Spawning induction for Latin American fishes

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Abstract

Aquaculture offers solutions to meet the growing global demand for fish, and reports from the UN-FAO indicate that aquaculture production in Latin America (LA) has grown at rates above the world average in recent years. One of the major constraints in the diversification of LA aquaculture is the control of reproduction in several popular native fish species for which difficulties in captive propagation have not yet been sufficiently overcome. This article reviews the use of hormone treatments to promote reproduction in females of these native fish species. LA has played a key role in the history of development of hormone administration, including the first hormonally induced spawning. That contribution is included in a historical overview of the discovery of the major hormones used in fish culture. The review provides a summary of difficulties to propagate females of various native fishes and the effects of administering hormones to enhance reproduction. Induced spawning of certain freshwater species was mainly achieved with pituitary extracts or human chorionic gonadotropin (hCG), although gonadotropin-releasing hormone analogues (GnRHa) treatments are being researched, and successful studies suggest that low doses may be more effective. Research on new and emerging aquaculture species has applied both gonadotropins (Gths) and GnRHa-based treatments, and GnRHa treatments have shown potential for marine species. However, native marine species new to aquaculture have also been conditioned to spawn spontaneously without hormones. Lastly, we proposed future lines of research to examine reproductive strategies and GnRHa-based hormone treatments to improve reproductive control for economically important fish species of LA.

Keywords: Fish, Latin American, Hormone, Pituitary extract, GnRH, reproduction.

1. General introduction

The rapid growth of the world's population presents significant challenges to provide large quantities of high-quality food for the 9.7 billion people expected to inhabit the world in 2050[1]. Fish consumption is expected to rise globally, with a large increase of 33% predicted for the Latin America (LA) region by 2030[2]. Capture fisheries have stagnated, especially in recent decades (1990-2020), a period when aquaculture has been the fastest-growing sector of food production[2,3]. Therefore, fish farming is expected to provide a significant proportion of the protein needed to feed the growing population.

Aquaculture appears to be the only solution to meet the increasing demand for fisheries products and to counteract the decline of wild fisheries. The Food and Agriculture Organization of the United Nations (FAO) reports that aquaculture production in the LA region has increased in recent years at rates above the world average[2]. However, this growth in LA aquaculture of fish production was mainly non-native fish species such as Atlantic salmon (*Salmo salar*), Nile tilapia (*Oreochromis niloticus*) and rainbow trout (*Oncorhynchus mykiss*)[2]. The production was characterised by both marine and freshwater species, with a dominance of Chilean salmon farming, which makes this country the largest fish producer (1,079,626 t in 2020) in LA[4]. The Chilean production was almost entirely non-native salmonids that represent 99% of the LA cultured fish production from the marine environment. Continental LA fish farming of freshwater species was dominated by the exotic species Nile tilapia, rainbow trout and common carp (*Cyprinus carpio*)[5]. Currently, Nile tilapia culture is the flagship for cultured fish production in many countries of LA, including Brazil, Colombia, Mexico and Honduras. In 2020, Brazil was the principal producer of freshwater fishes in the Americas, with 629,000 t[4], being the eighth largest producer in the world. The Brazilian Fish Farming Association (Peixe BR) published the production of 841,000 t in 2021, with a growth of 45% (means of 5.6%/year) over the last eight years[6]. Elsewhere in Latin America, cultured finfish production from the freshwater environment has shown sustained increases, and in 2020, Colombia produced 174,067 t, Mexico 82,975 t, Peru 60,988 t and Honduras 38,700 t[2]. The proportions amongst species groups of this LA production from the freshwater environment were, 61.8% corresponded to tilapias (601,498 t), 9.9% to rainbow trout (96,553 t) and 25.2% to native species (245,366 t)[2]. Among the native freshwater species produced in LA, the group of medium-to-large

characiforms, called “round fishes” because of their shape, are highlighted, since they are the third-largest group of cultured freshwater fishes in the region after tilapias (1st) and rainbow trout (2nd). Moreover, these species are also important for aquaculture production in Colombia, Perú, Bolivia, and Venezuela[2]. Nonetheless, according to the most recent report of the IBGE (Brazilian Institute of Geography and Statistics), the national aquaculture production of some native fish species decreased slightly between the years 2017 and 2021, with reduction of 10.2%. In contrast to exotic species, whose production increased 25.6% in this period[7]. The reasons for the contrasting production dynamics of these species are complex and multifactorial[6,8]. Both social (preference for animal protein) and economic (high price of fish meat) aspects influence markets and aquaculture production. The annual per-capita consumption of fish in LA was relatively low at 10.5 kg.year^{-1}, in 2021 compared to a world mean consumption of 20.3 kg.year^{-1} [2,9] and there is no standardized technological packages in LA to ensure stable production of native finfish. In particular, the unstable and uncertain supply of fish fry[8] represents a bottleneck for increasing production and reducing production costs of round fishes and other native species in LA.

Unlike fishes such as the Nile tilapia and several salmonid species, most native LA species, like the round fishes mentioned above, present difficulties in artificial propagation under farming conditions (see **Section 6** and **Table 1** for specific information) and require hormonal treatments to induce spawning. Therefore, one of the major bottlenecks in the diversification of LA aquaculture is the control of fish reproduction[10], which limits seed production and consequently the aquaculture production of native species. The administration of hormones has proven to be fundamental in aquaculture for new aquaculture species where reproduction is uncontrolled[11]. The use of hormones to improve fish reproduction is widespread worldwide and has become a reliable routine in many regions and fish species.

However, these practices and hormone treatments, require meticulous care and a comprehensive knowledge of the reproductive strategies of the respective species. Consequently, the study of the reproductive characteristics of each particular species is essential for the proper selection of treatments to be used in the management of captive propagation. It is well known that fishes exhibit a variety of reproductive strategies[12], and knowledge of these differences is critical to diversifying fish culture. Species diversification represents a key strategy for sustainable aquaculture development[13]. For

several years, diversification of European fish culture has been considered a political, social and economic priority of European Union (EU) members. However, major efforts are still required in LA and the Caribbean to diversify fish culture. Several emerging fish species still need to be domesticated, and in this sense, networking through projects such as the CYTED (www.cyted.org) network named LARVAplus (larvaplus.org) that promotes the exchange of knowledge and experience to benefit the development of the LA aquaculture industry in order to meet to the continually rising demand for seafood in these countries.

The objective of this review is to identify the difficulties faced in artificial propagation of emerging native LA aquaculture species, describe the use of hormone treatments to overcome these difficulties and how improvements could be made to provide solutions that increase the supply of high-quality eggs and larvae for these species that are candidates for diversification of the LA aquaculture sector.

2. Latin American pioneers in the use of hormones to induce fish reproduction

Latin America played a key role in the history of hormone administration to control fish reproduction. A brief historical review of the discovery of the major hormones used in fish reproduction shows that the first-ever studies initiated in LA in the 1930s[14,15,16], many decades before the large increase in fish culture production accomplished from 1990 through to 2020 (**Figure 1).** These early LA studies gave the bases and focus for the development of procedures to spawn fishes that, with further refinement and in combination with other technological advances, contributed to the massive expansion of fish culture during the last 40 years.

Insert Figure 1

The first successful experiments to hormonally induce fish to spawn eggs that hatched to produce larvae were made in 1930 in Argentina by intraperitoneal injection with pituitary extracts (PE) to a viviparous fish species, *Cnesterodon decemmaculatus*[14]. Only a few years later, further studies were conducted in Brazil to successfully induce spawning in a catfish, *Prochilodus argenteus*, by applying a similar PE treatment[15,16] (**Figure 2**). During the following decades, these pioneering studies stimulated work around the world that both improved the use of PEs and examined what

158 constituents in the PEs induced ovulation. The use of PEs was developed into the
159 hypophysation treatments that are still used today for important aquaculture species
160 such as the carps and catfishes[11,17,18].

161 In parallel to work on hypophysation, research also focused on the
162 gonadotropins as the hormone with in the PEs that activated spawning. Experiments
163 with mammalian hormones led to the development of a method to induce channel
164 catfish (*Ictalurus punctatus*) to spawn with human chorionic gonadotropin (hCG)[19] and
165 introduced an exogenous hormone, hCG, that has been and is still used for the
166 reproductive control of many different species. Research also examined the purification
167 of fish Gths and, despite of complications of species-specificity, important advances
168 were made. For example, the induction of reproduction with purified gonadotropins
169 was performed in cyprinids in Israel[20].

170 The first report of the use of prostaglandins in fish ovulation dates from 1975,
171 when ovulation was induced in goldfish (*Carassius auratus*)[21]. During the same period,
172 the role of gonadotropin-releasing hormone analogues (GnRHa) in fish was discovered
173 in China by a collaboration team using hormones in fish culture[22,23]. In the 1980s,
174 hormonally induced spawning with GnRHa was successfully achieved in LA round
175 fishes, such as *P. mesopotamicus*[24] and *C. macropomum*[25]. Since 2004, the kisspeptin
176 system has been associated with reproduction in fishes[26] and five years later, the effect
177 of kisspeptin peptides on stimulating luteinizing hormone secretion was demonstrated
178 by injection of exogenous kisspeptins into adult female goldfish[27].

179 In conclusion, hormonal manipulation of spawning began in LA during the early
180 1930s with the administration of PEs, and advances with other hormonal preparations
181 were not made until the second half of the 20th century, when aquaculture and related
182 research on reproductive control of fishes increased rapidly worldwide to establish three
183 principal hormone induction treatments based on PE, hCG and GnRHa that have made

an important contribution to the massive expansion in fish culture from the 1990s to the 2020s.

Insert Figure 2

3. Brain - pituitary – gonadal axis: an overview

3.1. Brain and pituitary

The brain-pituitary-gonadal (BPG) axis is considered the main regulator of reproduction in vertebrates, and the pituitary gland (hypophysis) is considered a key link between the nervous and the endocrine systems (**Figure 3**). The pituitary gland has two lobes, the neurohypophysis (NH) and the adenohypophysis (ADH). In teleost fishes, the NH consists of an infundibular stem suspended from the ventral region of the hypothalamus and whose distal part interdigitates, like tree branches, into the three regions of the ADH: the anterior most part, called the rostral *pars distalis* (RPD), the middle part called the proximal *pars distalis* (PPD), and the caudal part, the pars intermedia (PI)[29,30]. Thus, the NH represents the neurohemal part and the ADH the glandular part, in which different cell types occupy specific positions. In contrast to tetrapods, two types of gonadotrophs (one producing luteinizing hormone, Lh and another one producing follicle-stimulating hormone, Fsh) can be distinguished in the middle part of the PPD in teleosts[31], although recent work on zebrafish (*Danio rerio*) has demonstrated the existence of some bi- and multi-hormonal cells[32]. In teleosts, the pituitary gland is directly innervated by neurons located mainly in the preoptic and hypothalamic regions.

The BPG axis regulates reproduction in vertebrates through a complex network of various neuropeptides, neurotransmitters, and pituitary hormones. Information, both external (temperature, photoperiod, social interactions, food availability, etc.) and internal (nutritional status, metabolic state, stress, etc.), is integrated within the brain and transduced into neural and neuroendocrine signals that control the reproduction[29]. The number of neuroendocrine factors known to be involved in the control of reproduction in fishes has gradually increased in recent decades[33-36]. Among them, gonadotropin-releasing hormones (GnRH) are the main stimulators of gonadotropins (Fsh and Lh) synthesis and release, and dopamine (DA) is considered the inhibitory factor although in some teleost species such as Atlantic croaker, *Micropogonoias*

undulates and sea bream *Sparus auratus* this physiological role of DA was not found [37, 59].

Fishes represent the largest group of vertebrates, with more than 35,000 species[38]. During evolution, whole-genome duplications occurred in teleost fishes[39], notably the three rounds (3Rs), estimated to have occurred 320-400 million years ago in an ancient teleost fish[40]. In addition, salmonid species have undergone a fourth tetraploidization (4R) episode[41]. In several fish species, up to three GnRH genes (*gnrh*) from the first two rounds (1R and 2R) have been observed and five GnRH receptor genes (*gnrhr*) from the 3R have been characterized[42,43]. From the 4R, six gnrhr paralogs have been distinguished in the Atlantic salmon genome[44]. GnRH is a decapeptide found in vertebrates, and a considerable number of variants have been identified. Many species express two or three GnRH variants encoded by different genes[45,46]. These GnRH variants are currently classified into three different types based on their amino acid sequence, neuroanatomical localization, embryological origin, and synteny: GnRH1, GnRH2, and GnRH3[45,47-50]. In vertebrates, GnRH1 is the most variable GnRH type; it is the hypophysiotropic variant and the main regulator of gonadotrophs. The most conserved type, GnRH2, also known as the "midbrain variant", has been described in all vertebrate groups[51] and plays a key role in reproductive behaviour (reviewed in Parhar et al.[52]). GnRH3 was thought to be unique to teleost fish species, although some reports suggest a more primitive origin[46,49]. This latter variant is considered a neuromodulator of olfactory and visual information related to reproduction[53,54], which also serves hypophysiotropic functions, especially in most cyprinids and salmonids[37,45]. The action of GnRHs on target cells is mediated by binding to specific membrane-bound GnRH receptors (Gnrhrs), localized in gonadotrophs, but also in other pituitary cells[55,56], brain, gonads, and other peripheral tissues, and which exert diverse physiological effects on a variety of targets[33,57].

Dopamine is a neurotransmitter widely distributed in the vertebrate central nervous system (CNS)[58]. In teleost fishes, this catecholamine is the major inhibitory regulator of GnRH secretion[59]. In goldfish, DA has been shown to inhibit both basal and GnRH-stimulated Lh release by acting directly on gonadotrophs, and blocking

synthesis of the peptide or its release from pituitary GnRH nerve terminals (reviewed in Dufour et al.[39]).

3.2. Ovary and oogenesis

Fishes have different reproductive strategies. Most fishes are gonochoristic, but many species change sex, and these decisions on sexual differentiation and timing of maturation are programmed at different sizes or ages to provide an enormous variety of strategies[60, 61]. In females, the ovary consists of germline-forming cells that produce haploid reproductive cells. The essential unit is the follicle, which consists of two layers of cells and the oocyte. The outer layer, named theca, is a connective tissue separated from the inner or granular layer by a basal membrane. The oocyte develops in the basal membrane surrounded by the follicular cells. The primordial germ cells (PGCs) develop outside the gonad and then migrate into the gonad[62]. After a series of mitotic divisions, the diploid oogonia undergo their first meiotic division and differentiate into primary oocytes and develop through to the start of oocyte maturation[63]. Finally, maturation of the oocyte (late vitellogenesis, migration of the germinal vesicle, hydration and ovulation) takes place[63-65].

The BPG axis controls gametogenesis by releasing Fsh and Lh into the blood[31,37,63,66] (**Figure 3**). The principal target of these hormones in females is the follicular cells of the ovaries, where the gonadotropin receptors are expressed[67,68]. By activating these receptors, gonadotropins control the ovarian synthesis of specific sex steroids, which play a crucial role in stimulating oogenesis[31,63]. Within oogenesis, two phases are considered crucial for female reproduction: vitellogenesis and maturation of the oocyte[63]. The endocrine pathways controlling these two phases are distinct and largely mediated by the different functions activated by each gonadotropin receptor[69]. In this sense, Fsh promotes estradiol synthesis and stimulation of vitellogenesis, while Lh triggers a switch in steroidogenesis from estradiol to a maturation-inducing hormone steroid (MIS), initiating oocyte maturation and ovulation[63,64]. An important aspect to consider in the activation of the different gonadotropin receptors is their promiscuous

ligand recognition, which seems to depend upon the species and the origin of the gonadotropins (homologous/heterologous)[70].

Insert Figure 3

4. Hormone treatments used in aquaculture

Hormones are considered as messengers responsible for chemical communication between different cell types, which express their identity and functionality through the displays of specific receptors[72]. These receptors are proteins specialized in molecular recognition that, after the binding of the hormone and the receptor, triggers a series of biochemical reactions within target cells and link those cells to specific biological responses[73]. The administration of exogenous hormones makes use of these pathways and has been shown to be effective in many captive fish species to stimulate gonadal development, promote reproductive behaviour and trigger spawning[74].

4.1. Aims of hormone treatments in aquaculture

There are two principal aims for the use of hormone treatments in cultured fish species. The first aim is sex reversal to produce monosex populations, by hormonally controlling the biological strategy, leading to production of sex differentiation in teleost species[75]. Monosex populations offer advantages for some species in aquaculture that have sexual dimorphisms of economic importance, such as growth rate, timing and age of maturation, body shape, and, in ornamental fish species, physical criteria such as colour pattern and fin shape. Alternatively, monosex populations avoid the problem found in some species of uncontrolled reproduction that is associated with reduced growth or poor product quality[76,77].

The second reason and focus of this review is to induce reproduction to obtain viable gametes in species that prove difficult to propagate under captive conditions[11]. For these species, the captive environment does not provide the correct cues for reproduction to progress through to spawning and this situation has been described as a reproductive dysfunction cause by the captive environment. Several captivity-induced reproductive dysfunctions have been described in females and these were classified into three types in relation to the progress of gametogenesis, oocyte maturation, ovulation

and spontaneous spawning[11,74,78-82]. Type 1 dysfunction is when oogenesis is arrested in the early stages before or as vitellogenesis initiates. Type 2 dysfunction occurs when oogenesis is arrested prior to or at the onset of oocyte maturation. Type 3 dysfunction occurs when oogenesis is complete with ovulation but spawning or the courtship are not completed to produce viable eggs and fertilise the ova. Despite of the recent success of studies focused on overcoming the Type 1 dysfunction[83,84], this type of dysfunction is not controlled in aquaculture, and species that exhibit Type 1 dysfunctions are either not cultured or reproduction is not controlled, and in those systems, aquaculture uses wild-caught juveniles, for example eels (*Anguilla anguilla* and *A. japonica*) and flathead grey mullet (*Mugil cephalus*). Little research has been conducted on the control of Type 1 dysfunction in LA. Type 3 dysfunction in species has not limited aquaculture. With the Type 3 dysfunction, the ova either remain in the abdominal cavity and become overripe, or are released by the female, but not fertilised by the male. This is usually a behavioural problem due to the absence of courtship or an environmental problem, for example, no spawning substrate[85]. This dysfunction is common in salmonids and is overcome simply by stripping and fertilising the ova using *in vitro* fertilisation techniques, as performed in LA aquaculture.

4.2. Common hormone treatments used in fish reproduction

The principal use of hormones to control fish reproduction in aquaculture is to overcome Type 2 reproductive dysfunction, which has been described as the most common reproductive dysfunction in fish culture[11]. This Type 2 dysfunction can be attributed to the captive environment not providing the cues to stimulate progress through oocyte maturation to spawning[11,85]. Therefore, the absence or inconsistency of oocyte maturation and ovulation is one of the most common reproductive dysfunctions in captive fishes, and the endocrine pathway that induces this stage in oogenesis deserves special attention in this review. The induction of this reproductive stage is mediated by the production of MIS (17a, 20b-dihydroxy-4-pregnen-3-one, 17a, 20b-DP, in most fish), which is triggered by the activation of the luteinizing hormone receptor / choriogonadotropin receptor (Lhcgr) on follicular cells by Lh[63,64]. Therefore, the hormone treatment should promote the increase in plasma levels of a hormone with high affinity for Lhcgr (naturally Lh) present in the follicle cells of the oocytes in the maturation process. The most common hormonal treatments applied in fish culture to

stimulate Lhcgr to advance reproduction and overcome Type 2 reproductive dysfunction involve the use of two classes of hormones that differ mainly by the level within the BPG axis (**Figure 3**) where their direct effect is exerted: (1) Hormones (as well as pituitary extracts) that stimulate the gonadotropin receptors, specifically the Lhcgr in the follicular cells of oocytes in the ovary to produce MIS or (2) hormones, the gonadotropin-releasing hormone agonists (GnRHa) that stimulate Lh release from pituitary gland (see **Section 3**. Brain - pituitary – gonadal axis: an overview).

4.2.1. Preparations that contain gonadotropins

The first important group of hormones commonly used to induce spawning, which directly stimulate the Lhcgr, are preparations that contain gonadotropins (Gths) that include pituitary homogenates, pituitary extracts, and mammalian Gths. These preparations contain Lh and other pituitary hormones, purified piscine Lh, or, in the case of mammalian Gths, purified human chorionic gonadotropin (hCG)[11,74]. The principal advantage of these hormone preparations is the direct action at the level of the gonad. The pituitary homogenates were the first preparations used by the aquaculture industry to promote reproduction, *i.e.*, oocyte maturation and spawning[14-16,86]. Currently, these extracts have been improved (carp pituitary extract -CPE and purified salmon Gth) and are more effective than the pituitary homogenates used in the past. Additionally, modern extracts and purified Gths are more convenient with quantified Gth levels and can be purchased (chemical and veterinary supplies) rather than made from sacrificed carp. However, some disadvantages remain, such as the risk of pathogen transmission, immune response to the large exogenous proteins and species-dependent specificity due to the different primary structures of the respective Gth forms[11,87]. These extracts are usually administered in two different doses, a smaller priming initial dose (10–20 % of the total dose) and a larger resolving dose administered 12–24 hours later[88]. The total dose necessary to induce spawning with pituitary extracts is expected to depend on their relative Gths concentration (mainly Lh). For instance, for the case of the widely used CPE that contains other pituitary hormones additionally to Gths, the total dose usually applied ranges between 5 and 6 mg.kg-1 of total female weight (see **Table 2**). Similarly, hCG has been used for hormonal induction to act directly on Lhcgr in the gonads of fish species. In this case, a single dose was often administered because of the longer residence time of hCG in the bloodstream compared to Gth extracts[89].

4.2.2. Gonadotropin-releasing hormones

The second important group used as hormone inducers are the gonadotropin-releasing hormone agonists (GnRHa) that stimulate the gonadotrophs in the pituitary to release Lh. The use of GnRHa to induce reproduction in fishes has achieved some progress compared to Gth preparations because it is a synthetic source that avoids disease transmission, is less species-dependent, and acts higher in the BPG axis to facilitate the release of Gths and other pituitary hormones necessary for reproduction from the pituitary gland[74]. However, a drawback is the short half-life when injected into an organism. The amino acid sequence of GnRHa affects its biological half-life, as the peptide sequence is broken down by enzymes between amino acid positions 5-6 and 9-10, substitutions of amino acids at these positions have been shown to increase the half-life and biological activity of the different GnRHa types[90]. For this reason, it is important to select a potent form of GnRHa and the most commonly used forms of GnRHa in aquaculture have amino acid substitutions at positions 6 and 9[18,90,91]. Also, in consideration of the short half-life, the reproductive strategy of each species must be considered to determine the most appropriate protocol for hormone administration, a single injection, multiple injections or controlled-release implants[72,74]. A single injection of GnRHa has produced a brief increase in circulating Lh that lasted 24 to 48 h, and has been shown to be sufficient to trigger spawning in species such as longfin yellowtail (*Seriola rivoliana*)[92]. However, some species require a double injection to induce Lh secretion and thus complete oocyte maturation and ovulation. On the other hand, in multiple-spawning species, GnRHa implants allow females to maintain stimulated the Lh releasing pathway for an extended period of time to induce serial spawning over a period of days or weeks[11,90]. These preparations of GnRHa can be obtained in powered form from numerous chemical suppliers (e.g. Sigma). Implants have a more limited availability, regional suppliers may be unreliable as markets are small to sustain a business, although an international supplier exits (https://syndel.com/product/ovaplant/) and implants can be made following published protocols[78]. Another consideration is that in some species, particularly some freshwater species, the GnRH signalling pathway to stimulate Lh release from the pituitary gland is inhibited by DA influence. This dual control of reproduction by DA and GnRH has led to the development of a method for spawning induction based on combined

treatments with a DA antagonist and GnRHa[39]. However, it is important to note that the inhibitory effects of DA have not been found in species such as Atlantic croaker (*Micropogonoias undulatus*)[93], striped bass (*Morone saxatilis*)[94], gilthead sea bream (*Sparus aurata*)[11] or European seabass (*Dicentrarchus labrax*)[95].

5. Perspective of new hormones to improve fish reproductive induction in aquaculture

Several studies have shown that other hormones not commonly used in aquaculture can successfully induce maturation and spawning in fishes. Among these hormones, recombinants Gths, kisspeptins and secretoneurins deserve special attention.

5.1. Recombinant Gths

Recombinant Gths (rGths) are a form of species-specific Gths produced from a species DNA sequence in cellular expression systems using technologies that have been applied to fish in the 21st century[96]. The Gths are large proteins that have a complex structure with two subunits, an alpha and a beta subunit, that are folded and glycosylated. The rGths have been shown to have higher bioactivity when single-chain structures with an hCG-based linker connect the two subunits produced in mammalian expression systems such as Chinese hamster ovary (CHO) cells that provide the required glycosylation[96]. The rGths have been successfully used in experiments as a single injection of recombinant Lh (rLh) to induce ovulation in fish with a Type-2 dysfunction nearing oocyte maturation[97,98], and as multiple weekly injections of both recombinant Fsh (rFsh) and rLh, to trigger maturation from the early stages of oogenesis to produce viable ova[83,84] and to trigger the spawning of eggs that ultimately produced millions of larvae (Type 1 dysfunction)[84]. These rGths offer excellent potential for reproductive control in aquaculture to provide solutions to both Type-1 and Type-2 reproductive dysfunctions. Until now, it has been challenging to provide solutions for Type-1 dysfunctions, as the solution requires long treatments that induce the entire oogenesis process from pre-vitellogenesis to spawning of good-quality eggs. These recent successful long-term rGth treatments are in the early stages of evaluation by the aquaculture sector.

5.2. Kisspeptin

In recent years, knowledge of the number of neuropeptides involved in the control of vertebrate reproduction has significantly increased[34]. One of the best-studied neuropeptides is kisspeptin. Particularly this peptide, compared with GnRH, can act at a higher, brain level of the BPG axis, where the administration of this hormone is expected to produce a more natural endocrine signalling response to stimulate downstream reproductive functions[10,99].

Kisspeptins are a group of peptides critical for puberty and maintenance of normal reproductive function. The use of this group of peptides as an exogenous hormone treatment is relatively recent and was initially employed to study the onset of puberty in various fish species. The literature on the use of this treatment to induce reproduction in fishes is scarce, although it has been reported that administration of kiss1 or kiss2 peptides can stimulate gonadotropin synthesis and release, depending on the gonadal stage and also on the method of administration[99-107].

This use as an exogenous hormone treatment is based on the involvement of kisspeptin in the control of reproduction that has been demonstrated in vertebrates, including numerous fish species[10,102]. In the case of kisspeptin, only one gene (kiss1) and one receptor (kiss1r) have been observed in mammals, whereas most fish species have two kisspeptin systems: kiss1 and kiss2 genes (and Kiss1 and Kiss2 peptides) and two cognate receptors, kiss2r and kiss3r[10,99,108]. In contrast to mammals, recent studies suggest that Kiss2 directly stimulates the gonadotrophs by neural signalling in teleost fishes[102]. Neurons responsible for kisspeptin expression are direct targets for the positive and negative feedback effects of steroids. This link between steroids and kisspeptin neurons regulates mRNA expression in different brain regions that play an important role in triggering puberty. Comparing the two kiss genes, the kiss1 peptide is also known as the Y-Y form due to its core amino acid sequence (aa) kiss1-10 (YNLNSFGLRY), whereas the kiss2-10 peptide in kiss2 has an F-F form (FNYNPFGLRF)[109]. These coexisting genes show low amino acid sequence identity; this is the case in medaka (*Oryzias latipes*) at 20% and in zebrafish at 25%[109]. This is one of the reasons that kiss2 has stronger gonadotropin-releasing activity than kiss1, suggesting a more dominant role in controlling the BPG axis in fish[100]. The sequence of kiss-10 was found to be highly conserved among all vertebrate species. This is the reason for the synthesis of this decapeptide for use as an exogenous hormone treatment (see review for more details, Wang et al.[99]). However, it has been demonstrated that the

kiss1 precursor contains a conserved sequence that is five amino acids contiguous to the decapeptide, showing that fish kiss1 genes produce a mature peptide of 15 amino acids (QDMSSYNFNSFGLRY-NH2)[110]. At the same time, the teleost kiss2 gene produces a peptide with 12 amino acids (SNFNFNPFGLRF-NH2) because two conserved basic amino acids are added to the decapeptide[110]. These two peptides exhibited high potency for the activation of kissr1 and kissr2, indicating that they are more potent reactors for kiss receptors than the corresponding to kiss1/2-10 peptides[111]. These results suggest that these peptides may provide better outcomes than kiss1/2-10 in exogenous hormone treatment[99].

5.3. Secretoneurin A

Secretoneurin a (SNa) and SNb are 31-34 amino-acid peptides derived from the proteolytic processing of the large secretogranin 2a (Scg2a) and Scg2b precursor proteins, respectively. The consensus central core sequence YTPQ-X-L-X8-EL defines the SN peptide family. As examples, the predicted sequences for SNa and SNb in the Amazonian toadfish *Thalassophryne amazonica* are TNENVEEKYTPQNLATLQSVFKELGKLTSAKATH and ATEDLDEQYTPQSLANLRSIFEELGRMPPAGQ. These are ~74% and ~80% identical to the corresponding zebrafish peptides, illustrating that the C-terminus of these peptides is variable.

In goldfish, SNa stimulates Lh release *in vivo* and directly and independently of GnRH *in vitro*[71]. SNa-producing magnocellular isotocin (IST; fish oxytocin) neurons project heavily into the goldfish posterior pituitary[112]. Other secretograninergic neurons (IST+SNa or SNa-only neurons) directly innervate anterior pituitary and terminate near the gonadotrophs. Intense SNa immunoreactivity in lactotrophs of the rostral pars distalis[112] indicates that SN of both neuroendocrine and paracrine origin controls Lh-producing gonadotrophs in the proximal pars distalis. In the South American weakly electric fish *Brachyhypopomus gauderio*, electric organ discharges that signal arousal, as well as dominant or subordinate status may be modulated by injections of SNa[113]. Critically, frameshift mutation of the *scg2a* and *scg2b* genes in the zebrafish *Danio rerio* reduces sexual activity, and leads to suboptimal ovulation and egg laying, fertility, and embryonic survival[114]. For example, spawning success in double-mutant within-lines crosses is ~6%. That is, reduced expression of *gnrh3* in the hypothalamus and

glycoprotein hormone *lhb* and *cga* subunits in the pituitary provides evidence of impaired hypothalamic-pituitary function in zebrafish carrying single and double *scg2a* and *scg2b* mutations. Injection of SNa, but not SNb, partially restored spawning in the *scg2a/scg2b* double mutants. It should be considered that SNa not only has direct effects on gonadotrophs, but also increases GnRH3 expression in both grouper (*Epinephelus coioides*)[115] and zebrafish brain[114], supporting the hypothesis that it is a novel reproductive hormone[36,71,114]. Thus far, the only example for which neuropeptide gene mutations significantly impair reproduction are the *scg2* mutants in zebrafish[36].

Despite the major importance of GnRH, DA, kisspeptin, and Scg2 in fish reproduction, other neuropeptides like neuropeptide Y (NPY), gonadotropin inhibitory hormone (GnIH), and tachykinin 3 (tac3) also appear to play roles in fish reproduction[33,34,116]. The rapid development of new methods to study genomics and genes, such as next-generation sequencing (NGS), RNA-seq, gene-editing methods and assembled genomes, are effective and useful tools to study and detect numerous genes that are critical for monitoring the effects of hormone treatments and reproduction.

6. Hormones used in some Latin American fish reproduction

The use of hormones in LA has followed similar trends and developments as described previously for world aquaculture. Most species in mass aquaculture production in LA do not require that maturation is artificially induced. However, a number of native species that are established in freshwater aquaculture or emerging in freshwater or marine aquaculture require hormone treatments to overcome the captivity-induced Type-2 reproductive dysfunction, which as in other parts of the world[11] is the most common reproductive disfunction in LA aquaculture (**Table 1**). Therefore, the most common hormones used in LA aquaculture are similar to previously described treatments that are based on Gth (see **section 4.2.1.** Preparations that contain gonadotropins) or GnRHa (see **section 4.2.2.** Gonadotropin-releasing hormones), which ultimately stimulate the Lhcgr on follicular cells[63,64] to induce oocyte maturation, ovulation and spawning (**Tables 2** and **3**).

In LA aquaculture, a wide range of hormone preparations have been used to overcome Type-2 dysfunctions, which include both preparations containing Gths, which appear to be more available, and also GnRH preparations. Standardized commercial preparations of powdered or whole carp pituitary have been used for the

artificial induction of some native fish species such as the round fishes (Genus Colossoma)[117,118]. The use of hCG, available in some pharmacies for use in human medicine[119], has also been used to induce spawning of *C. macropomum*[120], *Pseudoplatystoma fasciatum*[121], and *Prochilodus* sp[122], among others. Since the discovery of the sequence of the mammalian peptide GnRH[123] and other similar peptides in fishes[124] such as -s-GnRH (salmon), various synthetic analogues with higher biological activity have been designed by replacing the amino acids in positions 5-6 and 9-10[90], and these analogues; have been used to induce spawning of *C. macropomum*[125], *P. mesopotamicus*[24] and *Rhamda quelen*[126]. However, GnRHa has been more commonly used in combination with dopamine antagonists that enhance the stimulatory effect of GnRH analogues, such as the preparations Ovaprim® (20 $\mu g.ml^{-1}$ sGnRH + 10 $mgml^{-1}$ domperidome). Ovaprim® at 10 $\mu g.kg^{-1}$ body weight of s-GnRH, has been successfully used to stimulate spawning of *Pseudoplatystoma punctifer*[127] and *P. fasciatum*[128], *R. quelen*[129] and *Prochilodus lineatus*[130]. More recently, Ovopel® (an implant with mGnRHa + metoclopramide) has also been used to induce ovulation in some farmed species[131]. However due to local supply difficulties, the use of pituitary glands or extracts from carp or other native species is still commonly used by fish farmers.

The largest group of species in terms of aquaculture production that requires hormone-induced spawning is by far the freshwater "round" and migratory species principally cultured in Brazil, as well as Perú, Colombia, Venezuela and Bolivia. Hormone treatments appear to be a viable solution to induce ovulation / spawning in captivity and produce the eggs, larvae, and fry necessary for aquaculture. However, reliable hormone induction treatments have not been developed for these LA species, despite of studies that identify the requirement of hormone-induced spawning and test hormone treatments that include hypophysation, Gth and GnRHa based treatments for a range of species (see **Tables 1, 2** and **3**), such as such as *P. mesopotamicus*[82], *Leporinus friderici*[80] and *Astyanax altiparanae*[132].

Migratory species such as the *C. macropomun* are usually induced with the same standardized CPE protocol of 5.5 $mg.kg^{-1}$ body weight, in two applications (10 % and 90 %, with a 12-h interval between applications) of fractionated doses of pituitary extracts, which have been used for many years[133-138]. One of the best-studied species is *P. mesopotamicus* has also been induced with hypophysation, but together with

prostaglandin administration to give excellent results[79,82,139] and increased the ovulation rate. However, while in many marine and freshwater species of the northern hemisphere, such as common carp, hypophysation has been replaced by synthetic products, such as the use of GnRH or the Linpe method combining GnRH with a dopamine inhibitor (domperidone, metoclopramide or pimozide)[11,18,23], there have been inconsistent results and few successful studies with the use of synthetic products in native LA fish.

In this context, the use of synthetic GnRHa has shown good ovulation and fertility rates in *C. macropomum*[131,140,141]. A single administration of 10 μg.kg^{-1} of sGnRHa resulted in 100% spawning rate but low fry survival[141]. Similarly, Muniz et al. [140], demonstrated that mGnRH induced 100% of the females with 40 to 80 μg.kg^{-1} in one or two injections. Recently, the application of Ovopel (mGnRHa + metoclopramide (MET), 4 μg.kg^{-1} and 7.2 μg.kg^{-1} respectively) in the same species resulted in 100% spawning rate, with satisfactory fertilization (71.4%) and hatching (56.8%) rates[131]. However, other studies using synthetic GnRHa reported that ovulation was followed by high embryonic mortality as one of the main problems described for *C. macropomum*[141], *L. macrocephalus*[142], and *L. friderici*[80]. The approach used in these studies has been to explore one or two doses of GnRHa often in combination with a dopamine antagonist, providing a successful treatment when it works, but leaves unanswered questions for example when hatching and larval survival were low. Comparing results amongst studies shows that using doses higher than 8 μg.kg^{-1} mGnRHa + 4 mg.kg^{-1} MET (Acuña and Rangel, 2009; Souza et al., 2018; 2020) had lower hatch rates, while studies using lower doses, 4 μg.kg^{-1} mGnRHa + 2 mg.kg^{-1} MET[131] and 2 μg.kg^{-1} GnRHa[125] in *C. macropomum*, had higher hatching rates. This may indicate that lower GnRHa doses provide improved hormone treatments for these species. The recent publication by Konzen- Freitas et al.[125] reported that buserelin acetate (synthetic GnRH) at a dose of 0.5 ml.kg^{-1} body weight, equivalent to 2 μg.kg^{-1} without dopamine antagonist promoted spawning in 40% of injected females and produced results similar to the use of the standardised CPE treatment.

The use of hormones for LA marine species has been similar to that in freshwater species. In terms of aquaculture production, marine aquaculture in LA is far behind that in freshwater, with low production of a few species new to aquaculture. Several of these marine species farmed in LA have been reproduced naturally in

captivity under favourable environmental and husbandry conditions. The spotted rose snapper (*Lutjanus guttatus*) has the highest aquaculture production of an LA marine species and was conditioned to obtain successful spontaneous spawning[143-145]. However, studies on *L. guttatus* initiated with recently caught fish that probably due to the stress of capture exhibited reproductive dysfunctions and did not spawn in captivity[91,146]. Early studies used preparations with Gth, CPE and hCG. Three mature females of *L. guttatus* (1.7-2 kg) were injected with 800 IU CPE kg^{-1} body weight, with egg production of 115,500 and 125,500, respectively[147]. Using hCG (Chorulon®)[148], 9 females of *L. guttatus* (604.9 ± 98.8 g) injected 1600 IU hCG kg^{-1}, with the first dose containing 56% of the total dose and the second dose administered 24 hours later containing 44%. Forty-seven thousand eggs were spawned, with fertilization rate and floating egg diameters of 90% and 0.857 ± 0.044 mm, respectively. The oocytes were 0.425 ± 0.020 mm in diameter at the time of first injection and 0.516 ± 0.070 mm at the second. Similar or better results have been reported with GnRHa in *L. guttatus* (**Table 3**), which can be administered by injections, but considering the asynchronous ovarian development and daily spawning, delayed-release implants have been reported to be more effective in achieving oocyte maturation and spawning. Studies conducted in Mexico used GnRHa implants in a dose-response approach and demonstrated that a dose of 240-280 µg GnRHa.kg^{-1} applied to females with an oocyte diameter of 440-550 µm produced 98,569 ± 23,860 eggs kg^{-1} with 85.2 ± 4.7 % hatching success[91]. Similar studies have shown that the required dose of hormone in *L. guttatus* decreases with increasing initial mean oocyte diameter[149]. Females with oocyte diameters ≥ 475-500 µm required doses between 75-100 µg GnRHa kg^{-1}. These studies resulted in mean total relative fecundity of 80-278 x 10^3 eggs kg^{-1} of body weight and a fertilization success of 51-85%[149].

The seriola species, *Seriola lalandi* and *S. rivoliana* that are new aquaculture species in LA, have also been conditioned to spawn spontaneously after some initial studies using hormone induction[92,150,151]. Other species with aquaculture potential, but little or no aquaculture production, have been successfully spawned with GnRHa, include leopard grouper (*Mycteroperca rosacea*) and common snook (*Centropomus undecimalis*) (**Table 3**) and to a lesser extent using CPE. For example, between 2002 and 2007, recently caught (38) and wild captive (59) leopard grouper females were treated with either hCG (CHORULON®, Intervet International, Boxmeer, The

Netherlands) or GnRHa (des-Gly10, [DAla6]-GnRHa)[152]. The hCG treatment was administered in two intramuscular injections, the first injection at a dose of 1000 IU.kg^{-1}, followed 24 h later with a resolving dose of 500 IU.kg^{-1}. The GnRHa treatments were administered in two intraperitoneal injections at two doses of 15 (5 μg.kg^{-1} followed 3 h later by 10 μg.kg^{-1}) or 150 μg.kg^{-1} (50 μg.kg^{-1} followed 3h later by 100 μg.kg^{-1}). Both hormones and all treatments induced the spawning of large numbers of fertilized eggs when the initial mean oocyte diameter was 498–523 μm. However, the total fecundity (5×10^6 eggs), fecundity of viable eggs (2×10^6 eggs) and fertilization (40%) of hCG-induced captive female was significantly higher than those from GnRHa groups and hCG treated wild animals[152].

All the studies considered in this work indicate that the hormonal experiments were performed on females at the advanced stage of vitellogenesis or even at the oocyte maturation stage (Type-2 dysfunction), and all of these females should have expressed the Lhr on the follicular cells of the leading clutch of oocytes. As mentioned earlier, the induction of this reproductive stage is mediated by the production of MIS (17a, 20b-dihydroxy-4-pregnen-3-one, 17a, 20b-DP, in most fishes), which is produced by the activation of the Lhcgr on follicular cells by Lh[63,64]. In this sense, the efficacy of a hormone treatments to induce reproduction in female fish depends not only on promoting the increase in plasma levels of a hormone with high affinity for Lhcgr (naturally Lh), but also on the presence of this receptor on the follicle cells of the oocytes in maturation targeted for induction. Knowledge of the Lhcgr expression profile during oogenesis is critical for implementing successful protocols for spawning induction in fishes. Nonetheless, this information is available only for a relatively limited number of fish species, of which only two, the pejerrey, *Odontesthes bonariensis*[153] and the greater amberjack, *Seriola dumerili*[154], are among LA species. However, since the expression profile of Lhcgr during oogenesis shows a similar pattern in all fish species studied so far, it can be assumed, as a general rule that the expression of this receptor in the ovary increases during the middle of vitellogenesis and reaches its highest levels between late vitellogenesis and oocyte maturation. Although caution is needed as, in particular, the continental freshwater species have evolved along different pathways or environments compared to species on other continents, and so variations in mechanism of maturational control may exist. However, oocyte size has been shown to effectively indicate the ovarian maturation stage and has

been considered a reliable parameter for determining the proper timing to implement a hormonal treatment to induce spawning in, fishes[18,91], perhaps maturational stage was not the principal reason that some studies in this review failed to successfully induce the spawning of good-quality eggs. Therefore, the unsuccessful treatments included in this review may be due to a failure to promote a proper plasma level of Lh (or a similar Lhr agonist) which depends on GnRHa type, the use of a dopamine antagonist, the dose used and on available pituitary Lh to induce the Lhr stimulatory pathway and thus the event of ovulation. In this aspect, low doses of GnRHa alone may provide successful treatments for freshwater species and could be a focus for future research in combination with description of strategies and maturational development of the BPG axis, in particular hormone production and binding to receptors in these organs.

Insert Table 1

Insert Table 2

Insert Table 3

7. GENERAL DISCUSSION AND CONCLUSIONS

Latin American aquaculture is dominated by production of introduced fish species that do not require hormonal interventions to obtain gametes or spawn. However, the native species that are cultured exhibit the same difficulties for artificial propagation observed in other species around the world. As in other areas, the species most commonly treated with hormone treatments exhibit captivity-induced Type-2 reproductive dysfunctions, in which oocyte development is arrested just prior to oocyte maturation. The same hormone treatments used around the world to overcome this dysfunction have been also used in LA, with a dominance of Gth-based treatments that act directly on the LH receptor (agonists of the Lhcgr). As we mentioned in the introduction, LA was the first region in the world to use pituitary extracts to induce ovulation in fishes and the use of pituitary extracts remains an important treatment to induce ovulation and spawning in this region. In addition to pituitary extracts, hCG is commonly used to induce spawning in LA. However, as this review has shown, these Gth-based treatments do not give completely satisfactory results, spawning responses can be variable, and often the proportion of females that ovulate is lower than desired.

For these reasons, the review notes that research continues to try to improve these protocols and test other hormone treatments such as GnRHa-based treatments.

One reason for the importance and dominance of the use of these two hormone treatments (PE and hCG) is that the farmers have more experience with the use of these preparations and can obtain the hormone preparations relatively easily from human or veterinary suppliers. This is a circular situation; the farmers prefer these treatments due to their experience and so suppliers provide these treatments. New treatments, for example GnRHa treatments, although available are more difficult to obtain and protocols are not available to promote these alternative treatments. Combined to this, studies with Gth-based and GnRHa-based treats have to date not provided a conclusive improved protocol and so the situation perpetuates. Therefore, as acknowledged and promoted by this review research needs to focus both on the continued development of hormone treatments with careful dose-response studies on females at the point of initiating oocyte maturation and the description of native species reproductive biology.

A comprehensive knowledge of the native species reproductive biology, strategies and the environmental requirements that stimulate reproductive development is lacking. Many of these native species (*Piractus mesopotamicus*, and others) have complex strategies that involve migrations of tens or hundreds of kilometres upstream to the headwaters of the rivers and few studies explore the details of the events that precede spawning in a natural environment. The described reproductive dysfunctions are induced by the captive environment[11,83] and an improved knowledge of these strategies and reproductive biology would aid the implementation of the correct environment and meticulous care needed to ensure the progression of maturation through to the final stages[85]. That females reach an adequate stage of development for the application of hormone treatments is critical to obtain successful spawning. In addition, an in-depth knowledge of the spawning environment would also aid the implementation of an environment to obtain spontaneous spawning without the need of hormones.

Another particular aspect of reproductive biology that would facilitate the development of hormone treatments for these species is the description of reproductive endocrinology. Aquaculture of native species in LA is dominated by freshwater species

735 that appear to have different strategies and hormone kinetics in relation to the
736 application of exogenous hormone treatments that may be complicated by undescribed
737 dopamine action, which is more common in freshwater species. These aspects may
738 render GnRHa treatments based on successes in other non-LA species ineffective.
739 However, studies cited in this review have shown that GnRHa treatments have been
740 used with success in LA and have potential for freshwater species. These uncertainties
741 may contribute to the slow uptake, as studies have provided contradicting results.
742 However, contradicting results, including negative results must be built upon to develop
743 effective spawning protocols. Successful studies indicate that the protocol was close to
744 optimal and that further studies can adjust and perhaps improve the response. While
745 unsuccessful studies indicate the incorrect application of a hormone, at the wrong stage
746 of ovarian development, or at the wrong dose (excessive or insufficient), indicating that
747 the protocol should be modified to improve the spawning response. In addition,
748 although the use of GnRHa treatment was established in Europe and Asia decades ago,
749 the slow uptake of GnRHa treatments in LA freshwater fishes is not unusual, as these
750 treatments are relatively new; in Europe, there also was a delay as studies were required
751 for treatments to be successfully translated to the industry. It also should be noted that
752 despite the use of GnRHa, treatments that act directly on the Lh receptor (PE and hCG)
753 are also still widely used in freshwater aquaculture in Europe and especially in Asia.
754 Finally, marine fish farming is still in its early stages in LA, and the use of GnRHa
755 treatments is widespread in research, suggesting that as marine fish farming develops,
756 the use of GnRHa treatments may also increase in LA. Consequently, broader use in
757 marine species could, influence the acceptance of GnRHa in freshwater aquaculture, as
758 has been observed in Europe.
759

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9. AUTHOR CONTRIBUTIONS

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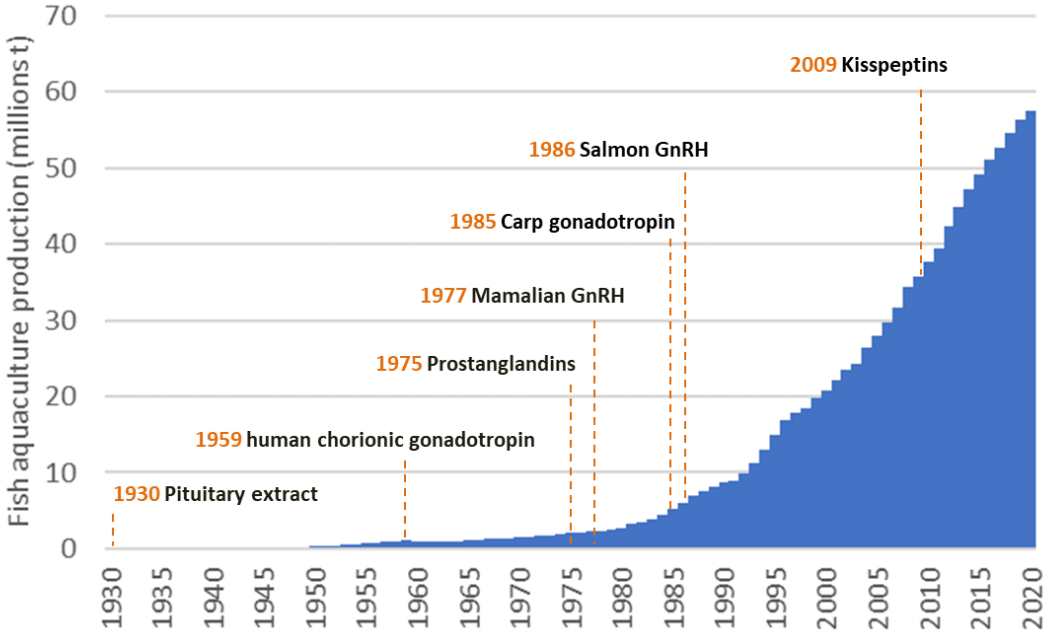
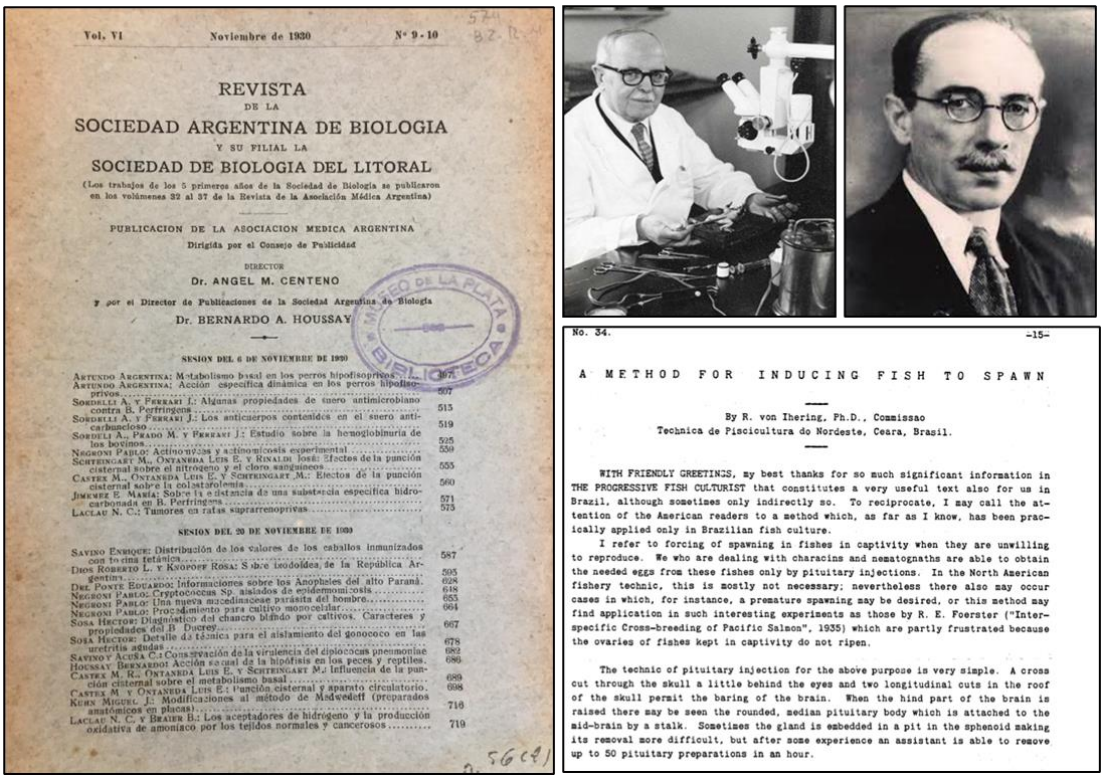


Figure 1. The increase in cultured fish production (production of diadromous, freshwater and marine fishes – no data from 1930 - 1950)[2] with dates of pioneering publications on induced fish spawning: use of pituitary extracts (PE) to induce spawning[14]; use of human chorionic gonadotropin (hCG)[19]; use of prostaglandins PG[21]. use of gonadotropin-releasing hormone analogues (GnRHa)[22]; use of carp gonadotropins[20]; use of salmon GnRH[28]; and use of kisspeptins[27]. While there would appear to be a connection between the timing of publications and the initiation of the large increase in world aquaculture production, but the causality is unclear. Skewed black line indicate stimulation and dotted line inhibition.



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No. 34. -15-

A METHOD FOR INDUCING FISH TO SPAWN

By R. von Ihering, Ph.D., Commissao
Technica de Piscicultura do Nordeste, Ceara, Brasil.

WITH FRIENDLY GREETINGS, my best thanks for so much significant information in THE PROGRESSIVE FISH CULTURIST that constitutes a very useful text also for us in Brazil, although sometimes only indirectly so. To reciprocate, I may call the attention of the American readers to a method which, as far as I know, has been practically applied only in Brazilian fish culture.

I refer to forcing of spawning in fishes in captivity when they are unwilling to reproduce. We who are dealing with characins and nematognaths are able to obtain the needed eggs from these fishes only by pituitary injections. In the North American fishery technic, this is mostly not necessary; nevertheless there also may occur cases in which, for instance, a premature spawning may be desired, or this method may find application in such interesting experiments as those by R. E. Foerster ("Interspecific Cross-breeding of Pacific Salmon", 1935) which are partly frustrated because the ovaries of fishes kept in captivity do not ripen.

The technic of pituitary injection for the above purpose is very simple. A cross cut through the skull a little behind the eyes and two longitudinal cuts in the roof of the skull permit the baring of the brain. When the hind part of the brain is raised there may be seen the rounded, median pituitary body which is attached to the mid-brain by a stalk. Sometimes the gland is embedded in a pit in the sphenoid making its removal more difficult, but after some experience an assistant is able to remove up to 50 pituitary preparations in an hour.

Figure 2: Two Latin American researchers (Bernardo Alberto Houssay from Argentina and Rodolpho Theodor Wilhelm Gaspar von Ihering from Brazil) pioneered the use of pituitary extracts to promote reproduction in fish. The first page of their studies is shown in the photograph, and copies of the full papers may be obtained from the corresponding authors of this article. Prof. Houssay (1887-1971) was also known for his discoveries about the role of pituitary hormones in sugar metabolism, which contributed to the development of an effective treatment for diabetes. He was awarded the Nobel Prize in 1947 for these studies. He was the first Latin American to be awarded the Nobel Prize. More information about his life and career can be found at this link (https://www.museohoussay.org.ar). Dr. von Ihering was a zoologist and biologist who is considered one of the founders of fish farming in Brazil**.**

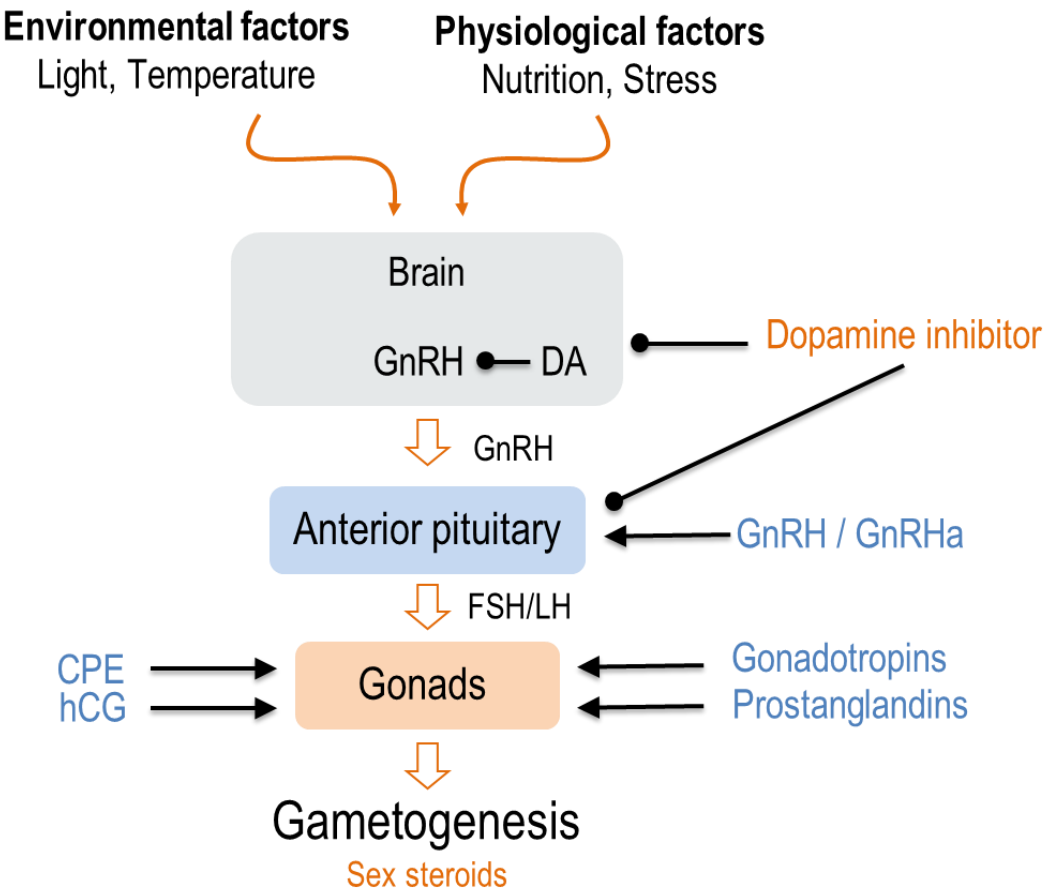

Figure 3: Neuroendocrine control of gonadal maturation in teleosts via the brain-pituitary-gonadal axis (BPG) and its integration with environmental and physiological factors. The black arrows and the filled circle lines indicate the hormones and an inhibitor most commonly used in aquaculture to control reproduction. For simplicity, the interactions of dopamines, secretogranins, and gonadotropin-inhibitory hormones, which are discussed in detail in other papers[33,34,36,39,59,71] are omitted. Abbreviations; dopamine (DA), follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), gonadotropin-releasing hormone agonists (GnRHa), human chorionic gonadotropin (hCG), luteinizing hormone (LH), carp pituitary extract (CPE).

Table 1. Difficulties in controlled propagation of females in some Latin America fish species. Such difficulties were classified according to the review of Zohar and Mylonas[11] as 1st: "Fail to undergo vitellogenesis", 2nd: "Failure of oocyte maturation" and 3rd: "Maturation complete - Failed release of eggs or release of overripe eggs and failed fertilisation".

Species /common name	Reproductive dysfunctions type	Observations	References
Marine species			
Lutjanus guttatus (Rose spotted snapper, pargo lunarejo)	2nd	Spawns naturally under suitable conditions in captivity. Under unfavourable captive conditions, hormone therapies can be used to induce captive snapper, to stimulate serial spawning and achieve spawning at the desired time.	*91,143,155*
Dormitator latifrons (Pacific fat sleeper)	2nd	No spawning without hormonal induction.	*156*
Scianops ocellatus (Red drum)	2nd	Breeds naturally with manipulation of temperature and photoperiod. Hormonal therapies are required if temperature and photoperiod are not manipulated.	*157*
Centropomus undecimalis (Common snook, robalo)	2nd	Spawns naturally with manipulation of temperature and photoperiod. Hormonal therapies are required if temperature and photoperiod are not manipulated.	*158-160*
Mycteroperca rosacea (Leopard grouper)	2nd	Natural spawning is very rare and is usually not fertilized by males. Hormone therapies are usually required for final maturation and spawning.	*152*
Freshwater species			
Leporinus friderici (Piau-três-pintas)	2nd	No spawning without hormonal induction. Induction protocols with low efficiency to spawning	*80*
Leporinus macrocephalus (Piauçu)	2nd	No spawning without hormonal induction.	*161,162*
Pseudoplatystoma fasciatum	2nd	No spawning without hormonal induction.	*121,128,163*

(Cachara, Bagre rayado, surubi)			
Pseudoplatystoma corruscans (Pintado, Surubi)	2nd	No spawning without hormonal induction.	*164,165*
Colossoma macropomum (Tambaqui, Cachama Negra, Guamitana)	2nd	No spawning without hormonal induction. Ovulation failed in some females. Sometimes mortality of embryos and larvae.	*131,141,166*
Piaractus brachypomus (Pirapitinga, Cachama branca)	2nd	No spawning without hormonal induction. Females exposed to oxygen-deficient waters show inhibition of spawning.	*167,168*
Piaractus mesopotamicus (Pacu, Pacú)	2nd	No spawning without hormonal induction. Unpredictable ovulation after spawning induction. Carp pituitary extract (CPE) can induce final maturation (germinal vesicle break down; GVBD) but not ovulation in some females.	*79,82*
Prochilodus lineatus (Curimba, Sábalo)	2nd	No spawning without hormonal induction. Low estradiol during vitellogenesis resulted in lower less yolk accumulation in eggs, and thus lower fertility and hatching rate.	*169,170*
Rhamdia quelen (Jundiá, bagre sapo)	2nd	No spawning without hormonal induction	*126,129,171*

Table 2. Summary of hormones used and their effects in females of selected Latin American freshwater fish species. Abbreviations are defined in the footnote.

Species (common name/s)	Hormone treatment (Method) *	Total Dose	Number of Females	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%) **	Reference
Leporinus friderici (Piau três pintas)	CPE (Two injections)	1.5 $mg.kg^{-1}$	10	100	75	85	80
		6 $mg.kg^{-1}$	10	80	<2	0	
	mGnRHa + MET (Two injections)	40 $\mu g.kg^{-1}$ GnRHa + 20 $mg.kg^{-1}$ MET	10	70	<2	0	
		12 $\mu g.kg^{-1}$ GnRHa + 6 $mg.kg^{-1}$ MET	6	50	<2	0	
Leporinus macrocephalus (Piauçu)	CPE (Two injections)	5.5 $mg.kg^{-1}$	10	71	63	59.07	142
	mGnRHa + MET (Single injection)	7 $\mu g.kg^{-1}$ GnRHa+ 10 $mg.kg^{-1}$ MET	9	100	6	0	
Pseudoplatystoma punctifer ex. *fasciatum* (Cachara, Surubí)	CPE (Two injections)	5.5 $mg.kg^{-1}$	9	100	53	No data	121
	CPE (Two injections)	5.5 $mg.kg^{-1}$	9	100	56	No data	
	hCG (Two injections)	15 $IU.g^{-1}$	7	100	55	No data	
	hCG (Two injections)	15 $IU.g^{-1}$	7	100	82	No data	
	CPE (Single injection) + hCG (Single injection)	0.5 $mg.kg^{-1}$ CPE + 5 $IU.g^{-1}$ hCG	5	0	No data	No data	
	sGnRHa + DOM (Two injections)	10 $\mu g.kg^{-1}$ sGnRHa + 5 $mg.kg^{-1}$ DOM	31	74	84	73.7	128
Colossoma macropomum (Cachama negra, Gamitana, Tambaquí)	hCG (Two injections)	30$IU.g^{-1}$	10	80	50	60	120
	CPE (Two injections)	5 $mg.kg^{-1}$	10	100	70	80	
	CPE (Two injections)	5 $mg.kg^{-1}$	6	100	61-88	26-53	172
	sGnRHa + DOM (Two injections)	10 $\mu g.kg^{-1}$ sGnRHa + 5 $mg.kg^{-1}$ DOM	3	100	11	20	141
	CPE (Two injections)	5 $mg.kg^{-1}$	3	100	63	68	

	CPE (Two injections)	5. 5 mg.kg^{-1}	8	88	62	56	
	mGnRHa + MET (Single injection)	4 μg.kg^{-1} mGnRHa + 2 mg.kg^{-1} MET	8	100	72	57	131
	mGnRHa + MET (Single injection)	8 μg.kg^{-1} mGnRHa + 4 mg.kg^{-1} MET	8	63	51	33	
Piaractus mesopotamicus (Pacu)	mGnRHa (Single injection)	5 μg.kg^{-1}	4	0	No data	No data	24
		50 μg.kg^{-1}	4	0	No data	No data	
			6	0	No data	No data	
		100 μg.kg^{-1}	3	100	No data	No data	
	mGnRHa (Two injections)	50 μg.kg^{-1}	6	83	No data	No data	
			3	100	No data	No data	
			3	67	No data	No data	
	mGnRHa (Two injections)	50 μg.kg^{-1}	3	100	No data	No data	
			3	67	No data	No data	
	CPE (Two injections)	6 mg.kg^{-1}	17	53	~90	~82	79
			12	83	~68	~64	
			4	25	~25	~22	
	CPE (Two injections) + PGF (with last CPE injection)	6 mg.kg^{-1} CPE + ~0.3 mg kg^{-1} PGF	4	100	~85	~84	
			5	100	~55	~62	
		6 mg.kg^{-1} CPE + ~0.8 mg kg^{-1} PGF	3	100	~92	~82	
Prochilodus lineatus (Curimatã, Sábalo)	CPE (Two injections)	5.5 mg.kg^{-1}	4	100	46	39	130
	sGnRHa + DOM (Two Injections)	10 μg.kg^{-1} sGnRHa + 5 mg.kg^{-1} DOM	4	100	48	39	
	sGnRHa + DOM (Two Injections)	10 μg.kg^{-1} sGnRHa + 5 mg.kg^{-1} DOM	6	100	52	48	
Rhamda quelen	CPE (Single Injection)	4 mg.kg^{-1}	8	88	82	No data	129

(Jundiá, Bagre sapo)	sGnRHa (Single injection)	10 μg.kg^{-1} sGnRHa	8	0	No data	No data	
	sGnRHa + DOM (Single injection)	10 μg.kg^{-1} sGnRHa + 5 mg.kg^{-1} DOM	8	88	82	No data	
	sGnRHa + MET (Single injection)	10 μg.kg^{-1} sGnRHa + 20 mg.kg^{-1} MET	8	38	74	No data	
	CPE (Single injection)	5mg.kg^{-1}	7	100	75	No data	
	CPE (Single injection) + Sperm (Single injection)	5 mg.kg^{-1} CPE + 2ml.kg^{-1} Sperm	7	85	82	No data	126
	CPE + Sperm (Single injection)	5 mg.kg^{-1} CPE + 2ml.kg^{-1} Sperm	7	71	76	No data	

The present table shows only species in which at least two different hormonal treatments were assessed, and ovulated female percentage data were provided (with n>2) in the references reviewed. Data in different rows for the same species involve different protocols or experimental replications of the same protocol. * For details of partial doses, time intervals and anatomical site of injections review each particular reference. Fertilization and hatching rate data represent the average condition of the spawned females in each treatment. ** Hatching values were calculated considering only fertilized eggs. CPE= Carp pituitary extract; hCG= Human chorionic gonadotropin; mGnRHa= Mammalian gonadotropin-releasing hormone analogue; sGnRHa= Salmon gonadotropin-releasing hormone analogue; MET= Metoclopramide (dopamine receptor antagonist); DOM= Domperidone (dopamine receptor antagonist). PGF= prostaglandin F.

Table 3. Summary of hormones used and their effects upon females of Latin American marine fish species.

Species (common name/s)	Hormone treatment (Method)*	Total Dose	Number of Females	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%) **	Reference
Lutjanus guttatus (Rose spotted snapper)	mGnRHa (Single implant)	50-150 μg.kg^{-1}	4	100	No data	28	[91]
		150-300 μg.kg^{-1}	4	75	No data	86	
		50-150 μg.kg^{-1}	5	100	44	No data	[149]
		100-200 μg.kg^{-1}	7	71	No data	No data	
		200-300 μg.kg^{-1}	6	100	61	No data	
		200-300 μg.kg^{-1}	3	100	No data	No data	
		200-300 μg.kg^{-1}	10	100	0	No data	
Seriola rivoliana (Longfin yellowtail)	GnRHa	20 μg.kg^{-1}	10	100	No data	79.86	[150]
			17	100	No data	84.29	
			9	100	No data	85.68	
			2	100	99.1	96.2	[92]
Seriola lalandi (Yellowtail kingfish)	GnRHa (Single implant)	500 g	7	100	90	No data	[151]
Lutjanus peru (red snapper)	HCG (2 injections)	500 IU/kg-1000 IU7Kg	2	100	No data	No data	[173]
Centropomus undecimalis (Common snook, robalo)	sGnRHa (Single implant)	90-150 μg.kg^{-1}	9	89	19	100	[158]
	mGnRHa (Single implant)	50 μg.kg^{-1}	43	81	57	78	[159]
	GnRHa (2-4 injections)	20-160 μg.kg^{-1}	11	82	97.3	No data	[174]

Sphoeroides annulatus (Bullseye puffer)	GnRHa (implant)	75 (<800g fish) or 150 (>800g fish) μg.fish^{-1}	11	82	90.2	No data	
Lutjanus novemfasciatus (Pacific cubera snapper)	GnRHa (2-3 injections)	20-40 μg.kg^{-1}	4	100	0-41.6	0-21.6	*175*
	GnRHa (implant)	36 μg.kg^{-1}	2	50	98	-	
*Mycteroperca rosacea (*Leopard grouper	hCG (Two injections)	1500 IU.kg^{-1}	7	86	44 - 100[b]	No data	*152*
			3	67	0 - 98[b]	No data	
			6	83	3 - 74[b]	No data	
			3	100	0	No data	
			7	71	0	No data	
			3	100	31 - 89[b]	No data	
			4	100	75 - 94[b]	No data	
	mGnRHa (Two injections)	15 μg.kg^{-1}	12	67	0 - 99[b]	No data	
		150 μg.kg^{-1}	13	69	0 - 41[b]	No data	

Data in different rows for the same species involve different protocols or experimental replications of the same protocol. * For details of partial doses, time intervals and anatomical site of injections review each particular reference. Fertilization and hatching rate data represent the average condition of spawned females in each treatment. [a] The average value was calculated as the mean values of the all fertilization value observed in the multiple spawning from each spawned female. [b] The range values are presented since the average value was not available. ** Hatching values were calculated considering only fertilized eggs. CPE= Carp pituitary extract; hCG= Human chorionic gonadotropin; mGnRHa= Mammalian gonadotropin-releasing hormone analogue; sGnRHa= Salmon gonadotropin-releasing hormone analogue; MET= Metoclopramide (dopamine receptor antagonist); DOM= Domperidone (dopamine receptor antagonist). PGF= prostaglandin F.