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NEUROENDOCRINE MECHANISMS OF ALTERNATIVE REPRODUCTIVE TACTICS IN FISH

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1. INTRODUCTION

With over 20,000 species described to date, teleost fish are the most diverse taxon of living vertebrates, representing a very successful lineage of recently evolved organisms (Nelson, 1994). Teleosts also exhibit the widest range of reproductive modes and mating systems across the vertebrates (Breder and Rosen, 1966; Thresher, 1984). The diversity in reproductive patterns ranges from gonochoristic species, to sequential hermaphroditic species (both protogynous and protandrous), to serial sex-changing species (e.g., goby *Trimma okinawae*; Sunobe and Nakazono, 1993) to simultaneous hermaphrodites (see Demski, 1987 for a review). Asexual reproduction also

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occurs in some parthenogenic species (e.g., the amazon molly *Poecilia formosa*; Schartl *et al.*, 1995). Although most species are external fertilisers, live-bearers also occur in phylogenetic independent lines (Goodwin *et al.*, 2002) and the mating system can vary from monogamous to polygamous to promiscuous (Turner, 1993). Also the patterns of parental care are the most diverse among vertebrates, with most species showing no care, to species with biparental, paternal, or maternal care (Sargent and Gross, 1993). This rich variation in reproductive modes is not only present at the interspecific level but also within species, with population differences in mating systems (e.g., sex-role reversal in courtship behaviour in lagunar populations of the peacock blenny, *Salaria pavo*, Ruchon *et al.*, 1995; Gonçalves *et al.*, 1996), to highly flexible breeding systems within the same population (e.g., monogamy vs. polygamy and biparental *vs* maternal care or paternal care in the St. Peter's fish, *Sarotherodon galilaeus*; Balshine-Earn and Earn, 1998; Fishelson and Hilzerman, 2002) to the occurrence of alternative sexual phenotypes within the same sex (e.g., Taborsky, 1994). This wide variation in reproductive patterns makes teleost fish the group of election for the study of the proximate causes of sexual plasticity in vertebrates, namely for alternative reproductive tactics.

2. ALTERNATIVE REPRODUCTIVE STRATEGIES AND TACTICS

2.1. Patterns of Variation

Intrasexual variation in reproductive behaviour is widespread among animals, occurring in many invertebrate taxa such as insects (Forsyth and Alcock, 1990), crustaceans (Shuster, 1992), cephalopods (Norman *et al.*, 1999), and in all vertebrate classes such as fish (Taborsky, 1994, 2001), amphibians (Perril *et al.*, 1978), reptiles (Sinervo and Lively, 1996), birds (Lank *et al.*, 1995), and mammals (Hogg, 1984; for general reviews see Arak, 1984 and Austad, 1984). In vertebrates, the intrasexual variation in reproductive behaviour has been mainly documented for males and it may be continuous or discrete (Rhen and Crews, 2002). Discrete polymodal behavioural phenotypes within adults of the same sex in a given species have been called *alternative reproductive tactics* (ART) (Brockmann, 2001). For the sake of brevity, this review will limit the focus to species that display ARTs. Continuous variation in sexual behaviour is beyond the scope of this review and has been published elsewhere (e.g., Crews, 1998; Oliveira *et al.*, 2005).

From an evolutionary point of view, a classic distinction is made between alternative strategies and tactics. According to Gross (1996), a strategy is a genetically based program, whereas a tactic is a phenotype that results from a strategy. Two main types of strategies are possible: alternative and conditional strategies (Gross, 1996; Brockmann, 2001). Alternative strategies are based on genetic polymorphism, are under frequency-dependent selection, and thus the alternative phenotypes have equal fitness. On the other hand, in conditional strategies the expression of alternative phenotypes is based on an individual's assessment of status-dependent cues, not genetic differences (i.e., status-dependent selection; Gross, 1996). In this case, fitness is not expected to be equal among alternative morphs. Theoretically, a third strategy is possible (a "mixed strategy") if frequency dependent selection can result in equal fitness outcomes for the alternative phenotypes. However, an empirical example of such a case has never been reported in the literature (Gross, 1996). According to this view, alternative phenotypes may represent either alternative strategies or alternative tactics, depending on the relative contribution of genetic and environmental factors for their evolution.

Based on the published empirical studies, conditional strategies (i.e., alternative tactics) appear to be more common than alternative strategies (Gross, 1996). However, this apparent prevalence of conditional strategies may result from the fact that this model seems to explain most observed cases of ART and genetic studies to unravel underlying genetic polymorphisms are only rarely conducted (but see Zimmerer and Kallman, 1989 and Ryan *et al.*, 1992). Moreover, the assumption of lack of heritability in the determination of the tactic switch-point (at which it pays off for individuals to switch from one tactic to the other) in conditional strategies has also been recently challenged by Shuster and Wade (2003), who have proposed that most cases of conditional strategies must have an underlying genetic polymorphism.

The new genetic approaches to the description of fish mating systems have also provided some interesting new data with major implications for the interpretation of the evolution and maintenance of alternative reproductive phenotypes. It has been shown that the alternative male morph may have higher fitness than the conventional tactic as a result of both an unsuspected higher reproductive success or by producing more viable offspring. In the bluegill sunfish, it has been shown that bourgeois males achieve lower egg fertilisation rates than parasitic males under sperm competition (Fu *et al.*, 2001). It has also been shown, both in bluegill sunfish and in Atlantic salmon, that the offspring of parasitic males grow faster and to a larger size than the offspring of bourgeois males (Garant *et al.*, 2002; Neff, 2004). Moreover, in field conditions bluegill sunfish fry from nests with a proportionally higher incidence of cuckoldry, are larger, and show a threefold increase in survival when faced with their major predator (Neff, 2004). Thus, the computation of relative fitness between alternative phenotypes, needed for the clarification of the evolutionary mechanisms underlying the

ART, should include not only the (lifetime) reproductive success of both male types but also the potential differential survivorship of their offspring.

Alternative reproductive phenotypes can also be classified based on the descriptive patterns of the observed behaviour (Brockmann, 2001). According to this classification scheme, ART can be categorised as fixed or plastic (Moore, 1991). In fixed alternative phenotypes, the individuals adopt one of the tactics for their entire lifetime. In plastic (or flexible) alternative phenotypes, individuals may change tactic during their lifetime. Within plastic ART, two subcategories can be further distinguished: irreversible sequential patterns, when individuals switch from one tactic to another at a particular moment in their lifetime (developmental switches), and reversible patterns, when individuals can change back and forth between patterns (Moore, 1991, Moore *et al.*, 1998; Brockmann, 2001; Figure 8.1).

Therefore, this chapter adopts a classification of ART based on observed patterns as recommended by Brockmann (2001) (i.e., fixed vs. sequential vs. reversible tactics; Figure 8.1).

2.2. Terminology of Alternative Reproductive Phenotypes

A vast number of terms have been used to describe alternative reproductive phenotypes (Table 8.1). Some of these terms fail to reveal the functional role of each tactic (e.g., type I vs. type II males in plainfin midshipman,

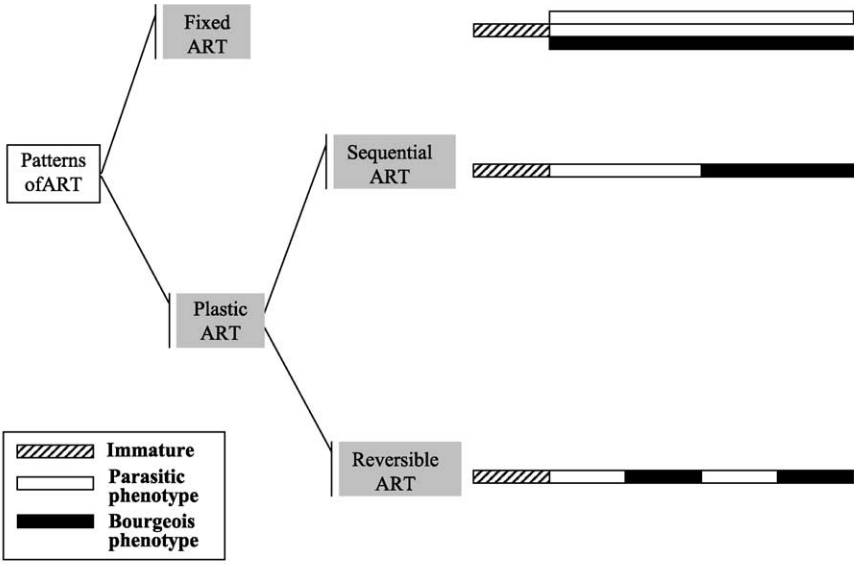

Fig. 8.1. Classification of alternative reproductive tactics (ART) based on observed patterns.

Table 8.1
Examples of the Diversity of Terms Used to Describe Alternative Reproductive Phenotypes in Male Fish

Conventional male type	Alternative male type
Territorial Nest-holder Parental Cuckold	Sneaker (most widely used term to name the alternative phenotype to designate a male that surreptitiously mates with a female within the territory of the conventional male; e.g. Gross, 1982; Taborsky, 1994)
Type I (term used in Batrachoids, e.g. Bass, 1993; Modesto and Canário, 2003a)	Female-mimic/ Pseudo-female (e.g. alternative males mimic females to achieve parasitic fertilisations, Taborsky, 1994, 1997)
Terminal phase (term used in Wrasses, e.g. Warner and Robertson, 1978)	Streaker (e.g. alternative males in pelagic spawning species that rush-in to join the mating pair, Warner and Robertson, 1978)
Courting (unifying term proposed by Brantley *et al.*, 1993b)	Satellite (e.g. alternative morphs associated with particular territories or conventional males, which usually tolerate their presence, Gross, 1982; Taborsky, 1994; Oliveira *et al.*, 2002b)
Bourgeois (functional unifying term proposed by Taborsky, 1997)	Opportunistic Cuckolder Type II (term used in Batrachoids, e.g. Bass, 1993; Modesto and Canário, 2003a) Initial phase (term used in Wrasses, e.g. Warner and Robertson, 1978) Non-courting (unifying term proposed by Brantley et al., 1993b) Parasitic (functional unifying term proposed by Taborsky, 1997)

Porichthys notatus; Bass, 1993), whereas others represent particular aspects of the expression of the tactic in a given species (initial *vs* terminal phase males in wrasses; Warner and Robertson, 1978). Therefore, the use of unified terms is highly recommendable to allow a comparative approach in the study of ART. However, the use of the pair of terms *territorial* versus *sneaker* that is present in several species (see Taborsky, 1994 for examples) is not appropriate because conventional male phenotypes do not need to necessarily be territorial (e.g., peacock blenny; Almada *et al.*, 1994). Brantley *et al.* (1993b) used the terms *courting* versus *noncourting* male morphotypes, but once again courtship behaviour is not present in all conventional reproductive phenotypes (e.g., peacock blenny; Almada *et al.*, 1995; Gonçalves *et al.*, 1996). Finally, Taborsky (1997) proposed the use of the terms *bourgeois* versus *parasitic* as the most adequate to describe the functional asymmetry in investment to gain access to a mate between the conventional and the alternative phenotype. Bourgeois males actively compete among themselves for the access to females, whereas parasitic males exploit the investment of bourgeois males to fertilise eggs (Taborsky, 1997). Thus, bourgeois males invest in mate attraction traits such as morphological ornaments (e.g., extension of the tail in swordtails, *Xiphophorus spp.*; Basolo, 1990), mating vocalisations (e.g., humming calls in plainfin midshipman, *Porichthys notatus*; Brantley and Bass, 1994; see also Chapter 2), the release of sex-pheromones (e.g., sex-pheromone producing anal gland in blennies; Laumen *et al.*, 1974; see also Chapter 9), or the elaboration and ornamentation of nests (e.g., Mediterranean wrasses; Lejeune, 1985). Conversely, parasitic males exploit the investment of bourgeois males in various possible ways: (1) they may try to approach the spawning site without being noticed, possibly even using female mimicry (e.g., adoption of female colours and courtship behaviours by sneaker males in the peacock blenny; Gonçalves *et al.*, 1996); (2) darting to the mating pair and releasing their sperm before the bourgeois male can react to its presence (e.g., streaking males in wrasses; Warner, 1984); or (3) cooperating with bourgeois males so that they tolerate their presence in the breeding grounds (e.g., satellite behaviour in rock-pool blenny, *Parablennius parvicornis*; Oliveira *et al.*, 2002b). Thus, without the initial investment by the bourgeois male, the alternative phenotype would not be functional.

It should be stressed here that the term *parasitic male* in this context merely indicates that these males exploit the investment of the bourgeois males in mate attraction, and it does not refer to the relationship between the alternative phenotypes, which can range from almost true mutualism when both types of males appear to benefit, to true parasitic where only the parasitic males appear to benefit (Taborsky, 1999, 2001).

2.3. Why Are Alternative Reproductive Phenotypes So Common in (Male) Fish?

Although ART are present in all vertebrate taxa (for a review see Caro and Bateson, 1986), teleosts are by far the taxon with the highest incidence of species with alternative reproductive phenotypes (Taborsky, 1994, 1999, 2001). The last published count of ART in fish identified 140 species of 28 different families (Taborsky, 1998; Figure 8.2), and new examples of species with ART are described annually, even among common temperate species that are studied on a regular basis such as Mediterranean parrotfish, *Sparisoma cretense* (de Girolamo *et al.*, 1999), grass goby, *Zosterisessor ophiocephalus* (Mazzoldi *et al.*, 2000), two species of triple fin blennies (Neat,

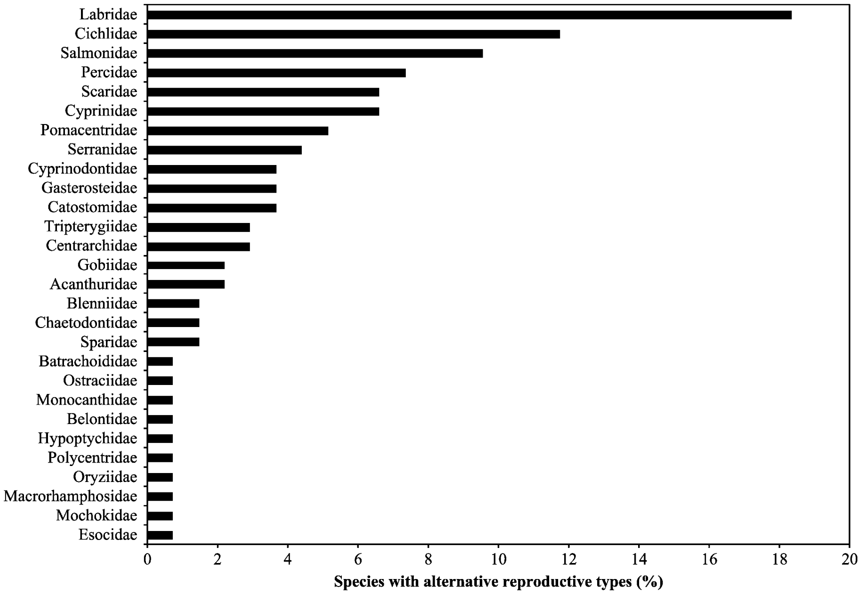

Fig. 8.2. Relative abundance (%) of alternative reproductive tactics in teleosts described in the literature up to 1998 distributed by taxonomic family. (Adapted from Taborsky, 1998.)

2001), black goby, *Gobius niger* (Mazzoldi and Rasotto, 2002), and dusky frillgoby, *Bathygobius fuscus* (Taru *et al.*, 2002). Thus, the numbers given by Taborsky (1998) should be regarded as an underestimate of the prevalence of ART among fish.

Three main factors have been identified that may predispose male fish to ART:

1. Indeterminate growth resulting in large size differences among sexually mature males;
2. External fertilisation permitting simultaneous parasitic spawning; and
3. Male parental care which provides parasitic males with a high payoff or benefit from breeding without incurring the high costs (in terms of time and energy allocation) associated with parental care (Taborsky, 1999).

Indeterminate growth can create a significant asymmetry among the sex competing for mates (usually the males). Taborsky (1999) estimated that the average difference between the largest and the smallest reproductive males for species for which data was available, and concluded that on average the largest breeding males were 18 times larger than their smallest counterparts.

With such extreme size differences among breeding competitors, smaller males may be more successful if they adopt a parasitic tactic than if they act as bourgeois males. However, this does not mean that parasitic males should always be the smallest individuals among their age class. The particular aspects of the parasitic tactic being adopted may determine the optimal relative body size for that specific tactic. For example, satellite males in the Azorean rock-pool blenny help the territorial nest-holder males to defend their breeding territory and thus are the largest of their age class (Oliveira *et al.*, 2002b). On the other hand, in the peacock blenny, sneaker males mimic females to get access to nests during spawning episodes, with larger sneakers being more easily detected by nest-holder males than smaller ones (Gonçalves *et al.*, 2005). Thus, in the peacock blenny, sneaker males are the smallest of their age class (Oliveira *et al.*, 2001f; Figure 8.3). Indeterminate growth also creates the need for the central nervous system to continue growing in adulthood (Zupanc, 2001). The consequent evolution of adult neurogenesis in teleosts in theory would have facilitated the plasticity of the neural circuits underlying reproductive behaviour during the lifetime of an individual and thus created the opportunity for the emergence of ART.

A second factor that may favor the occurrence of ART among fish is the prevalence of external fertilisation, which allows simultaneous parasitic spawning, not possible in species with internal fertilisation (Taborsky, 1999). Nevertheless, ARTs are also present in teleosts with internal fertilisation. This paradox may be explained by the fact that the evolution of ART in live-bearing teleosts is accompanied by the evolution of specialisations in the parasitic morph that increase the probability of fertilisation. For example, in poeciliids—in which males may inseminate females after courting them (bourgeois tactic) or by using forced copulations (parasitic tactic; for a review of alternative reproductive behaviour in poeciliid fish, see Farr, 1989)—an association between the frequency at which each behaviour is used and male morphology is present both at the intra- and interspecific levels. In guppies (*Poecilia reticulata*), males with longer gonopodia engaged significantly more often in forced copulations than males with shorter gonopodia (Reynolds *et al.*, 1993); in the sailfin molly (*Poecilia latipinna*), small males, specialised in gonopodial thrusts, have higher relative gonopodium lengths than large males (R. F. Oliveira, D. M. Gonçalves, and I. Schlupp, unpublished data, 2000). Also, there is an interspecific association among poeciliids between gonopodium length and the use of gonopodium thrusts rather than courtship displays (Rosen and Tucker, 1961).

Finally, because paternal care is widespread among teleosts (Sargent and Gross, 1993), in species with male parental care, parasitic males would benefit from a high payoff by being able to breed without incurring the high costs (in terms of time and energy allocation) associated with parental care.

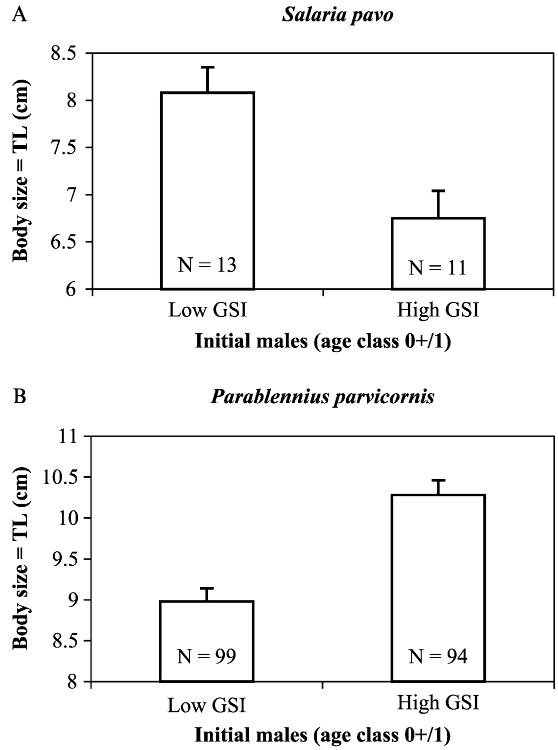

Fig. 8.3. Investment in gonadal tissue by the young-of-the-year (age class 0+ and 1) in two closely related blenniid species differs according to different types of alternative reproductive tactics. (A) In the peacock blenny, *Salaria pavo*, the parasitic tactic consists of female-mimicry and the smaller individuals among the 0+/1 year-old are the ones that display the tactic. (B) In the Azorean rock-pool blenny, *Parablennius parvicornis*, the parasitic tactic consists of behaving as territory satellites of nest-holders helping them in territorial defense and the larger individuals of the 0+/1 year-olds are the ones that exhibit the tactic. (Adpated from Oliveira *et al.*, 2001f.)

However, in both wrasses and cichlids, the two fish families with the highest prevalence of ART (see Figure 8.2), there was no association between the occurrence of ART and the mode of parental care (based on data compiled by Taborsky, 1994, 1999).

In summary, among the three factors usually used to explain the high incidence of ART in fish, high intrasexual size differences among reproductive competitors seems to be the most powerful factor. This may result from the fact that ART may have evolved in response to scenarios of high levels of intrasexual selection (but see Jones *et al.*, 2001). Apart from these ultimate factors there are also proximate factors that might help to explain the high numbers of fish species with ART, namely the high variability and lability of sex determination and differentiation mechanism and the brain bipotentiality of fish. These two factors will be discussed below (see Sections 3 and 4, respectively).

The occurrence of ART described above considers male alternative phenotypes. Reports of female ART are less common, which can be due to two factors: (1) most of the studies so far have focused on alternative male behaviours, possibly because in most fish species female sexual behaviour is less conspicuous than male behaviour (Breder and Rosen, 1966; Thresher, 1984); and/or (2) because intrasexual selection is usually higher among males than among females, indeterminate growth and its associated pattern of high intrasexual size differences would mainly have consequences for the competing sex, that is the males, because females seldom compete for breeding resources or for males, except in species with sex-role reversal (e.g., some Syngnathidae; Berglund and Rosenqvist, 1993). Nevertheless, female alternative reproductive behaviours have also been documented (Henson and Warner, 1997). For example, in the blueheaded wrasse (*Thalassoma bifasciatum*), pair-spawning sites and group-spawning sites exist, and females have a consistent pattern of their preferred mode of mating: some prefer group-spawnings, whereas others prefer pair-spawnings (Warner, 1987, 1990). Interestingly, almost all large-size females prefer pair-spawning irrespective of their previous preference at smaller size (Warner, 1985). This data suggests a mixed situation with fixed ART among smaller females followed by sequential ART when they grow older.

Despite the interest of female ART, this chapter will concentrate on male ART in the following sections and will address the proximate mechanism underlying the expression of discrete alternative phenotypes.

3. SEX DETERMINATION, SEXUAL DIFFERENTIATION, AND ALTERNATIVE REPRODUCTIVE PHENOTYPES

Historically, the conceptual framework of sex determination and sexual differentiation in vertebrates has been greatly influenced by the mammalian model of genotypic sex determination (GSD). In mammals, the sex-determining gene (*Sry*) in the Y chromosome induces the differentiation of a testis from the primordial bipotential gonad, and the differentiated testis then produces hormones that will start a cascade of events, which will simultaneously promote the masculinisation of some traits and the defeminisation of others, leading to sexual differentiation (Zarkower, 2001). In the differentiated gonad, Sertoli cells produce Müllerian inhibiting substance

(MIS) that promotes the regression of the Müllerian ducts, whereas Leydig cells produce testosterone (T) that differentiates a set of male traits: (1) the Wolffian ducts differentiate into male accessory reproductive organs (e.g., vas deferens, seminiferous tubules); (2) the differentiation of the male genitalia from the genital bud induced by dihydrotestosterone (DHT), which results from the local metabolisation of T by the enzyme 5-α-reductase; and (3) the organisation of the brain (hypothalamus/preoptic area) and the pituitary gland in a male fashion, that translates into a tonic release of gonadotropins from the pituitary and in the expression of male sexual behaviour (mounting and intromission) later in adulthood. In XX embryos, the lack of *Sry* allows ovary differentiation, and in the absence of testicular androgens and MIS, female traits differentiate: (1) the lack of the repressor effect of MIS allows the Müllerian ducts to develop into the female reproductive accessory organs (i.e., uterus and fallopian tubes), and the lack of T leads to the regression of the Wolffian ducts; (2) in the absence of DHT, a female genitalia develops; and (3) in the absence of T, a female brain organisation emerges that results in a female pattern of cyclic gonadotropin release from the pituitary, and in female typical behaviour (sexual receptivity; e.g., lordosis in rodents). Thus, in mammals the cascade of events that leads to morphological and behavioural sex differentiation is mainly orchestrated by gonadal sex steroids.

In fish, sex determination is more labile than in mammals. Cases of genotypic sex determination (GSD) and environmental sex determination (ESD) have been described in fish, and GSD mechanisms are more flexible and open to environmental influences in fish than in mammals (Devlin and Nagahama, 2002). GSD mechanisms in fish are very diverse and range from polygenic systems to systems with dominant sex-determining factors mixed with autosomal controls, to sex chromosomes with either heterogametic males (XY) or females (ZW) (Devlin and Nagahama, 2002). Cytogenetical data revealed that sex chromosomes are present in approximately half the species in which they were searched for (Devlin and Nagahama, 2002). However, this number may be underestimated because many related species have been investigated thus promoting a phylogenetic bias in the outcome (Devlin and Nagahama, 2002). Nevertheless, it is interesting to note that the number of species that display male heterogamety is twice the number of those with female heterogamety (Devlin and Nagahama, 2002). Although a sex-determining gene has not yet been found in fish, a strong candidate has recently been identified on the Y chromosome of medaka. Like the mammalian *Sry* gene, this gene (*DMY,* belonging to the DM-domain gene family, named after the *Dsx* and *mab–3* male specific regulatory genes in *Drosophila* and *Caenorhabditis elegans*, respectively) is expressed only in somatic cells of the male gonad at the time of sex determination (Matsuda *et al.*, 2002).

There are two main classes of ESD in fish: one is where sex is regulated by temperature (i.e., temperature sex determination [TSD]), with high temperatures promoting male differentiation and more females being produced at low temperatures; and the second is where sex is regulated by social factors (behavioural sex determination [BSD]). Both of these may occur before (primary ESD or BSD) or after (secondary ESD or BSD) sexual maturation (Nakamura *et al.*, 1998; Strüssman and Nakamura, 2002; Godwin *et al.*, 2003). Primary BSD has been documented in the Midas cichlid, *Cichlasoma citrinellum*, where the larger brood mates differentiate as males and the smaller as females, an effect that was demonstrated to be due to relative size by experimentally reversing the within-broods relative body sizes (Francis, 1990, 1992). Also, in the paradise fish, *Macropodus opercularis*, some evidence for prematurational BSD is present. In an artificial selection experiment for divergent lines of social dominance, Francis (1984) found that the high-dominance line consisted almost entirely of males and the low-dominance line of females. Thus, social dominance seems to be affecting sexual differentiation in this species. Postmaturational BSD has been more widely documented and comprises cases of functional sex-change in adulthood (Francis, 1992; Godwin *et al.*, 2003).

Sex steroids have been implicated in all three mechanisms of fish sex determination (i.e., GSD, TSD, and BSD; Devlin and Nagahama, 2002; Godwin *et al.*, 2003). In primary sex differentiation of species with GSD, endogenous estrogen acts as an ovarian inducer (Nakamura *et al.*, 2003), with aromatase playing a key role in all fish species investigated so far such as rainbow trout, *Oncorhynchus mykiss* (Guiguen *et al.*, 1999), Nile tilapia, *Oreochromis niloticus* (Guiguen *et al.*, 1999; Kown *et al.*, 2001), Mozambique tilapia, *Oreochromis mossambicus* (Tsai *et al.*, 2000), Japanese flounder, *Paralychthys olivaceus* (Kitano *et al.*, 1999; Trant *et al.*, 2001), and zebrafish, *Danio rerio* (Trant *et al.*, 2001). The involvement of androgens as testicular inducers is less clear and seems to vary among species (e.g., Govoroun *et al.*, 2001; Liu *et al.*, 2000; Nakamura *et al.*, 2003). Apart from steroids, other factors have been involved in fish sexual determination and differentiation including genes already known to be involved in sex differentiation pathways in other vertebrate and invertebrate taxa (e.g., *DMRT1*, *Sox9*, *DAX1*; see Baron and Guiguen, 2003 and references therein). Thus, a complete synthesis of fish sex determination and differentiation pathways is still difficult to achieve, which in part may result from the high diversity of sex-determining mechanisms found among teleosts.

Sex-changing species with BSD after sexual maturation are relevant for the understanding of the differentiation of alternative phenotypes within the same sex (more than any species with other ESD mechanisms) because they also exhibit a phenotypic reorganisation in adulthood. In these species, an association between changes in circulating sex steroid levels and sex change is present. In protogynous species (i.e., sequential hermaphrodites with female-to-male sex change), the levels (plasma concentrations or synthesis rates of *in vitro* incubations of gonadal tissue) of 11-ketotestosterone (KT), the most potent androgen in fish (Borg, 1994), increase but estradiol concentrations decline during female-to-male sex change, such as in saddleback wrasse, *Thalassoma duperrey* (Hourigan *et al.*, 1991) and stoplight parrotfish, *Sparisoma viride* (Cardwell and Liley, 1991). However, in protandrous fish (male-to-female sex change), the opposite changes occur, such as in anemonefishes, *Amphiprion melaropus, A. frenatus* (Godwin and Thomas, 1993; Nakamura *et al.*, 1994), and black porgy, *Acanthopagrus schlegeli* (Lee *et al.*, 2001). Similarly, KT levels are generally higher in the male phase and E2 levels in the female phase in both protogynous species such as saddleback wrasse, *Thalassoma duperrey* (Hourigan *et al.*, 1991), bambooleaf wrasse, *Pseudolabrus japonicus* (Morita *et al.*, 1997), blackeye goby, *Coryphopterus nicholsii* (Kroon and Liley, 2000), red grouper, *Epinephelus morio* (Johnson *et al.*, 1998), Hong Kong grouper, *Epinephelus akaara* (Tanaka *et al.*, 1990), and black seabass, *Centropristis striatus* (Cochran and Grier, 1991), and in protandrous species such as black porgy, *Acanthopagrus schlegeli* (Chang *et al.*, 1994), goldlined seabream, *Rhabdosargus sarba* (Yeung and Chan, 1987), and seabass, *Lates calcarifer* (Guiguen *et al.*, 1993).

In general, T levels are not reliable indicators of the sex phase in sequential hermaphroditic fish (Devlin and Nagahama, 2002). Moreover, in simultaneous hermaphrodites, KT levels tend to be positively correlated with the adoption of a male role by the individual (Cheek *et al.*, 2000). However, socially induced behavioural sex change can apparently occur in gonadectomised individuals in the bluehead wrasse, *Thalassoma bifasciatum* (Godwin *et al.*, 1996), which indicates that although sex steroids might be playing a major role in morphological sex differentiation in sequential hermaphroditic fish, they are not needed for behavioural sex change. The occurrence of BSD in sequential hermaphrodites also stresses the fact that the brain must have primacy over the gonads in the decision-making process of sexual differentiation. As Francis (1992) phrased it: "the only way the behaviour can affect the gonads is through the brain." Thus, one fundamental difference between teleost and mammalian sex differentiation is the fact that, although in mammals the differentiation of the gonad will lead the whole process of sexual differentiation including the sexual differentiation of brain and behaviour, mainly through organisational actions of sex steroids (but see Carruth *et al.*, 2002), in fish the brain seems to be the initial site of sex differentiation and the pattern of brain sex differentiation will then determine gonadal sex differentiation (Francis, 1992; Grober, 1998; Reavis and Grober, 1999). This crucial difference may explain the extreme sexual plasticity found among fish and the environmental and social influences on teleost sexuality that are present even after sexual maturation both in sex-changing and gonochoristic species.

How can the understanding of the sex differentiation mechanism in fish contribute to the understanding of the differentiation of alternative reproductive phenotypes? First, GSD mechanisms might be seen as homologous to the mechanisms underlying fixed ART, whereas ESD can be regarded as homologous to the differentiation of plastic ART, because the former impose lower degrees of freedom in the making of the sexual phenotype (i.e., there is a genetic constraint in the development of the alternative phenotypes) than the latter. Second, the sequential (irreversible) versus reversible patterns within plastic ART can be seen as a parallel to primary versus secondary patterns of ESD, respectively. Thus, the study of sex determination and differentiation mechanisms may provide important hints for the investigation of the proximate mechanisms of alternative reproductive tactics. To investigate the potential relationship between the types of ART displayed and the modes of sex determination in fish, data were compiled on these two parameters from the literature, which is summarised in Table 8.2. The following conclusions can be drawn from an analysis of Table 8.2:

1. Fixed ART are equally distributed among GSD and ESD, but most species listed with fixed ART and GSD (i.e., 60%) are salmonids, whereas species with fixed ART and ESD are centrarchids (also 60% of the species). These results suggest that fixed tactics do not have necessarily to be based on genetic mechanisms and can rely both on genetic and on environmental mechanisms, depending on the historical evolutionary pathway of the ART on a specific phylogenetic group. This implies that the same type of ART might have evolved separately in different teleost taxa using different mechanisms, which were probably constrained by the existing sex-determining mechanism of each species.
2. All but one of the species displaying plastic irreversible ART have ESD, which suggests that individuals from species with ESD are more prone to respond to environmental cues during their adult lives and thus maintain their phenotypic plasticity during their lifetime. This allows them to reorganise (or redifferentiate) their phenotyes at a postmaturational life stage.
3. Reversible ARTs are also equally distributed among GSD and ESD, but again a phylogenetic bias is present (i.e., 50% of species with reversible ART and GSD are cichlids and 50% of the species with reversible ART and ESD are sticklebacks). This result might sound

Table 8.2
Relationship Between Sex Determination Mechanisms and Alternative Reproductive Phenotypes in Fish

		Sex determining mechanism	
		GSD	ESD
Alternative reproductive tactic or strategy	Fixed	Coho salmon, *Oncorhynchus kisutch*	Bluegill sunfish, *Lepomis macrochirus*
		Pacific salmon, *Oncorhynchus tshawytscha*	Longear sunfish, *Lepomis megalotis*
		Atlantic salmon, *Salmo salar*	Spotted sunfish, *Lepomis punctatus*
		Guppy, *Poecilia reticulata*	Plainfin midshipman, *Porichthys notatus*
		Swordtail, *Xiphophorus nigrensis*	Corkwing wrasse, *Symphodus melops*
	Plastic irreversible (Sequential)	Rainbow wrasse, *Coris julis*	Rock-pool blenny, *Parablennius parvicornis*
			Peacock blenny, *Salaria pavo*
			Grass goby, *Zosterisessor ophiocephalus*
			Black goby, *Gobius niger*
			Common goby, *Pomatoschistus microps*
			Sand goby, *Pomatoschistus minutus*
			Dusky frillgoby, *Bathygobius fuscus*
			Stoplight parrotfish, *Sparisoma viride*
			Blueheaded wrasse, *Thalassoma bifasciatum*
			Mediterranean wrasse, *Symphodus ocellatus*
	Plastic reversible	Sailfin molly, *Poecilia velifera*	Three-spined stickleback, *Gasterosteus aculeatus*
		Mozambique tilapia, *Oreochromis mossambicus*	Fifteen-spined stickleback, *Pungitus pungitus*
		St. Peter's fish, *Sarotherodon galilaeus*	Damselfish, *Chromis chromis*
		Sergeant major, *Abudefduf saxatilis*	Dusky farmerfish, *Stegastes nigricans*

Sources: sex-determining mechanisms based on fish cytogenetic data presented in Devlin, R. H., and Nagahama, Y. (2002) and in Froese and Pauly (2004); type of ART based on Taborsky (1994, 1999, 2001) and references therein.

> odd because reversible ART represents the extreme expression of phenotypic plasticity, and thus a prevalence of ESD could be expected. However, it should be stressed that in all cases listed in Table 8.2 in this category (reversible ART), the differences between alternative phenotypes are mostly behavioural with little or no morphological differentiation between the two male types (e.g., three-spined stickleback, *Gasterosteus aculeatus*; Jamieson and Colgan, 1992). In fact, in most cases listed, the same individual can adopt one of the alternative tactics depending on context, such as nest density in breeding colonies of pomacentrids (Tyler, 1989; Picciulin *et al.*, 2004) or internest distance in sticklebacks (Goldschmidt *et al.*, 1992). Thus, high levels of phenotypic plasticity, which result in facultative reversible tactics, might require rapid and transient changes in neural activity (Zupanc and Lamprecht, 2000; Hofmann, 2003) independent of hormone-induced changes in gene expression and usually underlie ARTs that are stable over longer time periods (i.e., fixed and sequential ART). Hence, reversible ART may have become independent from a sex-differentiation mechanism ruled by sex hormones.

Interestingly, using examples from all vertebrate groups, Crews (1998) came to a different conclusion: he argued that among vertebrates all species with fixed tactics displayed GSD, but species with plastic tactics might display either GSD or ESD. The discrepancy between Crews' results and those presented here might reflect the fact that all the nonpiscine species (i.e., two amphibia, five reptiles, one bird, and three mammals) included in his analysis had GSD (see Table 1 in Crews, 1998). Furthermore in that study, a uniform taxon level of analysis was not used (i.e., in most cases the species level was used but in some other cases families were entered as the taxonomic unit, such as wrasse, angelfish, and parrotfish; see Table 1 in Crews, 1998). This is understandable because the author was providing examples rather than making a comprehensive coverage of all published studies. Unfortunately, these might have confounding effects for a subsequent quantitative analysis.

The analogy between processes of sexual differentiation (i.e., males *vs* females) and the differentiation of discrete alternative reproductive phenotypes within the same sex made it also plausible to consider a role for sex steroids in the differentiation of intrasexual alternative phenotypes. The view of sex steroids as candidates for the proximate control of ART becomes even stronger if one considers the fact that they are one of the pillars of fish reproduction because they are involved in the control of sexual maturation, in the development of secondary sex characters, and in the expression of reproductive behaviour (Borg, 1994).

4. SEX STEROIDS AND ALTERNATIVE REPRODUCTIVE PHENOTYPES

4.1. Patterns of Sex Steroids in Species with Alternative Reproductive Tactics

Sex steroids are good candidates to play a key role in the expression of male ART in fish considering their involvement in teleost sex differentiation described above. Also, sex steroids can be considered as intercellular honest signals (*sensu* animal communication theory; Bradbury and Vehrencamp, 1998) sent by the gonads as they mature to the rest of the body, to coordinate the expression of sexual behaviour and the differentiation of sexual characters with the availability of mature gametes to be released (Oliveira and Almada, 1999). Among the sex steroids, the androgens are a first choice because they are deeply involved in male reproductive physiology (e.g., Borg, 1994).

4.1.1. ANDROGENS AND ART

A first review of the available data on androgen levels in species with ART was given in Brantley *et al.* (1993b). These authors found that alternative phenotypes differ in their circulating 11-ketotestosterone (KT) levels, with bourgeois males having significantly higher levels of circulating KT than parasitic males, and that there was no clear pattern regarding testosterone (T): it was higher in bourgeois males in some species, higher in parasitic males in others, and there was also a species in which no differences were found between the two morphs. The six species surveyed were not closely related to each other, and thus phylogenetical bias is probably not present in their qualitative analysis. The author has updated the database used by Brantley *et al.* (1993b) with the data that has been published since then. Currently data on 16 species from nine different teleost families is available (Table 8.3). A reanalysis of the data yields similar results to those obtained by Brantley *et al.* (1993b): there is a strong association between the ART and KT, but not with T. In 13 out of the 16 species (81.3%) with ART for which sex steroid levels (or synthesis rates of *in vitro* incubations of gonadal tissue) are available, KT is higher in the bourgeois than in the parasitic males (Table 8.3).

The three exceptions to this rule are two species with reversible ART (monogamous vs. sequential polygamous males in the St. Peter's fish, *Sarotherodon galilaeus* and large courting males vs. small courting males in the sailfin molly, *Poecilia velifera*) and one species with sequential ART (breeders vs. sexually active helpers in the cooperative brooder cichlid *Neolamprologus pulcher*). Two kinds of factors that may help to explain these exceptions will be discussed below.

Table 8.3

Comparison of Sex Steroid Levels Between Bourgeois and Parasitic Males, in Relation to the Type of Alternative Tactic and the Presence/Absence of Intrasexual Dimorphism

FAMILY/ Species	Alternative phenotypes	Intrasexual dimorphism	KT	T	E2	DHP
BATRACHOIDIDAE						
Lusitanean toadfish, *Halobatrachus didactylus*[1]	Fixed?	+	B > P	B = P	B = P	B = P
Plainfin midshipman, *Porichtys notatus*[2,3]	Fixed: Type I calling vs. Type II non-calling males	+	B > P	B<P	B = P	—
BLENNIIDAE						
Peacock blenny, *Salaria pavo*[4,5]	Sequential: Nest-holders vs. female-mimic sneakers	+	B > P[a]	B > P[a]	—	—
Rock-pool blenny, *Parablennius parvicornis*[6]	Sequential: Nest-holders vs. satellites	+	B > P	B = P	—	—
CENTRARCHIDAE						
Bluegill sunfish, *Lepomis macrochirus*[7,8]	Fixed: Parentals vs.sneakers and satellites	+	B > P	B = P	—	—
Longear sunfish, *Lepomis megalotis*[9]	Fixed?	+	B > P[b]	B = P[b]	—	—
CICHLIDAE						
Princess of Burundi, *Neolamprologus pulcher*[10,*]	Sequential: breeders vs. helpers	—	B = P[c]	B = P[c]	—	—
Mozambique tilapia, *Oreochromis mossambicus*[*, 11,12]	Reversible: territorial courting vs. non-territorial female-mimics	+	B > P[d]	B > P[d]	—	B > P[d]
St. Peter's fish, *Sarotherodon galilaeus*[13,*]	Reversible: monogamous vs. polygynous males	—	B = P	B = P	—;	B = P
LABRIDAE						

Corkwing wrasse, *Symphodus melops*[14,15]	Fixed: Territorial vs. female-mimics	+	B > P	B < P	B<P	—
Rainbow wrasse, *Coris julis*[16]	Sequential: Initial phase vs. terminal phase males	+	B > P[f]	—	—	—
Saddleback wrasse, *Thalassoma duperrey*[17]	Sequential: Initial phase vs. terminal phase males	+	B > P	B = P	B = P	B = P
POECILIIDAE						
Sailfin molly, *Poecilia velifera*[18,*]	Reversible: large courting vs. small non-courting males	—	B = P[c]	B = P[c]	—	—
SALMONIDAE						
Atlantic salmon, *Salmo salar*[19]	Fixed: mature parr vs. anadromous males	+	B > P[e]	B<P[e]	—	B = P[e]
SCARIDAE						
Stoplight parrotfish, *Sparisoma viride*[20]	Sequential: Initial phase vs. terminal phase males	+	B > P	B > P	B<P	—
SERRANIDAE						
Belted sandfish, *Serranus subligarius*[21]	Reversible: streakers vs. pair spawners in a simultaneous hermaphrodite	—	B > P	—	—	B > P

KT, 11-ketotestosterone; T, testosterone; E2, estradiol; DHP, 17α, 20β-dihydroxy–4-pregen–3-one; B, bourgeois; P, parasitic.

[a] Testicular androgen levels (ng steroid/g of tissue).

[b] Values extrapolated from graph in Knapp, 2004, Figure 3.

[c] Steroid levels in fish holding water (ng steroid/h/g body mass).

[d] Urinary sex steroid levels (ng steroid/ml urine).

[e] Values for late summer, when GSI values peak.

[f] In vitro gonadal production from $[^{14}C]$ T incubation.

[1] Modesto and Canário, 2003a; [2] Brantley *et al.*, 1993b; [3] Bass, 1992; [4] Oliveira *et al.*, 2001b; [5] Gonçalves *et al.*, 1996; [6] Oliveira *et al.*, 2001c; [7] Kindler *et al.*, 1989; [8] Neff *et al.*, 2003; [9] Knapp, 2004; [10] Oliveira *et al.*, 2003; [11] Oliveira and Almada, 1998a; [12] Oliveira *et al.*, 1996; [13] Ros *et al.*, 2003; [14] Uglem *et al.*, 2000; [15] Uglem *et al.*, 2002; [16] Reinboth and Becker, 1984; [17] Hourigan *et al.*, 1991; [18] R. F. Oliveira, D. M. Gonçalves and I. Schlupp, unpublished data, 2000; [19] Mayer *et al.*, 1990; [20] Cardwell and Liley, 1991; [21] Cheek *et al.*, 2000. *Data based upon lab/pond studies that remain to be verified in the field.

First, differences in androgen (KT) levels may be less important in species with ART that lack major tactic-specific morphological specialisations (e.g., the expression of male secondary sex characters in bourgeois males) because KT has been demonstrated to be the most potent androgen in the induction of secondary sex characters in male teleosts (Borg, 1994). Indeed, in all three species, the main differences between the alternative male types are behavioural and of relative body size in the sailfin molly and in *N. pulcher*. No major morphological differences are present between these alternative morphs. Thus, differential KT levels are not necessary to induce or to maintain tactic-specific morphological characters. Therefore, if this hypothesis is correct, an association between the degree of dimorphism among the alternative male phenotypes and the differential variation in KT levels among alternative morphs is to be expected. In fact, from all listed species for which the ART involves a morphological intrasexual dimorphism (apart from differences in body size), the KT levels are higher in the bourgeois than in the parasitic male, and irrespective of the ART they display (Table 8.3). This suggests that androgens may play a major role in morphological differentiation between the alternative phenotypes, but will not be essential for the occurrence of behavioural sexual plasticity among fish. Two cases listed in Table 8.3 are worth mentioning in this context: the Mozambique tilapia, *Oreochromis mossambicus,* that exhibits a reversible ART and displays differential KT levels between the two male types, and the simultaneous hermaphrodite, *Serranus subligarius*, that exhibits differences in KT levels depending on the mating behaviour displayed (pair-spawning *vs* streaking) in the absence of sexual polymorphism (Cheek *et al.*, 2000). In the Mozambique tilapia, although the tactic is reversible, it is more constant in time and a morphological differentiation between territorial courting males and subordinate female-mimic males is present (Oliveira and Almada, 1998b). In *S. subligarius*, no polymorphism is present but in this simultaneous hermaphrodite, pair-spawning behaviour is associated with a larger body size, although no association was found between KT levels and testis mass (Cheek *et al.*, 2000).

Second, in species with a high degree of behavioural phenotypic plasticity, such as the case of species of reversible ART in which individuals can very rapidly switch reproductive tactics according to local or temporal conditions, differences in androgen levels underlying these rapid changes in behaviour are not necessarily expected, with changes on the activity of neural pathways being a more parsimonious explanation (Hofmann, 2003; but see Oliveira *et al.*, 2001g; Remage-Healey and Bass, 2004). This could explain the lack of differences in KT levels between alternative male types both in the St. Peter's fish and in the sailfin molly. In the St. Peter's fish, Fishelson and Hilzerman (2002) found that in captivity the mating system is very flexible and males varied their reproductive behaviour very quickly according to local conditions. For example, in environments with female-biased operational sex ratios (OSR), the expression of polygyny is promoted and conversely male-biased OSR facilitates the expression of monogamous behaviour in *S. galilaeus* males (Ros *et al.*, 2003). Also, in the sailfin molly, although there is a trend for a specialisation of large males in courtship and of small males in gonopodial thrusting, both small and large males can use both behaviours facultatively (Schlupp *et al.*, 2001). Finally, in the case of *N. pulcher*, male helpers may share the paternity of the brood (Dierkes *et al.*, 1999) and queue for vacated territories to become breeders (Balshine-Earn *et al.*, 1998). Thus, they are sexually active and must be ready for rapid behavioural changes once the opportunity for a nest takeover occurs.

An inspection of Table 8.3 also shows that in all species with fixed ART, KT levels are always higher in the bourgeois male morph. In fact, the incidence of the differences in KT levels among alternative phenotypes does not seem to be independent of the type of ART present in the species (Table 8.3). This fact prompted the author to analyze the dataset on androgens and ART in a quantitative way and compute, for each species with an available direct or an indirect measurement of androgen circulating levels (this criteria excluded *C. julis* and *S. subligarius* from this analysis), the ratio between the androgen levels in the bourgeois and in the parasitic male (e.g., bourgeois average KT level/parasitic average KT level). Data was collected from the references listed in Table 8.3, and in cases in which the original data was not in the text or in tables, the published figures were scanned to extrapolate the data from the graphs using the UnGraph software package (Biosoft, 1998). If one plots these ART relative androgen levels for each species against its type of ART, there is a trend for a positive association between the degree of phenotypic plasticity (with fixed ART being considered high, sequential ART intermediate, and reversible ART low in phenotypic plasticity) and the magnitude of the difference in KT levels between the male morphs. That is, species with fixed ART tend to display larger differences in KT levels between their alternative male types than do species with plastic ART. For T such a trend is not observed. These results suggest differential roles for the two androgens in the expression of phenotypic plasticity in fish.

Depending on each species, the alternative male types may differ from each other in a set of phenotypic traits, namely in their reproductive behaviour, in the differentiation of secondary sex characters, in the relative investment in gonadal development, and in the differentiation of accessory reproductive organs (e.g., testicular glands in blenniids; Patzner and Lahnsteiner, 1999). The available evidence shows that among androgens KT is more potent in eliciting all characters of this constellation of tactic-specific traits:

1. Androgens and reproductive behaviour: Although there is a large body of literature on the role of androgens in the expression of reproductive behaviours (for reviews see Liley and Stacey, 1983; Borg, 1994), there are few studies that have tested the specific role of KT. In species with male parental care, circulating levels of KT are higher in the courting phase than in the parental phase (Borg, 1994; Oliveira *et al.*, 2002a), and in castration and hormone replacement experiments KT is more effective than T in restoring the different aspects of male reproductive behaviour including territoriality, nest construction, and courtship, such as in the three-spined stickleback, *Gasterosteus aculeatus* (Borg, 1987) and bluegill sunfish, *Lepomis macrochirus* (Kindler *et al.*, 1991). In addition, KT treatment in field conditions is also effective in promoting male "bourgeois" behaviour in the rock-pool blenny (*Parablennius parvicornis*, Ros *et al.*, 2004b). These examples suggest that indeed KT plays a role in the expression of reproductive behaviours, namely in the establishment of a reproductive territory and/or in courtship behaviours.
2. Androgens and secondary sex characters: There is a large body of literature that shows that most of the morphological traits exhibited by bourgeois males are androgen-dependent, and that KT is the most potent androgen in inducing the differentiation of these characters, such as the sonic motor system in Type I male batrachoids (Brantley *et al.*, 1993a; but see Modesto and Canário, 2003b) and sex-pheromone producing anal gland in blenniids (Oliveira *et al.*, 2001b,d,e). Another aspect that supports this view is the fact that in gonochoristic species without sexual dimorphism males present very low levels of KT, such as the *Sardinops melanostictus* (Matsuyama *et al.*, 1991) and *Syngnathus typhle* (Mayer *et al.*, 1993).
3. Androgens and gonadal allocation: A trait that differs considerably among alternative male types is the relative size of the testis. In most species studied, parasitic males have higher gonadosomatic indices (GSI; gonad weight/body weight × 100) than bourgeois males, indicating a higher relative gonadal investment by the former male type (Taborsky, 1994, 1998; but see Tomkins and Simmons, 2002 for a critical assessment of the use of GSI values to measure relative gonadal investment in species with ART). Besides gonadotrophins, androgens also participate in the regulation of spermatogenesis. However, KT and T seem to play different roles: KT stimulates later stages of this process, whereas T is involved in the negative-feedback mechanisms needed to control KT-dependent spermatogenesis (Schulz and Miura, 2002). Consequently, a balance between T and KT is needed for the control of spermatogenesis (Schulz and Miura, 2002).

> This suggests that the ratio between KT and T should be more informative than absolute androgen levels for the understanding of differential gonadal allocation between alternative male types. Data was gathered from the literature on GSI values for 9 out of the 14 species for which we have both KT and T levels, and thus a KT:T ratio can be computed. For these nine species (*P. notatus, H. didactylus, L. macrochirus, S. melops, S. pavo, P. parvicornis, T. duperrey, O. mossambicus* and *S. galilaeus*) there was a nonsignificant trend for the magnitude of the difference in the KT:T to be negatively correlated with the magnitude of the difference in GSI values between alternative male types (Rs = –0.63, P = 0.067). That is, in species for which the magnitude of the difference in KT levels between bourgeois and parasitic is larger, there is a smaller difference in relative gonad size. This also means that among parasitic males a higher GSI is associated with a lower KT:T ratio, which probably allows them to have larger testis without a linked expression of the secondary sex characters and of bourgeois male behaviour.

The results reported above (Figure 8.4) also suggest that in fixed ART a higher magnitude of differences in KT levels between phenotypes might be the result of early (i.e., prematurational), long-lasting, and irreversible actions of KT in the differentiation of the ART, resembling the organisational

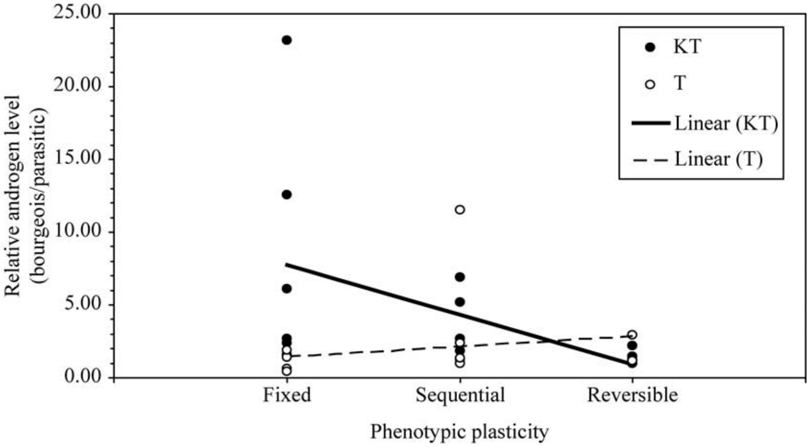

Fig. 8.4. Relative androgen levels between bourgeois and parasitic males (i.e., ratio between the androgen concentrations in the bourgeois male over the androgen concentration in the parasitic male) in teleost species with different types of alternative reproductive tactics. KT, 11-ketotestosterone; T, testosterone; solid line, linear fit curve for KT values; dashed line, linear fit curve for T values.

effects of sex steroids in mammalian sex differentiation. In the other extreme, the low magnitude in KT ratios between morphs in species with reversible ART would reflect the transient nature of this type of tactic, which parallels the activational effects of steroids on behaviour (see Section 4.2 for a further discussion of this hypothesis).

4.1.2. Estrogens and ART

Data on estrogen levels in species with ART is scarce (Table 8.3). This paucity of data probably reflects the focus of research on male ART. Only in five studies has estradiol (E2) been measured. Among the Batrachoididae (i.e., the plainfin midshipman and the Lusitanian toadfish), E2 levels do not differ between males of both phenotypes, with the majority of males having undetectable levels (Brantley *et al.*, 1993b; Modesto and Canário, 2003a). However, in the midshipman, a large proportion of type I males (ca. 50%) display low E2 levels during the breeding season (Sisneros *et al.*, 2004). The functional significance of this finding is unclear. Recent work found that aromatase levels in the testis of midshipman is very low or even undetectable, contrasting to high concentrations found in the brain (Forlano *et al.*, 2001), which suggests the brain as one potential source for these circulating low levels of E2 found in type I males during the breeding season.

In the saddleback wrasse, E2 levels were detectable at similar low levels both in initial and terminal phase males, and no seasonal variation was detected (Hourigan *et al.*, 1991). Finally, both in the stoplight parrotfish and in the corkwing wrasse the parasitic males have significantly higher E2 levels than the bourgeois males (Cardwell and Liley, 1991; Uglem *et al.*, 2002). Because in both species parasitic males mimic females, in particular their colouration pattern, it has been proposed that the higher levels of E2 found in parasitic males may have a feminising function (Uglem *et al.*, 2002). This hypothesis is supported by the fact that suppression of E2 synthesis is needed for sex and colour change in protogynous hermaphroditic fish (Nakamura *et al.*, 2003).

4.1.3. Progestogens and ART

Progestogens are very diverse among teleost species and have been associated with the acquisition of sperm motility and spermiation in male salmonids and cyprinids (Scott and Baynes, 1982; Asahina *et al.*, 1990, 1993). A pheromonal role has also been described for these compounds (e.g., Dulka *et al.*, 1987; Chapter 9). The progestin 17,20β-dihydroxy-4-pregen-3-one (17,20βP) has been reported to rise to high levels at times when males are expressing courtship behaviour such as in rainbow trout, *Oncorhynchus mykiss* (Liley *et al.*, 1986), Atlantic salmon (Mayer *et al.*, 1990), demoiselles, *Chromis dispilus* (Barnett and Pankhurst, 1994), brown trout, *Salmo trutta* (Olsén *et al.*, 1998), and the exogenous administration of 17,20βP to castrates restored courtship behaviour in male rainbow trout, a species in which androgen replacement therapy is inefficient (Mayer *et al.*, 1994).

Data on progestogens is available for six species with ART (Table 8.3). There is no clear pattern with progestogens being higher in the bourgeois males than the parasitic males in two species (Mozambique tilapia and belted sandfish, Table 8.3) and no differences being present in the remaining four species (Lusitanian toadfish, saddleback wrasse, St. Peter's fish and Atlantic salmon; Table 8.3). However, the progestogen(s) measured varied from species to species. In the Lusitanian toadfish, although type I and type II males showed no differences in the levels of progestogens, 17,20β,21-trihydroxy-4-pregen-3-one (17,20β21P) peaked in the breeding season, whereas 17,20α-dihydroxy-4-pregen-3-one (17,20αP) levels remained stable over the year, and 17,20βP was undetectable in the plasma (Modesto and Canário, 2003a). In the saddleback wrasse, initial and terminal phase males showed similar levels of 17,20βP that peak in the breeding season (Hourigan *et al.*, 1991), whereas in the St. Peter's fish there was no difference between monogamous and polygynous males in 17,20βP levels (Ros *et al.*, 2003). Finally, in the Atlantic salmon, 17,20βP levels did not differ between male morphs, but a peak of this progestin has been observed in large males during the spawning season (Mayer *et al.*, 1990). Thus, 17,20β21P in the toadfish, 17,20βP in the saddleback wrasse, and 17,20βP in the Atlantic salmon may play a role in male reproductive function, probably in spermiation. The lack of differences in 17,20βP between alternative male morphs in the Atlantic salmon, where levels have been associated with the expression of courtship behaviour, might be explained by physiological constraints imposed by 17,20βP effects on spermiation in both morphs.

In the Mozambique tilapia, territorial males have higher levels of both 17,20αP and 17,20βP than nonterritorial female mimicking males, but only 17,20αP plasma concentrations increase in the presence of females, when courtship behaviour is expressed by the males (Oliveira *et al.*, 1996). This result suggests that in male tilapia 17,20αP may play a major role in spawning behaviour and/or spermiation (potentially induced by the presence of the females).

Finally, in the belted sandfish, a simultaneous hermaphrodite fish, 17,20β21P levels increased with increasing body size, whereas 17,20βP concentrations did not vary with size. Because larger individuals preferentially play the male role during spawning and get involved in pair spawning rather than in streaking attempts, 17,20β21P rather than 17,20βP seems to be associated with male reproductive behaviour (Cheek *et al.*, 2000).

In summary, different progestogens appear associated with male reproductive function in the different species analysed. However, for most species it is difficult to disentangle potential effects of progestins on male courtship behaviour from effects on spermiation, which may co-occur in time. Therefore, only by carefully planned experiments can these two effects be isolated in future studies.

4.2. Organisational and Activational Effects of Sex Steroids in the Differentiation of Alternative Reproductive Phenotypes: The Relative Plasticity Hypothesis

Moore (1991) proposed a conceptual framework for the hormonal basis of alternative reproductive tactics that has been named the *relative plasticity hypothesis*. The rationale behind this hypothesis is that the effects of hormones in the differentiation of ART are equivalent to their effects in primary sex differentiation, and thus the hormonal basis of ART would have a parallel in the activational-organisational effect of hormones (Arnold and Breedlove, 1985) depending on the plasticity of the tactics. The relative plasticity hypothesis predicts an organisational role of hormones in the expression of fixed alternative phenotypes and an activational role in the case of plastic alternative phenotypes (Moore, 1991; Moore *et al.*, 1998). Two predictions can then be extracted from this hypothesis:

1. In species with plastic ART, hormone levels should differ between adult alternative morphs, whereas in species with fixed ART adult hormone profiles should be similar among alternative morphs, except when morphs are affected by different social experiences (Moore, 1991).
2. In species with plastic ART, the effect of hormone manipulations on phenotypic differences in behaviour and morphology should be effective in adults but not during early development (i.e., activational effect), whereas in fixed ART hormone manipulations should be effective during early development but not in adults (i.e., organisational effect) (Moore, 1991).

The first prediction of the relative plasticity hypothesis is flawed in a Popperian sense as it cannot be disproved. In fact, Moore (1991) explicitly mentioned that in fixed ART adult hormone profiles should be similar among alternative male phenotypes, except if the two male types experience different social environments (which is expected in individuals opting for alternative modes of reproduction). Thus, both positive and negative associations, and even the lack of a relationship between sex steroid levels and the ART type, all find support from it. Therefore, a test of the first prediction is inconclusive. Nevertheless, it may be mentioned that this first prediction of Moore's hypothesis is not confirmed for KT by the dataset presented here, because differences in KT levels between male types are present in most species irrespective of whether their tactics are fixed or plastic (i.e., among species with plastic ART, this prediction would be apparently confirmed in *S. pavo*, *P. parvicornis*, *S. viride, T. duperrey, C. julis*, and *O. mossambicus*, but not in *N. pulcher*, *S. galilaeus*, and *P. velifera*; in species with fixed ART, the prediction would only be confirmed in *S. subligarius* and rejected in *P. notatus*, *L. macrochirus*, *S. melops*, and *S. salar*; see Section 4.1. and Table 8.3 for details).

In contrast, the second prediction does not suffer from epistemological flaws and hence a test of the allowable. Unfortunately, few hormone manipulation experiments have been conducted in teleost species with ART. To my knowledge, only in four species have the effects of androgens on parasitic males been tested so far. These consisted of three species with plastic ART: the peacock blenny (Oliveira *et al.*, 2001d), the rock-pool blenny (Oliveira *et al.*, 2001e), and the sailfin molly (R. F. Oliveira, I. Schlupp and D. M. Gonçalves, unpublished data, 2000); and of one species with fixed ART, the plainfin midshipman (Lee and Bass, 2004). In the three species with plastic ART, the exogeneous administration of androgens to parasitic males did not induce a clear behavioural switch towards a bourgeois tactic. A 7-day androgen implant treatment failed to induce bourgeois behaviours but failed to promote male typical behaviour (e.g., establishing a nest and attracting females to it) in parasitic (i.e., sneaker/satellite) males of both blenniid species (Oliveira *et al.*, 2001d,e). However, in the peacock blenny, the KT treatment resulted in the inhibition of sneaking behaviour and female nuptial colouration in sneaker males (Oliveira *et al.*, 2001d). In the sailfin molly, an immersion treatment with methyl-testosterone did not induce small males to display courtship behaviour (R. F. Oliveira, I. Schlupp and D. M. Gonçalves, unpublished data, 2000). On the other hand, the same treatments were efficient in promoting the expression of "bourgeois" morphological traits. In both blennies, the treatment with KT induced the differentiation of a sex-pheromone producing anal gland, the differentiation of a gonadal accessory gland (i.e., testicular gland) involved in sperm maturation and steroidogenesis, and the development of a male genital papillae (Oliveira *et al.*, 2001d,e). Thus, the second prediction of the relative plasticity hypothesis is partially confirmed for species with plastic ART, in relation to the differentiation of morphological characters typical of bourgeois males. In the plainfin midshipman, type II males that received an intraperitoneal silastic implant of KT neither show an increase in the size of the brain sonic motor nucleus, which controls the courtship calls typical of type I males, nor expressed the type I male territorial or courtship behaviours. Nevertheless, this treatment increased the sonic muscle mass and the frequency of sneaking behaviour in type II males (Lee and Bass, 2004). Thus, the prediction of the relative plasticity hypothesis is again partially fulfilled (the lack of response to hormone manipulations in adulthood in a species with fixed ART) but KT still induces the development of the sonic muscle. Taken together, this set of results suggests that irrespective of the ART type KT is more efficient in differentiating morphological than behavioural alternative reproductive traits.

A second generation of the relative plasticity hypothesis was subsequently proposed to accommodate the diversity of mechanisms that occur in ART (Moore *et al.*, 1998). In particular, the revised version of the relative plasticity hypothesis places emphasis on the distinction between reversible and irreversible phenotypes among plastic tactics and between conditional and unconditional fixed tactics. According to the new version of this hypothesis, the plastic reversible tactics would be the true equivalents to activational effects of hormones and thus the original predictions of the relative plasticity hypothesis would only apply to this type of alternative tactic. Conversely, plastic irreversible ART would represent an intermediate situation between organisational and activational effects; that is, a postmaturational reorganisation effect, in which the phenotypical outcome is immediately produced (Moore *et al.*, 1998). Thus, hormone differences needed to differentiate the two alternative phenotypes need not be permanent and may only be present during the transitional phase. Among the fixed ART, the distinction between conditional and unconditional fixed tactics has no consequences for the predictions concerning the endocrine mechanisms of ART, with organisational actions being predicted in both cases (Moore *et al.*, 1998).

The predictions of the second generation of the relative plasticity hypothesis remain untested for most species with ART, except for the main species studied by Moore and collaborators, the tree lizard (*Urosaurus ornatus*; Moore *et al.*, 1998; Knapp *et al.*, 2003; Jennings *et al.*, 2004). The available data on fish androgens does not support the new predictions generated by the second generation of the relative plasticity hypothesis, because KT differences are still present in species with plastic irreversible ART (Table 8.3), where differences would only be expected during the transitional period between the alternative phenotypes. Moreover, the magnitude of these differences is even larger in species with plastic irreversible ARTs than in species with plastic reversible ART (Figure 8.4), where pure activational effects (and thus clear differences is hormone profiles) have been predicted.

In conclusion, although the relative plasticity hypothesis represents a major effort to develop a conceptual framework for the study of the endocrine mechanisms underlying ART, it does not seem to be generalised across vertebrate taxa. At least in teleost fish, a taxon that contains most described cases of ART, the relative plasticity hypothesis does not fit the available data. One of the major reasons for this mismatch may reside in the fact that this hypothesis is deeply based in the mammalian paradigm of sex differentiation, which is not applicable to all other vertebrate taxa, in particular those with nongenetic sex determination mechanisms.

4.3. Androgens and Alternative Reproductive Tactics: Cause or Consequence?

So far, this Chapter has interpreted the association between KT and ART as a proof for the involvement of KT in the differentiation of ART. However, androgen levels not only influence vertebrate reproductive physiology and behaviour but can also be influenced by the social environment in which the animal lives (Wingfield *et al.*, 1990; Oliveira *et al.*, 2002a).

4.3.1. Social Interactions Feed Back to Influence Hormone Levels

Several studies have shown the effects of social interactions on the short-term modulation of androgen circulating levels (for a review see Oliveira, 2004). In teleost fish, endocrine responses to social and sexual stimuli have been documented. For example, the presence of ovulated females induces a rise in sex steroid and gonadotropin levels and an increase in milt production in male salmonids (Liley *et al.*, 1986, 1993; Rouger and Liley, 1993). Anosmic salmonid males (i.e., rainbow trout, *Onchorhynchus mykiss*, and kokanee salmon, *Onchorhynchus nerka*) in the presence of sexually active females have lower levels of sex steroids and a lower sperm production than males with intact olfactory epithelia, which suggests that chemical signals may play an important role in this social modulation of hormone levels (Liley *et al.*, 1986, 1993; Rouger and Liley, 1993). In the Mozambique tilapia, males are sensitive to the sexual maturity stage of females courting more intensively ovulated females (Silverman, 1978), an effect that also seems to be mediated by chemical signals emitted by receptive females. It has also been shown that male Mozambique tilapia experience a rise in KT following courtship (Borges *et al.*, 1998). Not only sexual stimuli but also male–male competition may induce an endocrine response in the participating individuals, a response especially sensitive in the case of androgens. In different vertebrate groups, including humans, short-term fluctuations in androgen concentrations related to social interactions have been demonstrated (Oliveira, 2004). However, it seems that the association between androgen levels and agonistic behaviour is stronger in periods of social instability (challenge), as is the case of the establishment of dominance hierarchies, the foundation of a new territory, the response to territorial intrusions, or the active competition with other males for access to females (Oliveira *et al.*, 2002a). During periods of social inertia, the levels of aggression drop to a breeding baseline and a decoupling between androgens and aggression may occur.

These results have been interpreted as an adaptation for the individuals to adjust their agonistic motivation to changes in the social environment in which they are living. Thus, male–male interactions would stimulate the production of androgens and the levels of androgens would be a function of the stability of the social environment in which the animal is placed. This hypothesis was first proposed by Wingfield and collaborators and is currently known as the *challenge hypothesis* (Wingfield *et al.*, 1990).

4.3.2. The Challenge Hypothesis

Wingfield and coauthors proposed that the variation in androgen levels could be more closely associated with temporal patterns of aggressive behaviour than with changes in reproductive physiology (challenge hypothesis; Wingfield *et al.*, 1990). According to this hypothesis, when the breeding season starts, androgen levels rise from a nonbreeding baseline to a higher breeding baseline which is sufficient for normal reproduction, that is for gametogenesis, the expression of secondary sexual characters, and the performance of reproductive behaviour. Androgen levels can further increase until they reach a maximum physiological level in response to such environmental stimuli as male–male interactions and/or the presence of receptive females (Figure 8.5A). However, above this breeding baseline any increase in androgen levels does not influence the development of secondary sex characters nor the expression of reproductive behaviour (Figure 8.5B). Thus, androgen patterns during the breeding season are predicted to vary between species according to the amount of social interactions to which the individuals are exposed. In monogamous species with high levels of parental care, androgen levels should increase above the breeding baseline only when males are challenged by other males or by mating. At other times, androgens should remain at the breeding baseline so that they do not interfere with paternal care. Conversely, androgen levels in polygynous males should be near physiological maximum throughout the breeding season due to high levels of male–male competition in this type of breeding system. Wingfield *et al.* (1990) reviewed the available literature on testosterone and aggression in free-living birds and the results supported the above predictions of the challenge hypothesis, which led the authors to suggest that this hypothesis may be valid not only for birds but for vertebrates in general. We have recently confirmed this hypothesis for teleost fish (Oliveira *et al.*, 2002a; Hirschenhauser *et al.*, 2004).

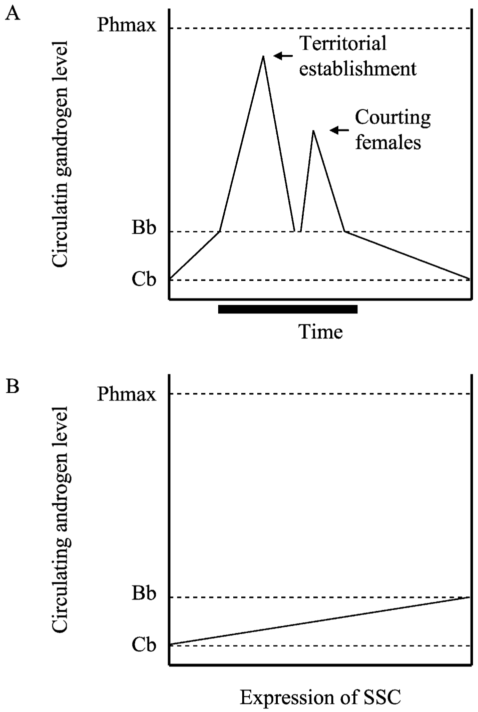

Fig. 8.5. Social influences on androgen levels. (A) Androgen levels rise from a constitutive baseline (Cb) to a breeding baseline (Bb) needed for the expression of reproductive behaviour, the differentiation of secondary sex characters (SSC) and spermatogenesis in breeding individuals; during the breeding season (marked by the black bar at the bottom of the graph) transitory androgen surges above the breeding baseline up to the physiological maximum (Phm) may be induced by social challenges posed by social interactions, either with competing males or with potential mates. (B) Theoretical variation in the expression of reproductive traits according to the challenge hypothesis (Wingfield *et al.*, 1990); note that above the breeding baseline further increases in androgen levels will have no effect in a subsequent increase in the expression of reproductive traits.

4.3.3. SOCIAL MODULATION OF ANDROGEN LEVELS IN ALTERNATIVE MALE PHENOTYPES

Considering the potential social influences on circulating androgen levels in fish, the differences in KT levels between alternative reproductive male types reported above (Section 4.1.1) might not reflect different hormone profiles due to an activational effect of KT on the expression of the bourgeois tactic, but rather merely reflect the responsiveness of this hormone to the expression of the tactic itself (i.e., they are a consequence and not a cause of the expression of alternative mating tactics). This can be the case if the alternative phenotypes experience different social environments, which is expectable since by definition bourgeois males defend resources to get access to mates and thus are expected to face higher levels of social challenges than parasitic males. There are few data available to test this hypothesis. However, three examples will illustrate how the social modulation of androgens may confound the interpretation of the data on KT levels on species with ART.

In the two closely related blenniid species with ART that the author and his collaborators have been studying, the peacock blenny and the rock pool blenny, there is a fundamental difference in the nature of the tactic used by parasitic males to achieve fertilisations. In the peacock blenny, parasitic males mimic female nuptial colouration and female courtship behaviour in order to get access to the nests when a spawning is taking place (Gonçalves *et al.*, 1996). In contrast, in the rock-pool blenny, parasitic males act as satellites of nest-holders taking a share in the defense of the breeding territory, engaging in a high number of agonistic interactions (Santos, 1985; Oliveira *et al.*, 2002b). Thus, the social challenge regime seems to be more asymmetric between male types in the peacock blenny than in the rock-pool blenny, where both male types participate in territorial defense. Thus, we predict that the magnitude of the difference in KT levels between bourgeois and parasitic males should be higher in the peacock blenny than in the rock-pool blenny. The available data is in accordance with this prediction because the difference in KT levels is fivefold higher in nest-holder males than in sneakers in the peacock blenny and approximately twofold higher in nest-holders than in satellites in the rock-pool blenny. Another piece of evidence comes from the study on the peacock blenny, in which KT levels in bourgeois males were shown to vary with the temporal variation in the rate of sneaking attempts (social challenges) by parasitic males (Oliveira *et al.*, 2001a).

In the St. Peter's fish, with facultative monogamous/polygynous males (Fishelson and Hilzerman, 2002), we tested the relationship between pair-bond intensity (i.e., quantitative measure of the partner preference when given a choice to interact with female partner vs. novel female) and KT levels, and found a significant negative correlation between KT levels and partner preference, suggesting that the more polygynous males had higher KT levels (Oliveira *et al.*, 2001h). In a second experiment, the effects of exogenous administration of androgens on pair-bonding were assessed by treating males with silastic implants of either T or vehicle only. Because in the first experiment a relationship between higher KT levels and a higher propensity to become polygynous emerged, it was predicted that androgen-treated males would display a lower partner preference than controls. Contrary to expectations, the androgen treatment had no effect on partner preference (Oliveira *et al.*, 2001h). The results from these two experiments suggest that the association between partner preference and androgen levels in St. Peter's fish is not due to a causal effect of androgens on partner preference, but they probably reflect variation in partner-preference behaviour observed among males (i.e., a raise in KT levels induced by the amount of interaction with the novel female). This interpretation is supported by data collected in Lake Kinneret (Israel), where KT levels of polygynous males did not differ from those of monogamous males (Ros *et al.*, 2003).

These three studies stress the fact that the differences in KT levels found between bourgeois and parasitic males may reflect, at least partially, differences in social experiences. This does not mean that these socially-induced androgen variations will not reflect in the behaviour subsequently adopted by the males, thus promoting the divergence between the two male types.

4.4. Steroid-Binding Globulins, Steroidogenic Enzymes, and Steroid Receptors

The previous sections have concentrated on differences in circulating levels of steroids. However, circulating hormones are just the "most visible" part of the endocrine system with the techniques that have been used more readily (i.e., hormone assays such as radioimmunoassay or enzyme-immunoassays). The activity of the steroidogenic pathways in the endocrine cells, the affinity to binding globulins in the plasma and local conditions in the target tissue, will all influence the action of the hormone (Knapp, 2004). Thus differences between morphs in total levels of circulating hormones are difficult to interpret per se and these other factors should also be considered and investigated.

4.4.1. Steroid-Binding Globulins

Binding globulins can regulate the availability of circulating steroids to target tissues, because only the free (unbound) fraction is biologically active. Despite their potential role in the modulation of endocrine mechanisms mediating the expression of reproductive traits (e.g., reproductive behaviour, morphological sex characters), to the author's knowledge, there is only one published study for all vertebrate taxa that documents differences in binding capacity of a steroid-binding globulin among alternative morphs (tree lizards; Jennings *et al.*, 2000). In teleost fish, sex hormone-binding globulins have been described in a number of species, and their affinity for different sex steroids varies across species (Pasmanik and Callard, 1986; Laidley and Thomas, 1994; Hobby *et al.*, 2000b) and with the reproductive cycle (Hobby *et al.*, 2000a). In most species studied, steroid hormone-binding globulins bind E2 with higher affinity than androgens, and among androgens T usually shows the highest relative affinity, whereas KT has a low affinity. For example, in zebrafish the relative binding affinity of KT is 10 times lower than that of T (Miguel-Queralt *et al.*, 2004), which indicates that KT is potentially more readily available to target tissues than T.

4.4.2. Steroidogenic Enzymes

Because in most teleosts studied to date, T is also found in females during the breeding season at higher or similar levels compared to males but KT has mainly been detected in males (Borg, 1994), some authors proposed that testosterone should be seen as a prohormone that can be metabolised into other biologically active steroids: KT in males and E2 in females (e.g., Bogart, 1987; Oliveira and Canário, 2001). The conversion of T into KT involves two steps catalysed by the enzymes 11-β-hydroxylase (11-β-H) and 11β-hydroxysteroid dehydrogenase (11-β-HSD). Testosterone, but not KT, can also be converted into E2 by a sequence of reactions in which the C19-methyl group is removed and the steroid A ring is aromatised. This cascade of reactions is catalysed by the cytochrome P450 aromatase complex (aromatase). Because both pathways have a common substrate and may be present in males in the same tissues, they may regulate each other's activity. For instance, one potential role for the high levels of aromatase found in the teleost brain (100- to 1000-fold higher than in the brain of mammals; Callard *et al.*, 1990) could be a downregulation of the KT biosynthetic pathway. By varying the local activity of these steroidogenic enzymes, an organism could dramatically change the availability of the biological active steroid in different target tissues. The occurrence or magnitude of the response of the target cell to the hormone depends on hormone levels in close proximity to targets, which can thus be different from circulating levels. 11β-H mRNA expression has been detected in the brain of terminal phase males of the saddleback wrasse after sex change, suggesting that levels of KT can indeed be increased at the target tissue (Morrey and Nagahama, 2000). Additionally, there is a shift in the expression of aromatase versus 11β-H in the gonad during sex change, with a downregulation of the former and an upregulation of the latter. The fact that in this species sex change is controlled by social factors, suggests that changes in steroidogenic enzyme activity may be influenced by social factors. Studies on steroid metabolism in the Siamese fighting fish (*Betta splendens*) further support this view, where subordinate individuals have lower expression of secondary sex characters and male display behaviour, both aggressive and sexual, and the activity of 11β-H in the testis is decreased (Leitz, 1987). This is consistent with different roles for the two androgens, with testosterone acting as a pool of androgens, which will allow the individual to shift behaviour according to the social context by a differential activation of aromatase and 11β-H.

In species with ART, a difference is thus expected in the activity of both enzymes between the two male types in tactic-sensitive tissues: bourgeois males are expected to upregulate 11β-H and downregulate aromatase and the opposite action is expected in parasitic males. Unfortunately, there is no data available on brain 11β-H expression in alternative reproductive phenotypes. In contrast, there is some evidence on brain aromatase that in general corroborates this hypothesis.

In the plainfin midshipman, aromatase activity in the hindbrain was three- to five-fold higher in type II males than in type I males (Schlinger *et al.*, 1999). Because only type I males produce a courting "humming" call that is controlled by a vocal motor circuit located in the hindbrain (Bass and Baker, 1990), these differences in aromatase activity may explain the differentiation of alternative male morphs regarding calling behaviour in this species. In the same species it has been found, using both immunocytochemistry and *in situ* hybridisation with specific probes for midshipman, that aromatase levels were high in the hindbrain sonic motor nucleus, in the periaqueductal gray area in the midbrain, and in the hypothalamus and telencephalon. More interestingly, this study shows that aromatase expression is almost exclusively located in glial cells and not in neurons (Forlano *et al.*, 2001). Thus, a role for glia in modulating brain steroid levels is plausible, and the ventricular location of most aromatase-immunoreactive cells would facilitate the bathing of the brain in E2 converted from T, and concomitantly would decrease the local availability of KT. Moreover, high levels of estradiol may be needed in the adult teleost brain because neurogenesis is present during adulthood (Zupanc, 2001) and estrogens are known to act as neurotrophic factors (e.g., Contreras and Wade, 1999; Dittrich *et al.*, 1999). In species with plastic ART, brain aromatase might also be important due to its role on synaptic plasticity that may underlie the reorganisation of neural circuits during the differentiation of alternative brain morphs.

The regulation of steroidogenesis in secreting tissues will also determine the amount of the steroid to be released in the plasma, and ultimately its availability to target tissues. Thus, as a crude exercise to assess the hypothesis raised above, the author has used the ratio KT:T (already used in Section 4.1.1.) as an indicator of the activity of the 11β-H + 11β-HSD pathway, and the E2:T ratio as an indicator of the activity of aromatase. The magnitude of the difference between bourgeois and parasitic male KT:T ratios is higher in species with fixed phenotypes, and lower in species with plastic reversible phenotypes. This suggests a higher upregulation of the 11-oxygenated androgens biosynthetic pathway in fixed ART species when compared to plastic ART species. These differences may be explained by the fact that the differentiation of divergent phenotypes in fixed species is not constrained by the subsequent phenotypic reorganisation that occurs in plastic species. Therefore, bourgeois males of fixed species might differentiate into more divergent phenotypes and 11-oxygenated steroids play a critical role in this process. For the ratio E2:T data is only available on five species and thus a clear pattern is not detectable. Nevertheless, if one compares the magnitude of the differences between tactics for both ratios, in all five species (*P. notatus, H. didactylus, S. viride, T.duperrey* and *S. melops*), the KT:T ratio is higher than the E2:T ratio, indicating a dominance of the 11-oxygenated-androgen pathway over the aromatisation pathway in the bourgeois tactic.

Recently, a model for the differentiation of alternative phenotypes based on glucocorticoid-androgen interactions has been independently proposed by Knapp *et al.* (2002), Knapp (2004) and Perry and Grober (2003). This model is based on the fact that the enzymes that participate in KT biosynthesis are also involved in the synthesis (11β-H) and inactivation (11β-HSD) of glucocorticoids. The functional significance of having the same enzymes catalysing parallel pathways is that they can be regulated by reciprocal competitive inhibition. In species with plastic ART or with socially controlled sex change, this commonality creates the possibility that the activity of these enzymes may mediate the transduction of social signals into endocrine ones that will modulate the adoption of a certain ART or sex change (Knapp *et al.*, 2002; Perry and Grober, 2003; Knapp, 2004). It is easy to conceive that parasitic males should have higher cortisol levels than bourgeois males, due to aggressive interactions among the two morphs, which would explain the lower levels of KT observed in parasitic males due to competitive inhibition of the KT-biosynthetic pathway. However, because inhibition of KT production does not affect T, a decoupling of the expression of male traits that are KT dependent from those that can be elicited by T is predicted. Moreover, inactivation of the KT pathway in parasitic males would also result in an accumulation of T, and thus in an increased availability of the substrate for the enzyme aromatase. Therefore, this would also predict a higher aromatase activity in parasitic males, which has been observed in midshipman type II males (Schlinger *et al.*, 1999). Data on cortisol levels in species with ART is very scarce. In the longear sunfish, parasitic males have both higher levels of cortisol and lower KT levels than bourgeois males, suggesting that parasitic males may have a lower activity of 11β-HSD both in the interrenal glands and in the testes relative to bourgeois males (Knapp, 2004). The role of glucocorticoids in phenotypic plasticity is further supported by the fact that in the bidirectional sex-changing goby, *Gobiodon histrio*, small males when stressed by competition with dominant males can change sex back to females (Munday and Jones, 1998). In accordance with this finding, a glucocorticoid responsive element has been identified as a putative promoter regulatory factor of the aromatase gene CYP19A1 (gonadal isoform) that promotes transcription of this gene, resulting in the expression of aromatase which subsequently enhances E2 synthesis involved in male-to-female sex change (Gardner *et al.*, 2003).

4.4.3. Steroid Receptors

The classic mechanism of steroid action involves the diffusion of the free (unbound) fraction of the circulating steroid into the target cell where it binds to intracellular receptors. The activated receptor is then translocated to the nucleus where it binds to specific genes acting as transcription factors. Thus, for steroids to exert their effects, their specific receptors have to be present in the target tissue.

In the brain, where the action of androgens is expected to modulate reproductive behaviour, unusually high levels of androgen receptors (AR) have been detected in goldfish, *Carassius auratus* (Pasmanik and Callard, 1988). The immunolocalisation of these AR, using an antibody to human/rat AR, identified numerous cells in the preoptic area and in the periventricular nuclei of the hypothalamus (Gelinas and Callard, 1997), which are brain key areas in the control of reproduction, namely activity of the hypothalamic-pituitary-gonadal (HPG) axis. A seasonal variation in AR levels was also found with a fivefold peak during the breeding season (Pasmanik and Callard, 1988). This suggests that brain AR expression can be regulated by sex steroids. Recently, a similar seasonal variation in a nuclear AR during the reproductive cycle in the brain of the Atlantic croaker (*Micropogonias undulatus*) was shown, which has been confirmed to be regulated by sex steroids (Larsson *et al.*, 2002).

To date, two different types of androgen receptors (AR1 and AR2; Sperry and Thomas, 1999, 2000; Ikeuchi *et al.*, 2001) and three forms of the estrogen receptor (ERα, ERβ, and ERγ; Hawkins *et al.*, 2001) have been identified both in the brain and in the gonads of teleost fish. All these receptors are ligand-activated transcription factors and thus mediate steroid genomic effects. Intriguingly, KT which has been shown to be the most potent androgen in fish in controlling reproductive function (see Section 3) is a poor ligand of known AR receptors (Pasmanik and Callard, 1988; Sperry and Thomas, 1999; Thomas, 2000). Two possible explanations to solve this paradox are the following:

1. Because KT has also a poor binding to steroid hormone-binding globulin, it is possible that the available fraction of KT to the target cell is relatively higher than that of its competitors that are better

ligands of the binding-globulin and thus are less available for the target cell. Therefore, the local kinetics of the receptor influenced by the relative availability of the ligands near the target may compensate KT for its low affinity to AR.

2. The KT actions on behaviour might be mediated by nongenomic mechanisms of steroid action that are mediated by a membrane steroid receptor. Evidence has accumulated over the years for rapid effects of steroids that are too fast to be mediated by the classic genomic mechanism that acts over a wider time scale (i.e., hours to days), suggesting the existence of specific receptors on the surface of target cells (Thomas, 2003). Recently, a membrane progestin receptor was identified that has characteristics of G protein-coupled receptors (Zhu *et al.*, 2003).

Therefore, it is possible that behavioural actions of KT on bourgeois males are being mediated by one of these two mechanisms. To the author's knowledge, the relative levels of brain steroid receptors between alternative reproductive phenotypes have only been documented for the protogynous wrasse, *Halichoeres trimaculatus* (Kim *et al.*, 2002). It was found using competitive reverse-transcription polymerase chain reaction that the levels of AR transcripts were significantly higher in the brain of terminal phase males than in initial phase males. No other significant differences in gene expression were observed either for AR in the gonads or for ER both in the brain and in the gonads. Thus, by varying the expression of AR in specific tissues (e.g., brain *vs* gonad), bourgeois males can both increase their sensitivity to circulating androgen levels in specific targets (the brain), and at the same time allow a compartmentalisation of the effects of androgens. In other words, deleterious effects can be avoided by varying AR densities in different tissues (Ketterson and Nolan, 1994). This mechanism hypothetically makes it possible to activate the expression of an androgen-dependent reproductive behaviour in bourgeois males without having the side effect of increasing spermatogenesis or the expression of a sex character, because the androgen action can be independently modulated at each compartment (brain *vs* gonad *vs* morphological secondary sex character).

5. NEURAL MECHANISMS OF ALTERNATIVE REPRODUCTIVE BEHAVIOURS

Historically, sex hormones were seen as causal agents of behaviour, acting directly on the display of a given behaviour. This classic view was the result of early studies of castration and hormone-replacement therapy that showed that some behaviours were abolished by castration and restored by exogenous administration of androgens (Nelson, 2000). Currently, this view has shifted towards a probabilistic approach and hormones are seen as facilitators of behavioural expression and not as deterministic factors (Simon, 2002). According to this paradigm, hormones may increase the probability of the expression of a given behaviour by acting as modulators of the neural pathways underlying that behavioural pattern. For example, the effects of androgens on the expression of aggressive behaviour in mammals are mediated by modulatory effects on central serotonergic and vasopressin pathways (Simon, 2002).

The phenotypic divergence between alternative male morphs is present at two main levels: sexual behaviour and morphological differentiation. Two main neurochemical systems have been studied that may help to understand the role of androgens on the differentiation of the ART at each of these two levels: gonadotropin-releasing hormone (GnRH), and arginine vasotocin (AVT) respectively. Both systems have been reviewed recently in the light of vertebrate sexual plasticity elsewhere (Foran and Bass, 1999; Bass and Grober, 2001). Thus, this section will only provide a brief update of these reviews, including data published more recently.

5.1. GnRH and the Differentiation of Alternative Reproductive Morphs

Across all vertebrates, GnRH plays a central role in the control of reproduction by orchestrating the functioning of the HPG axis. Nine different forms of GnRH have been identified to date in vertebrates (Parhar, 2002). Of these, three have been found in the brains of different fish species: salmon-GnRH (sGnRH or GnRH3), chicken-GnRH (cGnRH-II or GnRH2) and seabream-GnRH (sbGnRH or GnRH1). In some species, the three forms are present (e.g., the African cichlid *Astatotilapia burtoni*; White *et al.*, 1995), whereas only two forms (sGnRH and cGnRH-II) have been found in other species (e.g., goldfish; Lin and Peter, 1996). The neural distribution of these three forms suggests multiple GnRH systems in the fish brain. GnRH neurons have been identified in the terminal nerve (TN), in the preoptic area (POA), and in the midbrain, and the three forms of GnRH show a differential localisation across these three systems. In teleosts with the three forms present, GnRH1 is located in the POA, GnRH2 in the midbrain region, and GnRH3 in TN area (White *et al.*, 1995). In species with only two forms present, GnRH2 still occurs in the midbrain and the second form, which can vary with species, is present in the POA and olfactory-forebrain areas. Thus some variation occurs in the form of GnRH present in the POA across fish species (Yu *et al.*, 1997). However, in more advanced teleosts (e.g., cichlids) where three GnRH forms are present, each GnRH variant might have their specific receptor (Parhar, 2003).

Unlike other vertebrates in which the GnRH-POA neurons communicate with the pituitary via the blood portal system, in teleosts a direct innervation of the pituitary by GnRH-POA fibers occurs (Peter *et al.*, 1990; Yu *et al.*, 1997). This anatomical peculiarity allows for a faster action of the cascade of events present in the reproductive axis; that is, GnRH secretion will be more rapidly translated in peripheral effects, such as sex-steroid secretion in the gonads and the consequent differentiation of reproductive traits (Foran and Bass, 1999).

Only four studies on GnRH systems addressing the occurrence of morph differences have been conducted in species with ART. These studies revealed differences either in the number or in the size of GnRH-immunoreactive (GnRH-ir) cells between alternative male phenotypes. For example, in the bluehead wrasse, terminal-phase males have larger numbers of GnRH-ir cells than the initial phase males or the females (Grober and Bass, 1991; Table 8.4). A change from initial male into a terminal-phase male is followed by an increase in the number of GnRH-ir cells in the POA (Grober *et al.*, 1991). On the other hand, in the plainfin midshipman (*Porichthys notatus*), there are no observable differences in cell number (except if corrected for body mass; see Foran and Bass, 1999), but type I males have larger cells than type II males (Grober *et al.*, 1994; Table 8.4). Because otolith readings indicate that type II males are younger than type I (Bass *et al.*, 1996), the difference in this species is that the neural system is activated early in life in type I males. In the platyfish, *Xiphophorus maculatus*, small noncourting males have larger numbers of cells than large courting males and no differences are found in cell size between the two morphs (Halpern-Sebold *et al.*, 1986; Table 8.4). In the grass goby, a species with sequential ART, both the number and the size of GnRH-ir neurons is higher/larger in nest-holders than in sneaker males (Scaggiante *et al.*, 2004; Table 8.4). In this species, the experimentally induced transition from sneaker to nest-holder is accompanied by an increase in the number of GnRH-ir cells in the preoptic area, whereas no changes are detectable in their size (Scaggiante *et al.*, 2004). Terminal nerve GnRH-ir neurons seem not to vary in respect to ART (Foran and Bass, 1999; Scaggiante *et al.*, 2004).

Thus, in the two species with fixed ART (i.e., midshipman and platyfish), the early sexual maturation of the parasitic males is matched with the maturation of the GnRH system as revealed by the fact that parasitic males have similar numbers of GnRH-ir cells to bourgeois males but at a smaller body size (Table 8.4). Similarly, in the two species with sequential ART (i.e., grass goby and bluehead wrasse), the number of GnRH-ir neurons is higher in bourgeois males than in parasitic males (Table 8.4) and there is

Table 8.4

Differences in GnRH and AVT Neurons Between Alternative Reproductive Types

Species	ART	Neuropeptide	Cell size	Cell number	mRNA expression/cell
Plainfin midshipman, *Porichthys notatus*	Fixed	GnRH[1, 2]	B > P	B = P*	—
		AVT[3]	B > P	B = P*	—
Platyfish, *Xiphophorus maculatus*	Fixed	GnRH[4]	B = P	B < P	—
Grass goby, *Zosterisessor ophiocephalus*	Sequential	GnRH[5]	B > P	B > P	—
Bluehead wrasse, *Thalassoma bifasciatum*	Sequential	GnRH[6]	B = P	B > P	—
		AVT[7]	—	B > P	B > P
Saddleback wrasse, *Thalassoma duperrey*	Sequential	AVT[8]	B > P	B > P	—
Peacock blenny, *Salaria pavo*	Sequential	AVT[9]	B = P*	B = P*	B < P
Rock-pool blenny, *Parablennius parvicornis*	Sequential	AVT[10]	B = P*	B = P*	—

*If corrected for body mass then B < P.

[1]Grober *et al.* (1994); [2]Foran and Bass (1999); [3]Foran and Bass (1998); [4]Halpern-Sebold *et al.* (1986); [5]Scaggiante *et al.* (2004); [6]Grober and Bass (1991); [7]Godwin *et al.* (2000); [8]Grober (1998); [9]Grober *et al.*, 2002;[10]Miranda *et al.*, 2003.

a significant increase in GnRh-ir cells during the transition from parasitic to bourgeois male (Grober *et al.*, 1991; Scaggiante *et al.*, 2004). In summary, for the four species studied, changes in the GnRH-POA neural system seem to play a role in the sexual maturation among juveniles of species with fixed ART or in the reorganisation of the reproductive phenotype in plastic species.

5.2. AVT and the Differentiation of Alternative Reproductive Behaviours

The hypothalamic neuropeptide arginine-vasopressin (AVP), or its homologue arginine-vasotocin (AVT) in nonmammalian vertebrates, influences the expression of social behaviours in a wide range of vertebrates, from teleosts to mammals (Goodson and Bass, 2001). Exogenous administration of these neuropeptides induces the expression of different types of reproductive behaviours across all vertebrate taxa (Goodson and Bass, 2001), including the male spawning reflex and courtship behaviour in fish (Pickford and Strecker, 1977; Bastian *et al.*, 2001; Semsar *et al.*, 2001; Salek *et al.*, 2002; Carneiro *et al.*, 2003).

Species with ART offer the possibility of investigating how AVT may be involved in the differential expression of reproductive behaviour between male types. In all species studied so far, an association has been found between the expression of alternative tactics and forebrain AVT (i.e., soma size, number of AVT-ir neurons or AVT mRNA expression per cell; see Table 8.4). However, this association is not linear. In the midshipman and in the two wrasses, bourgeois males exhibit courtship behaviour and concomitantly have AVT-POA neurons with larger soma sizes (Foran and Bass, 1998; Grober, 1998) or with a higher mRNA expression on a per-cell basis (Godwin *et al.*, 2000) than parasitic males. In the peacock blenny, in which sex-role reversal occurs in courtship behaviour (i.e., females and sneaker males are the major courting phenotypes; Almada *et al.*, 1995) and thus the expression of courtship behaviour is decoupled from the bourgeois tactic, AVT mRNA expression is higher in both females and sneaker males than in nest-holders, suggesting that mRNA levels are correlated with the expression of courtship behaviour rather than with the alternative morph (Grober *et al.*, 2002). Finally, no differences were found between alternative male morphs in the rock-pool blenny both in the absolute size and number of AVT-ir cells in the POA (Miranda *et al.*, 2003). Interestingly, in this species the parasitic morph consists of satellite males that help the nest-holder males in the defense of the breeding territory and to some extent in female attraction (Santos, 1985; Oliveira *et al.*, 2002b), and thus the behavioural differentiation between morphs is smaller in this species than in the other studied species. Therefore, the reported differences in the AVT-POA system between alternative sexual morphs are clearly associated with the expression of "morph" typical behaviour.

Fewer studies have manipulated AVT by exogenous administration, either intraventricular or systemic, in fish species with ART. Using this approach, three species with alternative morphs have been studied to date: the midshipman, the bluehead wrasse, and the peacock blenny (Goodson and Bass, 2000; Carneiro *et al.*, 2003; Semsar and Godwin, 2003). These studies will be briefly reviewed below.

In the midshipman, administration of AVT directly in the anterior region of the hypothalamus has different effects in eliciting vocal activity in the two male morphs (Goodson and Bass, 2000). Both male morphs and females produce grunt vocalisations in an agonistic context (not to be confounded with the courtship humming calls emitted exclusively by type I males). However, type I males emit long trains of grunts associated with the defense of their nests, whereas both type II males and females produce short grunts in nonreproductive contexts. The central administration of AVT inhibits the production of electrically stimulated grunts in type I males, but not in type II males and females. Interestingly, isotocin (the teleost homologue of oxytocin) had the opposite effect, inhibiting vocal behaviour in type II males and females but not in type I males (Goodson and Bass, 2000).

In the bluehead wrase, AVT systemic injections increase courtship behaviour in both territorial and nonterritorial terminal-phase males but only increase aggressive behaviour and territoriality in nonterritorial TP males in field conditions (Semsar *et al.*, 2001). Moreover, the administration of an AVP V1 antagonist both to nonterritorial terminal-phase males and to females prevented them establishing breeding territories in vacated areas (Semsar and Godwin, 2003). However, exogenous AVT treatment did not increase the expression of display behaviours, typical of terminal-phase males, in an inhibitory social context, that is, in the presence of terminal-phase males in the field (Semsar and Godwin, 2003). In fact, AVT-treated initial-phase males continued to participate in group spawns in the reef. Interestingly, treating females with KT did not increase their responsiveness to AVT but induced the expression of terminal-phase male colouration and courtship behaviour, suggesting that courtship behaviour in females is KT-dependent (Semsar and Godwin, 2003). Thus, the behavioural effects of AVT in the bluehead wrasse seem to be dependent on the sexual phenotype.

In the peacock blenny, AVT treatment of sneaker males increased the time spent in female nuptial colouration and the frequency of the femalelike courtship behaviour displayed towards nest-holder males. In contrast, sneakers treated with AVT and presented with a gravid female failed to express male courtship behaviour (Carneiro *et al.*, 2003). Accordingly, AVT induced the expression of both nuptial colouration and courtship behaviour in females, but failed to promote any expression of male courtship behaviour in nest-holders (Carneiro *et al.*, 2003). As already mentioned above, KT treatment of sneaker males in this species inhibited the expression of female-courtship behaviour in sneaker males, but had no effect on the number or on the soma size of AVT-POA neurons (Oliveira *et al.*, 2001d), suggesting that the inhibitory effect of KT on AVT-dependent femalelike courtship behaviour is probably regulated by changes in mRNA expression. This hypothesis remains to be tested, but is supported by studies on other vertebrates species that present sex differences and/or seasonal variation in AVP brain elements. These results suggest that plasticity in AVP-POA neurons is regulated by T or by one of its metabolites (E2, DHT) acting on AVP mRNA expression and not on AVP-ir (Goodson and Bass, 2001 and references therein).

Thus, both in the midshipman and in the peacock blenny, AVT seems to activate neural mechanisms underlying behavioural patterns shared by parasitic males and females, whereas in the bluehead wrasse AVT is associated with the expression of courtship behaviour typical of bourgeois males. These within- and between-species variations in the effects of AVT in courtship behaviours indicate that a comparative approach using closely related species that vary in mating systems can be a very rewarding approach to this field in the future.

5.3. The Sexually Bipotential Brain of Teleost Fish

One interesting point related to the neural mechanisms underlying behavioural and morphological phenotypic plasticity is the question of whether alternative morphs possess separate brain mechanisms controlling their divergent behaviour or if both mechanisms are present and can be differentially activated depending on the tactic that is being expressed by the animal. The former situation would be expected in fixed and sequential ART and the latter in reversible ART.

As seen above in teleosts, the display of male sexual behaviour has been associated with KT and AVT, whereas female sexual behaviour is stimulated by prostaglandins, in particular by prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) (Stacey, 1987). In teleosts, there is ample evidence that brain sexual bipotentiality is maintained through adulthood. A series of studies performed in one gonochoristic (goldfish) and in one gynogenetic (crucian carp, *Carassius auratus langsdorfii*) species have elegantly elucidated this point. In the goldfish, sexually mature males treated with $PGF_{2\alpha}$ display female sexual behaviour, including a pseudospawning (Stacey and Kyle, 1983). In both species, KT-treated females display male sexual behaviour when paired with conspecific ovulated females (Stacey and Kobayashi, 1996; Kobayashi and Nakanishi, 1999). More interestingly, KT-implanted females also showed female spawning behaviour when injected with $PGF_{2\alpha}$ (Kobayashi and Nakanishi, 1999). These results indicate that induced behavioural masculinisation of females and feminisation of males is reversible. Thus, sexual plasticity is present even in species that do not display it in natural situations, as is the case of sex-changing species. Finally, it should be mentioned that in the parthenogenic Amazon molly (*Poecilia formosa*) spontaneous masculinisation occurs naturally with some individuals exhibiting pseudomale behaviour and a differentiation of a rudimentary gonopodium (Schlupp *et al.*, 1992).

These results are particularly interesting in the context of ART because they suggest that alternative male morphs would have the potential to express both alternative reproductive behaviours. However, exogenous administration of KT to parasitic males fails to induce the display of bourgeois male behaviour in species with either fixed or sequential ART (i.e., midshipman, peacock blenny, rock-pool blenny, see Section 2.1.). Therefore, ART species may have tactic-specific constraints that prevent them expressing bipotential behaviours even when manipulated experimentally.

6. INTEGRATING PROXIMATE AND ULTIMATE QUESTIONS IN THE STUDY OF ALTERNATIVE REPRODUCTIVE TACTICS

Negative genetic correlations and life-history tradeoffs can be better understood if their proximate mechanisms are known (Sinervo and Svensson, 1998). Hormones are good candidates to play a major role as physiological mediators of life-history tradeoffs because they may have opposite effects on two or more traits (Ketterson and Nolan, 1992; Sinervo and Svensson, 1998). Phenotypic plasticity is a life-history trait that might have evolved to allow animals to shift resources from one life-history stage to another, (e.g., from reproduction into growth or vice versa, in a condition- or frequency-dependent fashion; West-Eberhard, 1989). These shifts between life-history stages may be controlled by endocrine mechanisms, and it is expected that in some cases the life-history tradeoffs and the associated phenotypic plasticity have the same underlying physiological mechanism (Sinervo and Svensson, 1998). Because androgens are both involved in the animal's investment in current reproduction and have multiple effects on different phenotypic traits, they are excellent candidates to orchestrate transitions between life-history stages. Thus, it is not surprising that an association between KT and the expression of ART has been found.

The costs associated with specific tactics imposed by their underlying physiological mechanisms are another potential constraint for their evolution.

Little research has been conducted on a cost/benefit analysis of ART in teleosts. Bourgeois males display a set of androgen-dependent behavioural traits that help them in competition with other males for resources or for the access to females (e.g., territoriality). Thus, costs associated with keeping androgen levels elevated should be associated with the bourgeois tactic. These include increased energy consumption, interference with immunocompetence, increased risk of predation, and a higher incidence of injuries from agonistic interactions (for a review on costs of elevated T levels see Wingfield *et al.*, 1999, 2001). For example, in the rock-pool blenny, a field study recently demonstrated that lymphocyte counts and the antibody response to a nonpathogenic antigen is lower in nest-holder males than in satellite males, and both immune parameters correlated negatively with KT levels (Ros *et al.*, 2004a).

Because hormones act on different target tissues, many traits may have a common underlying physiological mechanism and thus can be phenotypically linked (Ketterson and Nolan, 1999; Ketterson *et al.*, 2001), as is the case with androgen-dependent traits such as the expression of male morphological characters, muscle hypertrophy, and the expression of reproductive behaviour. Thus, it is likely that selection acting on any one of these traits will affect the others, so that beneficial traits may evolve indirectly as exaptations (Gould and Vrba, 1982). According to Ketterson and Nolan (1999), one way to distinguish between adaptations and exaptations in hormone-dependent traits would be to assess whether these traits arose either in response to selection on circulating hormone levels, or whether they arose in response to variation in the responsiveness of the target tissues to invariant hormone levels (Figure 8.6). In the former case, selection probably did not act on all correlated traits and thus the ones that subsequently conferred an advantage to its carriers should be viewed as exaptations (Figure 8.6). In the latter case, selection probably acted independently on target-tissue sensitivity to constant hormone levels (e.g., by varying density of receptors or the expression of enzymes for particular biosynthetic pathways; Figure 8.6). Although a mixed scenario may occur, in which both circulating levels and target-tissue sensitivity are under selection, this dichotomy provides us with a framework to address the issue of endocrine-mediated adaptive traits. In this respect, alternative reproductive tactics that involve the differential development of androgen-dependent traits within the same phenotype, such as the differentiation of larger testis in parasitic males without displaying secondary sex characters, suggests a compartimentalisation of androgen effects on different target tissues that can be achieved by varying the densities of androgen receptors (AR) in different targets (e.g., gonadal *vs* secondary sex characters). Therefore, ARTs that involve compartimentalisation effects evolved most probably as adaptations,

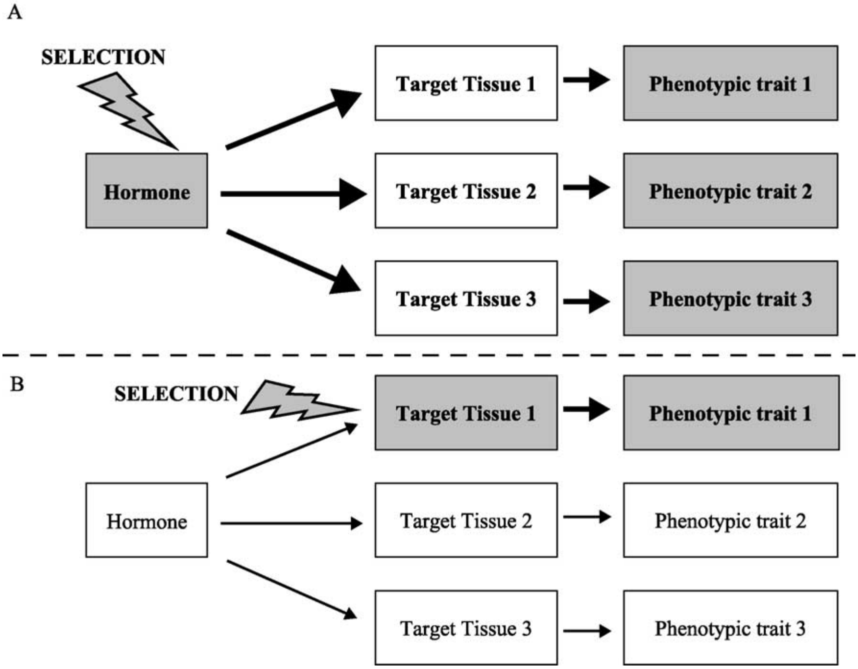

Fig. 8.6. Evolution of endocrine-mediated adaptive traits. (A) Exaptation: trait 1 is being selected by increasing the circulating level of the hormone that controls its expression. If this trait is linked to other traits (traits 2 and 3) because their expression is controlled by the same hormone, then traits 2 and 3 will also be selected, due to the selected increase in hormone levels. Traits 2 and 3 are exaptations. (B) Adaptation: trait 1 is being selected by increasing the expression of receptors to the hormone that controls its expression in a specific target tissue. Although trait 1 is linked to other traits (traits 2 and 3) because their expression is controlled by the same hormone, traits 2 and 3 will not be selected because hormone levels will not be subject to selection.

whereas ARTs that involve no compartimentalisation effects (e.g., some reversible tactics, such as the facultative use of sneaking behaviour by nest-holder males in sticklebacks) represent exaptations. Thus, this question stresses the importance that studies of proximate mechanisms may have to help to understand the evolution of alternative reproductive phenotypes.

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REFERENCES

Almada, V. C., Gonçalves, E. J., Santos, A. J., and Baptista, C. (1994). Breeding ecology and nest aggregations in a population of *Salaria pavo* (Pisces: Blenniidae) in an area where nest sites are very scarce. *J. Fish Biol.* **45,** 819–830.

Almada, V. C., Gonçalves, E. J., Oliveira, R. F., and Santos, A. J. (1995). Courting females: Ecological constraints affect sex roles in a natural population of the blenniid fish, *Salaria pavo*. *Anim. Behav.* **49,** 1125–1127.

Arak, A. (1984). Sneaky breeders. *In* "Producers and Scroungers" (Barnard, C. J., Ed.), pp. 154–194. Croom Helm, London.

Arnold, A. B., and Breedlove, S. M. (1985). Organisational and activational effects of sex steroids on brain and behaviour: A reanalysis. *Horm. Behav.* **19,** 469–498.

Asahina, K., Aida, K., and Higashi, T. (1993). Biosynthesis of 17α,20α-dihydroxy-4-pregnen-3-one from 17α-hydroxyprogesterone by goldfish (*Carassius auratus*) spermatozoa. *Zool. Sci.* **10,** 381–383.

Asahina, K., Barry, T. P., Aida, K., Fusetani, N., and Hanyu, I. (1990). Biosynthesis of 17α, 20α-dihydroxy-4-pregnen-3-one from 17α-hydroxyprogesterone by spermatozoa of the common carp, *Cyprinus carpio*. *J. Exp. Zool.* **255,** 244–249.

Austad, S. N. (1984). A classification of alternative reproductive behaviours, and methods for field testing ESS models. *Am. Zool.* **24,** 309–320.

Balshine-Earn, S., and Earn, D. J. D. (1998). On the evolutionary pathway of parental care in mouthbrooding cichlid fishes. *Proc. Roy. Soc. London B* **265,** 2217–2222.

Balshine– Earn, S., Neat, F., Reif, H., and Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behaviour in a cooperatively breeding fish. *Behav. Ecol.* **9,** 432–438.

Barnett, C. W., and Pankhurst, N. W. (1994). Changes in plasma concentrations of gonadal steroids and gonad morphology during the spawning cycle of male and female demoiselles *Chromis dispilus* (Pisces: Pomacentridae). *Gen. Comp. Endocrinol.* **93,** 260–274.

Baron, D., and Guiguen, Y. (2003). Gene expression during gonadal sex differentiation in rainbow trout (*Oncorhynchus mykiss*): From candidate genes studies to high throughout genomic approach. *Fish Physiol. Biochem.* **28,** 119–123.

Basolo, A. (1990). Female preference for male sword length in the green swortail, *Xiphophorus helleri* (Pisces: Poeciliidae). *Anim. Behav.* **40,** 332–338.

Bass, A. H. (1992). Dimorphic male brains and alternative reproductive tactics in a vocalising fish. *TINS* **15,** 139–145.

Bass, A. H. (1993). From brains to behaviour: Hormonal cascades and alternative mating tactics in teleost fishes. *Rev. Fish Biol. Fisher.* **3,** 181–186.

Bass, A. H., and Baker, R. (1990). Sexual dimorphisms in the vocal control system of a teleost fish: Morphology of physiologically identified neurons. *J. Neurobiol.* **21,** 1155–1168.

Bass, A. H., and Grober, M. S. (2001). Social and neural modulation of sexual plasticity in teleost fish. *Brain Behav. Evol.* **57,** 293–300.

Bass, A. H., Horvath, B. J., and Brothers, E. B. (1996). Nonsequential developmental trajectories lead to dimorphic vocal circuitry for males with alternative reproductive tactics. *J. Neurobiol.* **30,** 493–504.

Bastian, J., Schniederjan, S., and Nguyenkim, J. (2001). Arginine vasotocin modulates a sexually dimorphic communication behaviour in the weakly electric fish *Apteronotus leptorhynchus*. *J. Exp. Biol.* **204,** 1909–1923.

Berglund, A., and Rosenqvist, G. (1993). Selective males and ardent females in pipefishes. Behav. *Ecol. Sociobiol.* **32,** 331–336.

Bogart, M. H. (1987). Sex determination: A hypothesis based on steroid ratios. *J. Theor. Biol.* **128,** 349–357.

Borg, B. (1987). Stimulation of reproductive behaviour by aromatizable and non-aromatizable androgens in the male three-spined stickleback, *Gasterosteus aculeatus*. *In* "Proceedings of the 5th Congress of European Ichthyologists" (Kullander, S. O. K., and Fernholm, B., Eds.), pp. 269–271. Swedish Museum of Natural History, Stockholm.

Borg, B. (1994). Androgens in teleost fishes. *Comp. Biochem. Physiol. C* **109,** 219–245.

Borges, R. A., Oliveira, R. F., Almada, V. C., and Canário, A. V. M. (1998). Short-term social modulation of 11-Ketotestosterone urinary levels in males of the cichlid fish *Oreochromis mossambicus* during male-female interaction. *Acta Ethol.* **1,** 43–48.

Bradbury, J. W., and Vehrencamp, S. L. (1998). "Principles of Animal Communication." Sinauer, Sunderland, MA.

Brantley, R. K., and Bass, A. H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish, *Porichthys notatus* (Teleostei, Batrachoididae). *Ethology* **96,** 213–232.

Brantley, R. K., Marchaterre, M. A., and Bass, A. H. (1993a). Androgen effects on vocal muscle structure in a teleost fish with inter- and intra-sexual dimorphisms. *J. Morphol.* **216,** 305–318.

Brantley, R. K., Wingfield, J. C., and Bass, A. H. (1993b). Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Horm. Behav.* **27,** 332–347.

Breder, C. M., and Rosen, D. E. (1966). "Modes of Reproduction in Fishes." Natural History Press, New York.

Brockmann, H. J. (2001). The evolution of alternative strategies and tactics. *Adv. Study Behav.* **30,** 1–51.

Callard, G. V., Schlinger, B. A., and Pasmanik, M. (1990). Nonmammalian vertebrate models in studies of brain-steroid interactions. *J. Exp. Zool.* **4** (Suppl.), 6–16.

Cardwell, J. R., and Liley, N. R. (1991). Hormonal control of sex and color change in the stoplight parrotfish, *Sparisoma viride*. *Gen. Comp. Endocrinol.* **81,** 7–20.

Carneiro, L. A., Oliveira, R. F., Canário, A. V. M., and Grober, M. S. (2003). The effect of arginine vasotocin on courtship behaviour in a blenniid fish with alternative reproductive tactics. *Fish Physiol. Biochem.* **28,** 241–243.

Caro, T. M., and Bateson, P. (1986). Organisation and ontogeny of alternative tactics. *Anim. Beh.* **34,** 1483–1499.

Carruth, L. L., Reisert, I., and Arnold, A. P. (2002). Sex chromosome genes directly affect brain sexual differentiation. *Nature Neurosci.* **5,** 933–934.

Chang, C. F., Lee, M. F., and Chen, M. R. (1994). Estradiol-17-β associated with the sex reversal in protandrous black porgy, *Acanthopagrus schlegeli. J. Exp. Zool.* **268,** 53–58.

Cheek, A. O., Thomas, P., and Sullivan, C. V. (2000). Sex steroids relative to alternative mating behaviours in the simultaneous hermaphrodite *Serranus subligarius* (Perciformes: Serranidae). *Horm. Behav.* **37,** 198–211.

Cochran, R. C., and Grier, H. J. (1991). Regulation of sexual succession in the protogynous black sea bass *Centropristis striatus,* Osteichthyes Serranidae. *Gen. Comp. Endocrinol.* **82,** 69–77.

Contreras, M. L., and Wade, J. (1999). Interactions between nerve growth factor binding and estradiol in early development of the zebra finch telencephalon. *J. Neurobiol.* **40,** 149–157.

Crews, D. (1998). On the organisation of individual differences in sexual behaviour. *Am. Zool.* **38,** 118–132.

de Girolamo, M., Scaggiante, M., and Rasotto, M. B. (1999). Social organisation and sexal pattern in the Mediteranean parrotfish *Sparisoma cretense* (Teleostei: Scaridae). *Mar. Biol.* **135,** 353–360.

Demski, L. (1987). Diversity in reproductive patterns and behaviour in fishes. *In* "Psychobiology of Reproductive Behaviour: An Evolutionary Perspective" (Crews, D., Ed.), pp. 1–27. Prentice-Hall, Englewood Cliffs, N.J.

Devlin, R. H., and Nagahama, Y. (2002). Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **208,** 191–364.

Dierkes, P., Taborsky, M., and Kohler, U. (1999). Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav. Ecol.* **10,** 510–515.

Dittrich, F., Feng, Y., Metzdorf, R., and Gahr, M. (1999). Estrogen-inducible sex-specific expression of brain-derived neurotrophic factor mRNA in a forebrain song control nucleus of the juvenile zebra finch. *Proc. Natl. Acad. Sci. USA* **96,** 8241–8246.

Dulka, J. G., Stacey, N. E., Sorensen, P. W., and van der Kraak, G. J. (1987). A sex steroid pheromone synchronises male-female spawning readiness in goldfish. *Nature* **325,** 251–253.

Farr, J. A. (1989). Sexual selection and secondary sexual differentiation in poeciliids: Determinants of male mating success and the evolution of female choice. *In* "Ecology and Evolution of Livebearing Fishes (Poeciliidae)" (Meffe, G. K., and Snelson, F. J., Jr., Eds.), pp. 91–123. Prentice Hall, Englewood Cliffs, NJ.

Fishelson, L., and Hilzerman, F. (2002). Flexibility in reproductive styles of male St. Peter's tilapia, *Sarotherodon galilaeus* (Cichlidae). *Env. Biol. Fish* **63,** 173–182.

Foran, C. M., and Bass, A. H. (1998). Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. *Gen. Comp. Endocrinol.* **111,** 271–282.

Foran, C. M., and Bass, A. H. (1999). Preoptic GnRH and AVT: Axes for sexual plasticity in teleost fish. *Gen. Comp. Endocrinol.* **116,** 141–152.

Forlano, P. M., Deitcher, D. L., Myers, D. A., and Bass, A. H. (2001). Anatomical distribution and cellular basis for high levels of aromatase activity in the brain of teleost fish: Aromatase enzyme and mRNA expression identify glia as a source. *J. Neurosci.* **21,** 8943–8955.

Forsyth, A., and Alcock, J. (1990). Female mimicry and resource defense polygyny by males of a tropical rove beetle, *Leistotrophus versicolor* (Coleoptera: Staphylinidae). *Behav. Ecol. Sociobiol.* **26,** 325–330.

Francis, R. C. (1984). The effects of bidirectional selection for social dominance on agonistic behaviour and sex ratios in the paradise fish (*Macropodus opercularis*). *Behaviour* **90,** 25–45.

Francis, R. C. (1990). Temperament in a fish: A longitudinal study of the development of individual differences in aggression and social rank in the Midas cichlid. *Ethology* **86,** 311–325.

Francis, R. C. (1992). Sexual lability in teleosts: Developmental factors. *Quart. Rev. Biol.* **67,** 1–18.

Froese, R., and Pauly, D. (Eds.) (2004). FishBase. http://www.fishbase.org. September 7, 2004.

Fu, P., Neff, B. D., and Gross, M. R. (2001). Tactic-specific success in sperm competition. *Proc. R. Soc. Lond. B* **268,** 1105–1112.

Garant, D., Fontaine, P.-M., Good, S. P., Dodson, J. J., and Bernatchez, L. (2002). The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). *Evol. Ecol. Res.* **4,** 537–549.

Gardner, L., Anderson, T., Place, A. R., and Elizur, A. (2003). Sex change strategy and the aromatase genes. *Fish Physiol. Biochem.* **28,** 147–148.

Gelinas, D., and Callard, G. V. (1997). Immunolocalisation of aromatase- and androgen receptor-positive neurons in the goldfish brain. *Gen. Comp. Endocrinol.* **106,** 155–168.

Godwin, J. R., and Thomas, P. (1993). Sex-change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *Gen. Comp. Endocrinol.* **91,** 144–157.

Godwin, J., Crews, D., and Warner, R. R. (1996). Behavioural sex change in the absence of gonads in a coral reef fish. *Proc. R. Soc. Lond. B* **263,** 1683–1688.

Godwin, J., Luckenbach, J. A., and Borski, R. J. (2003). Ecology meets endocrinology: Environmental sex determination in fishes. *Evol. Dev.* **5,** 40–49.

Godwin, J., Sawby, R., Warner, R. R., Crews, D., and Grober, M. S. (2000). Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. *Brain Behav. Evol.* **55,** 77–84.

Goldschmidt, T., Foster, S. A., and Sevenster, P. (1992). Inter-nest distance and sneaking in the three–spined stickleback. *Anim. Behav.* **44,** 793–795.

Gonçalves, D. M., Matos, R., Fagundes, T., and Oliveira, R. F. (2005). Do bourgeois males of the peacock blenny, *Salaria pavo*, discriminate females from female-mimicking sneaker males? *Ethology* **111,** 559–772.

Gonçalves, E. J., Almada, V. C., Oliveira, R. F., and Santos, A. J. (1996). Female mimicry as a mating tactic in males of the blenniid fish *Salaria pavo*. *J. Mar. Biol. Ass. UK* **76,** 529–538.

Goodson, J., and Bass, A. H. (2000). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature* **403,** 769–772.

Goodson, J., and Bass, A. H. (2001). Social behaviour functions and related anatomical characteristics of vasotocin/ vasopressin systems in vertebrates. *Brain Res. Rev.* **35,** 246–265.

Goodwin, N. B., Dulvy, N. K., and Reynolds, J. D. (2002). Life history correlates in the evolution of live-bearing in fishes. *Phil. Trans. R. Soc. B* **357,** 259–267.

Gould, S. J., and Vrba, E. S. (1982). Exaptation: A missing term in the science of form. *Paleobiology* **8,** 4–15.

Govoroun, M., McMeel, O. M., D'Cotta, H., Ricordel, M. J., Smith, T., Fostier, A., and Guiguen, Y. (2001). Steroid enzyme gene expression during natural and androgen-induced gonadal differentiation in the rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Zool.* **290,** 558–566.

Grober, M. S. (1998). Socially controlled sex change: Integrating ultimate and proximate levels of analysis. *Acta Ethol.* **1,** 3–17.

Grober, M. S., and Bass, A. H. (1991). Neuronal correlates of sex/role change in labrid fishes: LHRH-like immunoreactivity. *Brain Behav. Evol.* **38,** 302–312.

Grober, M. S., George, A. A., Watkins, K. K., Carneiro, L. A., and Oliveira, R. F. (2002). Forebrain AVT and courtship in a fish with alternative reproductive tactics. *Brain Res. Bull.* **57,** 23–25.

Grober, M. S., Jackson, I. M. D., and Bass, A. H. (1991). Gonadal steroids affect LHRH preoptic cell number in a sex/role reversing fish. *J. Neurobiol.* **2,** 734–741.

Grober, M. S., Laughlin, C., Fox, S., and Bass, A. (1994). GnRH cell size and number in a teleost fish with two male reproductive morphs: Sexual maturation, final sexual status and body size allometry. *Brain Behav. Evol.* **43,** 61–78.

Gross, M. R. (1982). Sneakers, Satellites and Parentals: Polymorphic mating strategies in North American sunfishes. *Z. Tierpsychol.* **60,** 1–26.

Gross, M. R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *TREE* **11,** 92–98.

Guiguen, Y., Jalabert, B., Thouard, E., and Fostier, A. (1993). Changes in plasma and gonadal steroid hormones in relation to the reproductive cycle and the sex inversion process in the protandrous seabass, *Lates calacarifer*. *Gen. Comp. Endocrinol.* **92,** 327–338.

Guiguen, Y., Baroiller, J. F., Ricordel, M.-J., Iseki, K., McMeel, O. M., Martin, S. A. M., and Fostier, A. (1999). Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). *Mol. Reprod. Dev.* **54,** 154–162.

Halpern-Sebold, L., Schreibman, M. P., and Margolis-Nunno, H. (1986). Differences between early- and late-maturing genotypes of the platyfish (*Xiphophorus maculatus*) in the morphometry of their immunoreactive luteinising hormone releasing hormone-containing cells: A developmental study. *J. Exp. Zool.* **240,** 245–257.

Hawkins, M. B., Thornton, J. W., Crews, D., Skipper, J. K., Dotte, A., and Thomas, P. (2001). Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleosts. *Proc. Natl. Acad. Sci. USA* **97,** 10751–10756.

Henson, S. A., and Warner, R. R. (1997). Male and female alternative reproductive behaviours in fishes: A new approach using intersexual dynamics. *Annu. Rev. Ecol. Syst.* **28,** 571–592.

Hirschenhauser, K., Taborsky, M., Oliveira, T., Canario, A. V. M., and Oliveira, R. F. (2004). A test of the 'challenge hypothesis' in cichlid fish: Simulated partner and territory intruder experiments. *Anim. Behav.* **68,** 741–750.

Hobby, A. C., Geraghty, D. P., and Pankhurst, N. W. (2000a). Differences in binding characteristics of sex steroid binding protein in reproductive and non reproductive female rainbow trout (*Oncorhynchus mykiss*), black bream (*Acanthopagrus butcheri*) and greenback flounder (*Rhombosolea tapirina*). *Gen. Comp. Endocrinol.* **120,** 249–259.

Hobby, A. C., Pankhurst, N. W., and Geraghty, D. P. (2000b). A comparison of sex steroid binding protein (SBP) in four species of teleost fish. *Fish Physiol. Biochem.* **23,** 245–256.

Hofmann, H. A. (2003). Functional genomics of neural and behavioural plasticity. *J. Neurobiol.* **54,** 272–282.

Hogg, J. T. (1984). Mating in bighorn sheep: Multiple creative male strategies. *Science* **225,** 526–529.

Hourigan, T. F., Nakamura, N., Nagahama, Y., Yamauchi, K., and Grau, E. G. (1991). Histology, ultrastructure, and *in vitro* steroidogenesis of the testes of two male phenotypes of the protogynous fish, *Thalassoma duperrey* (Labridae). *Gen. Comp. Endocrinol.* **83,** 193–217.

Ikeuchi, T., Todo, T., Kobayashi, T., and Nagahama, Y. (2001). Two subtypes of androgen and progestogen receptors in fish testes. *Comp. Biochem. Physiol. B* **129,** 449–455.

Jamieson, I. G., and Colgan, P. W. (1992). Sneak spawning and egg stealing by male three-spine sticklebacks. *Can. J. Zool.* **70,** 963–967.

Jennings, D. H., Moore, M. C., Knapp, R., Matthews, L., and Orchinik, M. (2000). Plasma steroid-binding globulin mediation of differences in stress reactivity in alternative male phenotypes in tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* **120,** 289–299.

Jennings, D. H., Painter, D. L., and Moore, M. C. (2004). Role of the adrenal gland in early post-hatching differentiation of alternative male phenotypes in the tree-lizard (*Urosaurus ornatus*). *Gen. Comp. Endocrinol.* **135,** 81–89.

Johnson, A. K., Thomas, P., and Wilson, R. R., Jr. (1998). Seasonal cycles of gonadal development and plasma sex steroid levels in *Epinephelus morio*, a protogynous grouper in the eastern Gulf of Mexico. *J. Fish Biol.* **52,** 502–518.

Jones, A. G., Walter, D., Kvarnemo, C., Lindström, K., and Avise, J. C. (2001). How cuckoldry can decrease the opportunity for sexual selection: Data and theory from a genetic parentage análisis of the sand goby, *Pomatoschistus minutus*. *Proc. Natl. Acad. Sci. USA* **98,** 9151–9156.

Ketterson, E. D., and Nolan, V., Jr. (1992). Hormones and life histories: An integrative approach. *Am. Nat.* **140,** S33–S62.

Ketterson, E. D., and Nolan, V., Jr. (1994). Hormones and life histories: An integrative approach. *In* "Behavioural Mechanisms in Evolutionary Ecology" (Real, L. A., Ed.), pp. 327–353. University of Chicago Press, Chicago.

Ketterson, E. D., and Nolan, V., Jr. (1999). Adaptation, exaptation, and constraint: A hormonal perspective. *Am. Nat.* **154,** S4–S25.

Ketterson, E. D., Nolan, V., Jr., Casto, J. M., Buerkle, C. A., Clotfelter, E., Grindstaff, J. L., Jones, K. J., Lipar, J. L., McNabb, F. M. A., Neudorf, D. L., Parker-Renga, I., Schoech, S. J., and Snajdr, E. (2001). Testosterone, phenotype and fitness: A research program in evolutionary behavioural endocrinology. *In* "Avian Endocrinology" (Dawson, A., and Chaturvedi, C. M., Eds.), pp. 19–40. Narosa Publishing House, New Delhi.

Kim, S. J., Ogasawara, K., Park, J. G., Takemura, A., and Nakamura, M. (2002). Sequence and expression of androgen receptor and estrogen receptor gene in the sex types of protogynous wrasse, *Heliochoeres trimaculatus*. *Gen. Comp. Endocrinol.* **127,** 165–173.

Kindler, P. M., Philipp, D. P., Gross, M. R., and Bahr, J. M. (1989). Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus*: Centrarchidae). *Gen. Comp. Endocrinol.* **75,** 446–453.

Kindler, P. M., Bahr, J. M., and Philipp, D. P. (1991). The effects of exogenous 11-ketotestosterone, testosterone, and cyproterone acetate on prespawning and parental care behaviours of male bluegill. *Horm. Behav.* **25,** 410–423.

Kitano, T., Takamune, K., Kobayashi, T., Nagahama, Y., and Abe, S. I. (1999). Supression of P450 aromatase gene expression in sex-reversed males produced by rearing genetically female larvae at a high water temperature during a period of sex differentiation in the Japanese flounder (*Paralichthys olivaceus*). *J. Mol. Endocrinol.* **23,** 167–176.

Knapp, R., Carlisle, S. L., and Jessop, T. S. (2002). A model for androgen-glucocorticoid interactions in male alternative reproductive tactics: Potential roles for steroidogenic enzymes. *Horm. Behav.* **41,** 475.

Knapp, R., Hews, D., Thompson, C. W., Ray, L. E., and Moore, M. C. (2003). Environmental and endocrine correlates of tactic switching by non–territorial male tree lizards (*Urosaurus ornatus*). *Horm. Behav.* **43,** 83–92.

Knapp, R. (2004). Endocrine mediation of vertebrate male alternative reproductive tactics: The next generation of studies. *Integr. Com. Biol.* **43,** 658–668.

Kobayashi, M., and Nakanishi, T. (1999). 11-ketotestosterone induces male-type sexual behaviour and gonadotropin secretion in gynogenetic crucian carp, *Carassius auratus langsdorfii*. *Gen. Comp. Endocrinol.* **115,** 178–187.

Kown, J. Y., McAndrew, B. J., and Penman, D. J. (2001). Cloning of brain aromatase gene and expression of brain and ovarian aromtase genes during sexual differentiation in genetic male and female Nile tilapia, *Oreochromis niloticus*. *Mol. Reprod. Dev.* **59,** 359–370.

Kroon, F. J., and Liley, N. R. (2000). The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholsi* (Teleostei: Gobiidae). *Gen. Comp. Endocrinol.* **118,** 273–283.

Laidley, C. W., and Thomas, P. (1994). Partial characterisation of a sex–steroid binding protein in the spotted seatrout (*Cynoscion nebulosus*). *Biol. Reprod.* **51,** 982–992.

Lank, D. B., Smith, C. M., Hanotte, O., Burke, T., and Cooke, F. (1995). Genetic polymorphism for alternative mating behaviour in lekking male ruff *Nature* **378,** 59–62.

Larsson, D. G., Sperry, T., and Thomas, P. (2002). Regulation of androgen receptors in Atlantic croaker brains by testosterone and estradiol. *Gen. Comp. Endocrinol.* **128,** 224–230.

Laumen, J., Pern, U., and Blüm, V. (1974). Investigations on the function and hormonal regulations of the anal appendices in *Blennius pavo*. *J. Exp. Zool.* **190,** 47–56.

Lee, J. S. F., and Bass, A. H. (2004). Effects of 11-ketotestosterone on brain, sonic muscle, and behaviour in type-II midshipman fish. *Horm. Behav.* **46,** 115–116.

Lee, Y. H., Du, J. L., Yueh, W. S., Lin, B. Y., Huang, J. D., Lee, C. Y., Lee, M. F., Lau, E. L., Lee, F. Y., Morrey, C., Nagahama, Y., and Chang, C. F. (2001). Sex change in the protandrous black porgy, *Acanthopagrus schlegeli*: A review in gonadal development, estradiol, estrogen receptor, aromatase activity and gonadotropin. *J. Exp. Zool.* **290,** 715–726.

Leitz, T. (1987). Social control of testicular steroidogenic capacities in the Siamese fighting fish *Betta splendens* Regan. *J. Exp. Zool.* **244,** 473–478.

Lejeune, P. (1985). Etude éco-étologique des comportements reproducteurs et sociaux des labridés Méditerranéens des genres *Symphodus* Rafinesque, 1810 et *Coris* Lacepede, 1802. *Cah. Ethol. Appl.* **5,** 1–208.

Liley, N. R., Breton, B., Fostier, A., and Tan, E. S. P. (1986). Endocrine changes associated with spawning behaviour and social stimuli in a wild population of rainbow trout (*Salmo gairdneri*) 1. Males. *Gen. Comp. Endocrinol.* **62,** 145–156.

Liley, N. R., Olsén, K. H., Foote, C. J., and van der Kraak, G. J. (1993). Endocrine changes associated with with spawning behaviour in male kokanee salmon (*Oncorhynchus nerka*) and the effects of anosmia. *Horm. Behav.* **27,** 470–487.

Liley, N. R., and Stacey, N. E. (1983). Hormones, pheromones, and reproductive behaviour in fish. *In* "Fish Physiology—Reproduction, Part B: Behaviour and Fertility Control" (Hoar, W. S., Randall, D. J., and Donaldson, E. M., Eds.), Vol. 9, pp. 1–63. Academic Press, New York.

Lin, X. W., and Peter, R. E. (1996). Expression of salmon gonadotropin–releasing hormone (GnRH) and chicken GnRH-II precursor messenger ribonucleic acids in the brain and ovary of goldfish. *Gen. Comp. Endocrinol.* **101,** 282–296.

Liu, S., Govoroun, M., D'Cotta, H., Ricordel, M. J., Lareyre, J.-J., McMeel, O. M., Smith, T., Nagahama, Y., and Guiguen, Y. (2000). Expression of cytochrome P45011β (11β-hydroxilase) gene during gondal sex differentiation and spermatogenesis in rainbow trout, *Oncorhynchus mykiss*. *J. Steroid Biochem. Mol. Biol.* **75,** 291–298.

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C. E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S., and Sakaizumi, M. (2002). DMY is a Y-specificDM-domain gene required for male development in the medaka fish. *Nature* **417,** 559–563.

Matsuyama, M., Adachi, S., Nagahama, Y., Kitajima, C., and Matsuura, S. (1991). Testicular development and serum levels of gonadal steroids during the annual reproductive cycle of captive Japanese sardine. *Jap. J. Ichthyol.* **37,** 381–390.

Mayer, I., Lundqvist, H., Berglund, I., Schmitz, M., Schulz, R., and Borg, B. (1990). Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17β-estradiol. *Can. J. Zool.* **68,** 1360–1365.

Mayer, I., Rosenqvist, G., Borg, B., Ahnesjö, I., Berglund, A., and Schulz, R. W. (1993). Plasma levels of sex steroids in three species of pipefish (Syngnathidae). *Can. J. Zool.* **71,** 1903–1907.

Mayer, I., Liley, N. R., and Borg, B. (1994). Stimulation of spawning behaviour in castrated rainbow trout (*Oncorhynchus mykiss*) by 17α,20β-dihydroxy-4-pregnen-3-one, but not by 11-ketoandrostenedione. *Horm. Behav.* **28,** 181–190.

Mazzoldi, C., and Rasotto, M. B. (2002). Alternative male mating tactics in *Gobius niger*. *J Fish Biol.* **61,** 157–172.

Mazzoldi, C., Scaggiante, M., Ambrosin, E., and Rasotto, M. B. (2000). Mating system and alternative mating tactics in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). *Mar. Biol.* **137,** 1041–1048.

Miguel-Queralt, S., Knowlton, M., Avvakumov, G. V., AL-Nouno, R., Kelly, G. M., and Hammond, G. L. (2004). Molecular and functional characterisation of sex hormone binding globulin in zebrafish. *Endocrinol.* **145,** 5221–5230.

Miranda, J. A., Oliveira, R. F., Carneiro, L. A., Santos, R. S., and Grober, M. S. (2003). Neurochemical correlates of male polymorphism and alternative reproductive tactics in the Azorean rock-pool blenny, *Parablennius parvicornis*. *Gen. Comp. Endocrinol.* **132,** 183–189.

Modesto, T., and Canário, A. V. M. (2003a). Morphometric changes and sex steroid levels during the annual reproductive cycle of the Lusitanean toadfish, *Halobatrachus didactylus*. *Gen. Comp. Endocrinol.* **131,** 220–231.

Modesto, T., and Canário, A. V. M. (2003b). Hormonal control of swimbladder sonic muscle dimorphism in the Lusitanian toadfish, *Halobatrachus didactylus*. *J. Exp. Biol.* **206,** 3467–3477.

Moore, M. C. (1991). Application of organisation-activation theory to alternative male reproductive strategies: A review. *Horm. Behav.* **25,** 154–179.

Moore, M. C., Hews, D. K., and Knapp, R. (1998). Hormonal control and evolution of alternative male phenotypes: Generalisations of models for sexual differentiation. *Am. Zool.* **38,** 133–151.

Morita, S., Matsuyama, M., and Kashiwagi, M. (1997). Seasonal changes of gonadal histology and serum steroid hormone levels in the bambooleaf wrasse *Pseudolabrus japonicus*. *Bull. Jpn. Soc. Sci. Fish./Nippon Suisan Gakkaishi* **63,** 694–700.

Morrey, C. E., and Nagahama, Y. (2000). 11β-hydroxilase and androgen receptor mRNA expression in the ovary, testis and brain of the protogynous hermaphrodite *Thalassoma duperrey*. *In* "Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish" (Norgerg, B., Kjesbu, O. S., Taranger, G. L., Andersson, E., and Stefansson, S. O., Eds.), pp. 157–159. Bergen, Norway.

Munday, P., and Jones, G. (1998). Bi-directional sex change in a coral-dwelling goby. *Behav. Ecol. Sociobiol.* **43,** 371–377.

Nakamura, M., Mariko, T., and Nagahama, Y. (1994). Ultrastructure and *in vitro* steroidogenesis of the gonads in the protandrous anemonefish *Amphiprion frenatus*. *Jap. J. Ichthyol.* **41,** 47–56.

Nakamura, M., Kobayashi, T., Chang, X.-T., and Nagahama, Y. (1998). Gonadal sex differentiation in teleost fish. *J. Exp. Zool.* **281,** 362–372.

Nakamura, M., Bhandari, R. K., and Higa, M. (2003). The role estrogens play in sex differentiation and sex changes of fish. *Fish Physiol. Biochem.* **28,** 113–117.

Neat, F. C. (2001). Male parasitic spawning in two species of triplefin blenny (Tripterigiidae): Contrasts in demography, behaviour and gonadal characteristics. *Env. Biol. Fish* **61,** 57–64.

Neff, B. D. (2004). Increased performance of offspring sired by parasitic males in bluegill sunfish. *Behav. Ecol.* **15,** 327–331.

Neff, B. D., Fu, P., and Gross, M. R. (2003). Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). *Behav. Ecol.* **14,** 634–641.

Nelson (1994). "Fishes of the World." John Wiley & Sons, New York.

Nelson, R. J. (2000). "An Introduction to Behavioural Endocrinology." Sinauer Associates, Sunderland, MA.

Norman, M. D., Finn, J., and Tregenza, T. (1999). Female impersonation as an alternative reproductive strategy in giant cuttlefish. *Proc. R. Soc. Lond. B* **266,** 1347–1349.

Oliveira, R. F. (2004). Social modulation of androgens in vertebrates: Mechanisms and function. *Adv. Study Behav.* **34,** 165–239.

Oliveira, R. F., and Almada, V. C. (1998a). Mating tactics and male-male courtship in the lek–breeding cichlid *Oreochromis mossambicus*. *J. Fish Biol.* **52,** 1115–1129.

Oliveira, R. F., and Almada, V. C. (1998b). Androgenisation of dominant males in a cichlid fish: Androgens mediate the social modulation of sexually dimorphic traits. *Ethology* **104,** 841–858.

Oliveira, R. F., and Almada, V. C. (1999). Male displaying characters, gonadal maturation and androgens in the cichlid fish *Oreochromis mossambicus*. *Acta Ethol.* **2,** 67–70.

Oliveira, R. F., and Canario, A. V. M. (2001). Hormones and social behaviour of cichlid fishes: A case study in the Mozambique tilapia. *J. Aquariculture and Aquat. Sci.* **9,** 187–207.

Oliveira, R. F., Almada, V. C., and Canario, A. V. M. (1996). Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. *Horm. Behav.* **30,** 2–12.

Oliveira, R. F., Almada, V. C., Gonçalves, E. J., Forsgren, E., and Canario, A. V. M. (2001a). Androgen levels and social interactions in breeding males of the peacock blenny. *J. Fish Biol.* **58,** 897–908.

Oliveira, R. F., Canario, A. V. M., and Grober, M. S. (2001b). Male sexual polymorphism, alternative reproductive tactics and androgens in combtooth blennies (Pisces: Blenniidae). *Horm. Behaviour* **40,** 266–275.

Oliveira, R. F., Canario, A. V. M., Grober, M. S., and Santos, R. S. (2001c). Endocrine correlates of alternative reproductive tactics and male polymorphism in the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*. *Gen. Comp. Endocrinol.* **121,** 278–288.

Oliveira, R. F., Carneiro, L. A., Gonçalves, D. M., Canario, A. V. M., and Grober, M. S. (2001d). 11-ketotestosterone inhibits the alternative mating tactic in sneaker males of the peacock blenny, *Salaria pavo*. *Brain Behav. Evol.* **58,** 28–37.

Oliveira, R. F., Carneiro, L. A., Canario, A. V. M., and Grober, M. S. (2001e). Effects of androgens on social behaviour and morphology of alternative reproductive males of the Azorean rock-pool blenny. *Horm. Behav.* **39,** 157–166.

Oliveira, R. F., Gonçalves, E. J., and Santos, R. S. (2001f). Gonadal investment of young males in two blenniid fishes with alternative mating tactics. *J. Fish Biol.* **59,** 459–462.

Oliveira, R. F., Lopes, M., Carneiro, L. A., and Canario, A. V. M. (2001g). Watching fights raises fish hormone levels. *Nature* **409,** 475.

Oliveira, R. F., Ros, A. F. H., Hirschenhauser, K., and Canario, A. V. M. (2001h). Androgens and mating systems in fish: intra- and interspecific analyses. *In* "Perspectives in Comparative Endocrinology: Unity and Diversity" (Goos, H. J., Rastogi, R. K., Vaudry, H., and Pierantoni, R., Eds.), pp. 203–215. Monduzzi Editore, Bologna.

Oliveira, R. F., Hirschenhauser, K., Carneiro, L. A., and Canario, A. V. M. (2002a). Social modulation of androgens in male teleost fish. *Comp. Biochem. Physiol.* **132B,** 203–215.

Oliveira, R. F., Miranda, J. A., Carvalho, N., Gonçalves, E. J., Grober, M. S., and Santos, R. S. (2002b). The relationship between the presence of satellite males and nest-holders' mating success in the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*. *Ethology* **108,** 223–235.

Oliveira, R. F., Hirschenhauser, K., Canario, A. V. M., and Taborsky, M. (2003). Androgen levels of reproductive competitors in a cooperatively breeding cichlid. *J. Fish Biol.* **63,** 1615–1620.

Oliveira, R. F., Ros, A. F. H., and Gonçalves, D. M. (2005). Intra-sexual variation in male reproduction in teleost fish: A comparative approach. *Horm. Behav.* in press.

Olsén, H. K., Järvi, J. T., Mayer, I., Petersson, E., and Kroon, F. (1998). Spawning behaviour and sex hormone levels in adult and precocious brown trout (*Salmo trutta* L.) males and the effect of anosmia. *Chemoecology* **8,** 9–17.

Parhar, I. (2002). Cell migration and evolutionary significance of GnRH subtypes. *Prog. Brain Res.* **141,** 3–17.

Parhar, I. (2003). Gonadotrophin-releasing hormone receptors: Neuroendocrine regulators and neuromodulators. *Fish Physiol. Biochem.* **28,** 13–18.

Pasmanik, M., and Callard, G. (1986). Characteristics of a testosterone-estradiol binding globulin (TEBG) in goldfish serum. *Biol. Reprod.* **35,** 838–845.

Pasmanik, M., and Callard, G. (1988). A high abundance androgen receptor in goldfish brain: Characteristics and seasonal changes. *Endocrinol.* **123,** 1162–1171.

Patzner, R. A., and Lahnsteiner, F. (1999). The accessory organs of the male reproductive system in Mediterranean Blennies (Blenniidae) in comparison with those of other blenniid fishes (tropical Blenniidae, Tripterygiidae, Labrisomidae, Clinidae, Chaenopsidae, Dactyloscopidae). *In* "Behaviour and Conservation of Littoral Fishes" (Almada, V. C., Oliveira, R. F., and Gonçalves, E. J., Eds.), pp. 179–228. ISPA, Lisboa.

Perril, S. A., Gerhardt, H. C., and Daniel, R. (1978). Sexual parasitism in the green tree frog, *Hyla cinerea*. *Science* **200,** 1179–1180.

Perry, A. N., and Grober, M. S. (2003). A model for social control of sex change: Interactions of behaviour, neuropetides, glucocorticoids, and sex steroids. *Horm. Behav.* **43,** 31–38.

Peter, R. E., Yu, K. L., Marchant, T. A., and Rosenblum, P. N. (1990). Direct neural regulation of the teleost adenohypophysis. *J. Exp. Zool.* **4,** 84–89.

Picciulin, M., Verginela, L., Spoto, M., and Ferrero, E. A. (2004). Colonial nesting and the importance of the brood size in male parasitic reproduction of the Mediterranean damselfish *Chromis chromis* (Pisces: Pomacentridae). *Env. Biol. Fish.* **70,** 23–30.

Pickford, G. E., and Strecker, E. L. (1977). The spawning reflex response of the killifish *Fundulus heteroclitus*; isotocin is relatively inactive in comparison with arginine vasotocin. *Gen. Comp. Endocrinol.* **32,** 132–137.

Reavis, R. H., and Grober, M. S. (1999). An integrative approach to sex change: Social, behavioural and neurochemical changes in *Lythrypnus dalli* (Pisces). *Acta Ethol.* **2,** 51–60.

Reinboth, R., and Becker, B. (1984). *In vitro* studies on steroid metabolism by gonadal tissues from ambisexual teleosts. I. Conversion of 14–C testosterone by males and females of the protogynous wrasse *Coris julis* L. *Gen. Comp. Endocrinol.* **55,** 245–250.

Remage-Healey, L., and Bass, A. H. (2004). Rapid, hierarchical modulation of vocal patterning by steroid hormones. *J. Neurosci.* **24,** 5892–5900.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. (1993). Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. *Anim. Behav.* **45,** 145–152.

Rhen, T., and Crews, D. (2002). Variation in reproductive behaviour within a sex: Neural systems and endocrine activation. *J. Neuroendocrinol.* **14,** 517–531.

Ros, A. F. H., Canario, A. V. M., Couto, E., Zeilstra, I., and Oliveira, R. F. (2003). Endocrine correlates of intra-specific variation in the mating system of the St. Peter's fish (*Sarotherodon galilaeus*). *Horm. Behav.* **44,** 365–373.

Ros, A. F. H., Bouton, N., Santos, R. S., and Oliveira, R. F. (2004a). Are aggressive fathers immunosupressed? Alternative reproductive tactics, androgens and immunocompetence in the Azorean rock-pool blenny. *Horm. Behav.* **46,** 120.

Ros, A. F. H., Bruintjes, R., Santos, R. S., Canario, A. V. M., and Oliveira, R. F. (2004b). The role of androgens in the trade–off between territorial and parental behaviour in the Azorean rock-pool blenny, *Parablennius parvicornis*. *Horm. Behav.* **46,** 491–497.

Rosen, D. E., and Tucker, A. (1961). Evolution of secondary sexual characters and sexual behaviour patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). *Copeia* **1961,** 201–212.

Rouger, Y., and Liley, N. R. (1993). The effect of social environment on plasma hormones and availability of milt in spawning male rainbow trout (*Oncorhynchus mykiss* Walbaum). *Can. J. Zool.* **71,** 280–285.

Ruchon, F., Laugier, T., and Quignard, J. P. (1995). Alternative male reproductive strategies in the peacock blenny. *J. Fish Biol.* **47,** 826–840.

Ryan, M. J., Pease, C. M., and Morris, M. R. (1992). A genetic polymorphism in the swordtail *Xiphophorus nigrensis*: Testing the predictions of equal fitnesses. *Am. Nat.* **139,** 21–31.

Salek, S. J., Sullivan, C. V., and Godwin, J. (2002). Arginine vasotocin effects on courtship behaviour in male white perch (*Morone americana*). *Behav. Brain Res.* **133,** 177–183.

Santos, R. S. (1985). Parentais e satélites: Tácticas alternativas de acasalamento nos machos de *Blennius sanguinolentus* Pallas (Pisces: Blenniidae). *Arquipélago, Sér Ciênc. Nat.* **6,** 119–146.

Sargent, R. C., and Gross, M. R. (1993). William's principle: an explanation of parental care in teleost fishes. *In* "Behaviour of Teleost Fishes" (Pitcher, T. J., Ed.), pp. 333–361. Chapman and Hall, London.

Scaggiante, M., Grober, M. S., Lorenzi, V., and Rasotto, M. B. (2004). Changes in the male reproductive axis in response to social context in a gonochoristic gobiid, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae), with alternative mating tactics. *Horm. Behav.* **46,** 607–617.

Schartl, M., Wilde, B., Schlupp, I., and Parzefall, J. (1995). Evolutionary origin of a parthenoform, the Amazon molly, *Poecilia formosa*, on the basis of a molecular genealogy. *Evolution* **49,** 827–835.

Schlinger, B. A., Greco, C., and Bass, A. H. (1999). Aromatase activity in the hindbrain and vocal control region of a teleost fish: Divergence among males with alternative reproductive tactics. *Proc. Roy. Soc. Lond. B* **266,** 131–136.

Schlupp, I., Parzefall, J., Epplen, J. T., Nanda, I., Schmid, M., and Schartl, M. (1992). Pseudomale behaviour and spontaneous masculinisation in the all-female teleost *Poecilia formosa* (Teleostei: Poeciliidae). *Behaviour* **122,** 88–104.

Schlupp, I., McKnab, R., and Ryan, M. J. (2001). Sexual harassment as a cost for molly females: Bigger males cost less. *Behaviour* **138,** 277–286.

Schulz, R. W., and Miura, T. (2002). Spermatogenesis and its endocrine regulation. *Fish Physiol. Biochem.* **26,** 43–56.

Scott, A. P., and Baynes, S. M. (1982). Plasma levels of sex steroids in relation to ovulation and spermiation in rainbow trout (*Salmo gairdneri*). *In* "Proceedings of the International Symposium on Reproductive Physiology of Fish" (Richter, C. J. J., and Goos, H. J. T., Eds.), pp. 103–106. PUDOC, Wageningen, Netherlands.

Semsar, K., and Godwin, J. (2003). Multiple mechanisms of phenotype development in the bluehead wrasse. *Horm. Behav.* **45,** 345–353.

Semsar, K. A., Kandel, F. L. M., and Godwin, J. (2001). Manipulations of the AVT system shift social status and related courtship and aggressive behaviour in the bluehead wrasse. *Horm. Behaviour* **40,** 21–31.

Shuster, S. M. (1992). The reproductive behaviour of α-, β- and γ-male morphs in *Paracerceis sculpta*, a marine isopod crustacean. *Behaviour* **121,** 231–258.

Shuster, S. M., and Wade, M. J. (2003). "Mating Systems and Strategies." Princeton University Press, Princeton.

Silverman, H. I. (1978). Changes in male courting frequency in pairs of the cichlid fish, *Sarotherodon (Tilapia) mossambicus*, with unlimited or with only visual contact. *Behav. Biol.* **23,** 189–196.

Simon, N. G. (2002). Hormonal processes in the development and expression of aggressive behaviour. *In* "Hormones, Brain and Behaviour, Vol. 1" (Pfaff, D. W., Arnold, A. P., Etgen, A. M., Farbach, S. E., and Rubin, R. T., Eds.), pp. 339–392. Academic Press, New York.

Sinervo, B., and Lively, C. M. (1996). The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380,** 240–243.

Sinervo, B., and Svensson, E. (1998). Mechanistic and selective causes of life-history trade-offs and plasticity: Evolutionary physiology of the costs of reproduction. *Oikos* **83,** 432–442.

Sisneros, J. A., Forlano, P. M., Knapp, R., and Bass, A. H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. *Gen. Comp. Endocrinol.* **136,** 101–116.

Sperry, T., and Thomas, P. (1999). Characterisation of two nuclear androgen receptors in Atlantic croaker: Comparison of their biochemical properties and binding specificities. *Endocrinol.* **140,** 1602–1611.

Sperry, T., and Thomas, P. (2000). Androgen binding profiles of two distinct nuclear androgen receptors in Atlantic croaker (*Micropogonias undulatus*). *Steroid Biochem. Mol. Biol.* **72,** 93–103.

Stacey, N. E. (1987). Roles of hormones and pheromones in fish reproductive behaviour. *In* "Psychobiology of Reproductive Behaviour: An Evolutionary Perspective" (Crews, D., Ed.), pp. 28–60. Prentice-Hall, Englewood Cliffs, NJ.

Stacey, N., and Kobayashi, M. (1996). Androgen induction of male sexual behaviours in female goldfish. *Horm. Behav.* **30,** 434–445.

Stacey, N. E., and Kyle, A. L. (1983). Effects of olfactory tract lesions on sexual and feeding behaviour in the goldfish. *Physiol. Behav.* **30,** 621–628.

Strüssman, C. A., and Nakamura, M. (2002). Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. *Fish Physiol. Biochem.* **26,** 13–29.

Sunobe, T., and Nakazono, A. (1993). Sex change in both directions by alteration of social dominance in *Trimma okinawae* (Pisces: Gobiidae). *Ethology* **94,** 339–345.

Taborsky, M. (1994). Sneakers, satellites, and helpers: parasitic and cooperative behaviour in fish reproduction. *Adv. Study Behav.* **23,** 1–100.

Taborsky, M. (1997). Bourgeois and parasitic tactics: Do we need collective, functional terms for alternative reproductive behaviours? *Behav. Ecol. Sociobiol.* **41,** 361–362.

Taborsky, M. (1998). Sperm competition in fish: 'Bourgeois' males and parasitic spawning. *TREE* **13,** 222–227.

Taborsky, M. (1999). Conflict or Cooperation: what determines optimal solutions to competition in fish reproduction? *In* "Behaviour and Conservation of Littoral Fishes" (Almada, V. C., Oliveira, R. F., and Gonçalves, E. J., Eds.), pp. 301–349. I.S.P.A., Lisboa.

Taborsky, M. (2001). The evolution of bourgeois, parasitic, and cooperative reproductive behaviours in fishes. *J. Hered.* **92,** 100–110.

Tanaka, H., Hirose, K., Nogami, K., Hattori, K., and Ishibashi, N. (1990). Sexual maturation and sex reversal in red spotted grouper, *Epinephelus akaara*. *Bull. Natl. Res. Inst. Aquacult.* **17,** 1–15.

Taru, M., Kanda, T., and Sunobe, T. (2002). Alternative mating tactics of the gobiid fish *Bathygobius fuscus*. *J. Ethol.* **20,** 9–12.

Thomas, P. (2000). Nuclear and membrane steroid receptors and their functions in teleost gonads. *In* "Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish" (Norgerg, B., Kjesbu, O. S., Taranger, G. L., Andersson, E., and Stefansson, S. O., Eds.), pp. 149–156. Bergen, Norway.

Thomas, P. (2003). Rapid, nongenomic steroid actions initiated at the cell surface: Lessons from studies with fish. *Fish Physiol. Biochem.* **28,** 3–12.

Thresher, R. E. (1984). "Reproduction in Reef Fishes." T.F.H. Publications, Neptune City.

Tomkins, J. L., and Simmons, L. W. (2002). Measuring relative investment: A case study of testes investment in species with alternative male reproductive tactics. *Anim. Behav.* **63,** 1009–1016.

Trant, J. M., Gavasso, S., Ackers, J., Chung, B. C., and Place, A. R. (2001). Developmental expression of cytochrome P450 aromatase genes (CYP19A) and CYP19B) in zebrafish fry (*Danio rerio*). *J. Exp. Zool.* **290,** 475–483.

Tsai, C. L., Wang, L. H., Chang, C. F., and Kao, C. C. (2000). Effects of gonadal steroids on brain serotonergic and aromatase activity during the critical period of sexual differentiation in tilapia, *Oreochromis mossambicus*. *J. Neuroendocrinol.* **12,** 894–898.

Turner, G. (1993). Teleost mating behaviour. *In* "Behaviour of Teleost Fishes" (Pitcher, T. J., Ed.), pp. 307–331. Chapman & Hall, London.

Tyler, W. A. (1989). Optimal colony size in the Hawaiian Sergeant, *Abudefduf abdominalis* (Pisces: Pomacentridae). *Pacific Sci.* **43,** 204.

Uglem, I., Rosenqvist, G., and Schioler Wasslavik, H. (2000). Phenotypic variation between dimorphic males in corkwing wrasse (*Symphodus melops* L.). *J. Fish Biol.* **57,** 1–14.

Uglem, I., Mayer, I., and Rosenqvist, G. (2002). Variation in plasma steroids and reproductive traits in dimorphic males of corkwing wrasse (*Symphodus melops* L.). *Horm. Behav.* **41,** 396–404.

Yeung, W. S., and Chan, S. T. (1987). A radioimmunoassay study of the plasma levels of sex steroids in the protandrous, sex-reversing fish *Rhabdosargus sarba* (Sparidae). *Gen. Comp. Endocrinol.* **66,** 353–363.

Yu, K. L., Lin, X. W., Cunha Bastos, J., and Peter, R. E. (1997). Neural regulation of GnRH in teleost fishes. *In* "GnRH Neurons: Gene to Behaviour" (Parhar, I. S., and Sakuma, Y., Eds.), pp. 277–312. Brain Shuppan Publishing, Tokyo.

Warner, R. R. (1984). Mating behaviour and hermaphroditism in coral reef fishes. *Am. Sci.* **72,** 128–136.

Warner, R. R. (1985). Alternative mating behaviours in a coral reef fish: a life-history analysis. *In* "Proceedings of the 5th International Coral Reef Conference," pp. 145–150. Tahiti.

Warner, R. R. (1987). Female choice of sites versus mates in a coral reef fish, *Thalassoma bifasciatum*. *Anim. Behav.* **35,** 1470–1478.

Warner, R. R. (1990). Male versus female influences on mating-site determination in a coral reef fish. *Anim. Behav.* **39,** 540–548.

Warner, R. R., and Robertson, D. R. (1978). Sexual patterns in the Labroid fishes of the Western Caribbean, I: The wrasses (Labridae). *Smithsonian Contributions to Zoology* **254,** 1–27.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.* **20,** 249–278.

White, S. A., Kasten, T. L., Bond, C. T., Adelman, J. P., and Fernald, R. D. (1995). Three gonadotropin-releasing hormone genes in one organismsuggest novel roles for an ancient peptide. *Proc. Natl. Acad. Sci. USA* **92,** 8363–8367.

Wingfield, J. C., Hegner, R. E., Dufty, A. M., and Ball, G. F. (1990). The "challenge hypothesis": Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* **136,** 829–846.

Wingfield, J. C., Jacobs, J. D., Soma, K., Maney, D. L., Hunt, K., Wisti-Peterson, D., Meddle, S., Ramenofsky, M., and Sullivan, K. (1999). Testosterone, aggression, and communication: Ecological bases of endocrine phenomena. *In* "The Design of Animal Communication" (Hauser, M. D., and Konishi, M., Eds.), pp. 257–283. MIT Press, Cambridge, MA.

Wingfield, J. C., Lynn, S. E., and Soma, K. K. (2001). Avoiding the 'costs' of testosterone: Ecological bases of hormone-behaviour interactions. *Brain Behav. Evol.* **57,** 239–251.

Zarkower, D. (2001). Establishing sexual dimorphism: Conservation amidst diversity? *Nature Rev.* **2,** 175–185.

Zhu, Y., Rice, C. D., Pang, Y., Pace, M., and Thomas, P. (2003). Cloning, expression, and characterisation of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation in fish oocytes. *Proc. Natl. Acad. Sci. USA* **100,** 2231–2236.

Zimmerer, E. J., and Kallman, K. D. (1989). Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. *Evolution* **43,** 1298–1307.

Zupanc, G. K. H. (2001). Adult neurogenesis and neuronal regeneration in teleost fish. *Brain Behav. Evol.* **58,** 250–275.

Zupanc, G. K. H., and Lamprecht, J. (2000). Towards a cellular understanding of motivation: Structural reorganisation and biochemical switching as key mechanisms of behavioural plasticity. *Ethology* **106,** 467–477.