

Ecology meets endocrinology: environmental sex determination in fishes

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INTRODUCTION

Van Valen (1973) characterized evolution as the control of development by ecology. Sex determination in fishes provides some clear examples of this “control” in operation. Teleost fishes show a remarkable variety of sex determination and differentiation patterns. These range from systems in which sex is determined by sex chromosomes, as in birds and mammals, to simultaneous hermaphrodites that alternate spawning as a female and male on a second to second basis. This extraordinary flexibility may result from a combined lack of developmental constraint on reproductive structures in many lineages and selection for sexual lability in the face of environmental unpredictability.

This review addresses environmental influences on sex determination and differentiation in fishes. There is a variety of documented environmental influences on sex determination (ESD) in fishes. We focus here on two classes of examples where the key environmental cues are of clear ecological relevance, the effects appear especially likely to be important as a normal part of the life history, and where there is evidence suggesting the sexual patterns observed represent adaptations that increase individual fitness. These classes are sex determination that is controlled by social interactions (behavioral sex determination [BSD]) (Crews 1993) and temperature-dependent sex determination (TSD). Sex determination controlled by social influences can occur before or after sexual maturation but appears to maximize the expected reproductive success of individuals in both cases. Here we first address BSD and then TSD in fishes. For each pattern of sex determination, we discuss selection pressures that appear to favor these patterns, examples of each, and what is known regarding the underlying physiological mechanisms. For more comprehensive and general reviews of patterns and mechanisms of sex determination in fishes, the reader is referred to several excellent reviews (Nakamura et al. 1998; Baroiller et al. 1999; Baroiller and D’Cotta 2001; Piferrer 2001).

The major focus in studies of physiological mediation of teleost sex determination is what is referred to by endocrinol-

ogists as the hypothalamo-pituitary-gonadal (HPG) axis (Fig. 1). This axis consists primarily of hypothalamic neurosecretory neurons producing gonadotropin-releasing hormone (GnRH), gonadotropins produced in and released from the pituitary gland (GtH I and GtH II), and the gonad as the major site of steroid biosynthesis with its steroid metabolizing enzymes, steroid hormone receptors, and a variety of other proteins that mediate steroid hormone action. One steroid biosynthetic enzyme that has been a particularly fruitful focus in correlative and manipulative studies of vertebrate sex determination is cytochrome P-450 aromatase. This enzyme catalyzes the conversion of androgens to estrogens (primarily testosterone to estradiol-17 β). Aromatase expression correlates with female determination in a variety of vertebrates, and aromatase-specific antagonists can block female development in fishes, amphibians, reptiles, and birds (Elbrecht and Smith 1992; Lance and Bogart 1992; Crews et al. 1994; Wennstrom and Crews 1995; Kitano et al. 1999; D’Cotta et al. 2001). Estradiol-17 β plays a central role in female reproductive physiology in fishes, whereas the androgen 11-ketotestosterone (11-KT) is crucial to gamete maturation and the expression of secondary sexual characteristics in males (Borg 1994; Brantley et al. 1993). Importantly, testosterone levels often do not differ between male and female fishes or are higher in females (Borg 1994). Because of the central role of aromatase in the biosynthesis of estrogens, it will be a focus in consideration of mechanisms by which environmental information leads to sex determination responses. More generally, our understanding of vertebrate sexual function indicates the HPG axis plays the key role in transducing environmental information into gonadal determination, differentiation, and maturation events. A general theme of this review is where and how this transduction may occur in the HPG axis.

BEHAVIOR-DEPENDENT SEX DETERMINATION

Sex change is widespread in nature, occurring in plants, invertebrates, teleost fishes, and at least one amphibian (Policansky

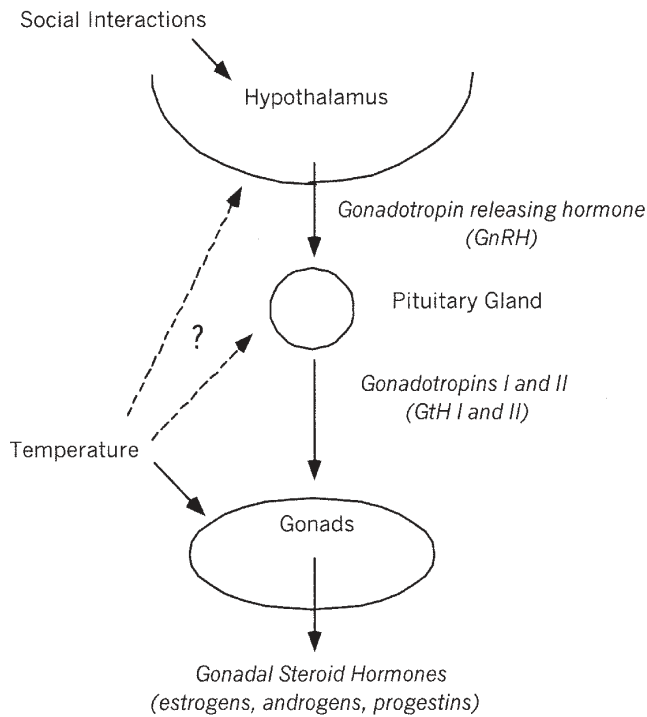


Fig. 1. The teleost hypothalamo-pituitary-gonadal (HPG) axis with likely sites of transduction for environmental signals. Gonadotropin-releasing hormone (GnRH) is homologous to luteinizing hormone-releasing hormone in mammals. GtH I and II are homologous to luteinizing hormone and follicle-stimulating hormone in mammals.

1982; Warner 1984; Grafe and Linsenmair 1989). There is, however, no evidence that any amniote vertebrate (mammals, birds, and reptiles) exhibits this reproductive pattern.

Sex change is a purely ontogenetic event in some species but in others is triggered by environmental stimuli such as interactions with conspecifics. Social control of sex change is best known from tropical coral reef fishes. This sex determination by social interactions in fishes is one of the better understood examples of sexual lability in any vertebrate group from the standpoint of ultimate causation. Although it has been criticized and refined in the time since it was first proposed, Ghiselin's size advantage model for sequential hermaphroditism remains the most widely accepted evolutionary explanation of the adaptive significance of sex change (Ghiselin 1969; Warner et al. 1975; Warner 1988; see Shapiro 1989 for dissenting opinion). Briefly, this model posits that if an individual can reproduce more effectively as one sex when small or young and as the other sex when larger or older, it should change sex at some point in its life history. The sex an individual initially differentiates as is termed the primary sex, whereas the sex of the individual after sex change is termed the secondary sex. The general expectation is that sex change should occur when the expected reproductive success as the secondary sex exceeds that of the primary sex (Fig. 2). This change in the relative expected reproductive success of the two sexes can change either predictably based on growth or other ontogenetic factors or unpredictably with social change.

Socially controlled functional sex change was first described in two coral reef fish species in the early 1970s. Two patterns of sex change are seen. Female to male sex change is referred to as protogyny ("first female"), whereas male to female change is termed protandry ("first male"). Fishelson (1970) showed that removal of males from harem groups of the fairly basslet *Anthias squamipinnis* induced female to male sex change in the largest female. Robertson (1972) sim-

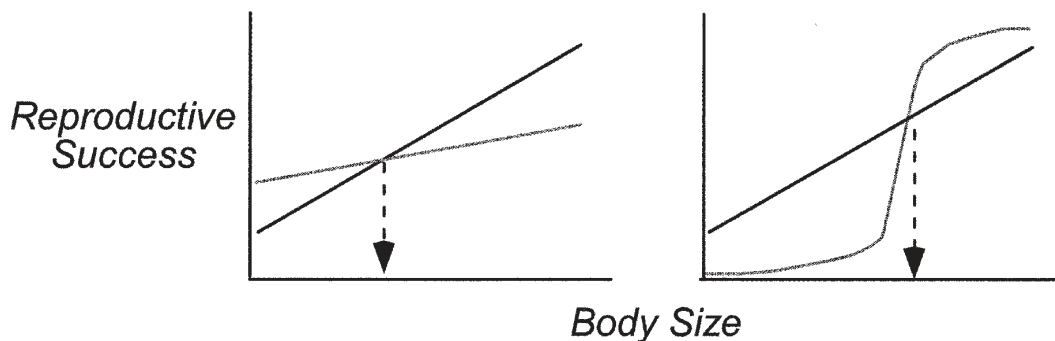


Fig. 2. Size advantage model of sex change. Female size-fecundity relationships are shown by black lines, whereas the relationships for males are shown by gray lines. The first panel depicts size-fecundity relationships that favor the evolution of protandry. Female size-specific fecundity exceeds male fecundity above the body size indicated by the arrow, and selection favors individuals who change sex at this point. This situation typically occurs in the absence of sexual selection favoring large males, and the male function does not show a steep increase at larger body sizes. The second panel depicts size-fecundity relationships favoring the evolution of protogyny, with selection favoring individuals who change sex at the body size where male fecundity exceeds that of females. This situation is common where strong sexual selection favors large males either through female preference or defense of resources critical to females. This mating advantage realized by large males is the reason for the steep increase in the male function upon attaining a body size that allows them to compete effectively with other males.

ilarly found that removal of dominant males from harem groups of the Indo-Pacific cleaner wrasse *Labroides dimidiatus* caused the largest females to become males within approximately 1 week. In both cases, females who changed sex to assume the dominant male role realized a near-immediate increase in reproductive success. This is because the fecundity of the sex changer now becomes equal to the combined fecundity of the remaining females in the group rather than only what their own would be had they remained female. Warner et al. (1975) addressed this change in reproductive success with sex change in the bluehead wrasse, *Thalassoma bifasciatum*. Large male bluehead wrasses realize high reproductive success through resource defense polygyny in which they monopolize spawning sites favored by females. Using both modeling approaches and estimates of reproductive success from natural populations, this work showed that sexual selection favoring large males should select for female to male sex change (see also Warner 1984, 1988). Socially controlled male to female sex change (protandry) appears to be much rarer in fishes, thus far being documented only in anemonefishes (Ross 1978a,b; Fricke 1979, 1983; Godwin 1994).

Since these initial descriptions, it has become clear that functional sex change is common in several families of fishes common on coral reefs (Serranidae, Labridae, Scaridae, Gobiidae, Cirrhitidae) and occurs in several others (Pomacentridae, Pomacanthidae) (Warner 1984). Most of the evidence for sex change in fish species is based on histological criteria in which gonads show structural remnants of previous female function (i.e., a remnant ovarian cavity) or examination of individuals undergoing sex change (Sadovy and Shapiro 1987). There are fewer cases where investigators have identified individuals of the primary sex and followed their transition to the secondary sex, and an even smaller number have shown that this process is under social control. The studies by Fishelson and Robertson were described above. Studies by Ross et al. (1983, 1990), Warner and Swearer (1991), Lutnesky (1994, 1996), Godwin (1994), and Cole and Shapiro (1995) have also documented social control of the sex change process. In most cases, removal of dominant individuals from social groups or housing potential sex changers with smaller conspecifics is the experimental manipulation used to induce sex change. Social influences on the sex change process were carefully dissected in the saddleback wrasse, *Thalassoma duperrey*, where both stimulation and inhibition were shown to be important (Ross et al. 1983). Larger conspecific individuals inhibit female to male sex change, whereas larger individuals of a sympatric congener do not. However, sex change in experimental pens (1 × 1 × 1 meter) also requires stimulation in the form of smaller conspecifics.

In tests of important cues regulating sex change, several studies suggest sex ratio and density effects on encounter rates are of crucial importance. Lutnesky (1994) found that

housing with smaller conspecifics in small pens and exceeding a critical threshold sex ratio of females to males in a large experimental pen (102 m²) induced female to male sex change in the angelfish *Centropyge potteri*. In contrasting several models, Lutnesky concluded that the absolute frequency of encounters with males and females was the critical proximate cue regulating sex change in *C. potteri*. Ross et al. (1990) found multiple sex changes occurred in experimental pens when the number of females housed together was increased, again suggesting the female to male sex ratio or encounter rates with females was the critical variable regulating sex change in the larger females rather than simply the presence of a larger individual. Cole and Shapiro (1995) reported similar results from groups of female gobies, *Coryphopterus glaucofrenum*. Working on natural reefs in the field, Warner and Swearer (1991) found that the number of female *Thalassoma bifasciatum* changing sex closely matched the number of large males experimentally removed.

Pheromonal mediation of sex change has been suggested in one gobiid species. Cole and Shapiro (1995) compared the occurrence of sex change in isolated female *C. glaucofrenum* exposed to water from female conspecifics, male conspecifics, female gobies of another species, or empty tanks as a control. The occurrence of sex change was higher for females exposed to water from female conspecifics than in the other treatments. The small size of gobies and consequently restricted home ranges facilitates laboratory investigations of sex change in what likely approach very naturalistic settings. Ross et al. (1983) did not find a stimulation of sex change in saddleback wrasses when females were exposed to chemical cues from a smaller female in experimental pens but visually isolated from them. Females who could see the smaller female did show sex change in these pens. Female bluehead wrasses begin to show increased “inspection” of other females upon attaining social dominance (Godwin, unpublished data). This inspection behavior involves a focal female placing her nares near the cloacal region of other females. It is therefore possible sex changers are increasing their exposure to female pheromonal cues at the beginning of the sex change process.

Although much less well characterized, social environment also influences sex determination before maturation in some teleost species. The best characterized example of this is the Midas cichlid, *Cichlasoma citrinellum* (family Cichlidae). Francis and Barlow (1993) found that larger individuals in broods developed as males, whereas their smaller broodmates developed as females. A difference in growth rate between individuals destined to become male versus female was ruled out in a simple experiment in which broods were divided by size. The larger individuals in both the large and small groups after this separation developed as males, whereas the smaller individuals developed as females, indicating that male determination was a result of large relative

size rather than the reverse. There is also evidence for protogynous sex change in cichlids (*Crenicara punctulata*) (Carruth 2000). It is likely relevant that this family of fishes is considered the sister family to the marine Pomacentridae, which includes both protogynous *Dascyllus* and protandrous *Amphiprion*. Cichlids are also part of the suborder Labroidei, which includes the wrasses and parrotfishes (Streelman and Karl 1997).

ENDOCRINOLOGY OF BSD

The physiological mechanisms regulating functional sex change in teleost fishes have been a fascinating but vexing problem in reproductive biology. Much has been learned about profiles of steroid hormones and the molecular events underlying change in the gonads, but progress in understanding the mechanisms by which a hermaphroditic fish is able to “transduce” a change in social situation into the series of neuroendocrine and cytological processes that result in sex change has been more limited. Consequently, consideration of molecular and neuroendocrine mechanisms below is biased toward changes occurring in gonadal tissue with less information about changes in other parts of the HPG axis.

Studies have shown sex in both hermaphroditic and gonochoristic teleost fishes can be altered through steroid hormone manipulations, with androgens typically inducing male determination whereas estrogen treatment induces female determination (Yamamoto 1969; Nakamura et al. 1998; Baroiller et al. 1999; Baroiller and D’Cotta 2001; Piferrer 2001). There are clear gonadal steroid hormone correlates of sex change in many species, but the patterns are much less clear in several others. Yeung and Chan (1987a,b) did not find clear correlations between sexual phase and plasma steroid profiles in their pioneering and comprehensive studies of the protogynous *Monopterus albus* and protandrous *Rhabdosargus sarba*. However, Godwin and Thomas (1993) showed that plasma levels of 11-KT declined as the testicular part of the gonad became an ovary in the anemonefish *Amphiprion melanopus*, whereas plasma levels of estradiol rose as the ovary developed (albeit not until the early events of ovary formation were already complete). These differences in plasma levels of 11-KT and estradiol in *A. melanopus* are paralleled by sex differences in gonadal synthesis of these two steroids in the closely related *Amphiprion frenatus* (Nakamura et al. 1994). Similar patterns are observed for estradiol synthesis by aromatase in another well-characterized protandrous species, the black porgy *Acanthopagrus schlegelii* (reviewed in Lee et al. 2001).

The gonadal steroid hormone correlates of protogynous sex change have been examined in a number of species following the initial studies by Yeung and Chan. Focusing only on two protogynous coral reef species for which there is con-

siderable behavioral and ecological information, both the Hawaiian saddleback wrasse (*Thalassoma duperrey*) and stoplight parrotfish (*Sparisoma viride*) show decreases in estradiol levels and increases in 11-KT with sex change (Nakamura et al. 1989; Cardwell and Liley 1991). These changes in plasma hormone levels are correlated with and due to changes in steroid synthesis in the gonads in *T. duperrey*. Production of the potent teleost androgen 11-KT depends on the enzyme 11 β -hydroxylase. This production increases with sex change and differs between male phenotypes in *T. duperrey*, being higher in the colorful terminal phase (TP) males than female-mimic initial phase males (Hourigan et al. 1991). Saddleback wrasses also show changes in the level and location (from thecal cells in the ovary to interstitial cells with sex change) of expression of another key steroidogenic enzyme, P-450 cholesterol side chain cleavage, over the course of sex change (Morrey et al. 1998).

Changes in steroid hormone profiles during sex change appear to be the primary influences on changes in external morphology. These gonadal steroid changes may also influence behavior, although the evidence for such effects is decidedly mixed. In 1955, Stoll (1955) showed that administering methyltestosterone, a synthetic androgen, could induce the development of dominant TP male colors in the bluehead wrasse. Since this demonstration, a variety of studies has demonstrated either correlative or experimental links between high levels of 11-KT and the development of male display characters or genital morphology. Brantley et al. (1993) presented androgen data from the plainfin midshipman (*Porichthys notatus*) and reviewed data from several other teleosts that exhibit alternate male phenotypes, including sex changing species. These species typically have one male phenotype that exhibits extensive development of secondary sexual characters and high levels of aggressive and courtship behavior (“bourgeois” males) (Taborsky 1997, 1998), whereas the other male phenotype does not. Although testosterone levels showed no consistent differences across these phenotype contrasts, 11-KT levels were consistently higher in the bourgeois males. Administering 11-KT is also sufficient to induce development of male secondary sexual characters in several species (e.g., Carlisle et al. 2000; Oliveira et al. 2001a,b,c). In the bluehead wrasse, exogenous 11-KT administered to gonadally intact females induced color change and neural changes but also induced sex change (Grober et al. 1991). Because sex change results in a suite of changes in steroid profiles, it cannot be definitively concluded that 11-KT rather than another hormonal change related to sex change accounted for the morphological effects in this study. The gonads are not necessary for behavioral sex change in the bluehead wrasse but are necessary for the development of the TP male coloration that accompanies sex change (Godwin et al. 1996), and 11-KT implants in ovariectomized females will induce the development of TP male coloration

(K. Semsar and J. Godwin, unpublished data). It is still possible that 11-KT acts to inhibit behaviors not typical of TP males in bluehead wrasses. This possibility is suggested by a study in the peacock blenny (*Salaria pavo*) in which 11-KT implants inhibited the display of sneaking behaviors (Oliveira et al. 2001c). It is important to note here that 11-KT may be more potent as an inducer of male typical characteristics because, unlike testosterone, it cannot be converted to an estrogen through aromatization. (See Forlano et al. 2001 for further discussion of this hypothesis and a potential example of how aromatization might prevent masculinization of the brain.)

Much remains to be learned about the neural mechanisms by which social cues are transduced into gonadal changes in both sex changing fishes and gonochoristic fishes that show social effects on sex determination. Attention has focused primarily on the gonadotropin system and especially the key regulator of gonadotropin secretion, GnRH. The GnRH system has been a focus because of its expression in a critical integrative area, the preoptic area of the hypothalamus, and its pivotal role in regulating reproduction in vertebrates. Gonadotropins can induce precocious sex change in some species, but the effects can also be specific to reproductive stage (Koulish and Kramer 1989; Yeung et al. 1993). Neuropeptide Y, a neuropeptide hormone expressed in the hypothalamus and thought to be a regulator of GnRH secretion, can also stimulate some signs of sex change in captive female bluehead wrasses, although relatively few females progressed beyond an early stage of gonadal change after 8 weeks of treatment (Kramer and Imbriano 1997). Complete transformation of the ovary into a functional testis can occur in 8–10 days in nature (Warner and Swearer 1991), but it should be noted that a lack of strong stimulation of sex change by neuropeptide Y in this study could be due to the stress and unusual pattern of exposure resulting from repeated injections.

Several sex changing species show differences in the GnRH system between sexual phases. These include the bluehead wrasse (Grober and Bass 1991), the ballan wrasse (*Labrus berggyla*) (Elofsson et al. 1999), and the anemonefish *A. melanopus* (Elofsson et al. 1997), with males showing greater numbers of GnRH cells in the preoptic area of the hypothalamus in each case. The GnRH system has also been well studied in relation to sexual plasticity in other fish species where there is dramatic phenotypic variation among males. Two notable examples here are the cichlid *Haplochromis burtoni* (Francis et al. 1992, 1993; Fernald 1995) and the plainfin midshipman *Porichthys notatus* (Grober et al. 1994; Foran and Bass 1999; Bass and Grober 2001).

A second neuropeptide that has received attention as a mediator of behavioral change during the sex change process is arginine vasotocin (AVT). AVT and its mammalian homologue arginine vasopressin (AVP) are important regulators of social behaviors in species from all classes of verte-

brates (see reviews by Moore 1992; DeVries 1995; Goodson and Bass 2001). AVT is expressed in the preoptic area of the hypothalamus, a critical integrative area for sexual behavior in both fishes and other vertebrates and is differentially expressed across phenotypes and with sex change in the goby *Trimma okinawae* (Grober and Sunobe 1996) and the bluehead wrasse (Godwin et al. 2000). The peptide also increases courtship behavior in TP male bluehead wrasses when given exogenously, whereas an AVT antagonist reduces it (Semsar et al. 2001). It is not yet clear how the expression of AVT is regulated. Expression of AVT and AVP in vertebrates other than fishes is regulated primarily by gonadal steroids (DeVries 1995; Moore 1992; Panzica et al. 2001). To date, there is no evidence of similar steroidal regulation in sex changing fishes or fishes that exhibit alternate male phenotypes. The rapidity of the sex change process in at least two species suggests that androgens of gonadal origin would not provide an appropriately rapid signal for up-regulation of the AVT system because behavior changes more rapidly than the gonads (Robertson 1972; Warner and Swearer 1991; Godwin et al. 1996). However, rapid actions of glucocorticoids and estrogens are known from the nervous system and could be important in rapid behavioral sex change (Wehling 1997; Borski 2000).

TEMPERATURE-DEPENDENT SEX DETERMINATION

TSD occurs in a variety of taxa. In vertebrates, TSD patterns are best documented in reptiles, including many lizards, turtles, and all crocodylian species (Crews, this issue). The molecular mechanisms underlying TSD are also best understood in reptiles, where the proximate temperature cue activates sex determination pathways that show strong similarities with those described in other vertebrates. Although fewer species of fishes have been documented to show a temperature sensitivity in sex determination that is clearly a normal part of the species life history (several species show effects at apparently abnormally high temperatures), there is some good evidence regarding the ecological significance of these patterns. An additional and particularly interesting feature of TSD in fishes is the presence of mixed genotypic sex determination (GSD) and TSD within single species. This intraspecific variation has been documented in both the Atlantic silverside *Menidia menidia* (Conover and Heins 1987) and in the Japanese flounder *Paralichthys olivaceus* (Yamamoto 1999). These systems provide particularly attractive models for understanding evolutionary transitions between ESD and GSD.

Charnov and Bull (1987) proposed that ESD is favored when the environments in which development will take place vary in terms of growth potential such that expected in-

dividual fitness under these varying growth conditions differs between the sexes. Under these conditions, individuals should differentiate as the sex with the greatest resulting expected fitness for the environment in which they develop. In other words, "If an individual finds itself in an environment where it can become a below average female or an above average male, selection will favor its becoming male because it can pass on more of its genes than if it were female" (Charnov and Bull 1987). An additional condition that Charnov and Bull postulate should favor the evolution or retention of environmentally sensitive sex determination is a patchy environment in which parents and offspring have limited ability to control or predict their place of development. Most marine fishes have planktonic larval stages lasting from weeks to months, and the settling environment appears to be at least largely determined by where larvae are carried on ocean currents. Consequently, these animals probably often lack the ability to control or predict critical features of the environment in which development will take place. Focusing on TSD, a patchy thermal environment may often arise for species with extended breeding activity in strongly seasonal areas or those inhabiting near-shore environments or estuaries.

The Atlantic silverside shows strong interpopulation variation in temperature effects on sex ratios (Conover and Heins 1987). This is a small short-lived species native to salt marshes, bays, and estuaries along the eastern coast of North America. Silversides are essentially "annuals," with very few fish living to 2 years of age. Individuals mature within a year and spawn in large schools. In southern populations from South Carolina to Virginia where growing seasons are longer (7–10 months), silversides exhibit pronounced TSD, with cooler water temperatures inducing female development and warmer water temperatures producing males. The resultant sex ratios are 70% to more than 80% female at 15°C and more than 80% male at 28°C. Females develop early in the year and are able to grow to large size. Males develop later and grow less before spawning occurs. Because fecundity is largely determined by body size in females, a long period of growth before spawning leads to higher reproductive success (Conover 1984). Male fecundity is apparently less limited by body size in this species, in part because spawning takes place in large groups where sperm competition appears to be intense and males do not monopolize mating opportunities with females. Consequently, individuals that grow less realize higher reproductive success as small males than they would as small females. In more northerly populations from New York, Massachusetts, and Prince Edward Island, TSD is less important and it is completely absent in a Bay of Fundy population in Nova Scotia. Growing seasons are 4–6 months in the New York, Massachusetts, and Prince Edward Island populations and only 3 months for the Nova Scotia population. This reduction in the period for growth results in less potential for large size differences between individuals

to develop and likely greater year to year environmental variation. Conover and Heins (1987) argue these factors reduce selection for TSD and increase selection for GSD.

Silversides also exhibit considerable within-population variation in sex response to temperature. Conover and Kynard (1981) found the proportion of female offspring resulting from rearing in warm water conditions varied from 0 to 51.8% in clutches from different mothers in a Massachusetts population. Considerable variation in TSD sensitivity can therefore exist within populations, and this temperature response should continue to be subject to and modifiable by selection.

At least two other atherinid fishes show strong temperature effects on sex determination, although the silverside is the only species known to show the population variation described above. The pejerrey, *Odontesthes bonariensis*, exhibits sex ratios varying from 100% female at 15–19°C to 100% male at 29°C (Strussmann et al. 1996a,b). A related atherinid, *Patagonia hatcheri*, shows slightly less dramatic effects of temperature with approximately 90% females at 13–15°C to approximately 30% at 25°C (Strussmann et al. 1997). Sex can be manipulated by estradiol over roughly the same developmental period as temperature exerts its effects in pejerrey, supporting the hypothesis that estrogens produced by aromatase are the transducers of temperature effects on gonadal differentiation (Strussmann et al. 1996c).

The other well-characterized examples of strong TSD effects in fishes are from the flounders, flatfishes of the order Pleuronectiformes. The best studied of these is the Japanese flounder or hirame, *Paralichthys olivaceus*. Based on studies including wild-type and all-female stocks derived through gynogenetic methods, Japanese flounder have a sex determination system that incorporates both temperature and genotypic influences, although apparently in a different way than observed in silversides. Figure 3 shows the sex determination patterns across rearing temperatures for three flounder species. The proportion of individuals developing as females peaks at approximately 50% at 20°C in wild-type Japanese flounder (*P. olivaceus*), with a preponderance of males developing at both higher and lower temperatures. The proportion developing as females approaches 100% in gynogenetic, all XX progeny, suggesting most or all XX individuals show TSD, whereas XY individuals exhibit GSD. This pattern of mixed TSD and GSD is reminiscent of the patterns discussed above for silversides, except that the major genetic contribution to sex determination responses in flounder appears to be whether or not offspring inherit a Y chromosome.

TSD may be common in flounders. The southern flounder (*Paralichthys lethostigma*) is native to the southeast Atlantic and Gulf of Mexico coasts of the United States. Our work with this species indicates a very similar TSD response to its congener, the Japanese flounder (Fig. 3). Southern flounder reared at 23°C show a sex ratio not different from 50:50,

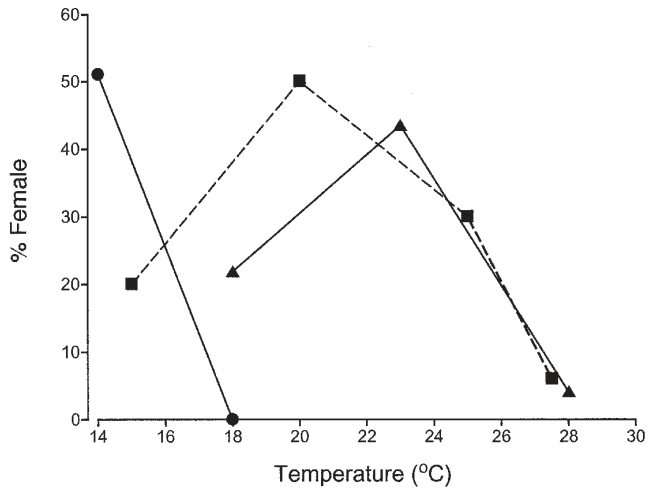


Fig. 3. Flounder sex ratios resulting from development at different temperatures. Depicted are data from barfin flounder (●), japanese flounder (■), and southern flounder (◆). References are in text. Japanese flounder values are means calculated from Yamamoto (1999).

whereas sex ratios are highly male biased at 18 and 28°C (Luckenbach et al. in press). The genus *Paralichthys* has several ecologically and economically important species, including halibut and summer flounder (Luckenbach et al. 2002). TSD may be common in the genus but has not yet

been investigated in these other species. Outside *Paralichthys*, the barfin flounder *Verasper moseri* is the only other flounder species documented to show TSD (Goto et al. 1999). Barfin flounder exhibit a 100% male sex ratio if moved from 14 to 18°C at a mean size of approximately 10 mm. As the size at which individuals are moved is increased in approximate 10-mm increments, the sex ratio approaches 50:50, becoming statistically indistinguishable from unity at 40 mm. It is interesting to note the difference in temperatures producing males in different flounders (Fig. 3), particularly in the Japanese species. It is likely relevant here that the barfin flounder has a more northerly distribution than *P. olivaceus*, inhabiting waters influenced by the cold Liman and Oyashio currents rather than the warmer Tsushima and Kuroshio currents that southern Japan experiences (Fig. 4). The cold-shifted TSD response shown by barfin flounder relative to *P. olivaceus* may be an adaptation to these colder temperatures.

Temperature also affects growth in southern flounder. In an initial study, we found that individuals reared at 23°C were significantly larger after 1 year than fish reared at either 18 or 28°C (Fig. 5). Females reach much larger adult sizes than males in southern flounder, so this faster growth at the temperature producing the greatest proportion of females is consistent with Charnov and Bull’s hypothesis for ESD because females should benefit more from rapid growth than males. It is also relevant that observations of southern flounder spawning in tanks suggest little competitive or aggressive interaction between males in which large size might provide an advantage (Smith et al. 1999). Importantly, the same initial study also suggests the body size of males and

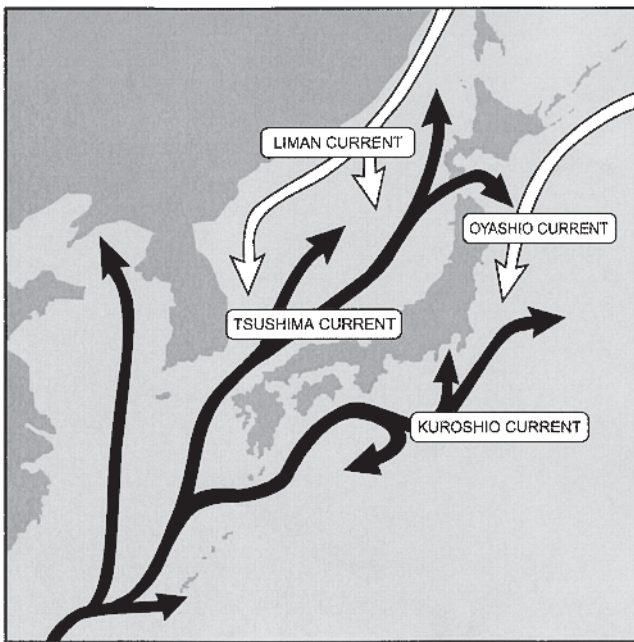


Fig. 4. Warm and cold currents in the area of Japan. The range of the Japanese flounder (*Paralichthys olivaceus*) extends from northern Japan south to Hong Kong. The range of the barfin flounder (*Verasper moseri*) extends from the Kuril Islands north of Japan south to approximately the point where the Oyashio and Kuroshio currents are shown meeting.

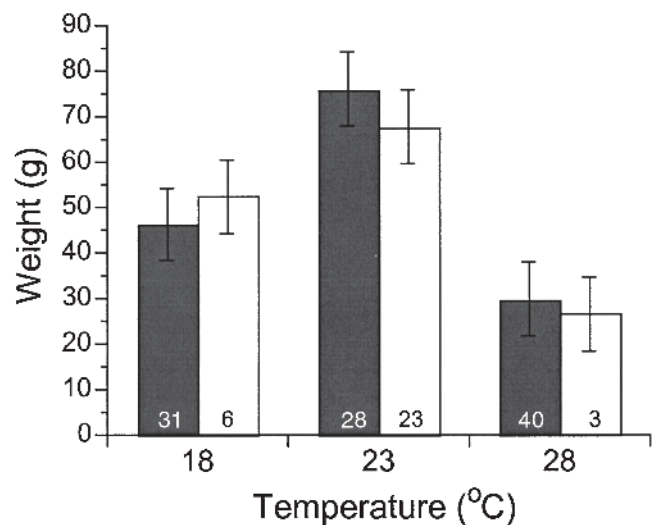


Fig. 5. Body sizes of southern flounder grown at different temperatures in captivity for 1 year (mean ± SEM). Fish grown at 23°C were significantly larger than fish grown at either 18°C or 28°C, but no sex difference in size was found in any of the 3 temperatures.

females does not differ within a rearing temperature (Fig. 5). This mirrors findings from the Atlantic silverside where female determining temperatures also produced faster growth, but no male to female size difference was found within a rearing temperature (Conover 1984). The lack of observed sex differences in growth within temperatures in both species is consistent with the Charnov and Bull hypothesis because it indicates that temperature rather than sex is the critical variable. Findings from southern flounder and silversides contrast with work in barfin flounder where moving juveniles from 14 to 18°C significantly enhanced growth in the first 150 days after fertilization, despite 18°C inducing more males to develop (Goto et al. 1999).

Information on the physiological mediators of TSD in fishes is limited, being available so far only for the Japanese flounder. Nevertheless, it appears likely that the underlying mechanisms show strong similarities to those described for fish showing BSD above and for TSD in the better studied reptilian examples. In particular, there is evidence that estrogen synthesis by the aromatase enzyme plays a key role in female sex determination. Aromatase mRNA expression in Japanese flounder becomes detectable as gonadal differentiation begins and only in individuals developing as females (Kitano et al. 1999). This is known from studies of all XX stocks reared at female determining temperatures. Rearing XX juveniles at male-determining temperatures eliminates aromatase expression. Tissue levels of estradiol are also significantly higher in XX offspring reared at a female determining temperature (18°C) than XX offspring reared at a male-determining temperature (27°C). No information is yet available regarding the physiological and molecular correlates of TSD in other flounder or atherinid species, although investigations are underway in southern flounder.

CONCLUSION AND DIRECTIONS

This review concentrated on a few examples of fish species that exhibit strong sex determination responses to the environment and for which the ecological relevance is reasonably clear. It is therefore far from exhaustive in terms of environmental effects on sex determination in this vertebrate group. However, it appears that the components of the endocrine system discussed are likely to be important mediators of this type of sexual lability across fishes. These mechanisms are becoming better understood in a few model systems, but the detailed means by which environmental variation is transduced into changes in these pathways remain obscure. It is, for example, not understood how a temperature signal leads to differences in aromatase gene expression. Elucidating these mechanisms should not only greatly improve our understanding of ESD, but of the environmental modulation of reproductive function generally.

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