11 Neuroendocrinology of Life History and Stress in Anemonefishes

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CONTENTS

11.1 INTRODUCTION

Environmental changes affect the evolution of complex life-history stages, notably phases of development, growth, reproduction, and senescence, each of them characterized by distinctive phenotypes (Wingfield 2008). These life-history transitions are under the control of the neuroendocrine system, which integrates both environmental cues and information provided by internal biological timers. The neuroendocrine system leads to the efficient synchronization of vital physiological functions with environmental cues (Wingfield 2015; Hazlerigg and Wagner 2006), allowing phenotypic plasticity in accordance with environmental changes.

In vertebrates, environmental cues (e.g., temperature, photoperiod, chemicals) as well as internal information (e.g., nutrition, metabolic signals) are integrated by the central nervous system (CNS), which results in the release of neuroendocrine signals notably from the hypothalamus. This triggers the downstream release of hormones by the anterior pituitary gland (i.e., the adenohypophysis) and, in turn, peripheral endocrine secretions that regulate morphological, physiological, and behavioral responses (Falcón et al. 2007). So far, most of the existing data regarding the impact of the environment on the fish life cycle derives from canonical models, such as zebrafish (*Danio rerio*) or medaka (*Oryzias latipes*) (both freshwater species), or aquaculture breeding species such as salmonids or the European sea bass (*Dicentrarchus labrax*) (Jonsson and Jonsson 2014). Hence, there is now a need to study alternative models such as anemonefish, in which very little data is available to date.

This chapter summarizes recent findings with regard to how the environment can trigger morphological, physiological, and behavioral responses that orchestrate the anemonefish life cycle at two main life-history steps: i) the post-embryonic phase transforming larvae into juveniles, called metamorphosis, and ii) the adult phase regarding sexual development, including protandrous (or male-to-female) sex change. We also link these two fascinating processes with the stress pathway. Finally, we give insights into how modifications associated with climate change could affect the ecology, physiology, and, eventually, survival of these fishes.

11.2 THE NEUROENDOCRINE SYSTEM IN FISH: HOW TO LIVE IN A FLUCTUATING ENVIRONMENT

In fish, as in all vertebrates, the brain constitutes the main interface between an individual's physiology and its environment. This allows organisms to maintain their homeostasis in a given environment and to face environmental fluctuations through acclimation. Following the pioneering work of Scharrer (1928), suggesting the presence of secretory cells in the brain of teleost fish, neuroendocrinology emerged as a new discipline in neurosciences (Cerdá-Reverter and Canosa 2009). Subsequently, the existence of the hypothalamo-pituitary system was established in fish, thus linking the CNS and the endocrine system which ensure the regulation of a variety of vital functions and processes. Both tightly linked systems contribute to adapting the response of the organism to changes in the environment and internal milieu, to ultimately control body homeostasis (Zohar et al. 2021; Vissio et al. 2021).

The hypothalamo-pituitary (HP) complex in teleosts is divided into three main areas: the hypothalamus (which

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is part of the diencephalon), the neurohypophysis (which derives from the ventral diencephalon and represents the neural compartment of the pituitary), and the adenohypophysis (which is the secretory part of the gland). The environmental information received by the hypothalamus is transferred through axonic projections to the neurohypophysis where neurosecretory fibres release various peptides (e.g., corticotropin-releasing hormone [CRH], thyrotropin-releasing hormone [TRH], or gonadotropin-releasing hormone [GnRH]) in the vicinity of the cells of the adenohypophysis (Figure 11.1) (Zohar et al. 2010). According to their localization within the pituitary, these cells synthesize at least eight different hormones, belonging to different families depending on their action. Four hypothalamo-pituitary (HP) axes can be thus defined: i) the hypothalamo-pituitary somatotropic (HPS) axis which consists in the secretion of growth hormone (GH), prolactin (PRL), and somatolactin (SL) controlling growth, but also osmoregulation; ii) the hypothalamo-pituitary gonadal (HPG) axis characterized by two gonadotropins, the follicle-stimulating hormone (FSH), and the luteinizing hormone (LH) which are involved in the regulation of sexual reproduction; iii) the hypothalamo-pituitary interrenal (HPI) axis consisting of peptides derived from a common precursor, the proopiomelanocortin (POMC), leading to the melanocyte-stimulating hormone (MSH), the adrenocorticotropic hormone (ACTH), and the β-endorphin, both involved in the control of stress and immunity in fish; and iv) the hypothalamo-pituitary thyroid (HPT) axis, characterized by the secretion of thyroid-stimulating hormone (TSH) mainly involved in teleost fish development and metamorphosis, but also in metabolism, reproduction, growth, and osmoregulation (Deal and Volkoff 2020; Zohar et al., 2021).

In this chapter, we review the available data concerning the involvement of the aforementioned neuroendocrine systems in two main transition phases shaping the life cycle of anemonefishes: metamorphosis and sex change.

11.3 ANEMONEFISHES AND THEIR ENVIRONMENT

Like most coral reef fishes, anemonefish exhibit a biphasic life cycle that includes a dispersive planktonic larval phase in the open ocean followed, after the recruitment phase, by a sedentary reef phase for the juveniles and adults (Roux et al. 2020). From an ecological point of view, one of the most pressing questions regarding the life history of anemonefish has been to decipher how anemonefish larvae manage to navigate within the pelagic environment and then orient themselves to a suitable benthic recruitment site. Although the exact mechanisms used by anemonefish larvae at this crucial step of their life cycle still remain unclear (Dixson et al. 2011), the behavioral capabilities of these and other reef fish larvae suggest they exhibit a strong determinism concerning their final settlement destination (Leis 2007) thanks to extraordinary swimming capabilities, endurance, orientation skills, and acute sensory abilities (Dixson et al. 2011).

During life transition periods, the neuroendocrine control system plays a crucial role: the fish brain (i.e., the hypothalamus) receives and integrates environmental information as well as tactile, visual, and chemical communication signals transmitted from the central and peripheral nervous systems. The detection of environmental signals stimulates the hypothalamic neurons to secrete peptides such as CRH. Nerve endings terminate in close association with corticotropic cells (cells secreting POMC) and thyrotropic cells (cells secreting TSH) of the adenohypophysis (Figure 11.1). Thus, environmental information is transmitted from the brain to peripheral organs thanks to a particular temporal pattern of secretion of several hormones that in turn control various physiological, developmental, and behavioral processes that lead to the final phenotype (Suzuki et al. 2020).

One of the pivotal components of the neuroendocrine system in teleosts is the hypothalamo-pituitary interrenal (HPI) axis. Once triggered by environmental stressors, the HPI axis releases glucocorticoid hormones (GCs) that have multiple regulatory effects on the organism's biology. GCs can interact with other hormonal pathways, among them: i) the HPT axis, leading to the release of thyroid hormones (TH) which control larval development and metamorphosis (Blanton and Specker 2007; Laudet 2011; Denver 2017); and ii) the HPG axis, which regulates the release of sex steroid hormones. Therefore, GCs play a crucial role in enabling vertebrates to cope with and respond to environmental factors in the wild. However, despite evidence that environmental variations activate the HPI axis, commonly referred to as the stress axis, direct links have rarely been established in anemonefish (Mills et al. 2020).

The settlement habitat of anemonefish is complex, it is composed of a sea anemone, a colony of congeners and a cortege of other reef species of invertebrates and vertebrates, among which some predators (Fautin and Allen 1992). Each part of this complex ecosystem constitutes a source of stress (positive or negative) either by direct visual detection (Casas and Saborido-Rey 2021), acoustic communication (Colleye and Parmentier 2012), or olfactive detection of the chemical cues produced (Barth et al. 2015). Like in many other coral reef fish (Atema et al. 2002; Lecchini et al. 2005; Gerlach et al. 2007; Leis et al. 2011), olfaction plays a critical role in locating settlement habitat after the larval dispersal stage in the orange anemonefish (*Amphiprion percula*) (Dixson et al. 2008). While the exact mechanisms (sensory receptors, communication molecules, etc.) that enable *A. percula* larvae to locate suitable settlement sites are still unknown, Dixson et al. (2008) suggest that an olfactory response to multiple chemical cues (e.g., those emanating from island vegetation, host sea anemones, and/or congeners) may play a role in the final choice of settlement. Therefore, a specific "olfactive landscape" might assist larvae in navigating to reefs where suitable settlement habitats can be found (Veilleux et al. 2013), afterwards triggering the required neuroendocrine signalling for subsequent morphological, physiological, or behavioral events during this life history transition. However, recent studies

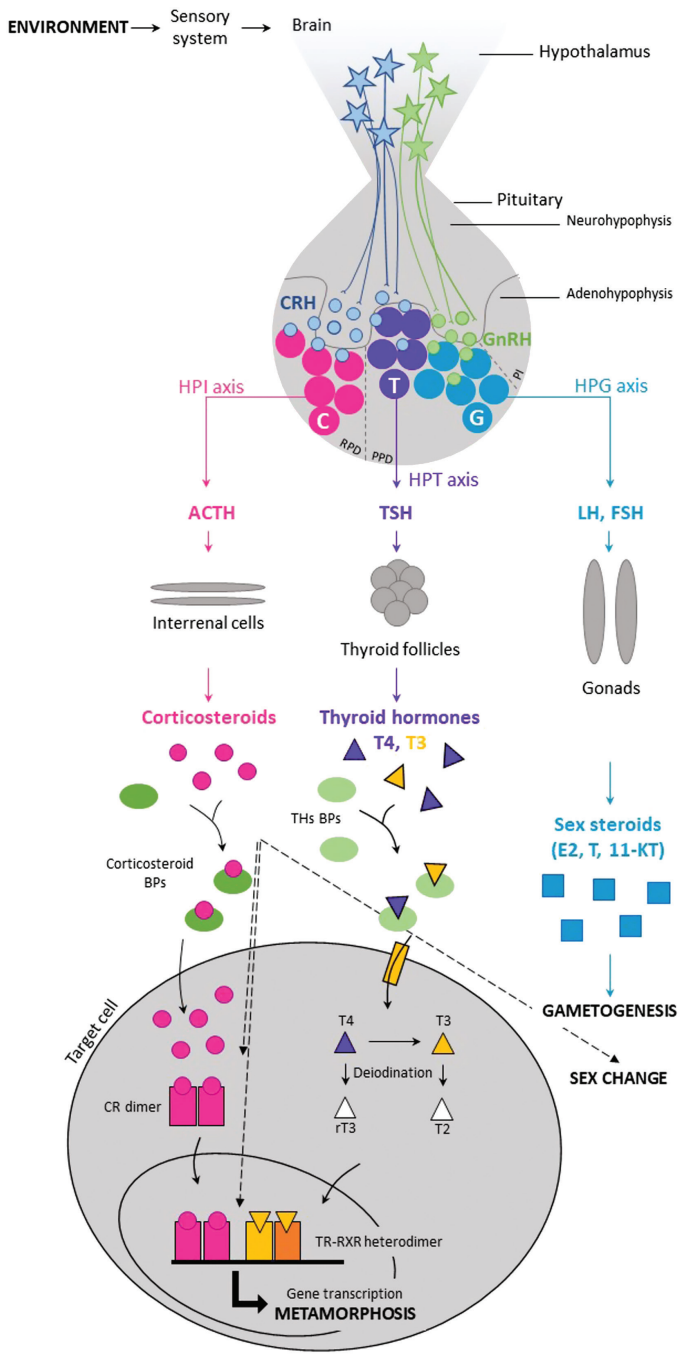

FIGURE 11.1 Illustration representing the HPI, HPT, and HPG axes in non-mammalian species (pink, purple, and blue, respectively). Environmental cues are perceived by sensory organs and processed in the brain, where corticotropin-releasing hormone (CRH) and gonadotropin-releasing hormone (GnRH) are secreted. In non-mammalian species, CRH is thought to control both the HPI and HPT axes. CRH acts on the pituitary cells to stimulate the synthesis and release of i) adrenocorticotropic hormone (ACTH) from corticotropic cells (C, pink circles) and ii) thyroid-stimulating hormone (TSH) from thyrotropic cells (T, purple circles). HPI axis: ACTH stimulates the secretion of glucocorticoids (GCs) by the interrenal cells. Once secreted, GCs are transported in the blood by corticosteroid binding proteins (BPs). GCs enter target cells where they bind to corticosteroid receptor dimers which then translocate into the nucleus where they bind to specific DNA regions to induce gene expression. HPT axis: TSH stimulates the production of thyroid hormones (THs, T4, and T3) by thyroid follicles. THs are transported to target cells *via* their specific BPs. Inside target cells, THs are substrates of deiodinase enzymes which can both activate (i.e., forming T3) or inactivate (i.e., rT3 and T2) THs. THs are transported to the cell nucleus where they bind to TR-RXR heterodimers, which activate gene transcription, *in fine* resulting in metamorphosis. HPG axis: GnRH reaches gonadotropic cells to induce synthesis and release of gonadotropins (LH and FSH). LH and FSH both reach the gonads where they stimulate gonadal maturation and function (i.e., synthesis of sex steroids: E2, T, and 11-KT). The balance between these sex steroids, in turn, regulates gametogenesis and controls sex change in hermaphroditic species. Finally, corticosteroids can influence both metamorphosis and sex change processes (dotted black lines). RPD: rostral *pars distalis*, PPD: proximal *pars distalis*, PI: *pars intermedia* of the adenohypophysis. Adapted from Denver (2017).

indicate that this landscape could be modified with global change, as indicated by i) behavioral experiments showing that anemonefish juveniles can discriminate between olfactive cues emanating from bleached and unbleached host anemones (Scott and Dixson 2016), and ii) the fact that some chemical cues degrade more quickly in conditions simulating global change (UV radiation and ocean acidification) compared to control conditions (Chivers et al. 2014). Therefore, modifications of the olfactive landscape could lead to misinterpretations by anemonefish larvae of the location of their settlement site or their ability to escape predation (Munday et al. 2009; Dixson et al. 2010; Biswal et al. 2021).

11.4 NEUROENDOCRINE CONTROL OF METAMORPHOSIS

As previously stated, very few species are used as models to investigate developmental biology, with anemonefish just emerging as a new model organism (Roux et al. 2020). As the signalling pathway underlying metamorphosis is strongly conserved among vertebrates, we will focus in this section on anemonefish whenever possible, and will otherwise refer more generally to other teleost fish and amphibians in which the mechanisms leading to metamorphosis have been more extensively described.

Metamorphosis is classically defined as an abrupt and irreversible life-history transition in which a larva transforms into a juvenile (Laudet 2011). In most marine teleost species, the life cycle is biphasic: pelagic larvae disperse into the ocean, then undergo metamorphosis that is concomitant with the recruitment to the adult habitat. During metamorphosis, larval features are lost and adult features develop, enabling the colonization of the new environment. This morphological transformation is thus accompanied by profound physiological and ecological changes (McMenamin and Parichy 2013; Bishop et al. 2006). More or less important alterations occur during metamorphosis, from a total remodelling of the body in flatfishes and amphibians to more subtle modifications in most teleost fishes, such as the development of fins, formation of scales, ossification, maturation of organs, as well as the acquisition of the adult color pattern (Laudet 2011). For example, in the anemonefish *A. ocellaris*, first the notochord bends, then soft rays appear in the anal and dorsal fins that later acquire their spines, pelvic fins grow, and finally, the color pattern develops, with the white bars appearing in a rostro-caudal gradient (Roux et al. 2019b).

In amphibians and in the teleost species studied so far, metamorphosis is triggered by the action of THs on thyroid receptors (TRs), which has been confirmed *in vivo* using either THs treatments, pharmacological treatments (using chemical compounds called goitrogens that block TH synthesis), or transgenic animals expressing a dominant-negative form of TRs (Inui and Miwa 1985; Schreiber et al. 2001; Buchholz et al. 2003; Salis et al. 2021; see McMenamin and Parichy 2013 for review). Recently, the importance of THs in metamorphosis has been extended to several coral reef fishes including *A. ocellaris* (Holzer et al. 2017; Salis et al. 2021). THs are the final product of the HPT axis, beginning in the brain. In mammals, thyrotropin-releasing hormone (TRH) stands at the top of this HPT axis. In non-mammalian species, however, it seems that CRH has taken up this role (Laudet 2011). CRH stimulates pituitary cells (i.e., thyrotropic cells) that consequently synthesize and release thyroid-stimulating hormone (TSH) into the global circulation. TSH reaches thyroid follicles that mainly produce thyroxine (T4, main form) and 3,5,3'-triiodothyronine (T3, biologically active form) (Laudet 2011; Denver 2017). T4 and T3 can both reversibly bind to circulating binding proteins (BPs) to be transported from the site of production (thyroid follicles) to target cells in peripheral tissues (Power et al. 2000). Thanks to transmembrane transporters, THs enter target cells in which they can be enzymatically activated or inactivated under the action of deiodinases (DIO), therefore regulating their intracellular availability in target tissues. This way, T4 can be activated into T3; T4 and T3 can be inactivated into rT3 and T2, respectively (Blanton and Specker 2007; Denver 2017). THs are then transported from the cytoplasm to the cell nucleus where they bind to thyroid hormone receptors (TRs). Teleost fish possess at least two TR genes named TRα and TRβ, with some species possessing additional copies of these genes (McMenamin and Parichy 2013). In teleosts, two distinct genes encoding TRα have been identified (TrαA and TRαB). Additionally, isoforms of TRβ have been described, resulting either from alternative splicing or transcription of two different genes (i.e., such as in the Japanese conger eel *Conger myriaster*) (Marchand et al. 2001; Kawakami et al. 2003a, 2003b; Galay-Burgos et al. 2008). TRs form dimers either with another TR or most often with a retinoid X receptor (RXR). These TR-RXR heterodimers act as transcriptional activators only upon THs binding which induces the transcription of target genes, giving rise to the transformations associated with metamorphosis (Sachs et al. 2002; Denver 2017) (see Figure 11.1). Generally speaking, whole-body TH levels, together with the expression of TSH and TRβ genes, increase at the onset of metamorphosis, peak during climax, and then decrease when the juvenile stage is reached (see Campinho 2019 for review). This general pattern has been confirmed in the coral reef fish *Acanthurus triostegus*, in which individuals being recruited show a rise in THs level and TRs expression, both dropping right after recruitment (Holzer et al. 2017). In other coral reef species (e.g., *Rhinecanthus aculeatus*, *Chromis viridis*, *Chaetodon lunula*, and *Ostorhinchus angustatus*) recruiting larvae also experience the same TH levels fluctuations, suggesting that TH-mediated metamorphosis could be generalized to all coral reef fishes (Holzer et al. 2017). Similar expression profiles of THs and TRs genes have been observed during the metamorphosis of *A. ocellaris* (Roux et al. 2019a).

Originally, CRH was known for its role in stimulating the HPI axis. By the end of the 20th century, although there was no doubt THs were essential for tadpole

metamorphosis, it was unclear which neurotransmitter could stimulate the HPT axis, as all attempts to induce TSH secretion with TRH injection were unsuccessful (reviewed in Ball 1981). Later on, *in vivo* experiments showed that in tadpoles experiencing environmental stress (pond drying, overcrowding, etc.) the time to complete metamorphosis was shortened, which is thought to maximize the probability of survival. Such metamorphosis acceleration could be reversed if the larvae were treated with a CRH antagonist (Newman 1992; Denver 1997). Concomitantly, *in vitro* and *in vivo* experiments showed that CRH could elevate TSH, THs, and stress-related hormone (glucocorticoids, GCs) levels while accelerating tadpole metamorphosis (Denver and Licht 1989; Denver 1993; Denver 1997). Therefore, CRH, stimulating both the HPI and HPT axes, cross-links environmental stress and TH homeostasis, procuring some developmental plasticity to larvae living in a fluctuating environment. Whether TRH and/or CRH stimulate the HPT axis in teleost fishes remains an open question; the regulation of the HPT axis might be species-specific (Larsen et al. 1998; Eales and Himick 1988; Geven et al. 2009; Galas et al. 2009; Rousseau et al. 2021).

Briefly, as soon as the HPI axis is stimulated, CRH is synthesized by the brain and reaches the pituitary to stimulate the synthesis and release of adrenocorticotropic hormone (ACTH) (derived from proopiomelanocortin, POMC) to the global circulation. ACTH then reaches its target cells in the interrenal gland to induce the synthesis of GCs, mainly corticosterone and cortisol, the latter being the main stress hormone in fish (Wendelaar-Bonga 1997). GCs are then transported to the blood bound to corticosteroid BPs and finally enter target cells where they bind to corticosteroid receptor (CR) dimers (glucocorticoid [GRs] or mineralocorticoid [MRs] receptors) that then translocate into the cell nucleus to induce gene transcription (Bury and Sturm 2007; Denver 2017). GCs can modulate various functions in fish, such as glycogen metabolism (energy is mobilized in a stressful situation) but also osmoregulation or blood pressure (see Mommsen et al. 1999 for a review). Additionally, GCs seem to have an important role during metamorphosis. Indeed, *in vivo* and *in vitro* experiments in frogs showed that GCs administered alone had no effect on the HPT axis but when administered together with T3, the interaction of both hormones strongly stimulated TRs and deiodinases gene expression, suggesting that tissue sensitivity to THs was increased (Bonett et al. 2010). Other reports showed that deiodinases activity could be enhanced thanks to this simultaneous treatment, increasing T3 bioavailability in target tissues, resulting in a rapid metamorphosis (Galton 1990; Kühn et al. 2005). Thus, stimulation of the HPI axis may accelerate metamorphosis *via* two different modes of action: i) centrally, stimulating the synthesis of CRH in the brain, therefore increasing the synthesis of TSH and stimulating the whole HPT axis; and ii) peripherally, increasing tissue sensitivity to THs at the peripheral level.

11.5 NEURAL REGULATION OF THE STRESS RESPONSE AND SEXUAL DEVELOPMENT

11.5.1 Neuroendocrinology of Plasticity in Teleost Fishes

Environmental factors (e.g., social cues) are known to be transduced into physiological changes that can promote alterations in the nervous system, notably affecting brain structure and functionality (Ebbesson and Braithwaite 2012). Neural plasticity is more pronounced in fishes compared to mammalian systems, and some brain processes such as neurogenesis can happen throughout the whole life (Zupanc et al. 2005). Although the remodelling of the fish brain following a stressful situation is not yet fully understood (reviewed by Sørensen et al. 2013), it has been hypothesized that cell proliferation in the brain might be, in part, influenced by the levels of GCs and their effect mediated by the corresponding receptors (i.e., GRs and MRs) (Montaron et al. 2003; Wong and Herbert 2005; reviewed by Sørensen et al. 2013). Such a link between the stress axis and neurogenesis is further reflected by the decrease in the proliferation of neural cells observed in fish exposed to chronic stress (i.e., social subordination) (Sørensen et al. 2013). This underscores a tight relationship between adaptation to social changes and neural plasticity, which results in a form of behavioral plasticity (Sørensen et al. 2011). Such malleability translates into an extraordinary capacity to adapt to stressful events of diverse magnitude, type, and/or duration. In fish, the stress response is regulated by the HPI axis. Under a situation of stress, activation of the HPI axis begins in the preoptic area of the hypothalamus, setting off a series of chain reactions, as described in the previous section, that ultimately promote the release of cortisol by the interrenal cells of the head kidney (Mommsen et al. 1999; Wendelaar Bonga 1997). The mechanisms underlying the stress response in fish have been extensively reviewed, including a few great examples such as Wendelaar Bonga (1997), Barton (2002), Flik et al. (2006), and Gorissen and Flik (2016), which should be referred to for further detail. Although not much is known yet regarding the molecular mechanisms involved in stress regulation in anemonefish in particular, they likely involve the same classical pathways. Like many other teleost fish, the yellowtail anemonefish *Amphiprion clarkii* expresses two glucocorticoid receptors, Gr1 and Gr2 (Zhang et al. 2020). Relatively high levels of *gr1* transcripts are found in the hypothalamus, cerebellum, and pituitary independently of the sex, whereas *gr2* peaks in the medulla oblongata of males only (Zhang et al. 2020). There are, however, no differences in *gr1* and *gr2* expression according to the sexual status of individuals (Zhang et al. 2020). Undoubtedly, further research into the relationship between environmental stressors and the elicited downstream reactions in the neuroendocrine system of anemonefishes will help improve our understanding of neural plasticity in these and other species. Moreover, addressing this lack of knowledge will shed light on how

fish and vertebrates in general may adapt to their changing environment.

Cortisol is released into the blood between 30 and 120 minutes following a stressful event (e.g., capture and/or restraint) in *Amphiprion chrysopterus* and *Amphiprion akallopisos*, levels similar to those observed in other fish (Mills et al. 2015, 2020). Interestingly, the maximum temperature encountered by *A. akallopisos* in their lifetime appears to modulate cortisol production in relation to stress in both male and female individuals, meaning that a correlation exists between increased cortisol release and previous exposure to high temperatures independent of sex (Mills et al. 2015). This exemplifies the tight link that exists between metabolism and stressful events in regulating cortisol production (Bessa et al. 2021). Basal cortisol levels reported in anemonefishes (Godwin 1994; Godwin and Thomas 1993a; Iwata et al. 2008, 2012; Mills et al. 2015, 2020) are also in line with what is generally found in other fish species. Such levels seem, nevertheless, modulated by the social (dominant versus subordinate) and reproductive (mature versus immature) status, although no clear and significant patterns have been observed (Godwin 1994; Godwin and Thomas 1993a; Iwata et al. 2008, 2012).

11.5.2 Social Control of Sex Change – a Superb Example of Sexual Plasticity

Social and sexual plasticity constitute some of the most extreme examples of phenotypic plasticity, and they become especially apparent in hermaphroditic fish (fish with the ability to change sex naturally during their life cycle, whose sexual fate is not fixed at birth). In some species, such as those belonging to the anemonefish subfamily, this incredible transformation is regulated by modifications in the social structure (reviewed in Olivotto and Geffroy 2017). Socially regulated sex change is a spectacular illustration of reproductive plasticity which has now been reported in multiple species (Choat and Robertson 1975; Jones and Thompson 1980; Liu et al. 2017; Thomas et al. 2019; Todd et al. 2019; Warner and Swearer 1991). While the gonadal and morphological alterations underlying sex change have now been well described in anemonefishes, the molecular cascade controlling this process remains to be fully understood, particularly at the level of the brain. However, it is in the brain where we may find the key to fully comprehending the triggering mechanisms underlying sex change, as visual cues the fish perceive (i.e., removal of the dominant female in the case of anemonefishes) set off a series of interactions, first promoting behavioral sex change (Fricke and Fricke 1977) and then followed by transformation of the gonads (Casas et al. 2016). For this reason, fulfilling this gap in knowledge will be essential to understanding the global picture of this astonishing process. While pieces of the puzzle are still missing, a number of signalling molecules associated with neural activity following the trigger of sex change in fish has been identified, including monoamine neurotransmitters (e.g., dopamine, norepinephrine, serotonin) and neuropeptides (e.g., gonadotropin-releasing hormone, arginine vasotocin, isotocin) (Godwin 2010; Lamm et al. 2015; Thomas et al. 2019). For example, arginine vasotocin (AVT) signalling seems of primary importance in the establishment of dominance in false clown anemonefish, *A. ocellaris* (Yaeger et al. 2014). Inhibiting this signalling pathway significantly reduces aggressive behaviors (bites and charges) as well as the number of *c-fos* positive cells (indicative of high neuroplasticity) in the preoptic area of the hypothalamus (Yaeger et al. 2014). Anemonefish also produce a variety of specific agonistic sounds that are likely involved in the establishment and maintenance of the social hierarchy (Colleye et al. 2011; Colleye and Parmentier 2012). Together, these agonistic sounds and acts have led to the straightforward hypothesis that stress, and more specifically cortisol, would be involved in socially controlled sex change. Recent results indeed pinpointed the essential role of this hormone in natural hermaphroditism, though mainly in protogynous fish (Goikoetxea et al. 2017). For instance, cortisol administration in *Halichoeres trimaculatus* successfully triggered sex change from female to male (Nozu and Nakamura 2015). Nevertheless, regarding protandrous sex change in anemonefishes, results have been inconclusive until now. For example, while serum cortisol levels were observed to increase during sex change in the cinnamon anemonefish *A. melanopus*, this rise took place in the late stages of the transformation (Godwin and Thomas 1993b), suggesting such fluctuation may have been a consequence rather than a cause of the redirection of sexual fate. In *A. ocellaris*, dominant (presumably future female) individuals tended to have less basal cortisol than subordinates after 180 days of inter-individual relationships, though the sex was not clearly established (Iwata et al. 2008). In another experiment involving groups of three immature false clown anemonefish, the dominant individual presented the highest level of cortisol after ten days of interaction, but this was unrelated to sex (Iwata et al. 2012). The fact that no clear patterns are observable could be due to the difficulty in obtaining reliable basal cortisol levels without disturbing the fish, but we believe that new matrices to measure cortisol (i.e., water, mucus, scales, faeces) will ultimately help in this direction for future studies (Sadoul and Geffroy 2019). It is also possible that other mechanisms (e.g., epigenetic) would be key transducers of socially induced sex change (Piferrer 2013; Todd et al. 2019).

11.6 ANEMONEFISH AS A MODEL TO UNDERSTAND MARINE FISH STRESS IN A CHANGING WORLD

The increasing use of anemonefishes as field and laboratory study species suggests their potential as a new biological marine model in multiple areas of research. For example, the recent review by Roux et al. (2020) presented the false anemonefish *A. ocellaris* as a promising fish model for developmental, ecological, and evolutionary biology. Furthermore, in the current landscape of climate change,

fish and other aquatic organisms are bound to suffer the intensifying consequences of thermal stress, as the temperature of water bodies on the planet increases (Laffoley and Baxter 2016). Therefore, understanding how such dramatic environmental changes will affect the stress physiology of fishes is of the utmost importance (Alfonso et al. 2021; Brierley and Kingsford 2009; Geffroy and Wedekind 2020).

Since the pre-industrial era, the release of greenhouse gases (carbon dioxide [CO_2], methane, etc.) into the atmosphere has drastically increased and has reached levels unprecedented during the last two million years (IPCC, 2021). As such, atmospheric CO_2 levels have increased from 280 ppm in 1800 to 410 ppm nowadays and could further increase to reach 900–1,000 ppm by 2100 (Meinshausen et al. 2011; IPCC 2021). Part of this atmospheric CO_2 is being absorbed by the oceans. This has two major consequences: i) the sea surface temperature (SST) rises and ii) the pH of the water decreases, a process known as "ocean acidification" (OA).

OA directly affects calcifying organisms (e.g., corals) because the availability of calcium carbonate ions is reduced. OA, together with other factors (pollution, overfishing, etc.), threatens the existence of corals and accelerates their global decline by creating a vicious circle. The decline of corals makes room for seaweed growth, which then becomes dominant in the reef and chemically repulses coral and fish larvae, limiting their recruitment (Dixson et al. 2014). Certainly, corals are essential for reef fish communities and their decline may seriously threaten the sustainability of coral reef fish communities.

Moreover, OA also directly affects the physiology and behavior of coral reef fishes, and anemonefishes are a valuable model to estimate its extent. As previously mentioned in this chapter, anemonefish larvae rely on their sensory capabilities (i.e., smell, hearing, and sight) to discover an adequate reef in which to settle. However, when larvae are raised at high CO_2 levels (i.e., end of the century CO_2 levels, simulating OA), their capabilities to discriminate between chemical cues are disrupted. Larvae become more attracted to chemical cues they usually avoid and lose the ability to discriminate between different types of chemical cues (parents vs non-parents; predators vs non-predators) (Munday et al. 2009; Dixson et al. 2010; Munday et al. 2010; Jarrold et al. 2017). Surprisingly, young coral reef fish can even become attracted to their predators' smell and reduce their antipredatory response when exposed to danger, showing a riskier behavior that could lead to death (Munday et al. 2010; Ferrari et al. 2011). Preference for specific acoustic environments is also thought to be altered in high CO_2 conditions, with larvae becoming deterred by the soundscape of settling habitats but attracted to the soundscapes of unfamiliar or dangerous environments (Simpson et al. 2011; Rossi et al. 2015, 2018). It is still unclear whether sound reception or sound processing by the brain could cause this phenomenon (Holmberg et al. 2019). Studies show that these alterations in sensory functions and behavior might be caused by impaired $GABA_A$ receptors function (Nilsson et al. 2012; Chivers et al. 2014; see Schunter et al. 2019 for more details). Importantly, it must be pointed out that some of the previously cited results are currently under debate (Clark et al. 2020; Clements et al. 2022).

Finally, coral reef fishes have to face an increased SST concomitant with OA. Even though the effects of high temperatures on coral reef fish have seldom been assessed, preliminary results seem to indicate an altered escape response of larvae exposed to a predation simulation, questioning their potential survival in the wild (Warren et al. 2017). Moreover, *in situ* experiments show that sea warming events cause stress in anemonefish (i.e., elevated cortisol circulating levels) (Mills et al. 2015; Beldade et al. 2017). At the genetic and physiological levels, it seems clear that a rise in temperatures creates stress in fish, with the expression of heat shock protein genes drastically increasing, which is associated with an increased CRH gene expression in the brain and increased cortisol circulating concentration (Currie et al. 2000; Madeira et al. 2017; Liu et al. 2018; Uchimura et al. 2019; Goikoetxea et al. 2021). As previously suggested in this chapter, the hypothalamic factor CRH may control both the HPI and HPT axes, themselves regulating the onset and continuity of metamorphosis. Consequently, one wonders whether larvae evolving at high temperatures would experience a precocious metamorphosis or not. Nonetheless, the combined effects of OA and increased SST on coral fish larvae have rarely been investigated to date and surely deserve more attention.

11.7 FUTURE DIRECTIONS

Throughout this chapter, we reviewed the current knowledge about the neuroendocrine control of metamorphosis and protandrous sex change in anemonefish and highlighted how stress could influence these processes. The use of anemonefish as model species is very recent and many questions regarding the biology and life history remain unanswered to date. Indeed, there is a need for basic characterization of the metamorphosis process in its globality, from the perception of environmental cues to the hormones secreted downstream the neuroendocrine cascades, leading to the physiological, morphological, and phenotypical changes transforming a larva into a juvenile. In regards to the stunning process of male-to-female sex change observed in multiple species of the anemonefish subfamily, further research using these remarkable fish as study models will shed light on the underlying mechanisms governing this transformation. Future studies may be able to finally discern the potential role of stress and epigenetic pathways involved in this process. The full characterization of the processes of metamorphosis and protandrous sex change will help us understand how anemonefish communities will be able to respond to future environmental challenges during key life-history steps.

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