Chapter 6

Stress and Reproduction

Meghan L.M. Fuzzen, Nicholas J. Bernier and Glen Van Der Kraak
University of Guelph, Guelph, ON, Canada

SUMMARY
This chapter explores the interactions between the hypothalamic–pituitary–interrenal (HPI) stress axis and the hypothalamic–pituitary–gonadal (HPG) reproductive axis and their effects on reproductive processes in teleost fishes. We review the evidence that stress and activation of the HPI axis affect reproduction and do so through actions on the central nervous system, pituitary, and gonads, and production of hepatic vitellogenin (Vtg). Moreover, we describe how stress affects reproductive development at different life stages including embryonic and larval stages, at puberty, and as adults. Collectively, based on the studies conducted to date, it is not possible to provide a generalized model of how stress affects reproduction in teleosts. Rather, the influence of stress on reproduction depends on the type of stressor, its intensity and duration, the sex of the fish, its developmental stage, its nutritional status, and its reproductive strategy. Corticosteroids are primary mediators of the stress response, yet they exhibit both stimulatory and inhibitory influences on reproductive development, which adds to the complexity of defining the role of stress on reproduction in teleosts.

1. INTRODUCTION

Stress is a common feature of life, and fishes, like all organisms, have evolved a suite of defense reactions to protect themselves against stimuli that pose a challenge to the maintenance of homeostatic equilibrium. A key component of the stress response in vertebrates is a reallocation of energy away from nonessential physiological functions, such as reproduction, and toward activities that contribute to the restoration of homeostasis. While this prioritizing of energy allocation is an integral component of allostasis, which represents part of the adaptive process for actively maintaining stability through change (McEwen & Wingfield, 2003), chronic inhibition of investment activities also can be maladaptive. The adverse consequences of chronic stress on reproduction have been well documented in several vertebrate groups including fishes (reviewed by Pankhurst & Van Der Kraak, 1997; Schreck, Contreras-Sanchez, & Fitzpatrick, 2001; Milla, Wang, Madiki, & Kestemont, 2009; Schreck, 2009; see also Volumes 2–5 in this series). In general, stress–reproduction interactions are complex (Figure 6.1) as the various mediators of the stress response can impact on a broad range of reproductive functions and behaviors.

This chapter focuses specifically on the interactions between the hypothalamic–pituitary–interrenal (HPI) stress axis and the hypothalamic–pituitary–gonadal (HPG) reproductive axis and the consequences of these interactions for reproductive processes. After a brief overview of the stress response and the regulation of reproductive functions in fishes, the chapter examines how stressors and the mediators of the stress response affect the key effectors of the HPG axis, including the hypothalamic gonadotropin-releasing hormones (GnRHs), the pituitary gonadotropins (GTHs), the gonadal sex steroids, and hepatic vitellogenin (Vtg). The chapter then reviews how stressors and stress hormones impact the development of the reproductive system and reproductive functions at the embryonic, larval, pubertal, and adult life stages. The effects of gender and reproduction on the activity of the HPI axis are discussed. Finally, we describe examples of fishes exhibiting an apparent resistance to stress during sexual maturation.

1.1. Effectors of the Stress Response

The stress response in fishes is mediated by the HPI axis and the autonomic sympathetic-chromaffin cell axis (Wendelaar Bonga, 1997). In mammals, and presumably also in fishes, stress-sensitive brain circuits with both excitatory and inhibitory neurotransmitters coordinate the activation of both stress axes (Ulrich-Lai & Herman, 2009). At the hypothalamic level, among the multiple stimulatory and inhibitory hypophysiotropic factors that may be involved in regulating the HPI axis, corticotropin-releasing factor (CRF) from the nucleus preopticus (NPO) is considered the primary signal (Lederis, Fryer, Okawara, Schonrock, & Richter, 1994; Bernier, Flik, & Klaren, 2009). Depending on the reproductive status of a fish, urotensin I (UI), a CRF-related peptide, and arginine vasotocin are additional

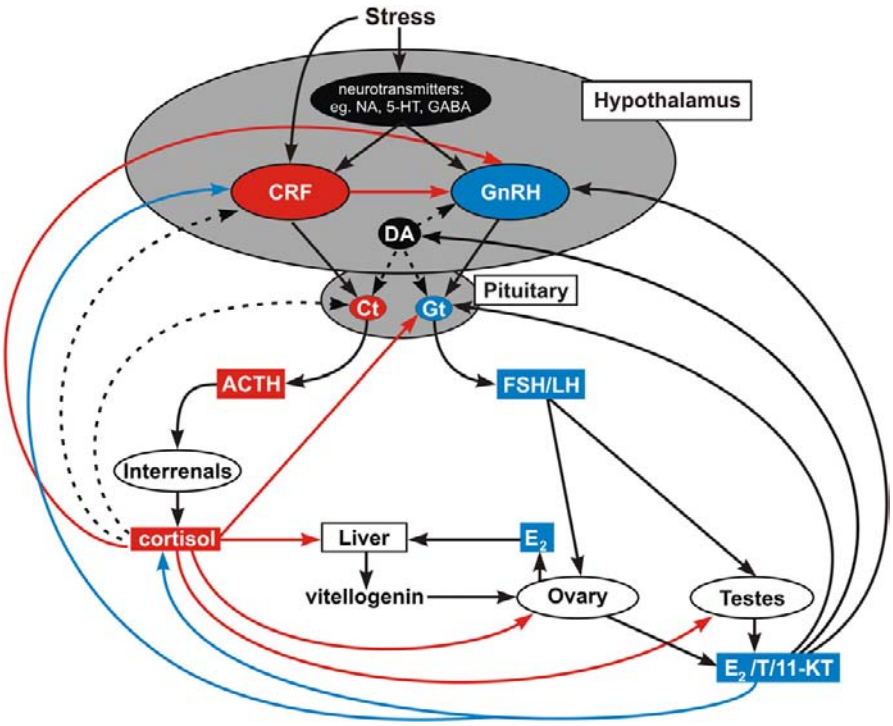

FIGURE 6.1 Overview of the major neuroendocrine signals and interactions between the hypothalamic–pituitary–interrenal (HPI) stress axis (in red) and the hypothalamic–pituitary–gonadal (HPG) reproductive axis (in blue) in teleosts. Neurotransmitters regulate the activity of both axes. Whereas activation of the HPI axis by stressors results in the production of cortisol by the interrenals, the HPG axis stimulates the production of the sex steroids, estradiol (E_2), testosterone (T), and 11-ketotestosterone (11-KT). Estradiol also stimulates the production of vitellogenin by the liver. Solid black arrows indicate stimulation. Dashed black arrows indicate inhibition. Red arrows indicate potentials effects of cortisol and corticotropin-releasing factor (CRF) on the HPG axis. Blue arrows indicate potential effects of sex steroids on the HPI axis. 5-HT, serotonin; ACTH, corticotropin; Ct, corticotropes; DA, dopamine; FSH, follicle-stimulating hormone; GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; Gt, gonadotropes; LH, luteinizing hormone; NA, noradrenaline. See color plate section.

hypophysiotropic factors that may play a significant role in the regulation of the HPI axis (Balment, Lu, Weybourne, & Warne, 2006; Westring et al., 2008). While CRF has multiple targets within the pituitary of teleosts, the hypophysiotropic role of CRF in the regulation of the hormone corticotropin (ACTH) secretion from the corticotropes is central to the workings of the HPI axis (Bernier et al., 2009). Circulating ACTH in turn is recognized as the principle regulator of corticosteroid synthesis in the interrenal cells of the head kidney and the stimulator of cortisol release during the acute phase of the stress response (Flik, Klaren, Van den Burg, Metz, & Huising, 2006; Aluru & Vijayan, 2008). Beyond its effects on reproduction, metabolism, growth, ionic balance, and the immune system, cortisol also can limit the magnitude and duration of the endocrine stress response in fishes via negative feedback effects on the gene expression of CRF in the NPO and the expression of the ACTH precursor molecule pro-opiomelanocortin (POMC) in the pituitary (Mommsen, Vijayan, & Moon, 1999; Norris & Hobbs, 2006; Bernier et al., 2009).

In the sympathetic-chromaffin cell axis, the chromaffin cells of the head kidney release the catecholamines adrenaline and noradrenaline in response to activation by acetylcholine and non-neuronal pathways (Reid, Bernier, & Perry, 1998). While most stressors elicit the secretion of cortisol (Barton & Iwama, 1991; Barton, 2002; Norris & Hobbs, 2006), circulating catecholamine levels generally do not change in response to mild or moderate stress but rise when fish experience acute life-threatening stressors (Perry & Bernier, 1999). Also, while noradrenaline of neuronal origin plays an important role in the neuroendocrine regulation of the HPG axis in fishes (Van Der Kraak, 2009), and sympathetic nerves are involved in the control of reproductive functions in mammals (Gerendai, Banczerowski, & Halasz, 2005), the role of catecholamines in the endocrine regulation of the HPG axis is poorly defined. Therefore, this review focuses on the interplay between the HPI and HPG axes, and the potential effects of humoral catecholamines on reproduction are not discussed.

1.2. Effectors of Reproductive Functions

The HPG axis regulates reproductive functions in fishes (see Chapter 2, this volume) as in all vertebrates (see Volumes 2–5 in this series). At the brain and pituitary levels, multiple GnRH forms and GnRH receptors play central roles in coordinating reproductive endocrinology and behavior in teleosts (reviewed by Van Der Kraak, 2009). Gonadotropin-releasing hormone originating from the preoptic area (POA) induces GTH release from the pituitary, whereas the GnRH expressed outside the POA contributes to diverse neuromodulatory functions including the regulation of sexual behaviors (Soga, Ogawa, Millar, Sakuma, & Parhar, 2005; Kah et al., 2007). Gonadotropin-releasing hormone stimulates the secretion of the GTHs—follicle-stimulating hormone (FSH) and luteinizing

hormone (LH)—from the pituitary gonadotropes (Dickey & Swanson, 2000; Vacher, Mananos, Breton, Marmignon, & Saligaut, 2000; Ando & Urano, 2005; Aizen, Kasuto, Golan, Zakay, & Levavi-Sivan, 2007). Whereas GnRH is probably the most important stimulator of GTH release, the control of GTH secretion is multifactorial and involves several additional stimulatory and inhibitory peptides and neurotransmitters (Trudeau et al., 2000; Chang et al., 2009; Van Der Kraak, 2009). Multiple peptides and neurotransmitters also are involved in regulating the production of GnRH in the POA. One key regulatory factor of the HPG axis in teleosts is dopamine (DA) (Dufour et al., 2005). Dopamine potently inhibits basal and GnRH-stimulated LH secretion in most teleost species. There is also evidence that DA can inhibit both the release of pituitary FSH and POA GnRH. While FSH promotes gametogenesis in females by stimulating the production of 17β-estradiol (E_2) and the incorporation of Vtg into developing oocytes, in males FSH stimulates Sertoli cell proliferation and testosterone (T) production, and maintains spermatogenesis. LH promotes final sexual maturation by stimulating gonadal steroidogenesis in both sexes, oocyte maturation and ovulation in females, and spermiation in males (Zmora, Kazeto, Kumar, Schulz, & Trant, 2007; Van Der Kraak, 2009). Beyond the role of GTHs, ovarian and testicular functions in teleosts are regulated by several other secondary hormones and growth factors (Van Der Kraak, Chang, & Janz, 1998; Van Der Kraak, 2009; see also Chapters 3 and 4, this volume). While these secondary endocrine and paracrine signals also may be affected by stressors, such interactions are beyond the scope of this review. In general, sex steroids participate in spermatogonial and oogonial proliferation and E_2 also plays a key role in the synthesis of the egg yolk precursor Vtg in the liver. Finally, E_2 and T exert positive and negative effects on the HPG axis. Whereas the negative feedback effects of sex steroids on GTH release are mediated by indirect effects on GnRH release via dopaminergic fibers, the positive feedback effects can be exerted either directly at the pituitary level or indirectly by effects on GnRH in the POA (Yaron et al., 2003; Levavi-Sivan, Biran, & Fireman, 2006; Van Der Kraak, 2009).

2. EFFECTS OF STRESS ON THE HYPOTHALAMIC–PITUITARY–GONADAL (HPG) AXIS

The HPI and HPG axes can interact at multiple levels in teleosts (Figure 6.1). While components of the two axes are located in separate nuclei and organ structures, there is crosstalk between the two axes at all levels and in both directions. In this section we will discuss the effects of the HPI axis on the HPG axis; the reverse interaction will be discussed in Section 4.

2.1. Effects of Stress on the Central Nervous System (CNS)

There is limited information available with respect to the effects of stress or stress hormones on the hypothalamic components of the HPG axis in fishes. Adult male tilapia (*Oreochromis niloticus*) exposed to chronic social stress are reproductively inactive and have decreased whole brain mRNA levels of GnRH-I, the main hypophysiotropic form in most teleosts including tilapia, and GnRH-II, but not GnRH-III (Ogawa, Soga, Sakuma, & Parhar, 2003; see also Chapter 2, this volume). Similarly, juvenile carp (*Cyprinus carpio*) fed cortisol have lower whole brain levels of GnRH-III peptide—the major hypophysiotropic form in carp, zebrafish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*), and masu salmon (*Oncorhynchus masou*)—and an associated decrease in plasma LH content and delayed testicular development (Consten, Bogerd, Komen, Lambert, & Goos, 2001). It is likely that the changes in GnRH expression are a direct result of corticosteroid actions in the brain and hypothalamus. For example, in rainbow trout GnRH neurons in the caudal telencephalon/anterior POA exhibit glucocorticoid receptor (GR) immunoreactivity (Teitsma et al., 1999). Further, glucocorticoid responsive elements (GREs) have been identified in the GnRH promoter of striped bass (*Morone saxatilis*) (Chow et al., 1998), tilapia (Farahmand, Rahman, Sohm, Hwang, & Maclean, 2003; Kitahashi, Sato, Sakuma, & Parhar, 2005), sea bream (*Sparus sarba*) (Hu et al., 2008), and zebrafish (Torgersen, Nourizadeh-Lillabadi, Husebye, & Alestrom, 2002). Overall, these results suggest that components of the stress axis may inhibit GnRH transcription and/or synthesis in fishes and more specifically that cortisol may directly regulate GnRH gene expression.

In addition to glucocorticoids, various hypophysiotropic factors that regulate the HPI axis can affect the HPG axis and reproduction. For example, GnRH transcription in mammals is inhibited by CRF (Belsham & Lovejoy, 2005; Kinsey-Jones, Li, Bowe, Lightman, & O'Byrne, 2006; Keen-Rhinehart et al., 2009), arginine vasopressin (Tellam, Mohammad, & Lovejoy, 2000), UI, and sauvagine (Tellam et al., 1998). Although the specific mechanisms by which these neuropeptides affect GnRH are unknown, there is evidence suggesting that CRF may act directly on the transcription of GnRH through CRF receptors (CRF-Rs) or indirectly through a β-endorphin-mediated pathway (Tellam et al., 2000). Whether the CRF system or other hypophysiotropic regulators of the HPI axis affect the production of GnRH in fishes is an area for future research.

Similarly, several neurotransmitters that contribute to the neurocircuitry of stress appear to be involved in the regulation of GnRH neurons and the control of reproduction (Dobson, Ghuman, Prabhakar, & Smith, 2003). For example, in the Atlantic croaker (*Micropogonias*

undulates) a decrease in GnRH gene expression in the POA and an inhibition of reproduction have been observed following exposure to hypoxic conditions. This response was associated with decreased brain serotonin (5-HT) levels and the effects of hypoxic conditions on GnRH are reversible with the pharmacological restoration of 5-HT (Thomas, Rahman, Khan, & Kummer, 2007). Whether 5-HT also contributes to the regulation of the HPI axis in hypoxic Atlantic croaker is not known, but there is compelling evidence implicating the serotonergic system in the regulation of the stress response in fishes (S. Winberg, Y. Winberg, & Fernald, 1997; Höglund, Balm, & Winberg, 2002).

Although intricate relationships between the control of the HPI axis and GnRH neurons have been identified, it is still unclear how these interactions are mediated. Interestingly, there is now evidence that the kisspeptin (Kp) receptor (Kiss1r)-signaling system is an essential component in the regulation of GnRH transcription in mammals (Popa, Clifton, & Steiner, 2008; Roa, Aguilar, Dieguez, Pinilla, & Tena-Sempere, 2008) and fishes (Kitahashi, Ogawa, & Parhar, 2009). This system may play an important role in mediating the suppressive effects of stressors on reproduction. In rats, different stressors and intracerebroventricular injections of CRF can downregulate the gene expression of Kp and/or Kiss1r within both the POA and the arcuate nucleus of the brain (Kinsey-Jones et al., 2009). Although GnRH was not measured in this study, the suppression of Kiss1r signaling was associated with a reduction in LH release from the pituitary (Kinsey-Jones et al., 2009). The impact of stressors, CRF-related peptides, and cortisol on the Kp system in fishes is also an area for future research.

2.2. Effects of Stress at the Level of the Pituitary

The impact of stress on LH secretion from the gonadotropes in fishes is equivocal. Chronic stress in rainbow trout (Bry & Zohar, 1980; Zohar, 1980) and acute stress in the white sucker (*Catostomus commersoni*) (Stacey, MacKenzie, Marchant, Kyle, & Peter, 1984) decrease plasma LH levels. Cortisol implants also reduce plasma LH levels in both male brown trout (*Salmo trutta*) and rainbow trout, and decrease pituitary LH content in male brown trout (Carragher, Sumpter, Pottinger, & Pickering, 1989). Pituitaries incubated *in vitro* from cortisol-fed juvenile carp release significantly less LH in response to GnRH stimulation than pituitaries from control fish (Consten, Lambert, & Goos, 2001). In contrast, plasma LH levels in cortisol-fed carp are either similar to (Consten, Lambert, Komen, & Goos, 2002) or higher than (Consten et al., 2001c) the levels from control fish. Similarly, acute confinement of male brown trout increase plasma LH levels (Pickering, Pottinger, Carragher, & Sumpter, 1987) and cortisol stimulates both LH production and LHβ transcript levels in juvenile European eel pituitary cells (*Anguilla anguilla*) (Huang et al., 1999). These conflicting results are likely due to differences between species as well as differences in both the type and duration of the stressors. In general, the direct actions of cortisol on LH secretion from carp pituitary cells *in vitro* (Consten et al., 2001c), and the presence of GR immunoreactivity in the large majority of LH-like pituitary cells in rainbow trout (Teitsma et al., 1999), suggest a direct action of cortisol at the pituitary level in fishes.

The FSH-like cells of the pituitary in rainbow trout also exhibit GR immunoreactivity (Teitsma et al., 1999) but, to our knowledge, only one study to date has measured the effect of cortisol on pituitary FSH gene expression in a fish and none have quantified the effects of stressors on plasma FSH levels. Immature common carp chronically fed cortisol-containing food pellets during the pubertal period have reduced pituitary FSHβ mRNA levels (Consten et al., 2001a). In general, there appears to be a significant gap in our understanding of how stressors and different components of the HPI axis affect FSH transcription and translation.

2.3. Effects of Stress on Hepatic Vitellogenesis

Since the initial reports showing that slow-release cortisol-containing implants cause marked reductions in circulating Vtg levels in rainbow trout (Carragher et al., 1989), many studies using *in-vivo* and *in-vitro* approaches have demonstrated the inhibitory effects of corticosteroids on Vtg biosynthesis (e.g., Pellisero et al., 1993; Mori, Matsumoto, & Yokota, 1998; Teitsma et al., 1998; Lethimonier, Flouriot, Valotaire, Kah, & Ducouret, 2000; Berg, Modig, & Olsson, 2004; Berg, Westerlund, & Olsson, 2004). The actions of cortisol could be a result of direct effects in the liver and interference with the estrogen-dependent induction of Vtg production or through indirect mechanisms leading to a reduction in E_2 levels. Studies with rainbow trout hepatocytes point to the former mechanism, as cortisol inhibits the expression of both the estrogen receptor (ER) and Vtg mRNA (Lethimonier et al., 2000; Leithmonier, Flouriot, Kah, & Ducouret, 2002). It seems that the ER may be the primary target as cortisol had a stronger inhibitory effect on ER mRNA expression compared to Vtg expression and did so with a higher sensitivity and a more rapid time course of action (Lethimonier et al., 2000). Other work showing that the promoter region of the ER contains GREs provides the functional basis for this effect (Teitsma et al., 1998). Further, activation of the GRE interferes with the actions of

a CCAAT/enhancer-binding protein β-like transcription factor that is involved in enhancing the transcription of the ER (Lethimonier et al., 2002). These studies are consistent with earlier work showing that cortisol implants reduce the number of hepatic ERs in rainbow trout (Pottinger & Pickering, 1990).

It is becoming clear that there are species differences in the manner in which cortisol affects Vtg synthesis. For example, cortisol caused dose-dependent inhibition of estrogen-induced Vtg levels in the plasma of Arctic char (*Salvelinus alpinus*) but, unlike what was reported for the rainbow trout, cortisol had no effect on Vtg mRNA levels (Berg et al., 2004a). This suggests that cortisol may have post-transcriptional effects.

In contrast to the inhibitory effects of cortisol on E_2-induced Vtg induction, cortisol has been shown to potentiate the effects of E_2 on the expression of the zona pellucida (eggshell) proteins in Arctic char (Berg et al., 2004b). These results indicate that Vtg and zona pellucida proteins in Arctic char are not regulated by the same mechanisms and this is reflected in a differential response to corticosteroids.

2.4. Effects of Stress on Gonadal Function

The ovary and testis of teleosts synthesize corticosteroids including cortisol, 11-deoxycortisol, corticosterone, and 11-deoxycorticosterone (DOC) (Fostier, Jalabert, Billard, Breton, & Zohar, 1983; Kime, 1993; Milla et al., 2009). The ovary and testis also contain GRs and mineralocorticoid receptors (MRs) (Takeo, Hata, Segawa, Toyohara, & Yamashita, 1996; Sturm et al., 2005; Milla et al., 2008). Finally, there is evidence that the promoter region of steroidogenic enzymes such as aromatase contain GREs, providing a functional link for the actions of corticosteroids in regulating gonadal gene expression (Gardner, Anderson, Place, Dixon, & Elizur, 2005). Despite the numerous studies showing that the gonads produce and respond to corticosteroids, there is considerable uncertainty as to the physiological roles that corticosteroids play in gonadal tissues (Milla et al., 2009).

Stress and/or cortisol reduce plasma sex steroid levels in a variety of teleost species (e.g., Pickering et al., 1987; Carragher et al., 1989; Jardine, Van Der Kraak, & Munkittrick, 1996; Clearwater & Pankhurst, 1997; Haddy & Pankhurst, 1999). Not surprisingly, many studies have evaluated the direct effects of corticosteroids on gonadal steroid biosynthesis. Although there was early work showing a direct suppressive effect of cortisol on E_2 production by rainbow trout ovarian follicles (Carragher & Sumpter, 1990), subsequent studies found that cortisol was not directly associated with the inhibition of steroid production by goldfish (*Carassius auratus*), carp, New Zealand snapper (*Pagrus auratus*), or zebrafish ovarian follicles (Pankhurst, Van Der Kraak, & Peter, 1995; Alsop, Ings, & Vijayan, 2009). Given the large number of studies with ovarian tissues, it seems unlikely that cortisol has a direct inhibitory effect on ovarian steroidogenesis. There have been far fewer studies with the testis and these studies suggest that there are developmental differences in the responsiveness of the testis to corticosteroids. For example, Consten et al. (2001a) showed that the cortisol agonist dexamethasone blocked *in-vitro* production of 11-ketotestosterone (11-KT) by rainbow trout testicular tissue from pubertal animals (120 days post hatch (dph)) but had no effect in adolescent animals (greater than 165 dph).

Recent studies point to another possible mechanism by which the stress axis may act directly at the level of the gonad to modulate steroid biosynthesis. This relates to work showing that the melanocortin 2 receptor (MC2R), which binds ACTH and is responsible for activating corticosteroid biosynthesis in the interrenal gland of fishes (Klovins et al., 2004; Aluru & Vijayan, 2008), is abundantly expressed in the ovary and testis of rainbow trout (Aluru & Vijayan, 2008). Corticotropin did not stimulate cortisol production in the zebrafish ovarian follicle (Alsop et al., 2009); however, ACTH did inhibit GTH-induced E_2 production by zebrafish follicles incubated *in vitro* (Alsop et al., 2009).

Corticosteroid levels in unstressed fishes are often highest around the time of spawning in females, which may reflect the energetic demands of this period. High levels of corticosteroids may also relate to the roles they play in oocyte maturation and ovulation. Corticosteroids promote meiotic maturation of full-grown ovarian follicles incubated *in vitro* (Figure 6.2a) (Goetz, 1983). It is unlikely that corticosteroids play a dominant role in this process *in vivo,* but they may promote the actions of progestins, which are the primary maturation-inducing steroids in fishes. Cortisol promotes hydration of the oocyte following oocyte maturation in rainbow trout (Figure 6.2b) (Milla, Jalabert, Rime, Prunet, & Bobe, 2006). Finally, a number of studies have shown that cortisol, 11-deoxycortisol, and DOC promote ovulation *in vivo* (Figure 6.2a) (Goetz & Theofan, 1979; Milla et al., 2009 for a review).

There is evidence that DOC, which acts through the MR (Sturm et al., 2005), may play a fundamental role in reproduction in male teleosts. High levels of DOC have been detected around the time of spermiation in rainbow trout (Campbell, Fostier, Jalabert, & Truscott, 1980; Milla et al., 2008), and MRs are expressed in the testes and vasa deferentia in rainbow trout with levels of expression increasing during the initiation of spermiation (Milla et al., 2008). *In-vitro* incubations of rainbow trout testis pieces showed that DOC and cortisol cause a decrease in both basal and GTH-stimulated production of 17α,20β-P (Figure 6.2c) (Milla et al., 2008). This steroid is linked to various effects on spermiation in males including milt

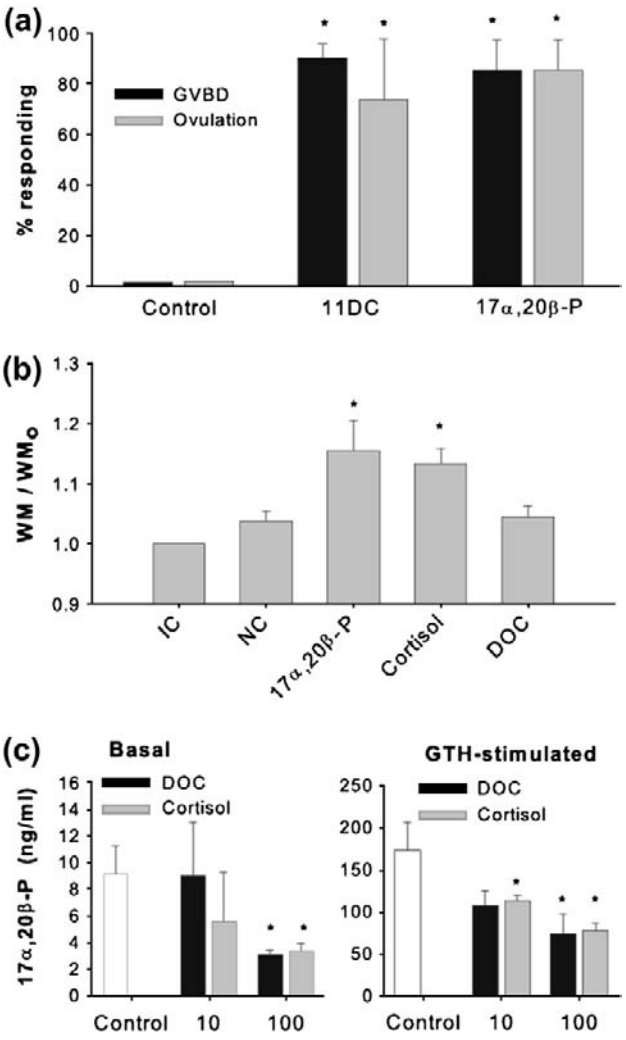

FIGURE 6.2 The effects of corticosteroids on various aspects of gonadal function in fishes. (a) Effects of 11-deoxycortisol (11-DC) and 17α, 20β-dihydroxyprogesterone (17α, 20β-P) (both at 31 ng/ml) on the proportion of yellow perch oocytes undergoing germinal vesicle breakdown (GVBD) and ovulation *in-vitro*. ∗, significantly different from the control. Redrawn from Goetz and Theofan (1979). (b) Effects of 17α, 20β-P (40 ng/ml), 11-deoxycorticosterone (DOC) (50 ng/ml), and cortisol (50 ng/ml) on the *in-vitro* hydration of rainbow trout ovarian follicles as assessed by the ratio of the initial wet mass of oocytes before (WM_o) and after (WM) steroid treatment. IC, initial control; NC, negative control; ∗, significantly different from NC. Redrawn from Milla, Jalabert, Rime, Prunet, and Bobe (2006). (c) Effects of graded concentrations of DOC and cortisol (ng/ml) on basal and gonadotropin (GTH)-stimulated production of 17α, 20β-P by rainbow trout testis fragments. ∗, significantly different from the respective controls. *Redrawn from Milla et al. (2008).*

production, hydration, and the ionic composition of the seminal fluid, although there are species differences in the specific responses (Milla et al., 2008). DOC and 17α,20β-P together significantly reduce the spermatocrit, suggesting that they act together to increase the hydration of the milt. At this point the nature of the interactions between DOC and cortisol with 17α,20β-P are not well understood. Collectively, these studies suggest that corticosteroids have effects on multiple processes associated with reproductive development and gamete maturation in fishes.

3. LIFE STAGE-SPECIFIC EFFECTS OF STRESS ON REPRODUCTION

The effects of stress on reproduction often vary among species depending on the type and intensity of the stressor, and the timing of application. Although many studies have evaluated the effects of stress on gamete formation in adults, it is also important to consider the effects of stressful events at earlier life stages. The embryonic, larval, and juvenile stages are all critical time points in the life history and reproductive biology of fishes.

3.1. Impact of Stress During Embryonic and Larval Stages

In teleosts, breeding females deposit cortisol into their eggs during gametogenesis. Utilization of cortisol begins shortly after fertilization, and cortisol is often completely depleted by the eyed stage of development (Auperin & Geslin, 2008). Stress during egg formation in females increases the quantity of maternal cortisol deposited into eggs (Stratholt, Donaldson, & Liley, 1997; McCormick, 1998). Although a few studies have found that an increase in maternal cortisol has no effect on embryonic growth or survival (Stratholt et al., 1997; Small, 2004), a larger number of studies show that elevated egg cortisol content has a negative impact on embryonic and larval development. For example, in tropical damselfish (*Pomacentrus amboinensis*), elevated maternal cortisol, either stress-induced or injected, is associated with a decrease in progeny size and increased variation in morphology (McCormick, 1998; 1999; McCormick & Nechaev, 2002; McCormick, 2006). High maternal cortisol levels are linked with low survivorship to the 'eyed up' stage in a study with masu salmon (Mingist, Kitani, Koide, & Ueda, 2007). Similar findings of decreased growth, increased mortality, and increased incidence of malformations are found in rainbow trout (Campbell, Pottinger, & Sumpter, 1992; 1994) and Atlantic salmon (*Salmo salar*) (Eriksen, Bakken, Espmark, Braastad, & Salte, 2006). Although the specific cellular mechanisms mediating the effects of elevated maternal cortisol on embryonic and larval development are not known, incubating eggs in cortisol induces similar effects (McCormick & Nechaev, 2002). There is evidence in mammals that exposure to elevated corticosteroids during development has adverse effects on gonadal development and reproductive behavior (Kaiser, Kruijver, Swaab, & Sachser, 2003; Ward et al., 2003; Meek, Schulz, & Keith, 2006; Piffer, Garcia, & Pereira, 2009).

Exposure of developing embryos to various types of stressor also leads to effects similar to those seen with increasing maternal cortisol deposits in eggs, but it is unlikely that effects of early life stress are mediated by the same mechanism since the stress axis in most fishes is not functional until after hatching (Barry, Malison, Held, & Parrish, 1995; Jentoft, Held, Malison, & Barry, 2002; Pepels & Balm, 2004; Alsop & Vijayan, 2008). Embryos exposed to stressful conditions hatch earlier, are smaller in mass and length, and have an increased frequency of deformities (Rombough, 1988; Nguyen & Janssen, 2002; Ciuhandu, Stevens, & Wright, 2005; Eriksen et al., 2006; Hassell, Coutin, & Nugegoda, 2008). The negative impacts of maternal cortisol on hatchling size and weight can be augmented when combined with a stress applied during early development. For example, relative to Atlantic salmon embryos with elevated egg cortisol or to others exposed to hyperthermia, embryos with elevated egg cortisol that were also exposed to hyperthermia had a smaller fork length and body mass at hatch (Eriksen et al., 2006). The mechanisms mediating these effects are unknown and are an area for future research.

Whether the size and weight reductions caused by early life exposure to stress have any impact on the reproductive capabilities of surviving progeny is still a matter of debate. There has been little research on effects of early life stress on subsequent sexual development and reproductive function of mature fishes. In zebrafish, exposure to hypoxic conditions (0.8 mg O_2/liter) from five hours post fertilization to four months post fertilization resulted in a higher incidence of males (74%) relative to the normoxic controls (62%) (Shang, Yu, & Wu, 2006). The skewed sex ratio of zebrafish exposed to hypoxic conditions during development is also associated with decreased gene expression of several steroid biosynthetic enzymes and an increase in the testosterone/estrogen ratio (Shang et al., 2006). In the future, the use of rapidly developing fish models such as zebrafish and medaka (*Oryzias latipes*) should offer new opportunities to evaluate the effects of adverse rearing conditions on reproductive function and to study the mechanisms responsible for the possibly lifelong effects of maternal and early life stress.

3.2. Impacts of Stress on Puberty

Prolonged cortisol treatment of common carp inhibits male pubertal development as measured by the first wave of spermatogenesis (Consten et al., 2001a; Consten, Keuning, Terlou, Lambert, & Goos, 2001b; Consten et al., 2001c; 2002a; 2002b). Juvenile carp around 60 dph were fed cortisol-treated food or received intraperitoneal implants of cortisol in cocoa butter. By approximately 90−100 dph, the cortisol-treated carp had a lower gonadosomatic index (GSI), impaired spermatogenesis, and lower plasma T and 11-KT levels as compared to controls. *In-vitro* studies confirmed that the latter effects were due to a decrease of the steroid production capacity of the testis (Consten et al., 2001b; 2002a; 2002b). Cortisol had modest effects on pituitary LH content and plasma LH levels, suggesting that the pituitary is not the primary site of the inhibitory effects of cortisol on testicular development (Consten et al., 2001a; 2001b). Rather, it seems likely that a decrease in androgen secretion may contribute to impaired maturation of gonadotropes. Interestingly, restoration of androgen levels in cortisol-treated carp did not result in testicular development similar to that of the control animals, suggesting that there also may be direct effects of cortisol on testicular development (Consten et al., 2002a).

It is not known whether puberty in male fishes is uniquely sensitive to the effects of corticosteroids. However, at least for the carp, the effects of cortisol are most pronounced during pubertal development. Adolescent carp having completed the first wave of spermatogonial development and having been treated with cortisol-containing food starting at 138 dph showed a diminished response to exogenous cortisol compared to pre-pubertal carp (Consten et al., 2002b).

Cortisol administration promotes testicular development during the early stages of spermatogenesis, while inhibiting spermatogenesis during the mature phase in a freshwater fish, *Notopterus notopterus* (Shankar & Kulkarni, 2000). Studies of Japanese eel (*Anguilla japonica*) testis in an organ culture system show that cortisol induced DNA replication in spermatogonia and potentiated the actions of 11-KT on spermatogonial proliferation (Ozaki et al., 2006). Additionally, cortisol induces 11-KT production in eel testicular fragments (Ozaki et al., 2006). Collectively these studies suggest, perhaps unexpectedly, that in some species corticosteroids may play a positive role in spermatogonial proliferation. To date, there are no studies examining the effects of stress on the early stages of sexual development in female fish.

3.3. Impacts of Stress on Adults

A number of studies have investigated the effects of stress and corticosteroids on reproductive development in sexually maturing fish. Most of these studies have shown that repeated stressful events delay ovulation. For example, redbelly tilapia (*Tilapia zillii*) fail to spawn in crowded holding tanks but the same fish will spawn soon after transfer to individual aquaria, coincident with increases in serum levels of E_2 and T (Coward, Bormage, & Little, 1998). These studies showed that, as the duration of time individuals were held under crowded conditions increased, so did the incidence of ovarian atresia. This increased rate of atresia was associated with depressed steroid hormone levels (Coward et al., 1998). Increased atresia has been

reported for vitellogenic follicles of the gurnard (*Chelionichthys kumu*) after confinement for up to 96 hours (Clearwater & Pankhurst, 1997). This observation contrasts with studies involving the striped trumpeter (*Latris lineate*), which showed that frequent handling of the broodstock resulted in a greater volume of eggs being produced by the handled fish (Morehead, Ritar, & Pankhurst, 2000). Similarly, adult female rainbow trout subjected to repeated acute handling stress during the later stages of reproductive development exhibited reduced egg size and a significant delay in ovulation, and males had lower sperm counts (Campbell et al., 1992). The same study also found significantly lower survival rates for progeny from stressed rainbow trout compared to progeny from unstressed control fish (Campbell et al., 1992). This is in sharp contrast to other studies showing that rainbow trout stressed during the period of final oocyte maturation and those that were stressed throughout vitellogenesis and oocyte maturation ovulated on average about two weeks earlier than controls (Contreras-Sánchez, Schreck, Fitzpatrick, & Pereira, 1998). This differs from the situation for rainbow trout stressed during the period of early vitellogenesis, which ovulated at the same time as controls. Absolute fecundity and fertilization were not significantly affected in any treatment group, but significant differences in relative fecundity were found. Stress applied early in vitellogenesis resulted in smaller eggs and swim-up fry (Contreras-Sánchez et al., 1998). Collectively these studies suggest that the response to stress varies markedly between species and depends on the time when the stress is applied and the nature of the stressor (Schreck et al., 2001).

Corticosteroids also may play a role in regulating reproductive behavior in terms of mobilizing energy at times of mate selection, spawning, and nest defense. Whereas some studies have shown that corticosteroids are higher during these energetically expensive times, the relationship to reproduction is poorly understood (Knapp, 2003; Neff & Knapp, 2009).

Several studies have investigated the effects of cortisol on reproductive function in adults. For example, sexually mature male brown trout treated with slow-release cortisol implants had significantly smaller testes, lower plasma levels of T, and reduced pituitary LH content compared to controls, although plasma 11-KT and LH were not different (Carragher et al., 1989). In contrast, testis size and plasma sex steroid levels in sexually maturing male rainbow trout were not affected by 36 days of cortisol treatment (Carragher et al., 1989). In other studies, sexually mature female brown trout implanted with cortisol for 18 days had smaller ovaries and reduced plasma levels of E_2, T, and Vtg (Carragher et al., 1989). Similarly, sexually mature female Mozambique tilapia (*Oreochromis mossambicus*) implanted with cortisol-containing pellets for 18 days had significantly depressed GSI, oocyte size, and serum T and E_2 levels (Foo & Lam, 1993).

Sex change is a common reproductive strategy in fishes (Devlin & Nagahama, 2002; see also Chapter 1, this volume) and corticosteroids have been hypothesized to play a role (Munday, Caley, & Jones, 1998; Perry & Grober, 2003; Frisch, Walker, McCormick, & Solomon-Lane, 2007). There are various ways in which this could operate but, for protogynous species (ones that first mature as a female and later change to a male), female fishes under suppressive male dominance and with high cortisol levels may be unable to change sex because the increased corticosteroids competitively inhibit the synthesis of 11-KT necessary for male development. Central to this issue are the overlapping actions of the enzymes 11β-hydroxylase and 11β-hydroxysteroid dehydrogenase, which are involved in the synthesis and deactivation of corticosteroids and also in the two-step synthesis of 11-KT from T (Kusakabe, Nakamura, & Young, 2003; Ozaki et al., 2006). It has been hypothesized that female fish (under suppressive male dominance) are unable to change sex because increased corticosteroid levels would inhibit 11-KT synthesis via substrate competition (Frisch et al., 2007). In an effort to test this hypothesis, female sandperch (*Parapercis cylindrica*) were treated with cortisol under conditions that were permissive to sex change. However, there was no effect of cortisol treatment on sex change or pattern of steroidogenesis, suggesting that increased corticosteroid has no effect on protogynous sex change in this species. (See Chapter 8, this volume for a discussion of hormonal regulation of sex change in fishes.)

4. EFFECTS OF SEX AND REPRODUCTION ON THE HYPOTHALAMIC–PITUITARY–INTERRENAL (HPI) AXIS

There is considerable evidence that components of the HPG axis can affect the activity of the hypothalamic–pituitary–adrenal (HPA) axis in mammals, the latter being the homolog of the HPI axis of fishes (Goel & Bale, 2009; Kudeilka, Hellhammer, & Wüst, 2009; Solomon & Herman, 2009). Although little research has been conducted on the effects of GnRHs or GTHs on the HPI axis, sex steroids do affect the latter. In general, the impact of sex steroids on the neuroendocrine stress response in fishes is of growing interest due to the large number of natural hormones and hormone mimics that are entering the aquatic environment (Arukwe, 2008; see also Chapter 13, this volume). To date, however, the results of studies on the impact of sex steroids on the stress response in fishes are equivocal and, although some studies suggest that E_2 exacerbates and 11-KT attenuates cortisol secretion, others suggest the opposite.

Noting larger increases in plasma ACTH and cortisol following a stressor in immature rainbow trout than in adult males, Pottinger, Balm, and Pickering (1995) first suggested that sex steroids may modulate the stress response in fishes and affect the activity of pituitary corticotropes. In a subsequent study, whereas sexually immature rainbow trout and immature brown trout of unknown sex given T and 11-KT implants had depressed stress-induced ACTH and cortisol levels, E_2-implanted fish were characterized by an enhanced cortisol stress response (Pottinger, Carrick, Hughes, & Balm, 1996). Immature Atlantic salmon exposed to E_2-containing water also had elevated plasma cortisol levels under both resting and stressed conditions (Lerner, Bjornsson, & McCormick, 2007). Interestingly, while the above studies suggest that E_2 stimulates cortisol production in fishes, mature female rainbow trout with naturally high plasma E_2 levels do not exhibit enhanced stress responsiveness (Pottinger et al., 1996). It has been suggested that high levels of T in both sexually maturing male and female fish may counteract the stimulatory effects of estrogens on the stress response (Pottinger & Carrick, 2000); however, this hypothesis has not been tested. In contrast, studies with juvenile gilthead seabream (*Sparus aurata*) (Teles, Pacheco, & Santos, 2005) and sea bass (*Dicentrarchus labrax*) (Teles, Pacheco, & Santos, 2006) have found that E_2 injections or immersion depress plasma cortisol levels. *In vitro*, the rate of cortisol synthesis induced by pregnenolone and ACTH was higher in immature rainbow trout than in mature males, mature males given 11-KT implants, or immature females treated with 11-KT (Young, Thorarensen, & Davie, 1996). However, McQuillan, Lokman, and Young (2003) reported no effect of 11-KT on the rate of cortisol synthesis from the interrenals of juvenile or mature rainbow trout and Chinook salmon (*Oncorhynchus tshawytscha*), and an inhibitory effect of E_2 on the ability of the head kidney to utilize pregnenolone for cortisol synthesis. McQuillan et al. (2003) also observed that immature and mature rainbow trout interrenals were insensitive to E_2, and that mature female Chinook salmon and rainbow trout were more sensitive to ACTH stimulation than mature males. Similarly, Barry, Riebe, Parrish, and Malison (1997) observed no effect of either E_2, T, or 11KT on basal cortisol secretion from incubated juvenile rainbow trout interrenals. Finally, Vijayan, Takemura, and Mommsen (2001) found no impact of E_2 exposure on the cortisol levels of male Mozambique tilapia, and Carrera et al. (2007) saw no change in the cortisol levels of gilthead seabream injected with E_2. While the reasons for these differing results are not known, differences in methodological approach (steroid dose, application, and duration) and species-specific differences, as observed between mammalian species (Young, Korszun, Figueiredo, Banks-Solomon, and Herman, 2008), are likely contributing factors. Despite the equivocal findings, and the obvious need for a more reductionist approach in order to decipher the specific mechanisms involved, results from the above studies clearly implicate sex steroids as potential modulators of the HPI axis in teleosts.

5. REPRODUCTION AND RESISTANCE TO STRESS

The chronic activation of the HPI axis that accompanies sexual maturation and spawning in Pacific salmon is paradoxical, as it appears to contradict the evidence presented in this chapter suggesting that the effectors of the HPI axis negatively impact reproductive functions in fishes. Characterized in numerous studies and species since the 1950s, the development of the gonads in Pacific salmon (genus *Oncorhynchus*) is accompanied by a sustained increase in plasma cortisol levels (for review see Dickhoff, 1989; Fagerlund, McBride, & Williams, 1995), hypertrophy and hyperplasia of pituitary corticotropes (Robertson & Wexler, 1962; Van Overbeeke & McBride, 1967), and elevated CRF and UI mRNA levels in the forebrain (Westring et al., 2008). Although resting plasma cortisol levels in immature Pacific salmon may not differ from those in other salmonids and typically lie below 10 ng ml^{-1}, during sexual maturation and migration the levels can reach several hundred ng ml^{-1} and remain elevated for weeks to months (Robertson & Wexler, 1959; McBride, Fagerlund, Dye, & Bagshaw, 1986; Carruth et al., 2000; Westring et al., 2008). Further, although the demands of the spawning migration (e.g., changes in salinity, water temperature, water flow, migration distance) can influence the magnitude of the cortisol surge in maturing semelparous and anadromous *Oncorhynchus* species such as sockeye salmon (*Oncorhynchus nerka*) (Macdonald et al., 2000; Hinch, Cooke, Healey, & Farrell, 2006), HPI axis activation is also observed in maturing adults that are nonmigrating. For example, sexual maturation in captive sockeye salmon (Patterson, Macdonald, Hinch, Healey, & Farrell, 2004), landlocked kokanee salmon (*O. nerka kennerlyi*) (Carruth et al., 2000), and iteroparous migrating and nonmigrating rainbow trout (Robertson & Wexler 1959; Robertson et al., 1961) is characterized by a significant increase in HPI axis activity. These results suggest that the stimulation of the HPI axis in mature Pacific salmon is at least partly due to an endogenous programmed event and not solely the consequence of migration and spawning (Dickhoff, 1989; Carruth, Jones, & Norris, 2002).

Interestingly, castration of sockeye and kokanee salmon reduces plasma cortisol levels, delays the activation of the HPI axis, and increases lifespan (Robertson, 1961; Robertson & Wexler, 1962; McBride & Van Overbeeke, 1969). Moreover, although the effects of sex

steroids on the HPI axis are complex and equivocal (see Section 4), several studies show that estrogens and androgens can stimulate the HPI axis in gonadectomized Pacific salmon (Donaldson & Fagerlund, 1969; Fagerlund & Donaldson, 1969; Van Overbeeke & McBride, 1971). Therefore, while the physiological processes associated with senescence may contribute to the activation of the HPI axis in semelparous salmon, considerable evidence also suggests that sex steroids participate in this process. Although the specific physiological roles of the hyperactive HPI axis in sexually maturing Pacific salmon are unknown, the catabolic effects of cortisol presumably contribute to the mobilization of energy reserves, which fuels development of the gonads and the migration to the spawning grounds. Carruth et al. (2002) suggested that the increase in plasma cortisol associated with sexual maturation and migration may enhance the ability of Pacific salmon to recall the imprinted memory of the home-stream chemical composition.

Although there is some evidence that the very high cortisol levels of salmon migrating through extreme conditions can be associated with a transient reduction in plasma sex steroid levels (Hinch et al., 2006), in general Pacific salmon successfully reproduce in the face of a prolonged surge in plasma cortisol. Thus, a marked and sustained increase in the HPI axis need not necessarily result in a suppression of the HPG axis and the energy-mobilizing properties of cortisol may take place without negatively affecting reproductive functions. Future studies on semelparous salmonids aimed at deciphering the cellular mechanisms by which the HPG axis overcomes the suppressive effects of the HPI axis during gonadal maturation could broaden our understanding of the interrelationships between the HPI and HPG axes in fishes.

6. CONCLUSIONS

Stressors in fishes can impact all levels of the HPG axis and physiological processes that are essential for reproduction, from fertilization to spawning. While both central and peripheral effectors of the HPI axis likely play key roles in mediating the effects of stress on reproduction, to date the majority of studies have focused on the impact of cortisol and relatively little is known about the influence of the CRF system and ACTH on the regulation of reproductive functions. In general, although equivocal findings have been reported, cortisol mainly has a negative impact on early development, gonadal differentiation, puberty, gametogenesis, and sexual behavior. In contrast, cortisol may exert positive effects during oocyte maturation and ovulation, and in some species gonadal maturation proceeds during chronic HPI axis hyperactivity.

Despite a recognition that the regulation of GTH secretion and gonadal functions in teleosts are multifactorial processes, to date most of the studies investigating the impact of stress on reproduction have focused exclusively on the primary effectors of the HPG axis, i.e., GnRH, GTHs, and sex steroids. Future studies are therefore needed to take into consideration the potential impact of stressors on the various secondary endocrine and paracrine factors that are involved in regulating the growth, differentiation, and function of the reproductive system. Finally, with few exceptions, studies into the interactions between the HPI and HPG axes in fishes have been carried out on seasonal oviparous (egg-bearing) species. Fishes are characterized by an amazing diversity of additional reproductive tactics, including continuous oviparous batch spawning, various forms of viviparity (live-bearing), and hermaphroditism. Exploiting the diversity of life-history strategies and reproductive tactics among fish species may offer unique opportunities among vertebrates to decipher the intricate relationships between the stress and reproductive axes.

ABBREVIATIONS

11-KT	11-ketotestosterone
17α,20β-P	17α,20β-dihydroxyprogesterone
5-HT	Serotonin
ACTH	Corticotropin
CRF	Corticotropin-releasing factor
CRF-R	Corticotropin-releasing factor receptor
DA	Dopamine
DOC	11-deoxycorticosterone
dph	Days post hatch
E_2	17β-estradiol
ER	Estrogen receptor
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GSI	Gonadosomatic index
GTH	Gonadotropin
HPA	Hypothalamic−pituitary−adrenal
HPG	Hypothalamic−pituitary−gonadal
HPI	Hypothalamic−pituitary−interrenal
Kiss1r	Kisspeptin receptor
Kp	Kisspeptin
LH	Luteinizing hormone
MC2R	Melanocortin 2 receptor
MR	Mineralocorticoid receptor
NPO	Nucleus preopticus
POA	Preoptic area
POMC	Pro-opiomelanocortin
T	Testosterone
UI	Urotensin I
Vtg	Vitellogenin

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