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Short Communication

Dopamine control of LH release in the tench (*Tinca tinca*)

Peter Podhorec [a,*], Magdalena Socha [b], Miroslawa Sokolowska-Mikolajczyk [b], Tomas Policar [a], Viktor W. Svinger [a], Borek Drozd [a], Jan Kouril [a]

[a] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25 Vodnany, Czech Republic
[b] Department of Ichthyobiology and Fisheries, University of Agriculture, ul. Prof. T. Spiczakowa 6, 30-199 Krakow-Mydlniki, Poland

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ABSTRACT

Tench (*Tinca tinca*) is apparently the only known member of the Cyprinidae in which ovulation is stimulated following administration of a low dose of GnRH analogue (GnRHa) without a dopamine inhibitor. This study evaluated LH release effectiveness of the most commonly used GnRHa and clarified whether LH secretion followed by ovulation is subject to inhibitory dopaminergic control in tench. Fish were intraperitoneally injected with three types of GnRHa, GnRHa with dopamine inhibitor metoclopramide (combined treatment), or the dopamine inhibitor metoclopramide alone. LH concentrations at five sampling times (0, 6, 12, 24, and 33 h) together with ovulation success and fecundity index were recorded. The combined treatment triggered an almost immediate LH release peak with a gradual decline, and resulted in a high ovulation rate. In contrast to the combined treatment, an application of GnRHa alone at 10 μg kg^{-1} induced gradual increase of LH concentrations with peaks close to ovulation time, and with high ovulation success. Significant differences in LH concentrations at 6 and 12 h and no differences in ovulation success were found between the combined and the GnRHa alone treatments. Metoclopramide alone induced a small increase in LH with no ovulation. The study presents clear evidence of dopaminergic control of LH release in tench, with a high ovulation rate obtained after application of GnRHa alone or in combination with dopamine inhibitor.

1. Introduction

The dual neuroendocrine control of luteinizing hormone (LH) secretion and ovulation is well established within the family Cyprinidae [27]. The main stimulatory and inhibitory factors of LH secretion from pituitary gonadotrophs are the gonadotrophin-releasing hormone (GnRH) and the catecholamine neurotransmitter dopamine (DA), respectively [42]. Gonadotrophin-releasing hormone regulates the synthesis as well as the release of LH required for stimulation of steroidogenesis with subsequent final oocyte maturation and ovulation [25]. Two forms of GnRH, cGnRH-II and sGnRH, have been identified in cyprinids [40], but native forms of GnRH have limited use in aquaculture due to their intense enzymatic degradation in fish [47]. Amino acid substitutions in positions 6 and 10 of the native GnRH chain have been shown to markedly improve enzymatic resistance of synthetic GnRH analogues (GnRHa) and facilitate their use in fish reproductive therapies [29]. Gonadotrophin-releasing hormone analogues vary in potency with respect to induction of LH release and ovulation [11].

Variable degrees of dopaminergic inhibition of LH release have been demonstrated in teleost fish irrespective of their taxonomic position, from a suggested role of DA as a puberty gatekeeper in juvenile eel [43], through less pronounced DA inhibition of final steps of gametogenesis in salmonids [26], to strong inhibition of a pre-ovulatory LH surge and ovulation in cyprinids [38]. DA inhibitory tone changes over the course of the cyprinid reproductive period, with the maximum inhibition of LH secretion at the final stages of gametogenesis [39]. In captivity, the natural pre-ovulatory decrease in dopaminergic inhibition is often disrupted by artificial conditions (temperature, water chemistry, spawning substrate, etc.), which leads to blocking of oocyte maturation and ovulation [24]. DA has a high capacity to block LH synthesis and release through disruption of intracellular GnRH signalling pathways [5], down-regulation of GnRH receptors [8], and inhibition of GnRH peptide synthesis [45] and release [46], as well as interference with other LH-stimulatory systems, e.g. the GABAergic system [33]. The discovery of DA inhibition meant a significant breakthrough in artificial reproduction of cyprinids, which do not undergo final oocyte maturation (FOM) and ovulation after administration of GnRHa alone [23,37,41]. A combined treatment with a GnRHa and a DA D2-receptor antagonist (DI) has been developed to eliminate the impact of DA on the reproductive axis and augment the stimulatory

* Corresponding author.
E-mail address: podhop01@vurh.jcu.cz (P. Podhorec).

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effect of the exogenous GnRHa [28,44]. Although cyprinids are considered typical of a fish group with decisively demonstrated DA inhibition of LH release and ovulation, it appears that this does not apply to tench (*Tinca tinca*). For successful hormonal induction of FOM and ovulation in female tench, a low dose of GnRHa is sufficient [10,17,32,35], contrary to what has been reported for all other cyprinids [31].

The present *in vivo* study was designed to verify whether in contrast to other cyprinid fish, dopamine is devoid of any capability to regulate LH release in the tench. Effectiveness of three GnRHa in inducing LH release and ovulation in tench was also evaluated.

2. Material and methods

2.1. Experimental animals

Mature female tench (924 ± 46 g body weight) were collected in the end of May, 2010 from a local fish producer and housed at the research fish facility at South Bohemia University in Vodnany. Prior to the experiment, females with soft and distended abdomens were catheterised to determine the stage of oocyte development. Females with $\geqslant$60% of the oocytes possessing migrating germinal vesicles were selected [9] and randomly divided into six groups (n = 10). Each group was kept separately in a well aerated flow-through plastic tank (700 L) and acclimated to conditions 21.8 ± 0.4 °C and a 16L:8D photoperiod.

2.2. Experimental design

All tested chemicals were dissolved in 0.9% NaCl solution and administered as a single intraperitoneal injection. The following treatments were applied after 2 days acclimatisation: (1) control treatment with 0.9% NaCl alone (Braun Melsungen AG); (2) [D-Ala^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1}, Bachem AG); (3) [D-Leu^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1}, Bachem AG); (4) [D-Arg^6, Pro^9, NEt]-sGnRH (10 μg kg^{-1}, Bachem AG); (5) water soluble DA D2 receptor antagonist metoclopramide (20 mg kg^{-1}, Sigma–Aldrich); and (6) combined treatment [D-Ala^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1}) + metoclopramide (20 mg kg^{-1}). Serial blood samples (400–500 μL) were collected by caudal venipuncture immediately prior to injection (0 h) and at 6, 12, 24, and 33 h post-injection. Plasma was separated by centrifugation and stored at −20 °C until determination of LH levels. Females were monitored for ovulation 24 h after injection and subsequently at 3 h intervals. Ovulation success (number of ovulated females within 48 h), latency period (time from injection to ovulation), fecundity index ([weight of stripped eggs/body weight before stripping] × 100), and LH concentrations were recorded.

Fish were anesthetized (0.03 mL L^{-1} clove oil) before manipulation. The experiment was conducted in accord to the principles of the Ethical Committee for the Protection of Research Animals at the University of South Bohemia.

2.3. LH determination

Plasma samples were assayed by heterologous ELISA previously established for common carp LH [16]. To validate the assay, serial dilutions of tench pituitary homogenate and plasma were made together with a common carp standard. All were found to be parallel with the sensitivity of the assay in the range 0.6–100 ng ml^{-1} and the intra- and inter-assay coefficients of variance at 5% and 9%, respectively.

2.4. Statistical analysis

For multiple comparisons among treatment groups, serum LH data were log-transformed prior to testing with one-way ANOVA followed by Tukey's HSD test. A χ^2 test was used to compare ovulation rates among the experimental groups. Significant differences were accepted with a P value of <0.05. All values are presented as means ± standard errors of the means (SEM).

3. Results

With the exception of the control group, all groups showed significantly increased LH concentrations 6 h post-treatment compared to pre-treatment basal levels (P < 0.05). The highest mean LH value at this sampling time was 45.42 ± 7.89 ng ml^{-1} recorded for the combined treatment, which was the highest concentration reached in this group over the course of the experimental period. Gonadotrophin-releasing hormone analogue treatments potentiated elevation of LH concentration with similar effectiveness; however the effect was significantly less pronounced than in the combined treatment group (P < 0.05) but significantly greater than the metoclopramide and control groups (P < 0.05). At 12, 24, and 33 h a progressive rise of all GnRHa treatments was observed, in contrast to a moderate decline in LH concentrations in the combined group, which however remained high. The highest mean LH concentration (47.45 ± 5.07 ng ml^{-1}) for any group over the entire experimental period was measured 33 h post-injection in the group treated with [D-Ala^6, Pro^9, NEthylamide]-mGnRH. Significant differences were detected in LH concentrations between the GnRHa groups and the combined treatment at 6 and 12 h (P < 0.05), all GnRHa containing treatments reached significantly higher LH levels than did the metoclopramide and control groups (P < 0.05). Metoclopramide treatment induced a slight LH increase differing from the control group at 6 and 12 h post injection (P < 0.05). Mean values for LH concentrations are presented in Fig. 1.

High ovulation success was obtained after combined (90%) and GnRHa (60–70%) treatments, with similar latency periods and fecundity indices, without significant differences between groups. No ovulation was recorded in the metoclopramide and control groups (Table 1).

4. Discussion

Hormonal induction of ovulation in tench is well-established in aquaculture production using a low dose of GnRHa alone [18,21]. To date there has been no investigation of DA inhibition on LH release in tench in spite of the apparent inhibitory tone in other cyprinid species. In cyprinids GnRHa alone generally does not induce an ovulatory response [1], with the exception of a minimal ovulation rate when administered at high doses [15,22]. The sensitivity of tench to hormonal treatment is similar to that of several marine fish species in which a low dose of GnRHa stimulates ovulation and which do not demonstrate dopaminergic control of LH release [2,20,34]. In some marine fish (e.g. Atlantic croaker; *Micropogonias undulatus*) the addition of a DA antagonist to hormonal treatment suppresses the effects of GnRHa on LH release while DA agonists potentiate it [7]. However, in tench the combined treatment composed of [D-Ala^6, Pro^9, NEt]-mGnRH and the DA D2-receptor antagonist metoclopramide triggered an almost immediate LH release peak with high LH concentrations throughout the experimental period inducing a high ovulation rate. This clear confirmation of DA inhibition in tench is surprising; especially in the light of GnRHa effectiveness in ovulation (60–70%) and the gradually elevated LH profile with values peaking close

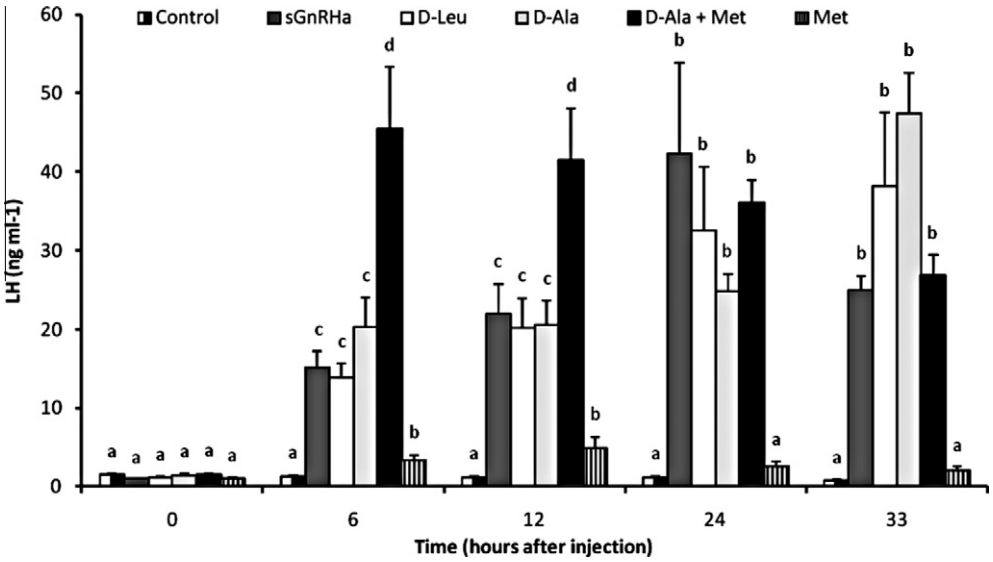

Fig. 1. Plasma levels (means ± SEM) of luteinizing hormone in female tench after 0.9% NaCl (control); [D-Arg^6, Pro^9, NEt]-sGnRH (sGnRHa); [D-Leu^6, Pro^9, NEt]-mGnRH (D-Leu); [D-Ala^6, Pro^9, NEt]-mGnRH (D-Ala); metoclopramide; and combined (D-Ala + Met) treatment. Different letter superscripts indicate significant differences within the same sampling time ($P < 0.05$).

Table 1
Ovulatory response of tench after GnRHas, combine, metoclopramide and saline solution treatment.

Group	Treatment	Female body weight (g)	Ovulation ratio	Latency period (h)	Fecundity index (%)
1	Physiological saline solution (0.9% NaCl)	977 ± 55	0/10^a		
2	Metoclopramide (20 mg kg^{-1})	930 ± 67	0/10^a		
3	[D-Arg^6, Pro^9, NEt]-sGnRH (10 μg kg^{-1})	945 ± 43	6/10^b	35.2 ± 2.1	6.1 ± 0.6
4	[D-Leu^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1})	885 ± 38	7/10^b	34.5 ± 1.8	5.3 ± 0.9
5	[D-Ala^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1})	915 ± 54	7/10^b	36.7 ± 1.6	6.6 ± 0.4
6	[D-Ala^6, Pro^9, NEt]-mGnRH (10 μg kg^{-1}) + metoclopramide (20 mg kg^{-1})	897 ± 45	9/10^b	34.9 ± 2.4	6.8 ± 1.1

Female body weight, latency period, and fecundity index are expressed as means ± SEM. Different letter superscripts indicate significant differences ($P < 0.05$).

to ovulation. These data are consistent with our earlier results showing that as little as 1 μg kg^{-1} of mGnRHa induced gradual elevation of LH with 63% of females ovulating [32]. Few studies have compared effects of GnRHa with DA antagonist versus GnRHa alone treatments in tench, although Pinillos et al. [30] reported an enhanced effect of GnRHa alone on secretion of 17,20β-dihydroxy-4-pregnen-3-one, 17,20α-dihydroxy-4-pregnen-3-one, and testosterone and ovulation success compared to a combined treatment. Although in the present study approximately 70% of females ovulated after GnRHa alone treatment (10 μg kg^{-1}) which is the mean ovulation rate obtained after combined treatment by Kujawa et al. [19], our and other studies have more commonly reported ovulation rates of 80% or more with GnRHa treatment [17,32]. The relatively lower LH mean concentrations in compare with our previous study [32] were detected in the current trial, although LH concentrations in some ovulated females exceeded detection limits of the assay (>100 ng ml^{-1}). The combined and GnRHa treatments were both associated with high ovulation rates, but GnRHa treatment led to a gradual LH release, while an instant LH surge was seen following the combined treatment. Peter et al. [27] suggested that the ovulatory response in goldfish (*Carassius auratus*) is dependent on both the magnitude of the LH concentration and the rate of increase in circulating LH levels. Immediate LH surge after the combined treatment was more effective in inducing ovulation than GnRHa-stimulated gradual increase despite comparable LH concentrations [4,37]. In tench we cannot confirm this, as an application of GnRHa alone induced completion of germinal vesicle migration and ovulation in the majority of females after a gradual increase of LH levels. Continuously elevated LH levels and high ovulation rates induced by a combined treatment have been detected also in common carp [9,22,23] and grass carp [12].

The most widely used GnRHas in tench artificial reproduction were tested for induction of pre-ovulatory LH surge and ovulation. However no difference in the effectiveness of [D-Arg^6, Pro^9, NEt]-sGnRH compared to [D-Ala^6, Pro^9, NEt]-mGnRH or [D-Leu^6, Pro^9, NEt]-mGnRH were detected. A difference was found in LH release kinetic stimulated by sGnRHa and by mGnRHas. Salmon GnRHa induced LH release peaking 24 h post-treatment in contrast to mGnRHa induced LH release peaking 33 h post-treatment. A possible explanation for earlier LH peaking after sGnRHa treatment in compare to mGnRHas treatments could be the hypophysiotropic function of native sGnRH in goldfish based on sGnRH high abundance in the pituitary [40]. Superiority of [D-Ala^6, Pro^9, NEt]-mGnRH previously shown in sea bass (*Dicentrarchus labrax*) [11], was not confirmed for the tench. All tested GnRHa induced similar LH concentrations and ovulation success and can be equally recommended for hormonal therapies in tench.

The great diversity of the family Cyprinidae has attracted scientific attention and resulted in many comprehensive phylogenetic studies [3,13,36]. However, despite considerable effort using morphological characters and molecular methods, the systematic position of the genus *Tinca* is still unclear [14]. Chen and Mayden [6] proposed that *Tinca* is a member of the terminal clade of cyprinids, the monophyly of which is highly supported. In conclusion, it

seems that tench may be representative of ancient cyprinids with several primitive family features conserved including neuroendocrine regulation of LH.

This study represents the first report of dopaminergic control of LH release in tench, which showed no difference in ovulation rates after administration of GnRHa alone and in combination with a DA inhibitor.

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References

[1] M. Arabaci, M. Sari, Induction of ovulation in endemic pearl mullet (*Chalcalburnus tarichi*), living in the highly alkaline Lake Van, using GnRHa ([D-Ser(tBu)^6, Pro^9-Net]-GnRH) combined with haloperidol, Aquaculture 238 (2004) 529–535.
[2] D.L. Berlinsky, W. King, T.I.J. Smith, R.D. Hamilton, J. Holloway, C.V. Sullivan, Induced ovulation of Southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants, J. World Aquacult. 27 (1996) 143–152.
[3] J. Briolay, N. Galtier, R.M. Brito, Y. Bouvet, Molecular phylogeny of Cyprinidae inferred from cytochrome b DNA sequences, Mol. Phylogenet. Evol. 9 (1998) 100–108.
[4] J.P. Chang, R.E. Peter, Effects of pimozide and des Gly^{10}, [D-Ala^6] luteinizing hormone-releasing hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration, and ovulation in female goldfish, *Carassius auratus*, Gen. Comp. Endocrinol. 52 (1983) 30–37.
[5] J.P. Chang, R.M. Jobin, A.O. Wong, Intracellular mechanisms mediating gonadotropin and growth hormone release in the goldfish, *Carassius auratus*, Fish Physiol. Biochem. 11 (1993) 25–33.
[6] W.J. Chen, R.L. Mayden, Molecular systematics of the Cyprinoidea (Teleostei: Cypriniformes), the world's largest clade of freshwater fishes: further evidence from six nuclear genes, Mol. Phylogenet. Evol. 52 (2009) 544–549.
[7] P.A. Copeland, P. Thomas, Control of gonadotropin-release in the Atlantic croaker (*Micropogonias undulatus*) – Evidence for lack of dopaminergic inhibition, Gen. Comp. Endocrinol. 74 (1989) 474–483.
[8] R. De Leeuw, H.R. Habibi, C.S. Nahorniak, R.E. Peter, Dopaminergic regulation of pituitary gonadotrophin-releasing hormone receptor activity in the goldfish (*Carassius auratus*), J. Endocrinol. 121 (1989) 239–247.
[9] S. Drori, M. Ofir, B. Levavi-Sivan, Z. Yaron, Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature, Aquaculture 119 (1994) 393–407.
[10] J. Fernandez San Juan, Tench (*Tinca tinca* L.) propagation in Spain. Induced spawning and larval development, Pol. Arch. Hydrobiol. 42 (1995) 63–67.
[11] M.A. Fornies, M. Carrillo, E. Mananos, L.A. Sorbera, Y. Zohar, S. Zanuy, Relative potencies of the forms of GnRH and their analogs on LH release in sea bass, J. Fish Biol. 63 (2003) 73–89.
[12] F. Glasser, T. Mikolajczyk, B. Jalabert, J.F. Baroiller, B. Breton, Temperature effects along the reproductive axis during spawning induction of grass carp (*Ctenopharyngodon idella*), Gen. Comp. Endocrinol. 136 (2004) 171–179.
[13] B. Hanfling, R. Brandl, Phylogenetics of European cyprinids: insights from allozymes, J. Fish Biol. 57 (2000) 265–276.
[14] S.P. He, R.L. Mayden, X.Z. Wang, W. Wang, K.L. Tang, W.J. Chen, Y.Y. Chen, Molecular phylogenetics of the family Cyprinidae (Actinopterygii: Cypriniformes) as evidenced by sequence variation in the first intron of S7 ribosomal protein-coding gene: further evidence from a nuclear gene of the systematic chaos in the family, Mol. Phylogenet. Evol. 46 (2008) 818–829.
[15] F.P. Heyrati, H. Mostafavi, H. Toloee, S. Dorafshan, Induced spawning of kutum, *Rutilus frisii kutum* (Kamenskii, 1901) using (D-Ala^6, Pro^9-NEt) GnRHa combined with domperidone, Aquaculture 265 (2007) 288–293.
[16] O. Kah, A. Pontet, J. Nunez Rodriguez, A. Calas, B. Breton, Development of an enzyme-linked immunosorbent assay for goldfish gonadotropin, Biol. Reprod. 41 (1989) 68–73.
[17] J. Kouril, T. Barth, J. Hamackova, M. Flegel, Induced ovulation in tench (*Tinca tinca* L.) by various LH-RH synthetic analogues: effect of site of administration and temperature, Aquaculture 54 (1986) 37–44.
[18] J. Kouril, Hormonally induced spawning of tench *Tinca tinca* (L.) females. (A review), Pol. Arch. Hydrobiol. 45 (1998) 415–433.
[19] R. Kujawa, D. Kucharczyk, A. Mamcarz, D. Zarski, K. Targonska, Artificial spawning of common tench *Tinca tinca* (Linnaeus, 1758), obtained from wild and domestic stocks, Aquacult. Int., doi: 10.1007/s10499-010-9366-z.
[20] B. Levavi-Sivan, R. Vaiman, O. Sachs, I. Tzchori, Spawning induction and hormonal levels during final oocyte maturation in the silver perch (*Bidyanus bidyanus*), Aquaculture 229 (2004) 419–431.
[21] O. Linhart, R. Billard, Biology of gametes and artificial reproduction in common tench (*Tinca tinca* L.). A review, Pol. Arch. Hydrobiol. 42 (1995) 37–56.
[22] T. Mikolajczyk, J. Chyb, M. Sokolowska-Mikolajczyk, W.J. Enright, P. Epler, M. Filipiak, B. Breton, Attempts to induce an LH surge and ovulation in common carp (*Cyprinus carpio* L.) by differential application of a potent GnRH analogue, Azagly-nafarelin, under laboratory, commercial hatchery, and natural conditions, Aquaculture 223 (2003) 141–157.
[23] T. Mikolajczyk, J. Chyb, P. Szczerbik, M. Sokolowska-Mikolajczyk, P. Epler, W.J. Enright, M. Filipiak, B. Breton, Evaluation of the potency of azagly-nafarelin (GnRH analogue), administered in combination with different formulations of pimozide, on LH secretion, ovulation and egg quality in common carp (*Cyprinus carpio* L.) under laboratory, commercial hatchery and natural conditions, Aquaculture (2004) 447–460.
[24] C.C. Mylonas, A. Fostier, S. Zanuy, Broodstock management and hormonal manipulations of fish reproduction, Gen. Comp. Endocrinol. 165 (2010) 516–534.
[25] Y. Nagahama, M. Yamashita, Regulation of oocyte maturation in fish, Dev. Growth Diff. 50 (2008) 195–219.
[26] W. Park, C.H. Lee, C.S. Lee, D.J. Kim, J.H. Kim, C.S. Tamaru, Y.C. Sohn, Effects of a gonadotropin-releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg quality in freshwater-exposed female chum salmon (*Oncorhynchus keta*), Aquaculture 271 (2007) 488–497.
[27] R.E. Peter, J.P. Chang, C.S. Nahorniak, R.J. Omeljaniuk, M. Sokolowska, S.H. Shih, R. Billard, Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish, Recent Prog. Horm. Res. 42 (1986) 513–548.
[28] R.E. Peter, H.R. Lin, G. Van der Kraak, Induced ovulation and spawning of cultured fresh-water fish in China – Advances in application of GnRH analogs and dopamine antagonists, Aquaculture 74 (1988) 1–10.
[29] R.E. Peter, K.L. Yu, Neuroendocrine regulation of ovulation in fishes: basic and applied aspects, Rev. Fish Biol. Fish. 7 (1997) 173–197.
[30] M.L. Pinillos, A.I. Guijarro, M.J. Delgado, P.C. Hubbard, A.V.M. Canario, A.P. Scott, Production, release and olfactory detection of sex steroids by the tench (*Tinca tinca* L.), Fish Physiol. Biochem. 26 (2002) 197–210.
[31] P. Podhorec, J. Kouril, Induction of final oocyte maturation in Cyprinidae fish by hypothalamic factors: a review, Vet. Med. 54 (2009) 97–110.
[32] P. Podhorec, M. Socha, M. Sokolowska-Mikolajczyk, B. Drozd, T. Policar, V. Stejskal, J. Kouril, Effective dose of mGnRHa for induction of ovulation in tench (*Tinca tinca* L.), Aquaculture 319 (2011) 184–187.
[33] J.T. Popesku, C.J. Martyniuk, J. Mennigen, H. Xiong, D. Zhang, X. Xia, A.R. Cossins, V.L. Trudeau, The goldfish (*Carassius auratus*) as a model for neuroendocrine signaling, Mol. Cell. Endocrinol. 293 (2008) 43–56.
[34] F. Prat, S. Zanuy, M. Carrillo, Effect of gonadotropin-releasing hormone analogue, GnRHa) and pimozide on plasma levels of sex steroids and ovarian development in sea bass (*Dicentrarchus labrax* L.), Aquaculture 198 (2001) 325–338.
[35] R. Rodríguez, J.D. Celada, M. Saez-Royuela, J.M. Carral, A. Aquilera, P.M. Melendre, Artificial reproduction in 1-year-old tench (*Tinca tinca* L.), J. Appl. Ichthyol. 20 (2004) 542–544.
[36] K. Saitoh, T. Sado, M.H. Doosey, H.L. Bart, J.G. Inoue, M. Nishida, R.L. Mayden, M. Miya, Evidence from mitochondrial genomics supports the lower Mesozoic of South Asia as the time and place of basal divergence of cypriniform fishes (Actinopterygii: Ostariophysi), Zool. J. Linn. Soc. 161 (2011) 633–662.
[37] M. Sokolowska, R.E. Peter, C.S. Nahorniak, C.H. Pan, J.P. Chang, L.W. Crim, C. Weil, Induction of ovulation in goldfish, *Carasius auratus*, by pimozide and analogs of LH-RH, Aquaculture 36 (1984) 71–83.
[38] M. Sokolowska, R.E. Peter, C.S. Nahorniak, The effects of different doses of pimozide and [D-Ala^6, Pro^9-N ethylamide]-LHRH (LHRHa) on gonadotropin-release and ovulation in female goldfish, Can. J. Zool. -Rev. Can. Zool. 63 (1985) 1252–1256.
[39] M. Sokolowska, R.E. Peter, C.S. Nahorniak, J.P. Chang, Seasonal effects of pimozide and des Gly^{10} [D-Ala^6] LH-RH ethylamide on gonadotrophin secretion in goldfish, Gen. Comp. Endocrinol. 57 (1985) 472–479.
[40] C. Steven, N. Lehnen, K. Kight, S. Ijiri, U. Klenke, W.A. Harris, Y. Zohar, Molecular characterization of the GnRH system in zebrafish (*Danio rerio*): cloning of chicken GnRH-II, adult brain expression patterns and pituitary content of salmon GnRH and chicken GnRH-II, Gen. Comp. Endocrinol. 133 (2003) 27–37.
[41] T. Szabo, C. Medgyasszay, L. Horvath, Ovulation induction in nase (*Chondrostoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone, Aquaculture 203 (2002) 389–395.
[42] V.L. Trudeau, Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, *Carassius auratus*, Rev. Reprod. 2 (1997) 55–68.
[43] B. Vidal, C. Pasqualini, N. Le Belle, M.C. Holland, M. Sbaihi, P. Vernier, Y. Zohar, S. Dufour, Dopamine inhibits luteinizing hormone synthesis and release in the juvenile European eel: a neuroendocrine lock for the onset of puberty, Biol. Reprod. 71 (2004) 1491–1500.
[44] Z. Yaron, A. Bogomolnaya, S. Drori, I. Biton, J. Aizen, Z. Kulikovsky, B. Levavi-Sivan, Spawning induction in the carp: Past experience and future prospects – A review, Isr. J. Aquac. -Bamidgeh 61 (2009) 5–26.

[45] K.L. Yu, R.E. Peter, Dopaminergic regulation of brain gonadotropin releasing hormone in male goldfish during spawning behaviour, Neuroendocrinology 52 (1990) 276–283.
[46] K.L. Yu, R.E. Peter, Adrenergic and dopaminergic regulation of gonadotropin-releasing hormone release from goldfish preoptic-anterior hypothalamus and pituitary in vitro, Gen. Comp. Endocrinol. 85 (1992) 138–146.
[47] Y. Zohar, A. Goren, M. Fridkin, E. Elhanati, Y. Koch, Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata*. 2. Cleavage of native salmon GnRH, mammalian LHRH, and their analogs in the pituitary, kidney, and liver, Gen. Comp. Endocrinol. 79 (1990) 306–319.