

Review

Social regulation of reproduction in male cichlid fishes



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ABSTRACT

Social interactions and relative positions within a dominance hierarchy have helped shape the evolution of reproduction in many animals. Since reproduction is crucial in all animals, and rank typically regulates access to reproductive opportunities, understanding the mechanisms that regulate socially-induced reproductive processes is extremely important. How does position in a dominance hierarchy impact an individual's reproductive behavior, morphology, and physiology? Teleost fishes, and cichlids in particular, are ideally-suited models for studying how social status influences reproduction on multiple levels of biological organization. Here I review the current knowledge on the reproductive behavioral and physiological consequences of relative position in a dominance hierarchy, with a particular focus on male cichlids. Dominant and subordinate social status is typically associated with distinct differences in activity along the entire hypothalamic–pituitary–gonadal axis. Further, when transitions in social status occur between subordinate and dominant individuals, there are plastic changes from whole-organism behavior to molecular-level gene expression modifications that occur quickly. These rapid changes in behavior and physiology have allowed cichlids the flexibility to adapt to and thrive in their often dynamic physical and social environments. Studies in cichlid fishes have, and will continue, to advance our understanding of how the social environment can modulate molecular, cellular, and behavioral outcomes relevant to reproductive success. Future studies that take advantage of the extreme diversity in mating systems, reproductive tactics, and parental care strategies within the cichlid group will help generate hypotheses and careful experimental tests on the mechanisms governing the social control of reproduction in many vertebrates.

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1. Introduction

In social animals, relative position in an established dominance hierarchy has important consequences for health, survival, and reproductive fitness (Sapolsky, 2005; Wingfield and Sapolsky, 2003). Dominance status in fishes may be influenced by several factors including relative body size or age, territory availability and quality, nutritional state and body condition, or overall group composition. Dominance is typically decided, however, by repeated agonistic interactions between individuals resulting in a consistent winner that is perceived by all members of the social group. Since hierarchical position is crucial for survival and reproduction, many animals, including cichlid fishes, have evolved the ability to predict their own position in the society, as well as the relative social rank of others, through observation and transitive

inference (Grosenick et al., 2007). Further, since environmental and social conditions are often dynamic, changes in an individual's relative social status can occur quickly and frequently within a population. How does an individual's social position influence his physiology and chances for reproduction? Cichlid fishes have become important model organisms for addressing this and related questions to further our understanding of the cellular and molecular mechanisms that regulate maintenance of social status positions in vertebrates.

Social rank can have profound impacts on an individual's reproductive behavior and physiology. In most cases, dominance is associated with increased reproductive opportunities, improved fitness, and an up-regulated reproductive axis compared to subordinate individuals. Notably, the influence of social rank has effects at every level of the reproductive axis from the brain to the testes, and at multiple levels of biological organization from whole animal behavior to molecular-level changes in gene expression (Maruska and Fernald, 2011b). In the sections below, I will review what is currently known about how social rank impacts reproductive behavior and physiology in male cichlids. I will focus primarily on the African cichlid *Astatotilapia burtoni*, an important and

Abbreviations: ATn, anterior tuberal nucleus; Ce, cerebellum; DL, lateral part of the dorsal telencephalon; POA, preoptic area; Vs, supra commissural nucleus of the ventral telencephalon; VTn, ventral tuberal nucleus; Vv, ventral nucleus of the ventral telencephalon.

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emerging model system for studying the social control of reproduction (Fig. 1), and make comparisons to other cichlid species where information is available. *A. burtoni* is a maternal mouthbrooding cichlid endemic to Lake Tanganyika, Africa. Males of this

species exist in two different phenotypes: dominant territorial males comprise ~10–30% of the population, are brightly colored, and aggressively defend a territory that serves as a food and spawning resource; and subordinate non-territorial males that

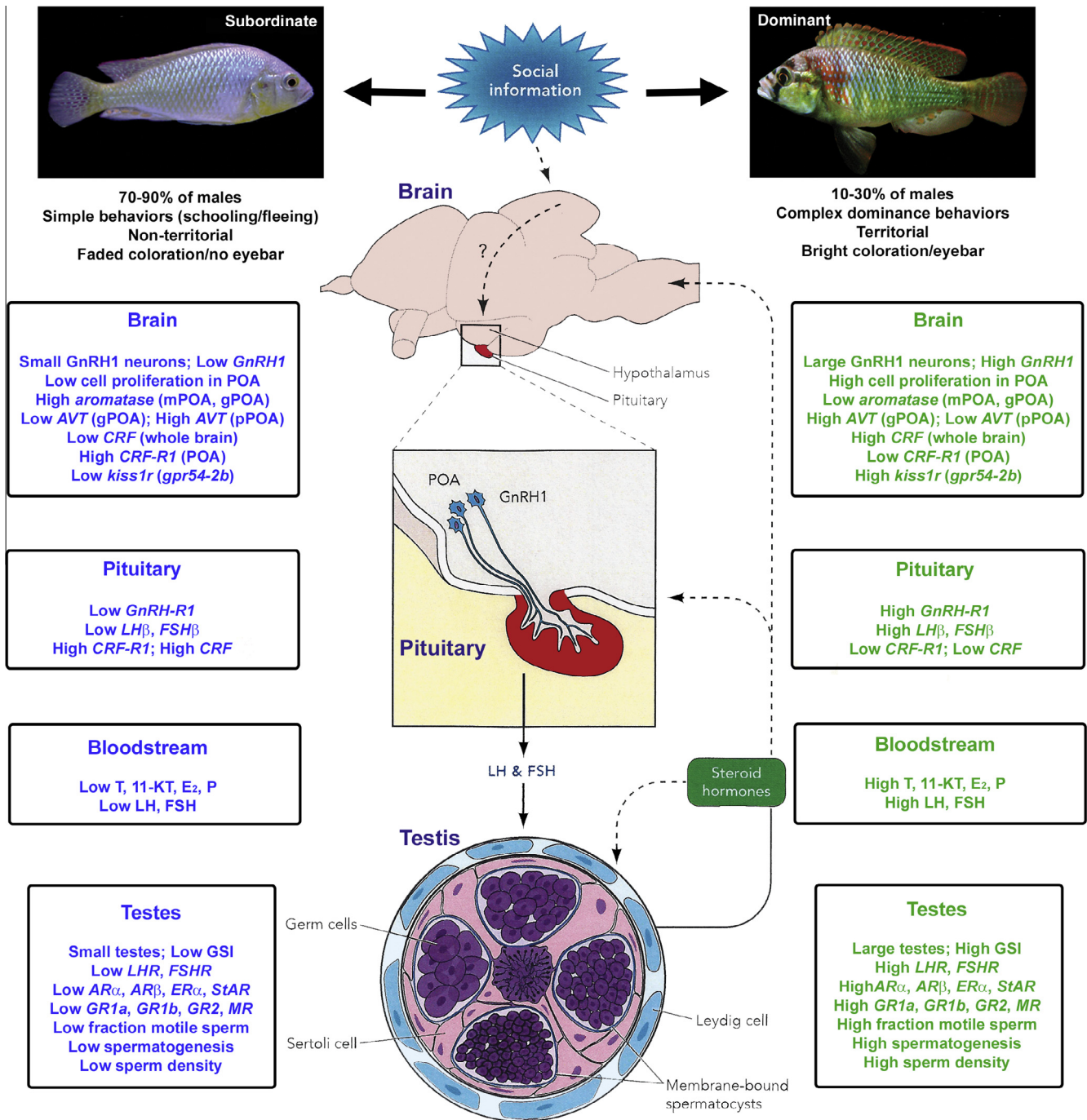


Fig. 1. Summary of social status differences in the hypothalamic–pituitary–gonadal (HPG) axis between dominant and subordinate male *Astatotilapia burtoni*. Dominant males (right) are brightly-colored, defend a territory, and exhibit many territorial and reproductive behaviors, while subordinate males (left), have more faded coloration similar to females, do not hold territories, and display submissive behaviors. Dominant males also have an up-regulated HPG axis compared to subordinate males. The HPG axis is also influenced by the social environment, and can rapidly change when males transition between lower-ranking and higher-ranking status, in both directions. Only those measures directly relevant to the HPG axis and reproduction are shown. Italicized genes indicate mRNA levels quantified via qPCR or *in situ* hybridization. Modified in part from Maruska and Fernald (2011b, 2013b) and data were compiled from the following studies (Carpenter et al., 2014; Chen and Fernald, 2006; Davis and Fernald, 1990; Greenwood et al., 2008; Grone et al., 2010; Huffman et al., 2012, 2013; Kustan et al., 2012; Maruska et al., 2012a, 2011, 2013b; Maruska and Fernald, 2010a, 2011a; O'Connell et al., 2013). **Abbreviations:** 11-KT, 11-ketotestosterone; AR α , AR β , androgen receptor subtypes α and β ; AVT, arginine vasotocin; CRF, corticotropin-releasing factor; CRF-R1, CRF receptor 1; E $_2$, 17 β -estradiol; ER α , estrogen receptor subtype α ; FSH β , β -subunit of follicle stimulating hormone; FSHR, FSH receptor; GnRH1, gonadotropin releasing hormone 1; GnRH-R1, GnRH receptor subtype 1; gPOA, gigantocellular POA; GR1a, GR1b, GR2, glucocorticoid receptor subtypes 1a, 1b, 2; *kiss1r*, kisspeptin receptor 1 (*gpr54-2b*), LH β , β -subunit of luteinizing hormone; LHR, LH receptor; mPOA, magnocellular POA; MR, mineralocorticoid receptor; P, progesterone; POA, preoptic area; pPOA, parvocellular POA; StAR, steroidogenic acute regulatory protein; T, testosterone.

comprise the majority of the male population, resemble and school with females, express primarily submissive behaviors, and do not defend a territory or typically court females (Fernald and Hirata, 1977) (Fig. 1). Males also rapidly and reversibly switch between dominant and subordinate phenotypes depending on the composition of the social group, often multiple times in their lifetime. These dominant and subordinate phenotypes, along with the socially-driven transformations, are all associated with distinct behavioral and physiological measures related to reproductive potential that will be discussed below (see Fig. 1 and also (Fernald, 2009, 2012; Fernald and Maruska, 2012; Hofmann and Fernald, 2001; Maruska and Fernald, 2013a,b) for reviews on *A. burtoni* social regulation).

2. Cichlids as model systems for studying how social status influences reproduction

Cichlids (family Cichlidae) are a large (~1600 validated species), diverse, and geographically widespread group of teleost fishes comprised of three major clades: African, Neotropical, and Malagasy/Indian (Friedman et al., 2013). Cichlid fishes are ideal vertebrate model systems for studying both proximate and ultimate mechanisms of how an animal's social environment, or relative rank in the population, can impact reproductive potential and function. For example, many cichlid species exhibit complex social behaviors associated with territoriality and reproduction, with dominance hierarchies common in one or both sexes, making them excellent subjects for testing hypotheses on the social control of reproduction. Cichlids are also relatively easy to manipulate socially, and are amenable to both laboratory and field-based studies. Further, cichlids are an important group for addressing comparative and evolutionary questions because they show incredible diversity in mating systems (e.g., monogamy, polygamy), parental care strategies (e.g., mouthbrooding, maternal, paternal, biparental, cooperative), and alternative reproductive tactics (e.g., sneaking, piracy) even within closely related species (Balshine-Earn and Earn, 1998; Kidd et al., 2012; Kuwamura, 1986; Sefc, 2011). The recent genome sequencing of five African cichlid species (Nile Tilapia *Oreochromis niloticus*; Lake Tanganyika *A. burtoni*; Lake Tanganyika *Neolamprologus brichardi*; Lake Malawi *Metriaclima (Maylandia) zebra*; Lake Victoria *Pundamilia nyererei*) is also advantageous, providing genomic and genetic tools to advance our understanding of the molecular basis of social behaviors and hypothalamic–pituitary–gonadal (HPG) axis function, as well as identify the substrates that have shaped their evolution.

Despite the wide suitability of cichlid fishes for studying the effects of social rank on reproduction, the majority of studies have concentrated on only a few representative species. For example, previous studies are dominated by work on African cichlids (e.g., *A. burtoni*, *O. mossambicus*, *O. niloticus*), with some information on Neotropical species (e.g., *C. dimerus*, *C. nigrofasciatus*), while almost nothing is known about reproductive behaviors and physiology in the Malagasy/Indian group.

3. Hypothalamic–pituitary–gonadal (HPG) axis in cichlids

Reproduction in all vertebrates, including fishes, is controlled by the conserved hypothalamic–pituitary–gonad axis. At the top of this axis are the GnRH1 neurons in the hypothalamic–preoptic area of the brain that integrate external signals from the environment with internal body information via excitatory and inhibitory neural inputs, and hormone regulators from other body systems, to control reproduction. In cichlids, and all teleost fishes, these GnRH1 neurons project directly to the pituitary gland, where GnRH1 peptide is released from axon terminals. This direct neural

innervation of the pituitary gland differs from that in mammals in which hypophysiotrophic GnRH1 is released into the median eminence and delivered to the anterior pituitary via a blood portal system. GnRH1 released to the pituitary then binds to G-protein-coupled GnRH receptors and stimulates the synthesis and secretion of the gonadotropin hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH in fishes are heterodimeric glycoproteins formed by a common α -subunit linked to a specific β -subunit, which determines the biological specificity and activity of the hormone (see Levavi-Sivan et al., 2010; Yaron et al., 2003 for reviews on fish gonadotropins and their receptors). In cichlids, LH and FSH are produced by separate cells that are concentrated in different regions of the pituitary. For example, in the tilapia *O. mossambicus*, LH cells are found in the peripheral region of the proximal pars distalis (PPD) while FSH cells are localized more centrally and adjacent to the hypothalamic nerve fibers ramifying in the PPD (Aizen et al., 2007a). In the tilapia, pituitary cells are also synchronized by coupling through gap junctions, which may facilitate complex patterns of pituitary hormone secretion (Levavi-Sivan et al., 2005). Notably, there are many other neurochemicals (e.g., dopamine, neuropeptide Y, GABA, glutamate, kisspeptin, serotonin, steroids) in addition to GnRH1 that positively or negatively influence gonadotropin synthesis and release either at the pituitary or upstream in the hypothalamus (Levavi-Sivan et al., 2010). Secreted LH and FSH then travel through the bloodstream to the testes (or ovaries in females) where they bind to G-protein coupled LH and FSH receptors from the family of rhodopsin-like receptors (family A). In fishes, LHR and FSHR are expressed in multiple testicular cell types (Garcia-Lopez et al., 2009, 2010), and studies in tilapia indicate that both LH and FSH can stimulate steroid synthesis and release, as well as promote spermatogenesis (Levavi-Sivan et al., 2010). Sex-steroid hormones primarily produced by the gonads also feedback to the brain, pituitary, and gonadal tissue to regulate HPG axis activity and behaviors. While there are still many aspects of HPG axis function and regulation that are not yet known in fishes (e.g., pulsatility of GnRH1 release; steroid feedback mechanisms), the conserved nature of this axis makes cichlids excellent models for understanding the evolution of the vertebrate reproductive system and how it responds to changes in social rank.

4. Male social status and reproduction

Cichlid fishes show extreme diversity in their social behaviors related to courtship and spawning, and territoriality and aggression, which stems from their diverse range of mating systems and parental care strategies (Sefc, 2011; Van Staaden and Smith, 2011). This diversity in social structure, therefore, also influences their reproductive behavior and physiology, but details on reproductive function are only currently available for a few select cichlid species. In systems in which food, shelters, or spawning substrate are limited resources, dominance hierarchies and territorial behaviors often develop, with the most dominant individuals successfully defending the prime real estate. While dominance hierarchies can exist in males, females, or both sexes within a single species, this review will focus on male social rank, which has received more experimental attention. Dominant males often display bright coloration patterns and complex social behaviors related to reproduction (e.g., digging and bower formation, chasing, nudging, courtship quivers, leading) and territory defense (e.g., chasing, biting, ramming, mouth-locking, frontal and lateral displays, border disputes). In contrast, subordinate individuals have a more limited behavioral repertoire to signal their submissive positions in the hierarchy (e.g., fleeing, hovering near surface). When opportunities arise for low-ranking males to rise in rank, the

changes from submissive to dominance behaviors and coloration often occur quickly. For example, in the African cichlid species *A. burtoni*, subordinate males intensify their body coloration and begin performing both reproductive and territorial aggressive behaviors in less than 10 min of perceiving a chance to rise in rank (Burmeister et al., 2005; Maruska and Fernald, 2010a). Conversely, when a dominant male is induced to fall in social rank and become subordinate, his coloration fades and he adopts more submissive behaviors within the same quick time frame (Maruska et al., 2013a). When territory space is limited, subordinate males must quickly recognize a vacant territory and signal new ownership to other males in the population, as well as signal their motivation to mate to the females in the group. Since subordinate males are typically reproductively suppressed, there must also be physiological changes along the HPG axis to prepare a newly ascended individual for increased reproductive opportunities.

4.1. Social status and circulating sex-steroids

Many studies have utilized cichlid fishes as models to test specific hypotheses on endocrine responses to different social contexts or how manipulations of steroids influence behavior (e.g., Antunes and Oliveira, 2009; Dijkstra et al., 2012; Oliveira, 2009; Oliveira et al., 2001; Sessa et al., 2013), but the focus here will be on examples related only to dominant and subordinate status (Table 1). In several cichlids, male social rank is associated with specific sex-steroid hormone profiles, and these steroid levels are typically a result of rather than a cause of the social position. For example, a study in male Mozambique tilapia showed that urinary concentrations of sex-steroids prior to establishment of social hierarchies were not good predictors of their subsequent social status, but steroid levels after group formation were positively correlated with dominance index (Oliveira et al., 1996). In *A. burtoni*, dominant males also have higher circulating plasma levels of testosterone (T), 11-ketotestosterone (11-KT; a fish-specific androgen), progesterone (P), and 17 β -estradiol (E₂) compared to subordinate males (Maruska et al., 2013a; O'Connell et al., 2013; Parikh et al., 2006b). When subordinate *A. burtoni* males are given a chance to rise in rank, however, they quickly show elevated plasma levels of T, 11-KT, E₂, and P within 30 min (Maruska and Fernald, 2010a). Conversely, during social descent from dominant to subordinate phenotype, there is evidence for decreased plasma androgen levels and increased cortisol levels also within 30 min in this species (Maruska et al., 2013a,b; Parikh et al., 2006a). These studies collectively illustrate that it is the social interactions related to establishment of dominance hierarchies that has important modulatory effects on sex-steroid levels in male cichlids, rather than the converse.

Even within a social status, other factors can also be associated with different steroid hormone profiles such as body coloration (e.g., plasma steroid differences in blue versus yellow dominant male *A. burtoni*; (Korzan et al., 2008), and prior social experience/interactions (e.g., prior social experience in tilapia influences steroid response to social isolation; (Galhardo and Oliveira, 2013). Exogenous administration of sex-steroids can also modulate aggressive and reproductive behaviors in some cichlid species, but the behavioral and physiological response may differ depending on the male's dominance status (Fernald, 1976; O'Connell and Hofmann, 2012; Sessa et al., 2013). For example, manipulations with sex-steroid receptor agonists and antagonists in *A. burtoni* showed that androgens and progestins influence courtship behaviors only in dominant males, but estrogens modulate aggressive behavior independent of social status (O'Connell and Hofmann, 2012). In the convict cichlid, treatment with the androgen receptor antagonist flutamide also decreased courtship but not aggressive behaviors in reproductive males (van Breukelen, 2013). Castration

experiments have also produced slightly different behavioral effects in different cichlid species. For example, castration in *A. burtoni* significantly lowered aggression levels while still allowing males to retain their dominance status over non-territorial individuals (Francis et al., 1992a). In *O. mossambicus*, however, castration had no effect on aggressive behaviors but abolished nest building and courtship behaviors (Almeida et al., 2014). These studies demonstrate that while there are specific hormone profiles typically associated with dominant and subordinate males, the steroid regulation of behaviors and physiology may be behavior-specific (e.g., aggressive vs. reproductive), status-dependent, and in some cases, species-dependent. Many of these results also highlight that aggressive and reproductive behaviors can be decoupled from gonadal-derived steroids, suggesting an important role of central neural mechanisms in regulating behaviors that are independent of circulating sex-steroids.

In the tilapia *O. mossambicus*, which has a similar mating and parental care strategy to *A. burtoni*, androgens are also higher in dominant compared to subordinate males (Oliveira et al., 1996). Interestingly, the effects of social isolation on circulating androgen levels also depends on previous social status in tilapia such that isolation causes a decrease in 11-KT levels in dominant males, and a non-significant trend for increasing 11-KT levels in subordinate males (Galhardo and Oliveira, 2013). This study highlights the important relationships between an individual cichlid's prior social experience, relative position in the dominance hierarchy, and physiological response measures crucial for reproduction. Higher circulating levels of androgens are also associated with dominant reproductive status in males of other cichlid species such as *O. niloticus* (Pfennig et al., 2012), *Pundamilia nyererei* (Dijkstra et al., 2007), and *Neolamprologus pulcher* (Taves et al., 2009). However, it should be noted that it is difficult to separate neural and hormonal differences solely associated with social rank from differences due to reproductive state in many of these species, and further studies are needed to fully understand the role of sex-steroid signaling in dominant versus subordinate phenotypes.

4.2. Social status and the brain

Since the brain ultimately controls both the expression of reproductive-related behaviors and the relative activity of the HPG axis, several studies have focused on neural differences associated with social rank (Table 1), or neural changes associated with transitions to higher or lower-ranking social positions. For example, when subordinate male *A. burtoni* are given an opportunity to rise in social rank, specific regions of the brain associated with processing social information (e.g., social behavior network, SBN; social decision making network, SDMN) are activated (measured via immediate early gene mRNA expression) within 30 min of this ascent (Maruska et al., 2013b). Several of these same brain regions are activated when males are experimentally induced to fall in rank from dominant to subordinate, although the activation pattern differs from that seen in ascending males (Maruska et al., 2013a). While the neural pathways that link the sensory perception of this gain or loss of status to IEG activation in different brain regions are not known, these experiments show that males recognize a change in relative social position very quickly, and then make the behavioral and physiological adjustments crucial for immediate survival and future reproductive opportunities.

In addition to IEG activation in the brain during social status transitions, there are also some differences in sex-steroid receptor expression in specific brain regions between dominant and subordinate males that may have important reproductive implications (Burmeister et al., 2007; Maruska et al., 2013a,b; O'Connell et al., 2013). For example, Maruska et al. (2013b) found higher mRNA levels of some steroid receptor subtypes in several SBN regions

Table 1
Summary of differences along the hypothalamic–pituitary–gonadal axis between dominant and subordinate male phenotypes in cichlids.

Measure/structure/gene	Species	Direction of difference	Reference(s)
<i>Brain (preoptic area only)</i>			
GnRH1 neuron soma size	<i>Astatotilapia burtoni</i> ; <i>Cryptoheros nigrofasciatus</i>	D > S	Davis and Fernald (1990), Chee et al. (2013)
GnRH1 mRNA	<i>A. burtoni</i>	D > S	White et al. (2002), Maruska and Fernald (2013a,b)
POA ER α mRNA	<i>A. burtoni</i>	D < S	Maruska et al. (2013a,b)
POA CRF-R1 mRNA	<i>A. burtoni</i>	D < S	Carpenter et al. (2014)
mPOA, gPOA aromatase mRNA	<i>A. burtoni</i>	D < S	Huffman et al. (2013)
pPOA AR β mRNA	<i>A. burtoni</i>	D > S	O'Connell et al. (2013)
pPOA AVT mRNA	<i>A. burtoni</i>	D < S	Greenwood et al. (2008)
gPOA AVT mRNA	<i>A. burtoni</i>	D > S	Greenwood et al. (2008)
pPOA AVT neuron soma size	<i>Cichlasoma dimerus</i>	D < S	Ramallo et al. (2012)
<i>Pituitary</i>			
LH, FSH mRNA	<i>A. burtoni</i> ; <i>Oreochromis niloticus</i>	D > S	Maruska et al. (2011), Golan and Levavi-Sivan (2013)
LH, FSH protein	<i>O. niloticus</i> ; <i>C. dimerus</i> (β -FSH only)	D > S	Golan and Levavi-Sivan (2013), Alonso et al. (2012)
GnRH-R1 mRNA	<i>A. burtoni</i>	D > S	Au et al. (2006), Maruska et al. (2013a,b)
# cells/pituitary	<i>O. niloticus</i>	D > S	Golan and Levavi-Sivan (2013)
# FSH cells/pituitary			
FSH cell granulation			
CRF mRNA	<i>A. burtoni</i>	D < S	Carpenter et al. (2014)
CRF-R1 mRNA	<i>A. burtoni</i>	D > S	Chen and Fernald (2008)
		D < S	Carpenter et al. (2014)
CRF-BP mRNA	<i>A. burtoni</i>	D < S	Chen and Fernald (2008)
AVT protein	<i>O. mossambicus</i>	D < S	Almeida et al. (2012)
<i>Testes</i>			
GSI	<i>A. burtoni</i> ; <i>O. niloticus</i> ; <i>C. nigrofasciatus</i> ; <i>C. dimerus</i> ; <i>O. mossambicus</i>	D > S	Maruska and Fernald (2011a,b), Golan and Levavi-Sivan (2013), Pfennig et al. (2012), Chee et al. (2013), Alonso et al. (2011, 2012), Oliveira and Almada (1998)
Spermatogonia B; spermatocytes; spermatids; sperm density; fraction motile sperm	<i>A. burtoni</i>	D > S	Maruska and Fernald (2011a,b), Kustan et al. (2012)
Interstitial (Leydig) cells	<i>A. burtoni</i>	D < S	Maruska and Fernald (2011a,b)
LHR, FSHR, AR α , AR β , ER α , StAR mRNA	<i>A. burtoni</i>	D > S	Maruska and Fernald (2011a,b), Huffman et al. (2012)
<i>vasa</i> , <i>sox2</i> , <i>dmc1</i> mRNA (germ-line specific genes), <i>amh</i> , <i>amhd rll</i> , <i>mrt1</i> mRNA (Sertoli-cell specific genes), <i>cyp19a1</i> mRNA	<i>O. niloticus</i>	D < S	Pfennig et al. (2012)
<i>Cyp11b</i> mRNA and protein; <i>sf1</i> mRNA	<i>O. niloticus</i>	D > S	Pfennig et al. (2012)
Leydig and myoid cell numbers; Tunica albuginea thickness	<i>O. niloticus</i>	D > S	Pfennig et al. (2012)
<i>Bloodstream</i>			
LH, FSH	<i>A. burtoni</i> ; <i>O. niloticus</i>	D > S	Maruska et al. (2011), Golan and Levavi-Sivan (2013)
11-KT	<i>A. burtoni</i> ; <i>O. niloticus</i> ; <i>O. mossambicus</i> ; <i>Pundamilia nyererei</i> ; <i>Neolamprologus pulcher</i>	D > S	Parikh et al. (2006), Maruska et al. (2013a,b), Pfennig et al. (2012), Golan and Levavi-Sivan (2013), Oliveira et al. (1996), Dijkstra et al. (2007), Taves et al. (2009)
T	<i>A. burtoni</i>	D > S	Parikh et al. (2006), Maruska et al. (2013a,b), Huffman et al. (2012)
E ₂ , P	<i>A. burtoni</i>	D > S	Maruska et al. (2013a,b), Maruska and Fernald (2010a,b,c), O'Connell et al. (2013)

Abbreviations: 11-KT, 11-ketotestosterone; AR α / β , androgen receptor subtypes α or β ; AVT, arginine vasotocin; CRF, corticotropin-releasing factor; CRF-BP, CRF binding protein; CRF-R1, CRF receptor 1; D, dominant; E₂, estradiol; ER α / β , estrogen receptor subtypes α or β ; FSH, follicle stimulating hormone; FSHR, FSH receptor; GSI, gonadosomatic index; LH, luteinizing hormone; LHR, LH receptor; P, progesterone; POA, preoptic area; S, subordinate; StAR, steroidogenic acute regulatory protein; T, testosterone; >, greater than; <, less than.

of the brain (e.g., AR α in Vv, Vs, VTn; ER α in DI, Vv, Vs, ATn, Ce; ER β in Vs) in dominant males, and fewer examples of higher mRNA levels in subordinate males (e.g., ER β in Vv and ATn; ER α in POA). O'Connell et al. (2013) also found more sex-related differences in steroid receptor levels in the brain than status-related differences within each sex. Further, subordinate males have higher aromatase (enzyme that converts testosterone to estradiol) expression in the magnocellular and gigantocellular regions of the preoptic area compared to dominant males, and inhibiting aromatase activity with fadrozole in dominant males decreases aggressive but not reproductive behaviors (Huffman et al., 2013). Thus, aromatase and estradiol may promote aggression in *A. burtoni* males via actions in the preoptic area and possibly other socially-relevant brain regions. In contrast to these stable social states, however, there were many more rapid changes in mRNA levels of sex-steroid

receptors in males transitioning between subordinate and dominant states, suggesting that transient changes in steroid sensitivity in the brain may help modulate behavioral and physiological adjustments required during social ascent (Maruska et al., 2013b). These data suggest that changes in relative steroid sensitivity during social ascent, manifested as transient changes in receptor expression in specific brain nuclei, may regulate how different nodes of the neural circuitry detect and respond to the salience of social inputs, which may be very different from the neural mechanisms that maintain stable dominant or subordinate social rank. Targeted neural manipulations of steroid signaling in subordinate, dominant, and transitioning male *A. burtoni*, along with relevant behavioral and physiological measures, are needed to fully understand how sex-steroids influence specific phenotypic attributes. Future comparative studies in other cichlids will also be

informative for elucidating mechanistic similarities and differences attributed solely to different social ranks.

4.2.1. Gonadotropin-releasing hormone (GnRH1) neurons

Cichlids are well known for their behavioral, morphological, and physiological reproductive plasticity, and this plasticity is also evident in the size and morphology of the GnRH1 neurons controlling the reproductive axis (Davis and Fernald, 1990) (Table 1). In male *A. burtoni*, GnRH1 neuron soma size is under social control, such that these cells grow and shrink as an animal transitions between dominant and subordinate status in both directions (Francis et al., 1993). Dominant males have larger GnRH1 neurons with increased dendritic complexity compared to subordinate males (Davis and Fernald, 1990; Scanlon et al., 2003). When a subordinate male gets a chance to ascend in status and become dominant, however, there are several rapid changes that occur in the GnRH1 neurons. First, the social opportunity is associated with rapid (20–30 min) induction of the immediate early gene (IEG) *egr-1* (a transcription factor-encoding gene; also called *zenk*, *zif-268*, *ngfi-a*, *krox-24*, *tis8*) in the preoptic area and in GnRH1 neurons (Burmeister et al., 2005; Maruska et al., 2013a,b). This molecular response is likely due to the recognition of the social opportunity because it is not elicited in males who are already dominant and performing similar behaviors. This type of molecular response to an opportunity may be conserved across vertebrates because socially-relevant reproductive stimuli are also known to induce IEG expression within GnRH1 neurons from fishes (Burmeister et al., 2005) to mammals (Gelez and Fabre-Nys, 2006; Meredith and Fewell, 2001; Pfaus et al., 1994). Second, there is an increase in GnRH1 mRNA levels in the brain at 30 min after ascent (Maruska and Fernald, 2013b), suggesting that GnRH1 production is increased and the reproductive axis is quickly stimulated. Castration of *A. burtoni* also causes hypertrophy of GnRH1 neurons, and subsequent studies showed that gonadal androgens, but not estrogens, act via a negative feedback mechanism to maintain GnRH1 neurons at a set point level that is determined by social cues (social set point hypothesis) (Francis et al., 1992b; Soma et al., 1996). Third, an increase in GnRH1 soma size is detected at 1 day after ascent (Maruska and Fernald, 2013b), and these neurons reach sizes typical of stable dominant male within 5–7 days (White et al., 2002). The importance of this change in GnRH1 soma size is unknown, but it may function in part to accommodate changes in synaptic inputs or variations in the cellular and molecular demands of the cell during the rise in social rank. Collectively, these studies suggest that suppressed males are well adapted to swiftly recognize and take advantage of an opportunity to gain a territory, become dominant, and reproduce.

A. burtoni is not the only cichlid species to show status-dependent plasticity in GnRH1 neuron soma size. In the sequentially monogamous Central American convict cichlid *Cryptoheros nigrofasciatus*, both sexes aggressively defend territories and exhibit a biparental substrate-brooding care strategy. Despite the different mating and parental care strategies between *A. burtoni* and *C. nigrofasciatus*, male convicts show similar status-related plasticity in their GnRH1 neurons. For example, similar to *A. burtoni*, the convict cichlid showed no differences in GnRH1 cell numbers between social states, but GnRH1 neuron soma size was larger in dominant territorial males compared to non-territorial subordinate males (Chee et al., 2013). Further studies that include other cichlid species with divergent mating and parental care strategies are needed to fully understand the relationship between male social status and GnRH1 neuronal plasticity.

4.2.2. Other candidate neuroendocrine regulators of reproduction

In addition to direct GnRH1 regulation of the HPG axis, other neuroendocrine regulators of reproductive behavior and

physiology are also influenced by social status (Table 1). For example, the nonapeptides arginine vasotocin (AVT; arginine vasopressin homolog) and isotocin (IT; oxytocin homolog) may also play important roles in reproductive plasticity associated with dominance hierarchies in cichlids. While studies on IT are limited, several recent studies have focused on the role of AVT in cichlid reproduction and social behaviors. For example, in *A. burtoni*, dominant males have higher levels of AVT mRNA expression (quantified by *in situ* hybridization) in the gigantocellular nucleus of the preoptic area, whereas subordinate males have higher AVT levels in the parvocellular nucleus, suggesting that these AVT neuronal subpopulations have different functions and regulatory mechanisms depending on social status (Greenwood et al., 2008). Further, AVT expression in the gigantocellular nucleus was positively correlated with aggressive and reproductive behaviors, whereas expression in the parvocellular nucleus was negatively correlated with these same behaviors. AVT expression in the parvocellular nucleus was also positively correlated with the tendency of subordinate males to flee from dominant males. Pharmacological blocking of the V1a AVT receptor (via Manning compound) in *A. burtoni* also decreased aggressive behavior and increased reproductive behavior in males ascending in social status, but had no effect on stable dominant or subordinate phenotypes (Huffman et al., 2014). Ascending male *A. burtoni* also showed higher mRNA levels of AVT and V1a2 receptor in the brain compared to both stable dominant and subordinate states (Huffman et al., 2014). Thus, while AVT signaling is complex and further complicated by its role in the stress axis, it likely modulates behavior by influencing specific motor, motivational, or sensory circuits in the brain.

In *C. dimerus*, non-territorial males also have larger parvocellular neurons compared to dominant territorial males (Ramallo et al., 2012), suggesting that AVT in the parvocellular nucleus may be associated with activation of submissive neural circuits and/or inhibition of dominance aggressive circuits. In the pituitary gland of *O. mossambicus*, levels of AVT peptide measured via HPLC were higher in subordinate males compared to dominant males, but there was no correlation with plasma cortisol levels (Almeida et al., 2012). This dissociated response between AVT and the stress axis also occurs in other fishes (Backstrom et al., 2011), and the authors suggest that higher pituitary AVT levels could function to maintain a heightened response to chronic social stress in subordinate fish. Studies in *C. dimerus* also demonstrate that AVT can induce gonadotropin secretion from pituitary cell cultures, as well as induce testicular androgen synthesis *in vitro* (Ramallo et al., 2012). Thus, there is evidence from several cichlids that AVT may act in the brain, pituitary, and testes to regulate reproductive physiology and behaviors related to dominant-subordinate social status. Collectively, these abovementioned studies reveal a complex relationship between AVT expression and social status, even within a single species, that likely involves differential functions among the three primary AVT neuronal phenotypes (parvo-, magno-, gigan-to-cellular). Comparisons of AVT receptor expression and abundance along the HPG axis in dominant and subordinate males should also provide important information towards understanding the role nonapeptides serve in socially-regulated reproduction across species.

The kisspeptin signaling system also plays a crucial role in controlling both GnRH1 neuron activity and the reproductive axis in fishes (Akazome et al., 2010; Gopurappilly et al., 2013; Oka, 2009). Kisspeptins, a group of RFamide peptides encoded by the *Kiss1*, and in some species, the *Kiss2* gene, act via their cognate G-protein coupled receptor GPR54 (=Kiss-R). Genome synteny analyses (Tena-Sempere et al., 2012; Um et al., 2010) have suggested that the kisspeptin receptor genes previously identified from tilapia (Parhar et al., 2004) and *A. burtoni* (Grone et al., 2010), as well as other teleost fishes, belong to the Kiss-R2

subfamily, suggesting that in teleosts, the Kiss2-Kiss-R2 is evolutionarily highly conserved and may be functionally equivalent to mammalian Kiss1-Kiss-R (Gopurappilly et al., 2013). There is some evidence for kisspeptin receptor expression in GnRH1 neurons in cichlids (Grone et al., 2010; Parhar et al., 2004), but this may be species-specific since colocalization of kisspeptin receptors in GnRH1 neurons is absent in some other fish species (Kanda and Oka, 2013). In *A. burtoni*, dominant males have higher levels of *kiss1r* (renamed *gpr54-2b*; Tena-Sempere et al., 2012) in whole-brain samples compared to subordinate males, but there was no difference in *gpr54-2b* expression in preoptic area GnRH1 neurons between social states quantified via *in situ* hybridization (Grone et al., 2010). In a more recent study, however, *gpr54-2b* mRNA levels in microdissected preoptic areas were higher at 30 min after social opportunity compared to both dominant and subordinate males (Hu et al., 2011), suggesting that the kisspeptin signaling system may be important during the social transition when suppressed males need to quickly up-regulate their reproductive behavior and physiology. A recent study in Nile tilapia also demonstrated that thyroid hormone (T_3) influences both kisspeptin and GnRH1 mRNA levels in the brain (Ogawa et al., 2013), suggesting that there are likely many more regulators of HPG axis function in cichlids that remain to be discovered.

The corticotropin-releasing factor (CRF) system also plays a role in regulation of the HPG axis. Dominant male *A. burtoni* have higher mRNA levels of CRF in whole brain samples compared to subordinate males, but levels of CRF-binding protein (CRF-BP) and the two CRF receptor subtypes (CRF-R1, CRF-R2) did not differ (Chen and Fernald, 2008). In the pituitary, where CRF protein stimulates the release of adrenocorticotrophic hormone (ACTH) and β -endorphin, CRF-R1 mRNA levels were twofold higher in dominant males, whereas CRF-BP mRNA levels that may block or limit CRF action are twofold higher in subordinate males (Chen and Fernald, 2008). These results suggest that during a prolonged period (3–4 weeks) of social stress, the CRF system in both the brain and pituitary is down-regulated in subordinate males, possibly as a homeostatic mechanism to maintain stable circulating cortisol levels. In a more recent study with finer neuroanatomical resolution, however, CRF and CRF-R1 mRNA levels were rapidly down-regulated in the preoptic area of ascending males at just 15 min after social opportunity compared to stable subordinates (Carpenter et al., 2014). The rapid time course of these changes (within minutes) suggests that the CRF system plays an integral role in physiological changes associated with shifts in social status. Since CRF typically has inhibitory effects on the neuroendocrine reproductive axis in many vertebrates, this attenuation of CRF activity may allow rapid activation of the reproductive axis and facilitate the male's transition to dominance. In other words, because dominance is the default status in *A. burtoni* there are likely active suppression mechanisms in subordinate males, and therefore, removal of the inhibitory CRF activity at ascent may disinhibit the HPG axis during a rise in social rank.

Differences in gene expression also exist in central and peripheral sensory processing regions between dominant and subordinate male *A. burtoni*, which has important implications for social communication related to reproduction. For example, in the olfactory bulbs, mRNA levels of *GnRH-R1* are higher in subordinate males, whereas levels of the sex steroid receptor subtypes (*AR α* , *AR β* , *ER α* , *ER β a*, *ER β b*) are higher in dominant compared to subordinate males, suggesting that olfactory sensitivity may change with social status (Maruska and Fernald, 2010b). mRNA levels of several steroid receptor subtypes (estrogen and corticosteroid receptors) in the main hearing organ of the inner ear (sacculle) are also higher in subordinate compared to dominant males, indicating that social status may also be linked to auditory processing capabilities (Maruska and Fernald, 2010c; Maruska et al., 2012a,b). Since both

chemosensory and acoustic signaling are important components of the multimodal communicative repertoire of this species, as well as other cichlids with dominance hierarchies (Barata et al., 2007; Longrie et al., 2013; Miranda et al., 2005), this molecular plasticity associated with social status has important functional implications for reproductive success.

4.3. Social status and the pituitary

The primary targets of the GnRH1 neurons are the gonadotropin-producing cells in the pituitary gland. The released GnRH1 peptide binds to GnRH receptors (members of the G-protein coupled receptor superfamily) on the gonadotrope cells to induce release and synthesis of the two gonadotropin hormones, LH and FSH. Cichlids typically contain multiple GnRH receptor types within the same species that may serve different functions, even within the pituitary gland. *A. burtoni* and tilapia, for example, both contain two types of GnRH receptors (Chen and Fernald, 2006; Levavi-Sivan et al., 2010). In male *A. burtoni*, pituitary mRNA levels of *GnRH-R1*, but not *GnRH-R2*, are socially regulated such that stable dominant males have higher levels compared to subordinate males (Au et al., 2006; Maruska et al., 2011). An increase in *GnRH-R1* levels occurs more slowly (days) during the social transition compared to changes in mRNA levels of other genes that occur within minutes to hours (Maruska and Fernald, 2011b). For example, pituitary mRNA levels of the IEGs *egr-1* and *cfos*, *LH β* , and *FSH β* are more rapidly increased at just 30 min after social ascent, suggesting that GnRH1 release from its axon terminals has quickly activated the pituitary gland (Maruska et al., 2011, 2013a,b). In tilapia, GnRH1 increases plasma levels of both LH and FSH (Aizen et al., 2007b; Levavi-Sivan et al., 2006). Furthermore, circulating levels of mature LH and FSH in *A. burtoni* are higher by 30 min after ascent, and positively correlated with mRNA levels in the pituitary, suggesting that GnRH1 activation of the pituitary stimulates both the release and synthesis of both gonadotropins (Maruska et al., 2011). Thus, within minutes of a social opportunity, the brain-pituitary portion of the HPG axis has been stimulated.

In the Neotropical cichlid *C. dimerus*, dominant reproductive males had higher pituitary levels of FSH β , but not LH β , compared to non-reproductive subordinate males (Alonso et al., 2011). Further, within non-reproductive males, there was also a positive correlation between pituitary FSH β levels and dominance index, suggesting that those individuals most likely to ascend in social status already have a greater substrate for increased HPG axis activation compared to lower ranking individuals within the social group. The lower levels of FSH β in less dominant non-reproductive males may also indicate an inhibition of FSH synthesis and/or release at the level of the brain or pituitary, possibly via mechanisms involving the stress hormone cortisol (Alonso et al., 2012).

A recent study in the Nile tilapia also showed that dominant males had higher circulating levels of FSH and LH compared to subordinate males (Golan and Levavi-Sivan, 2013). Further, pituitaries in dominant male tilapia also had higher gonadotropin protein content and mRNA levels compared to subordinates (Table 1). Using flow cytometry, Golan and Levavi-Sivan (2013) also found that pituitaries of dominant males contained more cells compared to pituitaries of subordinate males, and that ~30% of this increase in cell number was attributed to FSH-producing cells. In addition, the FSH cells in dominant males had increased granularity, an indication of more active gonadotropin synthesis. The higher FSH production in dominant male tilapia, therefore, is likely due to a combination of increased mRNA production, cell granularity, and higher FSH cell numbers. A similar scenario may exist for the LH cells, but requires further study. Nevertheless, gonadotroph hyperplasia and increased production by individual cells appears to help promote the elevated HPG axis activity seen in dominant male

tilapia, and may be a mechanism that exists in other cichlids with dominance hierarchies.

4.4. Social status and the testes: GSI, spermatogenesis, sperm quality, gene expression

In addition to small GnRH1 neurons and low HPG axis activity, subordinate male cichlids also typically have small testes, although this depends on the type of mating system (e.g., in species with alternative reproductive tactics, sneaker or parasitic males often have greater gonadal investment and/or higher sperm quality than nesting territorial males; see below). In subordinate male *A. burtoni*, however, despite having a low gonadosomatic index (GSI) and small testes, they continue to produce sperm during the suppression period, and therefore retain viable sperm for fertilization (Kustan et al., 2012; Maruska and Fernald, 2011a). This is significant, and adaptive, because it allows reproductively suppressed males to immediately spawn with females when rising in rank and obtaining a territory, without having to wait 5–7 days for the testes to grow or the 10–11 days required for new sperm production (Kustan et al., 2012; Maruska and Fernald, 2011a). In fact, behavioral experiments showed that these suppressed subordinate males can successfully spawn and fertilize eggs within minutes to hours of social ascent (Kustan et al., 2012). During the subordinate to dominant male social transition, the morphological and structural changes in testicular cell composition and relative testes size takes several days, whereas many molecular changes in the testes are detected more quickly (Huffman et al., 2012; Maruska and Fernald, 2011a). For example, social opportunity in *A. burtoni* triggers rapid (minutes to hours) changes in mRNA levels of some receptor types (FSHR, androgen receptors, corticosteroid receptors), as well as slower (days) changes in other receptor types (LHR, estrogen receptors, and aromatase, the enzyme that converts testosterone to estradiol) (Maruska and Fernald, 2011a). This rapid transcriptional response in the most downstream component of the HPG axis highlights the sensitivity and plasticity of the entire reproductive system to social information. Thus, there are measurable changes in transcriptional activity from the brain to the testes, all within minutes of a social opportunity. Furthermore, the swift molecular changes in the testes raise the alternate possibility that there could be other signaling pathways that perhaps bypass the linear cascade from brain GnRH1 release to pituitary LH/FSH release to testicular gonadotropin receptor activation, but this hypothesis requires future testing.

In other cichlids such as the Nile tilapia (Golan and Levaviv-Sivan, 2013; Pfennig et al., 2012), Mozambique tilapia (Oliveira and Almada, 1998), and the convict cichlid (Chee et al., 2013), dominant territorial males also have higher GSI compared to non-territorial males (Table 1). In non-reproductive males of the Neotropical cichlid *Cichlasoma dimerus*, higher GSI is also correlated with a higher dominance index (Alonso et al., 2012). This suggests that non-dominant males may adjust their reproductive investment in relation to their perceived position in the social hierarchy and likelihood of rising in social rank when given an opportunity. This relationship between position in the dominance hierarchy and reproductive physiological investment likely exists in many cichlid species with similar social structures, which makes evolutionary sense when spawning resources (e.g., territories or mates) are limited and chances for social ascent are frequent.

In the Nile tilapia, dominant males also show hyperplasia of the tunica albuginea, the epithelium and connective tissue layer surrounding the testis, due to numerous cytochrome-P450-11 β -hydroxylase (Cyp11b)-expressing myoid cells that are required for 11-KT synthesis (Pfennig et al., 2012). The myeloid cells of the tilapia tunica albuginea form a contractile network, similar to the structural arrangement termed the ‘Y’ zone in the wrasse

(Koulish et al., 2002), presumably facilitating testicular contraction to release mature sperm during spawning. Dominant males also had more steroid-producing Leydig cells compared to subordinate males, with corresponding greater levels of plasma 11-KT. Further, similar to subordinate male *A. burtoni*, subordinate Nile tilapia preserve their capability for spermatogenesis, have increased mRNA levels of germ-line specific (*vasa*, *sox2*, *dmc1*) and Sertoli-cell specific (*amh*, *amhd rll*, *mrt1*) genes, indicating that while they have reduced steroidogenic potential (fewer Leydig cells and lower *cyp11b* levels) they are primed and ready for reproduction when presented with an opportunity.

In some cichlids, reproductive investment associated with male social status is more complex, and can be related to relative risk of sperm competition that is dependent on mating system and population breeding structure. For example, in the cooperatively breeding *Julidochromis ornatus*, in which some males mate monogamously and others reproduce as dominant breeders or helpers within cooperative groups, males tend to increase their testis investment (measured by relative testis size) under elevated risk of sperm competition (Awata et al., 2006). In cooperatively breeding groups, which have a high risk of sperm competition, dominant male breeders with male helpers increase their investment in testis size compared to other male breeders, but male helpers are not reproductively suppressed and invest more in testes size than male breeders. This higher investment in male helper testes size was also positively correlated with the number of offspring sired, providing direct fitness benefits and perhaps dictating the helper's propensity to cooperate with brood care behaviors. Another example of cooperative breeding that shows a different pattern of reproductive investment is *Neolamprologus pulcher*, which lives in groups consisting of a dominant breeding pair and several subordinate male and female helpers that assist in territory defense and brood care. In this species, male breeders have larger testes, faster and longer-duration swimming sperm, and a higher percentage of motile sperm compared to helpers (Fitzpatrick et al., 2006). Large helpers, however, had sperm that were physiologically equivalent to breeders, but their small testes suggest they are somewhat reproductively suppressed with lower overall fertilization potential. In the Lake Tanganyikan *Lamprologus callipterus* (a shell brooder), in which males display nesting bourgeois and dwarf parasitic tactics, GSI is ~5-times higher in dwarf parasitic males compared to nesting males but these parasitic males do not invest energy in courtship behaviors (Schutz et al., 2010). Thus, in some cichlids with alternative reproductive tactics, there are cases in which gonadal investment and HPG axis activity are decoupled from dominance behaviors (see Oliveira et al., 2008; Taborsky and Brockmann, 2010 for reviews). While the mechanisms that regulate this decoupling are not well understood in most species, recent brain transcriptome studies in other fishes with alternative reproductive tactics have revealed distinct molecular signatures of male dimorphisms (e.g., sneaker vs. nesting territorial) (Schunter et al., 2014).

Thus, in addition to social hierarchies in which dominant males typically invest more in reproduction, the complex social structures involving cooperatively breeding species with multiple male phenotypes offer additional insights into social control of reproductive physiology. Interestingly, in many of the abovementioned cases, there is evidence for males near the bottom of the dominance hierarchy, regardless of whether gonad investment is high or low, to engage in sneak fertilizations and/or to maintain some spermatogenic potential. Thus, many low-ranking individuals may still sire offspring in a suppressed subordinate state, while also being able to quickly transition to a dominant breeding status when the appropriate social opportunity arises. Future transcriptome analyses in cichlids with different reproductive tactics should

yield important insights on the molecular modules sub-serving these alternative phenotypes.

5. Conclusions and future directions

In this review, I have summarized the relevant information on the differences in reproductive and endocrine physiology between dominant and subordinate male social status in cichlid fishes (Table 1 and Fig. 1). While our existing knowledge on the effects of social rank on reproductive behavior and physiology is currently dominated by studies in only a few select species, the suitability of cichlid fishes as models for understanding how the social environment, and changes in relative rank, can influence the reproductive axis from the brain to the testes is evident. As we move forward, it is also important to recognize that individual males within most cichlid populations are not just 'dominant' or 'subordinate'. Instead, they hold a relative status position within a larger dominance hierarchy with some individuals around them of higher status, and others of lower status. This relative social rank is also influenced by prior experience and constantly altered by daily social interactions and fluctuations in internal state, which collectively also impacts HPG axis function. Nevertheless, what remains enigmatic in our understanding of the links between social status and reproductive functions are many of the mechanistic details. For example, what are the regulatory mechanisms that mediate the changes at different levels of the HPG axis when males transition between subordinate and dominant status? Most elusive is an understanding of the neural pathways from reception of an external social cue about an individual's position in the dominance hierarchy that lead to behavioral, phenotypic and molecular-level changes throughout the HPG axis. Further, what role do mechanisms such as epigenetics, microRNA regulation, chromatin remodeling, post-transcriptional and post-translational modification, and others play in the regulation of reproductive behavior and physiology associated with social rank? In terms of social regulation of the HPG axis, there are also several unknowns, including the identity of neural inputs upstream of the GnRH1 neurons and how they might be regulated by social status, and what role extra-neural neuropeptides (e.g., neuropeptides acting outside the brain, such as direct AVT actions on the gonads), gonadotropins, and other signaling chemicals might have on reproductive function. We have only begun to explore the links between social position and reproductive fitness using this diverse group of ideally-suited fishes, and with the recently sequenced genome of several African species, cichlids will likely be important organisms for addressing future questions on the proximate and ultimate mechanisms underlying socially-regulated reproductive function. Notably, the high diversity of cichlid fishes will allow careful and focused experimental designs with appropriate species to address targeted hypotheses on how reproductive behaviors and physiology are influenced by an individual's position within a dominance hierarchy.

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