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Hormones and Spawning in Fish[1]

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Abstract

Fish integrate their reproductive activities with seasonal environmental cycles. Certain en-vironmental factors such as temperature, photoperiod and rainfall act as cues for the approach-ing favorable season for reproduction. Signals from environmental cues and endogenous physi-ological cycles input to the neuroendocrine system, which in turn regulates pituitary and go-nadal functions. One of the main reasons for lack of ovulation and spawning in a number of cultured fish is failure of the pituitary to release gonadotropin (GtH-II), one of the hormones involved in the regulation of reproduction. GtH-II secretion in many teleosts is under a dual control, with release being stimulated by gonadotropin releasing hormone (GnRH), and inhibi-tion by dopamine on the actions of GnRH, as well as spontaneous release of GtH-II. The inhibi-tory actions of dopamine on GtH secretion can vary in potency between species. In goldfish, carps and catfish, dopamine inhibition is very strong: injection of a dopamine blocker, such as domperidone (DOM), results in potentiation of the actions of GnRH analogs, leading to a large release of GtH-II and ovulation. In bream and loach, dopamine inhibition is weak, injection of a high dose of GnRH analog alone is effective in stimulating GtH-II release and ovulation; how-ever, the combination of DOM with GnRH analog results in potentiation of the GtH-II response and shortening of the response time from injection to ovulation. In seabream, sciaenids and some other marine fish, GtH-II secretion is not under dopaminergic inhibitory control, they are sensitive to GnRH analogs and can be induced to ovulate and spawn by multiple injections or chronic administration of GnRH analogs.

Introduction

Most species of teleost fishes are seasonal breeders, and only a few breed continuously. Among the seasonal breeders, there is variation in the time of year when breeding occurs. Fish integrate their reproductive activities with seasonal environmental cycles; and certain environmental factors, such as temperature, photoperiod and rainfall, act as cues for the approaching season which is favorable for reproduction. Signals from environmental cues and endogenous physiologocal cycles input to the neuroendocrine system, which in turn regulates pituitary and gonadal function.

One of the main reasons for lack of ovulation and spawning in a number of cultured fish is failure of the pituitary to release gonadotropin (GtH-II), one

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of the hormones involved in the regulation of reproduction. For example, in cultured common carp (*Cyprinus carpio*, Cyprinidae), adding males and floating vegetation as a spawning substrate to the holding pond in the evening usually results in spontaneous ovulation and spawning the following morning. We have found a pronounced surge of GtH-II, during which GtH-II levels increase about 10-fold above basal levels, last about 12 h and are synchronized with the photoperiod. Serum GtH-II levels remain low during the course of sampling in control fish and in fish that failed to ovulate. In general, a pre-ovulatory surge of GtH-II is responsible for ovulation and spawning of fish.

Traditional methods of induced spawning for cultured fish are based on the injection of GtH-II from different sources, including crude extract of carp pituitary gland (CPE), partially purified fish GtH-II and mammalian GtH, especially human chorionic gonadotropin (HCG) (Lam 1982; Donaldson and Hunter 1983; Peter et al. 1988a). However, a number of problems have been found with the use of this technique: 1) fish GtH-II is generally of high species specificity, thus, for example, carp or salmon GtH-II are ineffective in gilthead seabream (*Sparus aurata*, Sparidae) (Zohar et al. 1987); HCG is routinely used to induce spawning in some fish, but is ineffective in others, such as grass carp (*Ctenopharyngodon idellus*, Cyprinidae) and black carp (*Mylopharyngodon piceus*, Cyprinidae) (Lin et al. 1986a); 2) when crude pituitary extracts are used to induce spawning, the fish is treated with a mixture of hormones which may have side efffects on gametogenesis or other functions; 3) the use of partially purified fish GtH-II still involves high costs, and HCG is also expensive; 4) the use of exogenous GtH-II might lead to an immune response in treated fish and cause subsequent refractoriness to the hormones (Peter et al. 1988a); 5) CPE and HCG are highly variable in potency and have a short storage life.

Problems associated with traditional methods led to the search for alternative hormonal treatments to induce ovulation and spawning in cultured fish. Recent advances in the understanding of the neuroendocrine regulation of GtH-II secretion in teleost fishes have led to the development of a new, highly efficient and effective technique for induced ovulation and spawning in cultured fish.

Neuroendocrine Regulation of Gonadotropin Secretion

Neuroendocrine regulation of GtH-II secretion in teleosts is mainly under a dual neurohormonal system. GtH-II release is stimulated by a gonadotropin-releasing hormone (GnRH) and inhibited by dopamine, which functions as a gonadotropin release-inhibitory factor (GRIF). Dopamine acts directly at the level of the pituitary to modulate the actions of GnRH as well as the spontaneous release of GtH-II, and inhibits release of GnRH (Peter et al. 1986, 1991). The surge of GtH-II, which is responsible for ovulation, may be regulated by a stimulation of GnRH and a release from inhibition by dopamine.

Recent studies indicate that some other neurohormones are also involved in the regulation of GtH-II secretion. Norepinephrine (NE) or serotonin (5-HT) treatment induces an increase in serum GtH-II levels in goldfish (*Carassius auratus*, Cyprinidae) (Chang and Peter 1984; Somoza et al. 1988); this may, in

part, be due to the stimulatory effects of NE or 5-HT on GnRH release (Yu et al. 1991; Yu and Peter 1992). NE also has a direct effect on GtH-II release (Chang et al. 1991). Neuropeptide Y (NPY) directly stimulates GtH-II release from dispersed and cultured goldfish pituitary cells; however, NPY also has stimulatory effects on GnRH release (Peng et al. 1990). Injection of GABA increases serum GtH-II levels in goldfish during the early stages of gonadal recrudescence, but not in fish that are prespawning or sexually regressing (Kah et al. 1990). GABA does not stimulate GtH-II release from dispersed goldfish pituitary cells in static or perifusion culture; however, it does have a stimulatory effect on GnRH release from goldfish pituitary fragments, which is explained by its stimulatory effects on GnRH release (Kah et al. 1990). The neuroendocrine regulation of GtH-II secretion in teleost fish is highly complex. A model of known neuroendocrine regulatory factors involved in the regulation of GtH-II secretion in teleosts is presented in Fig. 1.

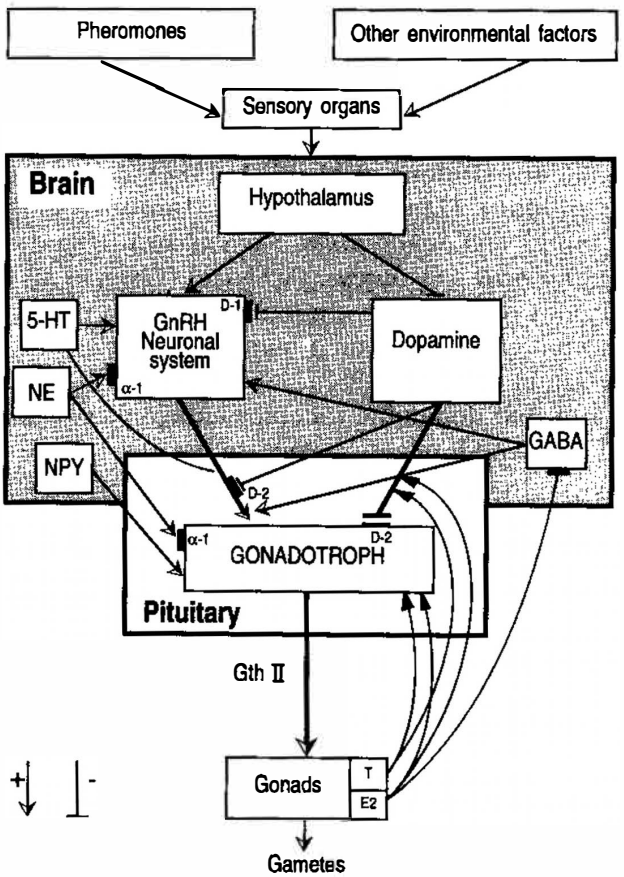

Fig. 1. Model for the neuroendocrine regulation of gonadotropin-II release in teleosts, adapted from Peter et al. (1991). A line with an arrow indicates a stimulatory effect; a line with a bar indicates an inhibitory effect. Abbreviations: α-noradrenergic receptor, α-1; estradiol, E2; Y-aminobutryic acid, GABA; gonadotropin-II, GTH-II; norepinephrine, NE; neuropeptide Y, NPY; serotonin, 5-HT; testosterone, T; type-1 dopamine receptor, D-1; type-2 dopamine receptor, D-2.

Studies on goldfish were the first to demonstrate the effects of blockage of dopamine actions on GtH-II release and ovulation by determining the response to (D-Ala^6, Pro^9-NEt)-mammalian GnRH (mGnRH-A) when given in combination with the dopamine receptor antagonist, pimozide. In these studies, injection of pimozide caused a marked potentiation of the GtH-II release response to mGnRH-A, and the combined injections of pimozide and mGnRH-A were highly effective in inducing ovulation (Chang and Peter 1983; Chang et al. 1984; Sokolowska et al. 1984). Reserpine, a drug which causes general depletion of catecholamines, also significantly potentiates the action of GnRH and its analogues. The drug, alpha-methyl-paratyrosine, and carbidopa, which block catecholamine synthesis at steps up to and including the production of dopamine, potentiate the actions of mGnRH-A on GtH-II release in goldfish (Peter et al. 1986) and Chinese loach (*Paramisgurnus dabryanus*, Cobitidae) (Lin et al. 1985,1986b); however, blocking the conversion of dopamine to norepinephrine with the drug, diethyldithiocarbamate, had no apparent effects on the responsiveness to mGnRH-A.

The presence of dopamine inhibition of GtH-II release has been extended to a number of other teleost species, including: African catfish (*Clarias gariepinus*, Clariidae) (De Leeuw et al. 1986), coho salmon (*Oncorhynchus kisutch*, Salmonidae) (Van Der Kraak et al. 1986), European eel (*Anguilla anguilla*, Anguillidae) (Dufour et al. 1984, 1988), Japanese eel (*Anguilla japonica*, Anguillidae) (Lin et al. 1990), rainbow and brown trout (*Oncorhynchus mykiss* and *Salmo trutta*, Salmonidae) (Billard et al. 1984), tilapia (*Oreochromis niloticus* x *O. aureus*, Cichlidae) (Gissis et al. 1988). It is clear that the GRIF actions of dopamine are found in a wide phylogenetic range of teleosts. Results from experiments on a number of species of cultured Chinese carp and loach (Lin et al. 1986a, 1987a, 1987b; Peter et al. 1986, 1987a, 1988a, 1988b) all confirm that dopamine receptor antagonists or drugs that block dopamine actions on GtH-II release potentiate the activity of GnRH analogues, and these combined treatments are highly effective in stimulating GtH-II secretion and ovulation.

The inhibitory actions of dopamine on GtH-II secretion can vary in potency between species. For example, in goldfish and common carp, dopamine inhibition of GtH-II secretion is strong, and mGnRH-A and (D-Arg^6, Pro^9-NEt)-salmon GnRH (sGnRH-A) stimulate only a modest increase in serum GtH-II levels and are ineffective in inducing ovulation (Peter et al. 1985, 1986, 1987b; Sokolowska et al. 1985a, 1985b; Lin et al. 1987a); administration of the dopamine receptor antagonist, pimozide (PIM), or domperidone (DOM) greatly potentiates the action of mGnRH-A and sGnRH-A on GtH-II release, and combined injections of PIM or DOM and mGnRH-A or sGnRH-A are highly effective in inducing ovulation and spawning in these species (Lin et al. 1988; Peter et al. 1988a, 1988b). On the other hand, in bream (*Parabramis pekinensis*, Cyprinidae) and Chinese loach, the dopamine inhibitory tone on GtH-II secretion is not so strong, and injection of a high dose of mGnRH-A or sGnRH-A alone overcomes the dopaminergic inhibition, and is effective in stimulating GtH-II release and ovulation; however, the combination of PIM with mGnRH-A resulted in potentiation of the GtH-II response and shortening of the response time from injection to ovulation (Lin et al. 1985, 1986a, 1986b).

In contrast, in some marine species, such as Atlantic croaker (*Micropogonias undulatus*, Sciaenidae) (Copeland and Thomas 1989), gilthead seabream (*Sparus aurata*) (Zohar et al. 1987) and striped bass (*Morone saxatilis*, Serranidae) (Sullivan et al. 1992), there is no evidence for a dopamine inhibitory regulation of GtH-II secretion or ovulation. In a series of experiments, the effect of injection of a number of dopamine agonists and antagonists on GtH-II secretion or ovulation response to mGnRH-A were tested and no evidence of inhibition was found. The apparent differences between species in the degree of GRIF activity exerted by dopamine suggests that the relative importance of dopamine and GnRH in regulating GtH-II secretion in teleosts may be altered through evolution.

Induction of Ovulation and Spawning in Cultured Fish by GnRH Analogues and Dopamine Antagonists

The combination of a GnRH analogue and a dopamine antagonist for induced ovulation and spawning in cultured fish is a highly effective procedure called the Linpe method (Peter et al. 1988a). To maximize the efficiency of this combined treatment, research was done to determine the most effective GnRH analogue and dopamine antagonist to be used to induce ovulation and spawning in cultured fish.

In a combination of in vivo and in vitro studies on structure-activity relations of agonist analogues of mGnRH and sGnRH, the analogue sGnRH-A was found to be the most potent in terms of GtH-II release in goldfish (Peter et al. 1985, 1987a), and to have the highest affinity to GnRH receptors in the pituitary of goldfish (Habibi et al. 1987, 1989) and catfish (De Leeuw et al. 1988). This analogue was also the most active in stimulating GtH-II release in vivo in common carp and Chinese loach (Lin et al. 1988, 1991), and it is highly resistant to enzymatic degradation by the pituitary, kidney and liver of gilthead seabream (Goren et al. 1987; Zohar et al. 1989). sGnRH-A appears to be highly advantageous for minimizing dosages of analogue for induced ovulation of cultured brood fish. However, it is apparent that the superactive analogue of mGnRH-A is also highly active in teleosts; in some fish, such as Atlantic salmon (*Salmo salar*, Salmonidae) and gilthead seabream (Zohar et al. 1989), mGnRH-A is apparently more potent than sGnRH-A for inducing GtH-II release and ovulation, although additional dose response studies are needed to confirm this.

A wide variety of drugs effective in inhibiting dopamine synthesis, or depleting catecholamines, or in blocking D-2 type dopamine receptors, can block the inhibitory actions of dopamine on GtH-II release, and potentiate the effects of GnRH in vivo (Peter et al. 1986, 1987a, 1987b; Goos et al. 1987; Omeljaniuk et al. 1987; Lin et al. 1988; Van Asselt et al. 1988). Choosing the best of such drugs for practical application involves consideration of differences in potency and undesirable side-effects that may occur due to central actions on the catecholaminergic system. Notably, the most potent and highly specific D-2 type dopamine receptor antagonist, DOM, does not cross the blood-brain barrier in goldfish (Omeljaniuk et al. 1987), nor presumably in other teleosts, and is

therefore assumed to be optimal for use in induced ovulation and spawning of cultured fish.

Based on experimental work and field trials (Lin et al. 1986a, 1986b, 1987a, 1987b, 1988, 1990, 1991; Peter et al. 1987b, 1988a, 1988b), Table 1 summarizes the latest information on effective dosages of mGnRH-A and sGnRH-A, and DOM for induced spawning of Chinese carp, catfish and loach in China. The effectiveness of the Linpe method was judged against several criteria (Peter et al. 1987a, 1988a, 1993): a high rate of ovulation occurs consistently from one group of brooders to another within each species; ovulation is complete rather than partial; the time to ovulation following injection is short and predictable; ovulated eggs are fertile and viable; and induction of ovulation by this technique does not affect subsequent reproductive cycles by the same broodfish. The results of the experiments and field trials on fish farms for each species of cultured fish were highly successful in meeting these criteria. The main advantages of the Linpe method compared to traditional methods include the following: reduced cost of the synthetic drugs, long stability of the drugs, they can be successfully applied to a great variety of species, high predictability of the time from injection to ovulation, decreased stress on broodstock because only a single injection is needed, and absence of side effects on subsequent reproductive cycles.

Table 1. Linpe method of induced ovulation and spawning of cultured fish in China.

Species	Water temperature (°C)	Treatment (single injection)	Time to ovulation or spawning following injection (h)
Common carp	20-25	DOM 5 mg + LHRH-A 10 ug·kg^{-1} or	16-14
		DOM 1 mg + sGnRH-A 10 ug·kg^{-1}	16-14
Silver carp	20-30	DOM 5 mg + LHRH-A 20 ug·kg^{-1} or	12-8
		DOM 5 mg + sGnRH-A 10 ug·kg^{-1}	12-8
Mud carp	22-28	DOM 5 mg + LHRH-A 20 ug·kg^{-1} or	6
		DOM 1 mg + sGnRH-A 10 ug·kg^{-1}	6
Bream	22-30	DOM 3 mg + LHRH-A 10 ug·kg^{-1} or	10-8
		DOM 3 mg + sGnRH-A 5 ug·kg^{-1}	10-8
Grass carp	18-30	DOM 5 mg + LHRH-A 10 ug·kg^{-1} or	12-8
		DOM 5 mg + sGnRH-A 5 ug·kg^{-1}	12-8
Bighead carp	20-30	DOM 5 mg + LHRH-A 50 ug·kg^{-1} or	12-8
		DOM 5 mg + sGnRH-A 20 ug·kg^{-1}	12-8
Black carp	20-30	DOM 3 mg + LHRH-A 10 ug·kg^{-1} plus 6 h later	
		DOM 7 mg + LHRH-A 20 ug·kg^{-1}	8-6
Chinese loach	18-30	DOM 1 mg + LHRH-A 20 ug·kg^{-1} or	14-11
		DOM 5 mg + sGnRH-A 10 ug·kg^{-1}	14-11
Labeo rohita	18-30	DOM 5 mg + LHRH-A 50 ug·kg^{-1} or	6
		DOM 5 mg + sGnRH-A 20 ug·kg^{-1}	6
Thai carp	25-29	DOM 5 mg + LHRH-A 20 ug·kg^{-1} or	8-7
		DOM 5 mg + sGnRH-A 20 ug·kg^{-1}	10-8
Chinese catfish	22-30	DOM 10 mg + LHRH-A 100 ug·kg^{-1} or	18-16
		DOM 10 mg + sGnRH-A 50 ug·kg^{-1}	18-16
Mandarin fish	22-30	DOM 10 mg + LHRH-A 100 ug·kg^{-1} or	20-15
		DOM 10 mg + sGnRH-A 50 ug·kg^{-1}	20-15

The Linpe method is rapidly gaining acceptance in fish farms in China, and has been commercialized by Syndel Laboratories, Inc., Vancouver, British Columbia, Canada, under the tradename Ovaprim. The Ovaprim spawning kit is especially formulated for use with salmonids, cyprinids and other freshwater cultured fish. It has been used successfully in a number of species in several countries and is gaining wide acceptance as the preferred method for induced ovulation and spawning of cultured freshwater fish. For example, in India, based on three years' (1988-90) field trials with Ovaprim for induced spawning of major Indian carp (catla, rohu, mrigal, fringe-lipped carp), silver carp, bighead carp and grass carp in various fish farms located in different agro-climate regions, Nandeesha et al. (1991) concluded that, in economic terms, the use of Ovaprim is advantageous. In trials on fish farms, the percentage of spawning success, the number of eggs obtained per kilogram body weight of brooders, the fertilization rate and hatching percentage, remained consistently higher with Ovaprim as compared to CPE or HCG treatment in almost all instances. To illustrate, in the trials of 1990, the average number of eggs obtained per kilogram body weight of a brooder was 114,000 with Ovaprim as compared to 85,000 with CPE; similarly, the average number of fry obtained per kilogram body weight of a brooder was 72,000 with Ovaprim as against 43,000 with CPE. This indicates that nearly 40% more fry can be obtained by using Ovaprim in place of commercial CPE.

Ovulation and spawning has been successfully induced by the Linpe method in the following species to date:

Belontiidae	
Trichogaster trichopterus	Degani et al. 1991
Characidae	
Pacu, *Colossoma bidens*	Lin et al. 1987a
Cichlidae	
Oreochromis niloticus X O. aureus	Gissis et al. 1988
Prochilodontidae	
Prochilodus scrofa	M. Little, unpubl. data
Clariidae	
Chinese catfish, *Clarias fuscus*	Lin et al. 1987a
Asian catfish, *C. batrachus*	Manickam and Joy 1989
African catfish, *C. gariepinus*	Lin et al. 1987a
Cobitidae	
Chinese loach, *Paramisgurnus dabryanus*	Lin et al. 1986b
Cyprinidae	
Common carp, *Cyprinus carpio*	Lin et al. 1986a
Goldfish, *Carassius auratus*	Peter 1982
Grass carp, *Ctenopharyngodon idella*	Lin et al. 1986a
Silver carp, *Hypophthalmichthys molitrix*	Lin et al. 1986a
Bighead carp, *Aristichthys nobilis*	Lin et al. 1986a
Black carp, *Mylopharyngodon piceus*	Lin et al. 1986a
Mud carp, *Cirrhinus molitorella*	Lin et al. 1986a
Bream, *Abramis brama*	Glubokov et al. 1991

Chinese bream, *Parabramis pekinensis*	Lin et al. 1986a
Catla, *Catla catla*	Nandeesha et al. 1991
Rohu, *Labeo rohita*	Nandeesha et al. 1991
Mrigal, *Cirrhinus mrigala*	Nandeesha et al. 1991
Fringe-lipped carp, *Labeo fimbriatus*	Nandeesha et al. 1991
Thai carp, *Puntius gonionotus*	Sukumasavin et al. 1992
Rainbow sharks, *Labeo erythrurus*	Shireman and Gildea 1989
Redtail black sharks, *Labeo bicolor*	Shireman and Gildea 1989
Gudgeon, *Gobio gobio*	Kestemont 1988
Silvery chub, *Xenocypris argentea*	Lin et al. (unpubl. data)
Gobiidae	
Mudskipper, *Boleophthalmus pectinirostris*	Zhang et al. 1989
Heteropneustidae	
Indian catfish, *Heteropneustes fossilis*	Manickam 1992
Salmonidae	
Coho salmon, *Oncorhynchus kisutch*	Van Der Kraak et al. 1986
Chum salmon, *O. keta*	Barranikova and Dyubin 1992
Siluridae	
Asian catfish, *Parasilurus asotus*	Lin et al. (unpubl. data)
Percichthydae	
Mandarin fish, *Siniperca chautsi*	Lin et al. 1987a
Petromyzonidae *(Cyclostoma)*	
River lamprey, *Lampetra fluviatilis*	Barranikova and Dyubin 1992

A number of additional salmonid species can be induced to ovulate and spawn with low dosages of Ovaprim (M. Little, Syndel Laboratories, pers. comm.). However, more research is needed to determine whether the Linpe method is appropriate for marine species that have an extended spawning season with daily or weekly spawning activity. In some marine teleost species, including those in which dopamine has not been shown to exert an inhibitory effect on GtH-II release, ovulation and spawning can be induced successfully by treatment with a superactive GnRH analog alone. Much research is being devoted to the mode of delivery of GnRH analogues for inducing ovulation and spawning of such species.

Superactive GnRH analogs, which are resistant to enzymatic degradation, should have prolonged biological half-lives in the circulation. However, in gilthead seabream, the mGnRH analogue tested disappeared from circulation about 1-2 h after injection, and the GtH-II surge caused by a single injection of the analogue lasted approximately 48 h, which may be insufficient time to induce ovulation in females that have not reached the final stage of maturation. Moreover, since gilthead seabream has asynchronous ovarian development and is a frequent spawner, a single injection of GnRH analogue may only induce partial spawning (Zohar 1988). Thus, a sustained slow release delivery system (cholesterol, cholesterol-cellulose or biodegradable polymer) offers one solution to overcome the short bioactive life of this peptide, and has been proven efficient in inducing constantly elevated GtH-II secretion and long term spawn-

ing, or accelerating and synchronizing final maturation and spawning in a variety of cultured fish such as rainbow trout (*Salmo gairdneri*, Salmonidae) (Crim et al. 1983), Atlantic salmon (Crim and Glebe 1984), milkfish (*Channos channos*, Chanidae) (Lee et al. 1986), sea bass (*Lates calcarifer*, Centropomidae) (Harvey et al. 1985; Almendras et al. 1988), gilthead seabream (Zohar 1988), herring (*Clupea harengus*, Clupeidae) (Carolsfeld et al. 1988) and largemouth bass (*Micropterus salmoides*, Centrarchidae) (Lin 1992, unpubl. data).

More recently, several studies demonstrated the feasibility of using the oral route to deliver hormone and catecholaminergic drugs to fish for inducing GtH-II release, ovulation and spawning. Suzuki et al. (1988) reported that oral intubation of a crude pituitary extract of chum salmon, *Oncorhynchus keta*, to goldfish induced ovulation in this agastric species. Thomas and Boyd (1989) reported that oral administration of mGnRH-A in the diet of spotted sea trout (*Cynoscion nebulosus*, Sciaenidae) induced spawning 32-38 h after feeding. Similar results have also been obtained following oral intubation of sablefish (*Anoplopma fimbria*, Anoplopomatidae) with mGnRH-A (Solar et al. 1990). McLean et al. (1991) demonstrated that mGnRH and its analogues are absorbed by the gut of coho salmon after oral administration, and assessed the biological activity of mGnRH-A following passage into circulation by examining GtH-II secretion in 17β-estradiol-primed juvenile salmon. Sukumasavin et al. (1992) reported that oral delivery of sGnRH-A and DOM to Thai carp (*Puntius gonionotus*) induced ovulation in 83-100% within 48 h of administration. This latter study provides evidence regarding the feasibility of orally inducing spawning in cultured fish that require a dopamine antagonist to potentiate the actions of GnRH analogue, due to a high dopamine inhibitory tone. All these preliminary studies with a variety of teleosts suggest that oral administration of superactive analogues of mGnRH-A or sGnRH-A, as well as dopamine antagonists in the diet or by intubation, have potential value in aquaculture as a reliable method of inducing ovulation and spawning in species that may be stress-susceptible, or in small ornamental fishes which cannot be injected easily. Although higher dosages of GnRH analogue or drugs may be required to induce ovulation and spawning by oral administration than by injection, these increased costs could be offset in many culture facilities by labor savings. However, additional studies are required to determine the dose-response relationships, pharmacokinetics and polypeptide protection methodologies in order to ascertain appropriate dosages, and to prove the efficacy of this approach for induced spawning of cultured fish.

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