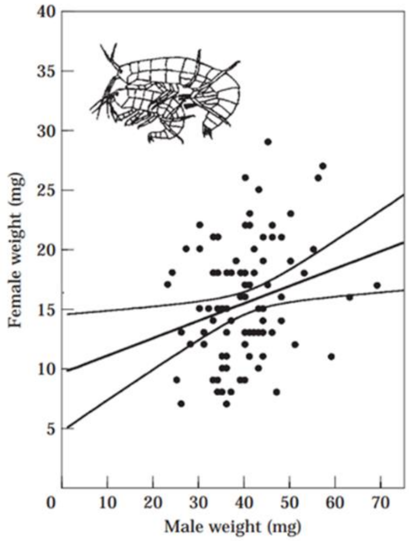

Figure 1. Relationship between male and female size in precopula pairs. The weight of females is plotted against that of the male with which they paired. Lines show the linear regression line of best fit (central line), and 95% confidence limits.

Figure 1: There is a significant positive relationship between male and female body size, this is known as positive size-assortative mating. (Credit: Hatcher, M., Dunn, A., & McCabe, J. 1997. Size and pairing success in *Gammarus diebeni*: can females be too big? Animal Behavior, 54, 1301-1308.)

This experiment set up 4 experimental populations each with 180 female shrimp, but with varying numbers of males to simulate competition for access to mates. In one population the sex ratio was 1:1 with 180 females and 180 males, in the second population the sex ratio was 1:2 with 180 females and 90 males, the third population the sex ratio was 1:3 with 180 females and 60 males, and then the fourth population was the closest to replicating the female biased sex ratios seen in the wild with a sex ratio of 1:5 with 180 females and only 36 males. For each population they measured the body size of each sex and measured female fecundity by the presence of eggs, with the goal of investigating if larger females produce a larger number of eggs. The results (Figure 2) revealed that egg production is dependent on female size, with larger females produces more eggs than smaller females, but that there is a tradeoff for female body size between fecundity and mate availability. The mating options for large females are low, which means that large females are always at a disadvantage because the males can always carry smaller females, but there are often a lot of females within the population which are too large for any male to mate with. It is the intermediate sized females that are the most likely to have the best reproductive success because it is much easier for them to encounter and mate with an appropriately sized male. In conclusion, differential reproductive success in body size and hatching season can reinforce ESD.

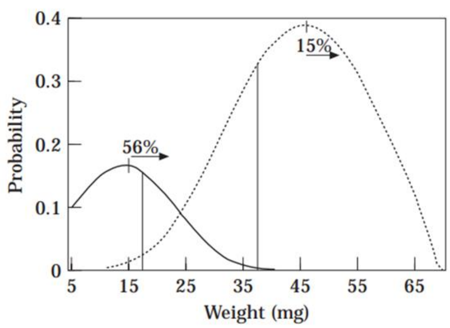

Figure 2. Relationship between pairing success and size. The lines indicate probability of pairing against wet-blotted weight. ——: Pairing probability for females: model equation is: logit $P = -3.01 + 0.20 \times$ (female weight) $- 0.004$ (female weight)^2. · · · ·: Pairing probability for males: general equation combining all sex-ratio treatments is: logit $P = -8.70 + 0.09 \times$ (male weight) $- 0.004 \times$ (male weight)^2. Vertical lines indicate the mean weight of females and males used in the experiment; figures marked at the turning points indicate the percentage of females and males that are described by the downslope in respective pairing curves.

Figure 2: The relationship between pairing success and the size of males and females. Unpaired males weigh less than males that successfully paired, successfully paired females weighed less than unsuccessfully/unpaired females. Maximal probability of pairing based on weight was: 15 mg for females (but 56% of females are larger than this) and 47 mg for males (but only 15% of males are larger than this), demonstrating that increasing size for males has a larger positive effect on reproductive fitness than increasing size of females. (Credit: Hatcher, M., Dunn, A., & McCabe, J. 1997. Size and pairing success in *Gammarus diebeni*: can females be too big? Animal Behavior, 54, 1301-1308.)

When is TSD maintained or removed?

Selection for TSD:

- The life history of species can counter harmful biased sex ratios. Such as in species with overlapping generations and long longevity, because then sex ratio fluctuations between generations may be balanced by multiple generations breeding with each other
- Sufficient genetic variation is necessary to buffer sex ratios against climate change so that a species can persist over the long term and quickly adapt to environmental changes.

Genetic variation for individual reactive norm with regards to: (1) thermal responsiveness, (2) pivotal temperature, (3) thermosensitive period, and (4) temperature-dependent expression of sex determining genes.

- TSD is favored when there is sufficient environmental variation in the temperature during the breeding/incubation season to maintain balanced sex ratios AND there is differential reproductive fitness. Differential reproductive fitness means that incubation temperature affects fitness differentially for males and females, and also that the fitness differential between sexes is significant enough to be maintained.
- Changes in environment can permit the evolution of TSD in otherwise GSD species when there is preexisting thermal sensitivity (such as when expression of the aromatase gene is temperature dependent and/or when the Dmrt1 gene has differential expression during the thermosensitive period) AND the sex chromosomes are not highly differentiated (otherwise the Y and W chromosomes will degenerate over generations and lead to GSD).
- Sex bias can be beneficial in some environments – for example if producing offspring of each sex is not equally costly.
- The environmental cue that is used for ESD is a reliable indicator of environmental conditions and selective forces.
- In species with sexually dimorphic body sizes.
- When reptile nests have thermal heterogeneity, meaning that the position within the mound determines the temperature. In crocodilians eggs that are clustered together will raise metabolic heat production to produce more males and isolated eggs produce more females.
- TSD might be maintained when it confers some sort of advantage to the offspring such as in cases with maternal nest site choice, group selection of sex ratio, and cultural inheritance of home or nest sites.

- TSD can also be maintained in a population if it is a neutral behavior (reproductive fitness is equivalent to GSD), this depends on the overlap of generations, life history strategy, longevity, and phylogenetics.
- TSD can be advantageous due to the effect of temperature on: differential fitness, maternal effects (nest site, differential dispersal), differential mortality/survival, fecundity and sexual size dimorphism, seasonal hatchling time, biased sex ratios for group structure.
- **Facultative Sex** Allocation can favor the evolution of TSD because then the females that produce the rarer sex have the advantage. For example, the Australian snow skink (*Niveoscincus microlepidotus)* and Ocellated skink (*Niveoscincus ocellatus*) are a viviparous skink species with facultative sex allocation, meaning that they produce male-biased litters when males are rare sex, and produce female-biased litters when females are limited. The Ocellated skink has genetic sex determination with an XX/XY system, but in the lowland population sex is determined from the interaction of temperature and genotype. Females in lowland populations of the Ocellated Skink are able to control the sex ratio of their offspring by maintaining a specific internal body temperature through controlling the amount of time they spend basking. When they limit basking, they are able to cool their internal body temperature and produce mostly males, and by engaging in unrestricted basking they can warm their body temperature and produce mostly females. This is an evolutionarily stable strategy because overall a population will produce offspring at a 1:1 sex ratio in the wild. Sex determination in upland Ocellated skinks is strictly GSD because there is not enough environmental variation during the reproductive period to produce a balanced sex ratio of offspring.

Selection against TSD:

- Biased sex ratios can lead to extinction of one sex and are not a stable strategy. At the 1:1 ratio the reproductive success for males and females is equal and an evolutionarily stable strategy. Female-biased sex ratios are not stable since this would result in males have higher reproductive success than females, which means that the genes that select for male offspring would spread through the population because they are advantageous, in that, the parent individual that produces the most males would have greater reproductive success.

Male-biased sex ratio not stable for the same reasons, because then selection would act on the parents to produce the female offspring because they are the rarer sex. The rarer sex always has the advantage in terms of differential reproductive fitness; thus selection would always act on parents to choose the rarer sex.

- Parental investment for one sex is significantly higher than for the other, such that the cost of producing male and female offspring is unequal, so if females are capable of maternal choice in nesting site, then both sexes may not be produced.
- Low genetic variation
- The life history strategy of the species can result in harmful biased sex ratios in cases of (1) continuous year-round breeding/growth seasons, (2) short or limited breeding/growth windows that do not have enough variation in environmental condition for sex ratios to be balanced or for one sex to have a differential advantage, and in (3) short-lived or non-overlapping generations because sex ratio fluctuations by climatic variation can lead to extinction for short lived species with non-overlapping generations.
- Climate change (increased or decreased thermal variability) can skew sex ratios
- Sex chromosomes are highly differentiated and heteromorphic. (YY and WW genotypes are lethal)
- The environmental cue that is tracked is not a reliable indicator of the selective forces which are acting on the offspring, or any other cases of poor phenotype-environmental matching.
- In cases of antagonistic pleiotropy where selection for one sex leads to negative selection for other sex.
- Sex phenotypic plasticity is not adaptive when = (1) magnitude of fitness differential is low and (2) the frequency at which eggs are laid in different environmental conditions with different selective factors is low.
- Extreme thermal variability resulting in skewed sex bias or not enough thermal variability

Questions for Discussion

Q1: How could the life history and physiology of a species select for/against ESD? Aspects to consider:

- Seasonal versus year-round breeding seasons
- Length of the growing season
- Differential advantage to females versus males having the larger body size
- Multi-clutching opposed to one breeding attempt per year (more variation to act on offspring)
- Egg laying vs live birth (difference in variation in thermal environment)
- Lifespan (short versus long lived species)
- Generational interaction (non-interacting or interacting)
- Dispersal patterns
- Mating system (Monogamous versus Polygynous versus Polyandrous) and the operational sex ratio.

Q2: What cues would selection favor/oppose as a reliable predictor of environmental conditions? Aspects to consider:

- Cues are reliable factors that change gradually and predictably over time. The reactive norm should evolve in a way that tracks changes in the environment.
- Switching phenotypes in response to cue/environmental threshold point is favored when phenotypic plasticity in the sex ratio response leads to higher fitness (which results in directional selection).
- Stressor signals (for poor habitat)
- Seasonal changes in (1) temperature, (2) photoperiod (daylight), (3) precipitation.

Q3: If we think about ESD as a form of phenotypic plasticity for sex determination then some environments would be more beneficial by providing a greater differential fitness advantage for a particular sex. What environments would be more and less likely to favor ESD? Aspects to consider:

- Environments that favor ESD: (1) plasticity is selected for in a variable environment because the variation of phenotypic plasticity allows offspring to be more successful in uncertain environments especially if the offspring vary in their level of fitness, (2) high dispersal rates,

(3) temporal variation in environment, (4) predictable variation in the fluctuating environment, (5) when phenotypic plasticity has a low cost and high benefits, and (6) adaptive to novel environments.

- Environments that do not favor ESD: (1) climate pattern creates a sex bias, (2) ESD cannot track environmental changes and shift the population reactive norm fast enough, (3) changes in the environment shift conditions beyond the limits of the viable incubation range or beyond a point where both sexes would be maintained, (4) environmental conditions are always the same and reoccurring incubation temperature will lead to favoring of the rare sex, (5) climate change results in inability to produce either males or females, and (6) low temporal variation such that incubation temperatures are stable throughout the year and widen the individual reactive norm to the point of leading to female heterogeneity.

Q4: How would maternal choice in nest site affect the stability of ESD/TSD? Aspects to consider:

- Biased sex ratio
- Possibly selection for matching sex to environment (favor rare sex hypothesis)
- Hormone levels matching the sex of egg. Ex: In the Painted Turtle the estrogen levels in the eggs increase over the breeding season, resulting the phenotypic reactive norm shifting due to varying egg hormonal concentrations across clutches.