

Population Consequences of Environmental Sex Reversal

SAMUEL COTTON*†‡ AND CLAUD WEDEKIND*

*Department of Ecology and Evolution, University of Lausanne, Biopôle, CH-1015 Lausanne, Switzerland

†Research Department of Genetics, Evolution & Environment, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, United Kingdom, email s.cotton@ucl.ac.uk

Abstract: *When sex determination in a species is predominantly genetic but environmentally reversible, exposure to (anthropogenic) changes in the environment can lead to shifts in a population's sex ratio. Such scenarios may be common in many fishes and amphibians, yet their ramifications remain largely unexplored. We used a simple model to study the (short-term) population consequences of environmental sex reversal (ESR). We examined the effects on sex ratios, sex chromosome frequencies, and population growth and persistence after exposure to environmental forces with feminizing or masculinizing tendencies. When environmental feminization was strong, X chromosomes were driven to extinction. Analogously, extinction of normally male-linked genetic factors (e.g., Y chromosomes) was caused by continuous environmental masculinization. Although moderate feminization was beneficial for population growth in the absence of large viability effects, our results suggest that the consequences of ESR are generally negative in terms of population size and the persistence of sex chromosomes. Extreme sex ratios resulting from high rates of ESR also reduced effective population sizes considerably. This may limit any evolutionary response to the deleterious effects of ESR. Our findings suggest that ESR changes population growth and sex ratios in some counter-intuitive ways and can change the predominant factor in sex determination from genetic to fully environmental, often within only a few tens of generations. Populations that lose genetic sex determination may quickly go extinct if the environmental forces that cause sex reversal cease.*

Keywords: environmental sex reversal, extinction, population growth, sex determination

Consecuencias Poblacionales de la Reversión de Sexo Ambiental Cotton & Wedekind

Resumen: *Cuando la determinación del sexo en una especie es predominantemente genética pero ambientalmente reversible, la exposición a cambios (antropogénicos) en el ambiente puede llevar a cambios en la proporción de sexos de una población. Tales escenarios pueden ser comunes en muchos peces y anfibios, pero sus ramificaciones están muy poco exploradas. Utilizamos un modelo simple para estudiar las consecuencias poblacionales (corto plazo) de la reversión de sexo ambientalmente inducida (RSA). Examinamos los efectos sobre la proporción de sexos, frecuencias de cromosomas sexuales y el crecimiento y persistencia de la población después de la exposición a fuerzas ambientales con tendencias feminizantes o masculinizantes. Cuando la feminización ambiental era fuerte, los cromosomas X eran llevados a la extinción. Análogamente, la extinción de factores normalmente ligados a machos (e.g., cromosomas Y) fue provocada por la masculinización ambiental continua. Aunque la feminización moderada fue benéfica para el crecimiento poblacional en la ausencia de efectos notables sobre la viabilidad, nuestros resultados sugieren que las consecuencias de RSA son generalmente negativas en términos del tamaño poblacional y la persistencia de cromosomas sexuales. Las proporciones de sexo extremas resultantes de las altas tasas de RSA también redujeron considerablemente el tamaño poblacional efectivo. Esto puede limitar cualquier respuesta evolutiva a los efectos deletéreos de RSA. Nuestros resultados sugieren que la RSA cambia el crecimiento poblacional y la proporción de sexos de manera contra intuitiva y que puede cambiar el factor predominante en la determinación del sexo*

‡Address for correspondence: University College London
Paper submitted January 29, 2008; revised manuscript accepted May 20, 2008.

de genético a completamente ambiental, a menudo en sólo unas decenas de generaciones. Las poblaciones que pierden la determinación genética del sexo pueden extinguirse rápidamente si cesan las fuerzas ambientales que provocan la reversión de sexo.

Palabras Clave: Crecimiento poblacional, determinación de sexo, extinción, reversión de sexo ambiental

Introduction

Sex determination in fishes and amphibians is often genetic, with many species relying on the segregation of heteromorphic sex chromosomes for assignment of gender (Wallace et al. 1999; Devlin & Nagahama 2002). Nonetheless, the phenotypic sex attained can often be modified by environmental influences after fertilization. For example, temperatures above or below normal during larval development can create biased sex ratios in many species (Wallace et al. 1999; Devlin & Nagahama 2002). Sex hormones are used widely to manipulate gender in the aquaculture and fisheries industries; estrogens can be used to change genetic XY males to XY females, whereas androgens can modify XX females to XX males (Pandian & Sheela 1995; Beardmore et al. 2001; Piferrer 2001). Although most investigation of phenotypic sex reversal has been conducted under the controlled conditions of the laboratory or hatchery, there is increasing recognition that environmental factors may induce sex reversal in natural populations. The consequences of such environmental sex reversal (ESR) have received little attention.

Sex hormones, hormone mimics, and endocrine-disrupting chemicals released into natural watercourses are also associated with biased sex ratios and sex-reversed individuals. Many effluents from domestic and industrial sources have endocrine-mimicking or disrupting properties (Jobling & Tyler 2003). Exposure of male fish to such effluent induces synthesis of vitellogenin, an egg-yolk protein usually expressed only in females (Sumpter & Jobling 1995), and populations inhabiting polluted rivers have a higher incidence of intersex and potential sex reversal (e.g., Penáz et al. 2005). Environmental feminization has been reported in Chinook salmon (*Oncorhynchus tshawytscha*; Nagler et al. 2001; Williamson & May 2002; Chowen & Nagler 2004); 84% of phenotypic females sampled from a wild population tested positive for a Y chromosome marker, whereas there was a complete absence of such markers in hatchery-reared fish from the same population. Masculinization is suspected in eelpout (*Zoarces viviparus*) located near a Swedish pulp mill. Male-biased sex ratios were reported in broods conceived downstream from the mill, suggesting that effluent is androgenic (Larsson et al. 2000). Sex ratios in upstream populations and those downstream of the mill during periods of shutdown did not differ from unity (Larsson & Förlin 2002; Förlin et al. 2004). Female mosquitofish (*Gambusia affinis holbrooki*) can also develop a male-like anal fin (which serves as a gonopodium, an intromittant or-

gan for internal fertilization) after exposure to pulp-mill effluent (Parks et al. 2001).

Fish exposed to hormones often display reduced reproductive performance (Vos et al. 2000; Jobling & Tyler 2003). Sex hormones, or sex hormone mimics, are believed to be responsible for gonadal deformities seen in both sexes of many species. For example, sex reversal may often be incomplete with individuals displaying gonadal characteristics of both sexes. Such intersex individuals are presumed to have reduced reproductive output relative to wild-type individuals (Jobling & Tyler 2003). Other symptoms of exposure to environmental hormones include delayed onset of sexual maturity, lower larval viability, reductions in gonadal growth, inhibition of spermiogenesis, and lowered egg production (Sumpter & Jobling 1995; Vos et al. 2000).

Two previous theoretical treatments have evaluated the consequences of ESR on sex-chromosome frequencies (Kanaiwa & Harada 2002; Hurley et al. 2004). They showed that environmental pressure to feminize or masculinize affected the phenotypic sex ratio (increasingly female or male biased, respectively) and the frequency of the Y chromosome in the population. Both analyses found that the Y chromosome goes extinct as the probability of masculinization of XX females approaches 0.5. Nevertheless, there is a pressing need to model the dynamics of how ESR, and associated fitness consequences, influences patterns of population growth, persistence, and effective population size (Jobling & Tyler 2003). For example, biased sex ratios have profound effects on population growth and genetically effective population size if females are the reproductively limiting sex (Wedekind 2002). Moreover, when individuals become phenotypically sex reversed from their genotype, populations have the potential to decline in size to extinction or to increase rapidly (Gutierrez & Teem 2006; Cotton & Wedekind 2007a, 2007b).

We extended a previous theoretical treatment (Hurley et al. 2004) and examined the effects on population composition, growth, and persistence after exposure to environmental forces with feminizing or masculinizing tendencies. We evaluated the effects of reduced fitness associated with such episodes of exposure.

Methods

We simulated the consequences on population growth of exogenous sex hormones, but analogous conclusions

can be drawn when sex reversal is induced by temperature, change in pH, or other environmental factors (Korpelainen 1990). We considered only short-term (≤ 100 generations) consequences because these are likely to be most relevant to conservation and management practices. We simulated 2 exposure scenarios: (1) environmental feminization after exposure to exogenous estrogens, in which a proportion p of genetic males (XY) turn into phenotypic females and (2) environmental masculinization after exposure to exogenous androgens, in which a proportion q of genetic females (XX) turn into phenotypic males.

We made a number of assumptions about the population. We assumed no genetic variability in how individuals respond to exposure to hormones and, ergo, that responses to hormones cannot evolve over this time period. (This condition seems to be fulfilled in some populations—C.W. et al., unpublished data; see also Discussion.) For simplicity, we also assumed the population is characterized by discrete generations. We further assumed an initial, natural sex ratio of 1:1, with sex being determined by male heterogamety (i.e., XY = male, XX = female). Females mate only once, and each contributes r offspring to the subsequent generation. Males mate with more than one female if the sex ratio becomes female biased (but see below for limitations of male mating ability), and mating among males and females is random with respect to their sex-reversal status. The population initially has N_{MXYt} XY males (M) and N_{FXXt} XX females (F) in

generation $t = 0$ (census population size, $N_C = N_M + N_F$).

With environmental feminization ($p > 0$), the recurrence equations for the frequencies of N_{MXY} , N_{FXX} , and N_{FXY} genotypes produced in generation $t + 1$ are

$$N_{MXY_{t+1}} = (0.5(1-p)a_t) + (0.5(1-p)b_t) + ((1-p)c_t) + (0.5(1-p)d_t) r_t, \quad (1)$$

$$N_{FXX_{t+1}} = [(0.5a_t) + (0.25b_t)] r_t, \quad \text{and} \quad (2)$$

$$N_{FXY_{t+1}} = [(0.5pa_t) + (0.5pb_t) + (pc_t) + (0.5pd_t)] r_t, \quad (3)$$

where $a-d$ are the expected mating frequencies between each genotype in generation t (Table 1). Matings between XY males and XY females result in one-quarter of progeny being of YY genotype, $1-p$ being males, and p being females (Fig. 1). Following Eqs. 1-3, the recurrence equations for the frequencies of N_{MYY} and N_{FYY} genotypes are

$$N_{MYY_{t+1}} = (0.25(1-p)b_t) + (0.5(1-p)d_t) + ((1-p)e_t) r_t \quad \text{and} \quad (4)$$

$$N_{FYY_{t+1}} = [(0.25pb_t) + (0.5pd_t) + (pe_t)] r_t, \quad (5)$$

where e is the expected mating frequency between YY individuals (Table 1).

Table 1. Mating frequencies of the different genotypes if the population experiences environmental sex reversal.

Sex reversal	Male genotype	Female genotype	Mating frequency*
Feminization ($p > 0$)			
	XY	XX	$a = \left(\frac{N_{MXY}}{N_{MXY} + N_{MYY}} \right) N_{FXX}$
	XY	XY	$b = \left(\frac{N_{MXY}}{N_{MXY} + N_{MYY}} \right) N_{FXY}$
	YY	XX	$c = \left(\frac{N_{MYY}}{N_{MXY} + N_{MYY}} \right) N_{FXX}$
	YY	XY	$d = \left(\frac{N_{MYY}}{N_{MXY} + N_{MYY}} \right) N_{FXY}$
	YY	YY	$e = \left(\frac{N_{MYY}}{N_{MXY} + N_{MYY}} \right) N_{FYY}$
Masculinization ($q > 0$)			
	XY	XX	$f = \left(\frac{N_{MXY}}{N_{MXY} + N_{MXX}} \right) N_{FXX}$
	XX	XX	$g = \left(\frac{N_{MXX}}{N_{MXY} + N_{MXX}} \right) N_{FXX}$

*Mating frequencies depict those as in Fig. 1. See text for definitions of variables.

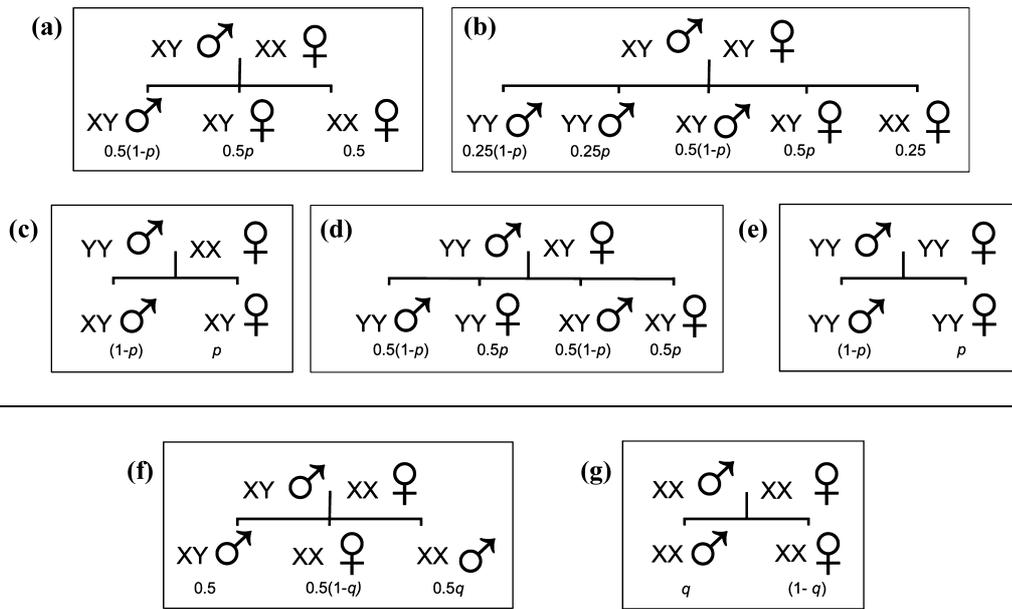


Figure 1. Progenies resulting from the mating of different genotype combinations following environmental sex reversal: (a) wild-type XY males and XX females, (b) wild-type XY males and sex-reversed XY females, (c) YY males and wild-type XX females, (d) YY males and sex-reversed XY females, (e) YY males and sex-reversed YY females, (f) wild-type XY males and wild-type XX females, and (g) sex-reversed XX males and XX females. The numbers associated with each mating type depict the proportion of each clutch of each sex or genotype contribution. p is the probability of sex reversal of genetic males into phenotypic females, and q is the probability of sex reversal of genetic females into phenotypic males.

A similar model was produced for circumstances in which individuals are exposed to androgens. With environmental masculinization ($q > 0$), the recurrence equations for the frequencies of N_{MXY} , N_{FXX} , and N_{MXX} genotypes in generation $t + 1$ are

$$N_{MXY_{t+1}} = [0.5f_i] r_t, \quad (6)$$

$$N_{FXX_{t+1}} = [(0.5(1 - q)f_i) + ((1 - q)g_i)] r_t, \quad \text{and } (7)$$

$$N_{MXX_{t+1}} = [(0.5qf_i) + (qg_i)] r_t, \quad (8)$$

where f and g are the expected mating frequencies between each genotype in generation t (Fig. 1; Table 1).

Population growth in one generation (ΔN) is density-dependent and contingent on deviation of the population from the optimal population size (N_{opt}), which is assumed to remain constant over time ($N_{\text{opt}} = 1000$ in our simulations):

$$\Delta N = \frac{N_{t+1} - N_t}{N_t} = k(N_{\text{opt}} - N_t), \quad (9)$$

k is the constant of proportionality that scales the population growth in one generation with the magnitude of the deviation of N_t from N_{opt} (Maynard Smith 1968). The number of parents in generation t therefore determines the number of offspring produced in generation $t + 1$.

This density dependence may be caused by reduced fecundity, fertility, or parental care in periods of high population density or by a reduction of resources available to the offspring generation as a result of overuse or exploitation by the parental generation. When N_t is small, the maximum reproductive rate of the population (R_{max}) is

$$R_{\text{max}} = kN_{\text{opt}} + 1, \quad (10)$$

and the rate of reproduction in generation t (R_t) is

$$R_t = \frac{N_{t+1}}{N_t} = kN_{\text{opt}} + 1 - kN_t. \quad (11)$$

Density dependence at the level of the individual is affected by scaling r_t , the mean per female output in generation t , by R_t . In our simulations, r_t follows the function $r_t = 2R_t i$, where i describes the effect of biased sex ratios on female reproductive output (see later). Because $R_t \leq R_{\text{max}}$, r_t is constrained within the limits of $0 \leq r_t \leq (2R_{\text{max}})i$. Under natural conditions with equal sex ratio expectations, this allows a maximal increase for each sex of R_{max} each generation.

If sex ratios become sufficiently female biased, males may be unable to mate (successfully) with every female in the population, either because they have insufficient sperm or because of spatial or temporal constraints on mating (e.g., Dewsbury 1982; Wedell et al. 2002). So a proportion of females will remain unmated or

unfertilized. For simplicity, we assumed that the probability of a male giving a successful mating (B) is a step function of the form

$$B = \begin{cases} 1, & M \leq M^* \\ 0, & M > M^* \end{cases}, \quad (12)$$

where $M = N_F/N_M$ and M^* is the maximum number of successful matings a male can perform. The probability of a female (\equiv proportion of females) receiving a successful mating (i) is thus 1, when $M^* \geq M$, and M^*/M , when $M^* < M$. If the population is male biased, we assumed no direct effect on female fitness of excess males.

We described the sex ratio with 2 measures, the proportion of phenotypic males in the population,

$$S_P = \frac{(N_{MXY} + N_{MYY} + N_{MXX})}{(N_{MXY} + N_{FXX} + N_{FXY} + N_{MYY} + N_{FYY})}, \quad (13)$$

and the genotypic sex ratio, S_G , defined as the proportion of Y chromosome carriers in the population:

$$S_G = \frac{(N_{MXY} + N_{FXY} + N_{MYY} + N_{FYY})}{(N_{MXY} + N_{FXX} + N_{FXY} + N_{MYY} + N_{FYY} + N_{MXX})}. \quad (14)$$

Effective population size

Inequality of male (N_M) and female (N_F) numbers reduces the genetically effective population size, N_e , by a factor $N_e = 4N_MN_F/(N_M + N_F)$ (Falconer & Mackay 1996). We examined some of the consequences for N_e of ESR and biased sex ratios with 2 estimates of N_e . We used the harmonic mean of N_e (\bar{N}_e) across generations $t = 1-100$ to account for variation in population census size (N_c) and provide an estimate of the average long-term effective population size:

$$\bar{N}_e = \frac{j}{\sum_{t=1}^j (N_e)^{-1}}, \quad (15)$$

where j is the total number of generations (Falconer & Mackay 1996; Vucetich et al. 1997). Following Vucetich et al. (1997), we also computed the long-term N_e expressed as a proportion of the long-term average census size through the ratio \bar{N}_e/\bar{N}_c , where \bar{N}_c is the arithmetic population size over generations $t = 1-100$.

Reduced Reproductive Potential Linked to ESR

We explored the consequences of sex-reversed individuals or those with an unnatural complement of sex chromosomes having lower reproductive success relative to wild-type individuals. We assigned independent fitness coefficients to chromosomally aberrant females (v) and males (w), scaling the number of progeny derived from a mating. For example, a mating between XY (or YY) females and XY males would produce vr_t offspring, between XX females and YY (or XX) males wr_t offspring,

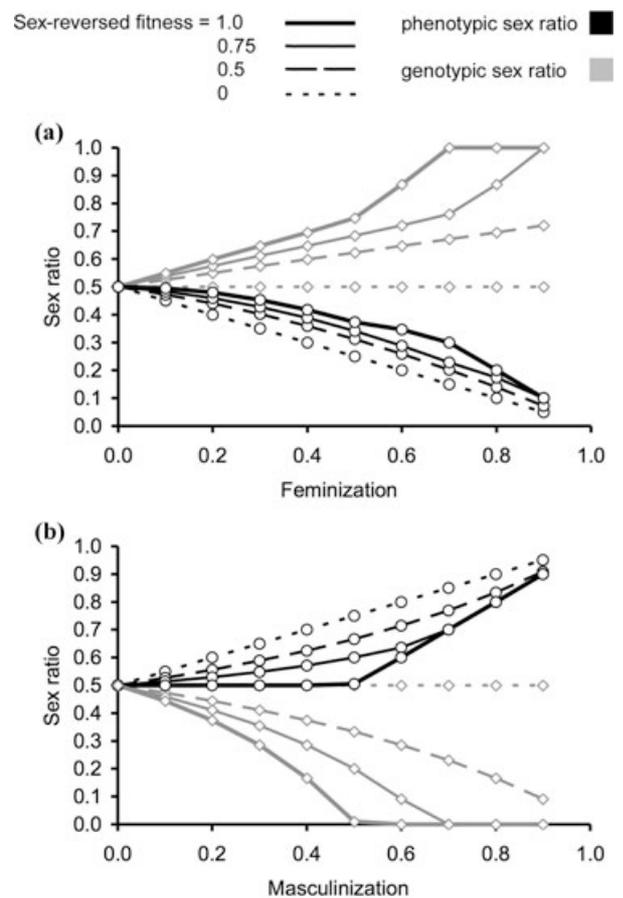


Figure 2. The effect of environmental sex reversal on the sex ratios of populations after $t = 100$ when a sex-reversing force is applied from generation $t = 1$ onwards: (a) environmental feminization ($p > 0$) under varying values of v , the sex-reversed female fitness coefficient and (b) environmental masculinization ($q > 0$) under varying values of w , the sex-reversed male fitness coefficient. Phenotypic sex ratios are the proportion of phenotypic males in the population, whereas genotypic sex ratios are the proportion of individuals carrying a Y chromosome.

and between XY females and YY males wvr_t offspring. Simulations were rerun over varying values of v and w to assess associated changes in population size and composition. A YY individual is often viable, but there are many instances when YY individuals have (near) zero fitness (Devlin & Nagahama 2002). The effect of YY inviability was simulated by setting w to zero in simulations of environmental feminization.

Results

Sex Ratios under Full Viability

We found marked consequences of the effects of exogenous hormones on sex ratios (Fig. 2). Environmental

feminization resulted in female-biased phenotypic sex ratios and an increase in the frequency of the Y chromosome (Fig. 2a). With environmental masculinization, the phenotypic sex ratio remained constant at 0.5 until $q = 0.5$, beyond which it became increasingly male biased (Fig. 2b).

As the pressure to feminize became large, genotypic sex ratios reached 1.0 (Fig. 2a) and all individuals exhibited YY genotypes (i.e., the X chromosome became extinct [data not shown]). Females in the population were maintained solely by environmental feminization of YY males. The frequency of the Y chromosome decreased rapidly with increasing environmental masculinization, and it was eliminated from the population once $q >$ approximately 0.5 (Fig. 2b).

Sex Ratios with Reduced Reproductive Potential

When sex-reversed individuals (or those with an unnatural complement of sex chromosomes, such as YY males) suffer reduced fitness, phenotypic sex ratios became more female biased with environmental feminization relative to those with full viability (Fig. 2a for v data; qualitatively similar patterns were observed for variation in w [results not shown]). Under environmental masculinization, phenotypic sex ratios became more male biased under moderate values of q , increasingly so as w declined (Fig. 2b).

When v or w decreased, the deviation of the genotypic sex ratio from equality declined and the probability of the Y chromosome becoming fixed or lost occurred at increasingly higher levels of environmental estrogens or androgens, respectively (Fig. 2). So extinction of X or Y chromosomes became less likely if ESR had deleterious effects on fitness. When YY males were inviable (i.e., $w = 0$), phenotypic sex ratios became increasingly female biased, but genotypic sex ratios were relatively insensitive to the effects of ESR (results not shown).

To understand these results, consider the feminizing case when the viability of sex-reversed (XY) females was zero (e.g., Fig. 2a). There was a linear relationship between the pressure to feminize (p) and the sex ratio (dotted black line in Fig. 2a) because the proportion of females in the population was 0.5 (those that are already females) $+0.5p$ (the proportion of females created via ESR of genetic males). Sex-reversed individuals did not contribute offspring to the next generation when their viability was zero, so the genotypic sex ratio (the proportion of individuals carrying a Y chromosome) did not change over time (dotted gray line in Fig. 2a). When the viability of sex-reversed individuals increased above zero, however, XY females contributed viable offspring to the next generation, and these additional female-origin Y chromosomes resulted in proportionately more males (both XY and YY) produced in future generations. So we saw less female-biased phenotypic sex ratios, but

increased genotypic sex ratios (>0.5). Similar, but less marked, dynamics were observed when the viability parameter affected YY males only. The reverse logic explained the sex-ratio patterns seen with masculinization (Fig. 2b).

Changes in Population Size with Full Viability

Environmental estrogens caused an increase in the proportion of females in the population, which elevated growth potential and effected an increase in population size when males were able to mate successfully with many females (Fig. 3a). In our simulations we used $M^* = 4$ to allow multiple mating, but qualitatively similar results were obtained with higher values of M^* (results not shown). With high pressure to feminize, X chromosomes became extinct after a few tens of generations, leading to a population of only YY individuals. Subsequently, the number of females in the population was determined solely by the conversion of YY males to YY females, and because $p > 0.5$, the population was female biased and hence grew more rapidly. When male mating rates were constrained (e.g., $M^* = 1$), female reproductive success was limited by male availability, so the number of mated females, and hence population size, declined as p increased (Fig. 3b). If $p >$ approximately 0.5, populations became extinct within a few tens of generations.

Exposure to exogenous androgens with low to moderate masculinizing capacity had little effect on equilibrium population size (Fig. 3c). Nevertheless, at high pressure to masculinize, insufficient numbers of females are produced to maintain the population, so the population declined, potentially to extinction (Fig. 3c).

To examine the population consequences of a cessation of ESR, we reset p or q to zero once populations had reached equilibrium (after 60 generations). Populations subjected to environmental feminization all crashed in size following an end to ESR and then recovered to initial (i.e., $t = 0$) composition and size if p was low to moderate, but became extinct if p was high and the X chromosome was extinct (Figs. 3a & 3b). Under environmental masculinization, populations suffering weak ESR briefly grew in size as a result of the temporarily increased number of XX females (Fig. 3c). Nevertheless, populations with moderate to high q crashed to extinction as the X chromosome became fixed and genetic variance for sex was lost (Fig. 3c).

Changes in Population Size with Reduced Reproductive Potential

If the female fitness coefficient v was <1.0 , populations showed fewer and less extreme changes in response to environmental feminization if males could mate multiply (e.g., $M^* = 4$). At low to moderate values of p , there were relatively few differences in population growth profiles

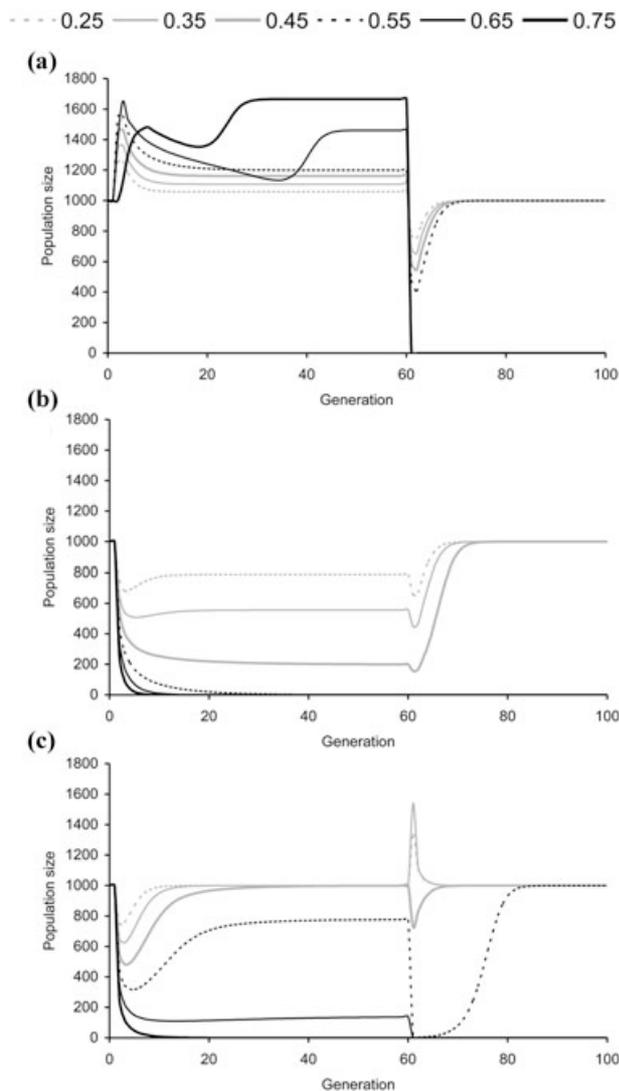


Figure 3. The effect of the strength of environmental feminization [(a) and (b), $p > 0$] and masculinization [(c) $q > 0$] on size and growth of populations with full viability when a sex-reversing force is applied from generation $t = 1$ until $t = 60$, when it ceased (i.e., p or $q = 0$): (a) males allowed to mate successfully with up to 4 females ($M^* = 4$) and (b) males constrained in their mating rate ($M^* = 1$). Initial model parameters: number of males ($N_{\text{MXY}} = 500$), number of females ($N_{\text{FXX}} = 500$), maximum reproductive rate ($R_{\text{max}} = 1.5$), density-dependence constant, $k = 0.0005$, sex-reversed female fitness coefficient ($v = 1.0$), sex reversed male fitness coefficient ($w = 1.0$).

at moderate values of v (Fig. 4a). If p was high, population size declined when v fell below 1 (Fig. 4a). When $v = 0$, sex-reversed individuals were produced but contributed no offspring to future generations, so population size did not change, except when p was large, and population sizes crashed owing to a paucity of reproducing

females (Fig. 4a). Similar, but less marked, dynamics occurred with variation in the male fitness coefficient, w (results not shown). Inviability of YY individuals per se had little specific effect on population size, just a marginal reduction compared with the case when w was small, except when p was very high (results not shown). When male mating was constrained ($M^* = 1$), reductions in sex-reversed viability caused populations to decline in size, and if $v = 0$, populations declined to extinction (Fig. 4b). Similar, but less extreme, patterns were observed with variation in w (results not shown).

Populations under environmental masculinization tended not to recover after the initial decline when $w < 1$, and XX males suffer reduced viability. If w was moderate, population sizes fell when q was low to intermediate, whereas at higher values of q the population rapidly became extinct (Fig. 4c). These patterns became stronger as w declined further (Fig. 4c).

When ESR ceased populations with $v < 1$ tended to revert to pre-ESR composition and size (Fig. 4). Nevertheless, when environmental feminization ($M^* = 4$) was strong, moderately reduced v (e.g., $v = 0.5$) resulted in some populations persisting when they would otherwise have gone extinct (Fig. 4a).

Effective Population Sizes

For simplicity, we only considered changes in N_e in populations whose members displayed full viability (i.e., v and $w = 1$) and ESR occurred during generations $t = 1$ –100. When males could mate with many females ($M^* = 4$), population growth associated with environmental feminization caused small increases in N_e (Fig. 5a). Nevertheless, the disparity between N_c and N_e increased with p , meaning that N_c reflected N_e less reliably when environmental feminization became stronger (Fig. 5b). When male mating rates were constrained ($M^* = 1$), we observed a rapid decline in both \tilde{N}_e and \tilde{N}_e/\tilde{N}_c as p increased (Figs. 5a & 5b).

Environmental masculinization had relatively little effect on estimates of N_e when $q < 0.5$ (Fig. 5a). Nevertheless, when $q > 0.5$ and phenotypic sex ratios shifted from equality, N_e fell rapidly, as did the ratio \tilde{N}_e/\tilde{N}_c (Figs. 5a & 5b).

Variation in R_{max}

Throughout our simulations $R_{\text{max}} = 1.5$, which allowed populations to grow by a maximum of 50% per generation. When $R_{\text{max}} < 1.5$, population growth was reduced, but not disproportionately affected by environmental feminization when males could mate multiply (results not shown). In contrast, reduced R_{max} had marked consequences on population growth with limited male mating following environmental feminization and environmental masculinization. Any reduction in reproductive output exacerbated the observed (Figs. 3b & 3c)

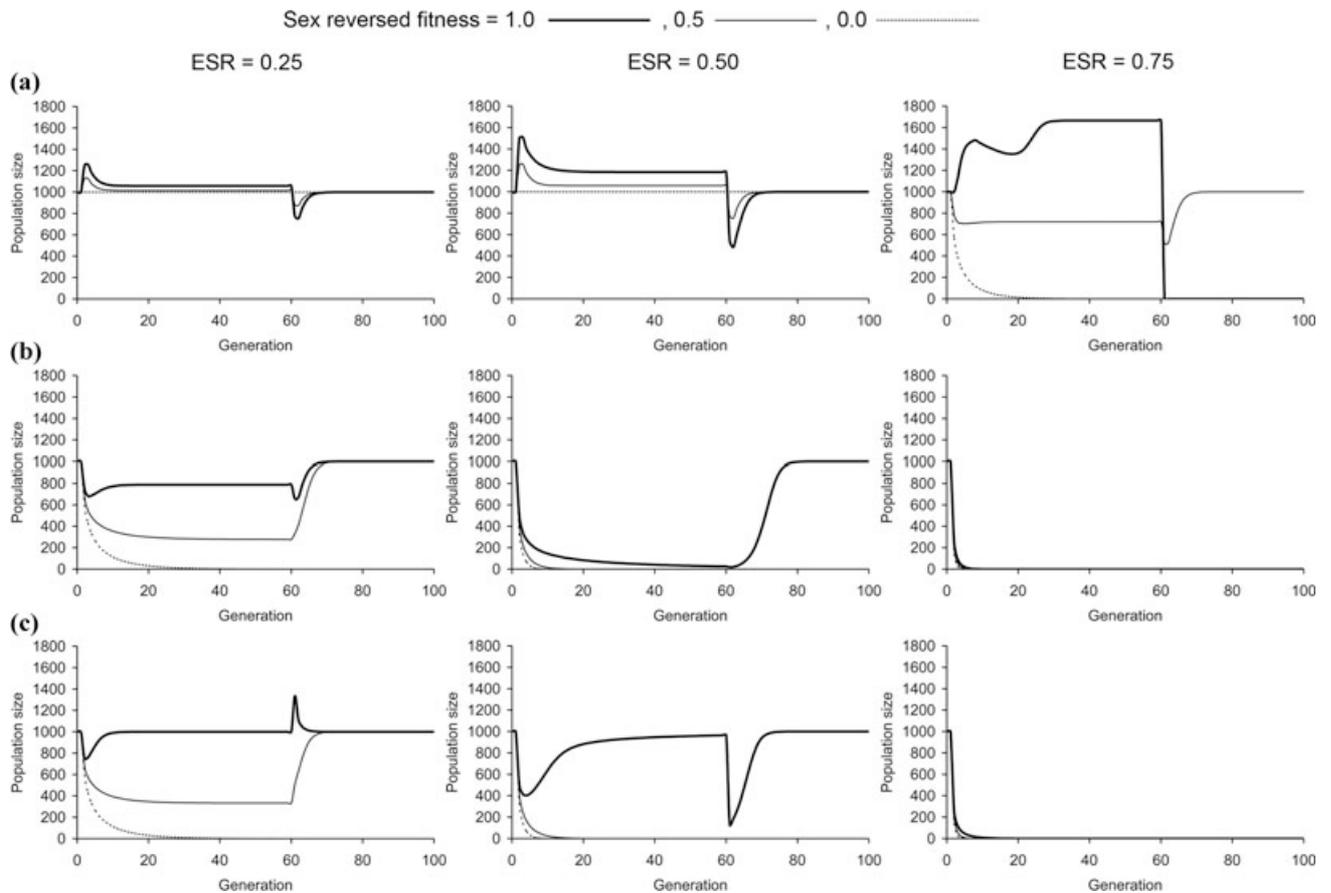


Figure 4. The effect of the strength of environmental sex reversal (ESR) (applied from generation $t = 1$ until $t = 60$ when it ceased [i.e., p or $q = 0$]) on size and growth of populations with reduced viability of sex-reversed or chromosomally aberrant individuals: (a) effect of v , the fitness coefficient of sex-reversed (XY or YY) females, on population growth profiles for environmental feminization when males can mate with many females ($M^* = 4$), (b) effect of v on population growth profiles under environmental feminization when male mating rates are constrained ($M^* = 1$), (c) effect of w , the fitness coefficient of XX males, on the size and growth of populations under environmental masculinization. Initial model parameters: $N_{MXY} = 500$, $N_{FXX} = 500$, $R_{max} = 1.5$, $k = 0.0005$ (other variables defined in legend of Fig. 3).

loss of population growth potential (results not shown). When $R_{max} > 1.5$, populations were more productive and therefore less susceptible to numerical extinction (results not shown). High values of R_{max} sometimes lead to cycling and extinction (results not shown), which suggests that our model may be unstable in populations with prolific growth rates. Nonetheless, phenotypic or genotypic sex ratios remained unaffected by R_{max} , so strong ESR could still cause a single sex chromosome to become fixed, leading to a loss of genetic variation for sex and effective extinction.

Discussion

There is increasing evidence that environmental changes (e.g., temperature, pH) or the release of hormones, hormone mimics, and endocrine-disrupting chemicals into

streams and lakes has marked consequences on sex determination and fitness in many species (Sumpter & Jobling 1995; Vos et al. 2000; Devlin & Nagahama 2002; Jobling & Tyler 2003). Although information on the physiological and reproductive consequences of such environmental changes is accumulating, there is a pressing need to examine the effect on population growth (both positive and negative) of ESR and to investigate the effect on population-growth processes of diminished reproductive potential in sex-reversed individuals. Here, we used a simple population-growth model to explore such cases when environmental changes cause feminization and masculinization. We expect analogous dynamics in species with female heterogamety, such as ZZ/ZW systems. We also believe that qualitatively similar outcomes will be likely when sex is determined by one or a few major genes, rather than by specific sex chromosomes.

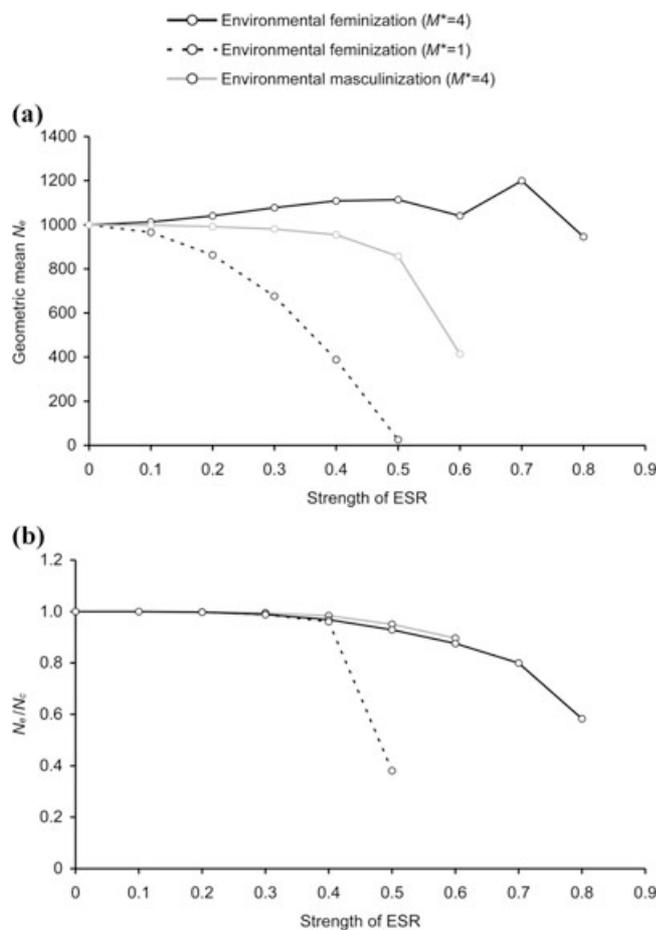


Figure 5. Effective population sizes that result from varying strengths of environmental sex reversal (ESR): (a) changes in mean effective population size (\bar{N}_e), the harmonic mean of N_e over $t = 1-100$, and (b) changes with ESR of \bar{N}_e/\bar{N}_c (N_c , census population size), the ratio of \bar{N}_e to the arithmetic mean population size over $t = 1-100$. Absence of a datum point indicates population is extinct. Initial model parameters: $N_{MXY} = 500$, $N_{FXX} = 500$, $R_{max} = 1.5$, $k = 0.0005$, $v = 1.0$, $w = 1.0$ (other variables defined in legend of Fig. 3).

As with a previous treatment in which a similar model was used (Hurley et al. 2004), our results showed that ESR had profound effects on the phenotypic sex ratio of a population and frequency of the sex chromosomes. As expected, environmental feminization caused a greater influx of female phenotypes than that expected by Mendelian inheritance of sex chromosomes, whereas masculinizing factors produced male-biased phenotypic sex ratios. Nevertheless, permanent population consequences often resulted from correlated shifts in genotypic frequencies. The frequency of genotypic (XX) females declined continually to extinction as the pressure to feminize increased, and, similarly, under even modest pressure to masculinize, the frequency of the Y chromo-

some declined rapidly to zero. Once populations became fixed for a single sex chromosome, no genetic variation for sex existed, and genders were maintained solely by ESR of a proportion (p or q) of the population. So populations that experienced high rates of sex reversal were severely threatened, and any apparent sign of health (i.e., high census size) was misleading. Should ESR cease, for example, through a clean-up of effluent, then populations would quickly go extinct. Even if ESR is only moderate, populations may still suffer large crashes in census size following cessation of ESR, which will create bottlenecks. If an environmental cleanup is to be implemented, care must be taken to counteract the paucity of one or other of the sex chromosomes, for example, by addition of wild-type individuals containing the lost chromosomes.

We extended our basic model and that of Hurley et al. (2004) to show that ESR has profound effects on population growth and persistence. In general, environmental feminization resulted in moderate increases in population size, as a result of the female-biased sex ratios and the consequent increase in reproductive potential. Nevertheless, this result was only observed when males were able to mate with all (or most) females in the population. When male mating rates were constrained, females remained unmated and populations declined rapidly in size, increasingly more so as ESR became stronger (see below). The male-biased sex ratios produced from environmental masculinization created a paucity of females in the population and thereby limiting population growth potential. As a result, populations under moderate masculinization declined in size, and those under extreme ESR approached and attained extinction.

Environmentally induced sex reversal has frequently been reported to cause reductions in the reproductive potential of females and males (Sumpter & Jobling 1995; Vos et al. 2000; Jobling & Tyler 2003; Kidd et al. 2007), so we evaluated the consequences of such effects on population growth and dynamics. Phenotypic sex ratios became more extreme when the viability of sex-reversed individuals decreased relative to that of wild-types, and populations declined to lower equilibrium sizes compared with those with full viability. So at first glance, reduced viability of sex-reversed individuals appeared deleterious to population growth and composition. Nevertheless, these negative effects in some cases may be counteracted by a favorable response of genotypic sex ratios. Lower viability of sex-reversed individuals or YY inviability reduces the disproportionate transmission of one sex chromosome caused when sex-reversed individuals reproduce. The outcome is a less extreme genotypic sex ratio bias in future generations and hence less potential for loss of a sex chromosome. This may be sufficient to preserve populations if ESR ceases.

Although moderate feminization can be beneficial for population growth in the absence of large viability effects, our results suggest that the consequences of ESR

are generally negative in terms of population size and the persistence of sex chromosomes. Changes in population size and sex ratios may engender further problems for the population. In our simulations we assumed initially that males were capable of fertilizing all females in a population. Nevertheless, if males are rare and unable to mate successfully with many females, then strong environmental feminization creates a paucity of males. This results in a proportion of females remaining unmated, thereby limiting population growth and leading to potentially rapid population decline. In our simulations we assumed that the maximal ability for male mating (M^*) was independent of w , the male sex-reversed fitness coefficient. Nevertheless, if XX or YY males have reduced fitness and this becomes manifest as reduced mating ability (e.g., reduced sperm production/fertility), then M^* may be lowered, leading to fewer females being successfully fertilized and rapid declines in population growth. In contrast, male-biased sex ratios may be detrimental if male harassment of females over mating negatively affects female fitness, which could be a force that leads to population extinction by itself (Rankin & Kokko 2007). This would exacerbate the deleterious effect of environmental masculinization on population viability. So mating behavior could have profound consequences for a population's ability to cope with and respond to ESR. Allee effects may also become an issue when individuals become scarce because fitness often declines as population sizes decrease below critical levels (Stephens et al. 1999).

Inequality of male and female numbers creates variance in reproductive success and reduces the genetically effective population size (Falconer & Mackay 1996). So in addition to changing population growth, ESR may precipitate an increased loss of genetic variation per generation. For instance, we found that even when there was population growth following strong environmental feminization, there was marked reduction in effective population size relative to the census size (Fig. 5b). We also assumed there was no mating structure in the population. Nevertheless, in harem-based mating systems N_e is reduced by a factor of $N_e = 4N_M N_F / (2N_M + N_F)$ (Nomura 2002), meaning that our simple estimate(s) of N_e would overestimate the true N_e under such scenarios. So populations with biased gender representation may lose proportionately more genetic variability than sexually balanced ones, and hence their ability to adapt to changing environments. This may be particularly relevant to any evolved responses to counteract ESR. Fisherian sex-ratio selection will favor the spread of mutations that counteract biased sex ratios (and hence ESR) and restore sex ratios to equality (Fisher 1930). But if N_e is small, such selection will be less effective at driving changes in the frequency of the mutant allele (Lande 1995).

There are parallels between our model of the consequences of ESR and those dealing with population dynamics under parasitic distortion of sex ratio. For exam-

ple, Hatcher et al. (1999) modeled the consequences of vertically transmitted feminizing parasites on population sex ratio and growth. They arrived at congruent conclusions to some of those presented here. For instance, they observed that parasitic feminizing agents increase or decrease equilibrium host density depending on male mating capacity; when males can mate multiply, strongly feminized populations tend to grow, whereas when males are constrained in their mating frequency, populations decline in size (Hatcher et al. 1999).

Environmentally induced sex reversal has been a matter of discussion for some time (Sumpter & Jobling 1995; Vos et al. 2000; Jobling & Tyler 2003; Hurley et al. 2004) and may become increasingly relevant with greater anthropogenic interference in watercourses and changing global and local temperatures. It is therefore important to understand the (short-term) population effects of ESR. Population fragmentation and migration barriers generally reduce the evolutionary potential of populations to adapt to changed environments. In our model we assumed populations are not genetically variable in their susceptibility to exogenous hormonal effects and as such their sex-determining systems cannot evolve in the face of environmental pressures. This simplifying assumption seems to hold over the short term (≤ 100 generations) in some populations (e.g., C.W. et al., unpublished data), but future work would profit by investigating this point. Our simulations suggest that ESR can have profound effects and, as a consequence of the mismatch between phenotypes and genotypes, some probably counter-intuitive consequences for population growth and persistence. We used a simple model of logistic population growth with nonoverlapping generations. Future theoretical attention would benefit from investigation of other modes of density dependence and the case with overlapping generations (e.g., Rankin & Kokko 2007). But perhaps more important, there is a pressing need to study the frequency of ESR in wild populations, its duration in number of generations, and its fitness consequences.

Acknowledgments

Financial support was provided by the Swiss National Science Foundation and a NERC Fellowship to S.C. The authors thank D. Rankin, J. Gutierrez, and an anonymous referee for helpful comments on the manuscript.

Literature Cited

- Beardmore, J. A., G. C. Mair, and R. I. Lewis. 2001. Monosex male production in finfish as exemplified by tilapia: applications, problems and prospects. *Aquaculture* 197:283–301.
- Chowen, T. R., and J. J. Nagler. 2004. Temporal and spatial occurrence of female Chinook Salmon carrying a male-specific genetic marker

- in the Columbia River watershed. *Environmental Biology of Fishes* **69**:427–432.
- Cotton, S., and C. Wedekind. 2007a. Control of introduced species using Trojan sex chromosomes. *Trends in Ecology & Evolution* **22**:441–443.
- Cotton, S., and C. Wedekind. 2007b. Introduction of Trojan sex chromosomes to boost population growth. *Journal of Theoretical Biology* **249**:153–161.
- Devlin, R. H., and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208**:191–364.
- Dewsbury, D. A. 1982. Ejaculate cost and male choice. *The American Naturalist* **119**:601–610.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*. 4th edition. Addison Wesley Longman, Harlow, Essex, United Kingdom.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon Press, Oxford, United Kingdom.
- Förlin, L., M. Adolfsson-Erici, and D. G. J. Larsson. 2004. Epidemiological studies indicate a cause-effect relationship between pulp mill effluent exposure and male-biased sex ratios in fish. Presentation at the 5th International conference on fate and effects of pulp and paper mill effluents, 2003. Pages 48–54 in D. Borton, T. J. Hall, R. P. Fisher, and J. F. Fisher, editors. *Pulp and paper mill effluent environmental fate and effects conference proceedings*. Extended abstracts. DEStech Publications, Lancaster, United Kingdom.
- Gutierrez, J. B., and J. L. Teem. 2006. A model describing the effect of sex-reversed YY fish in an established wild population: the use of a Trojan Y chromosome to cause extinction of an introduced exotic species. *Journal of Theoretical Biology* **241**:333–341.
- Hatcher, M.J., D. E. Taneyhill, and A. M. Dunn. 1999. Population dynamics under parasitic sex ratio distortion. *Theoretical Population Biology* **56**:11–28.
- Hurley, M. A., P. Matthiessen, and A. D. Pickering. 2004. A model for environmental sex reversal in fish. *Journal of Theoretical Biology* **227**:159–165.
- Jobling, S., and C. R. Tyler. 2003. Endocrine disruption in wild freshwater fish. *Pure and Applied Chemistry* **75**:2219–2234.
- Kanaiwa, M., and Y. Harada. 2002. Genetic risk involved in stock enhancement of fish having environmental sex determination. *Population Ecology* **44**:7–15.
- Kidd, K. A., P. J. Blanchfield, K. H. Mills, V. P. Palace, R. E. Evans, J. M. Lazorchak, and R. W. Flick. 2007. Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America* **104**:8897–8901.
- Korpelainen, H. 1990. Sex ratios and conditions required for environmental sex determination in animals. *Biological Reviews* **65**:147–184.
- Lande, R. 1995. Mutation and conservation. *Conservation Biology* **9**:782–791.
- Larsson, D. G., and L. Förlin. 2002. Male-biased sex ratios of fish embryos near a pulp mill: temporary recovery after a short-term shutdown. *Environmental Health Perspectives* **110**:739–742.
- Larsson, D. G., H. Hallman, and L. Förlin. 2000. More male fish embryos near a pulp mill. *Environmental Toxicology and Chemistry* **19**:2911–2917.
- Maynard Smith, J. 1968. *Mathematical ideas in biology*. Cambridge University Press, Cambridge, United Kingdom.
- Nagler, J. J., J. Bourne, G. H. Thorgaard, and D. D. Dauble. 2001. High incidence of a male-specific genetic marker in phenotypic female Chinook Salmon from the Columbia River. *Environmental Health Perspectives* **109**:67–69.
- Nomura, T. 2002. Effective size of populations with unequal sex ratio and variation in mating success. *Journal of Animal Breeding and Genetics* **118**:297–310.
- Pandian, T. J., and S. G. Sheela. 1995. Hormonal induction of sex reversal in fish. *Aquaculture* **138**:1–22.
- Parks, L. G., C. S. Lambright, E. F. Orlando, L. J. Guillette, G. T. Ankley, and L. E. Gray. 2001. Masculinization of female mosquitofish in a kraft mill effluent-contaminated Fenholloway River water is associated with androgen receptor agonist activity. *Toxicological Sciences* **62**:257–267.
- Penáz, M., Z. Svobodová, V. Barus, M. Prokes, and J. Drastichova. 2005. Endocrine disruption in a barbel, *Barbus barbus* population from the River Jihlava, Czech Republic. *Journal of Applied Ichthyology* **21**:420–428.
- Piferrer, F. 2001. Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture* **197**:229–281.
- Rankin, D. J., and H. Kokko. 2007. Do males matter? The role of males in population dynamics. *Oikos* **116**:335–348.
- Stephens, P. A., W. J. Sutherland, and R. P. Freckleton. 1999. What is the Allee effect? *Oikos* **87**:185–190.
- Sumpter, J. P., and S. Jobling. 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives* **103**:173–178.
- Vos, J. G., E. Dybing, H. A. Grei, O. Landefoged, C. Lambré, J. V. Tarazona, I. Brandt, and A. D. Vethaak. 2000. Health effects of endocrine-disrupting chemicals of wildlife, with special reference to the European situation. *Critical Reviews of Toxicology* **30**:71–133.
- Vucetich, J. A., T. A. Waite, and Nunnery, L. 1997. Fluctuating population size and the ratio of effective to census population size. *Evolution* **51**:2017–2021.
- Wallace, H., G. M. I. Badawy, and B. M. N. Wallace. 1999. Amphibian sex determination and sex reversal. *CMLS Cellular and Molecular Life Sciences* **55**:901–909.
- Wedekind, C. 2002. Manipulating sex ratios for conservation: short-term risks and long-term benefits. *Animal Conservation* **5**:13–20.
- Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology & Evolution* **17**:313–320.
- Williamson, K. S., and B. May. 2002. Incidence of phenotypic female Chinook Salmon positive for the male Y-chromosome-specific marker *OtY1* in the Central Valley, California. *Journal of Aquatic Animal Health* **14**:176–183.