

# Spawning induction in Kutum, *Rutilus frisii kutum* (Kamensky), with different hormones: Analysis of hormone profiles and induced spawning success

Bahram Falahatkar, Samaneh Poursaeid, Hadi Ershad Langroudi, Iraj Efatpanah, Bahman Meknatkhah, Mehdi Rahmati

Received – 02 March 2013/Accepted – 13 June 2013. Published online: 31 December 2013; ©Inland Fisheries Institute in Olsztyn, Poland  
Citation: Falahatkar B., Poursaeid S., Langroudi H.E., Efatpanah I., Meknatkhah B., Rahmati M. 2013 – Spawning induction in Kutum, *Rutilus frisii kutum* (Kamensky), with different hormones: Analysis of hormone profiles and induced spawning success – Arch. Pol. Fish. 21: 271-281.

**Abstract.** The objective of this study was to evaluate the effectiveness of different hormones on spawning success and sex steroid changes in Caspian Kutum, *Rutilus frisii kutum*. Groups of five fish were injected intramuscularly as follows: 2 mg kg<sup>-1</sup> common carp pituitary extract (CPE) as the positive control, 100 IU kg<sup>-1</sup> human chorionic gonadotropin (hCG), 2 µg kg<sup>-1</sup> luteinizing hormone releasing hormone analog (LHRHa<sub>2</sub>), 10 µg kg<sup>-1</sup> synthetic analogue gonadoliberin (sGnRHa), 0.2 mL kg<sup>-1</sup> Ovaprim which is a complex of salmon GnRH analogue with a dopamine receptor antagonist (domperidone), a combination of 2 µg kg<sup>-1</sup> LHRHa<sub>2</sub> and 100 IU kg<sup>-1</sup> hCG, a complex of 10 µg kg<sup>-1</sup> sGnRHa and 100 hCG IU kg<sup>-1</sup>, and saline solution as the negative control. Blood samples were taken simultaneously with injections and after ovulation. The results

showed that no fish ovulated in the negative control group. In the CPE treatment group, 4 of 5 fish ovulated. In the other experimental groups; no response was observed in the hCG group, while the females injected with sGnRHa-alone, LHRHa<sub>2</sub>-alone, and sGnRH+hCG had low spawning percentages. Of the hormones tested, the combination of sGnRHa+domperidone was the most effective spawning inducer and the greatest spawning success was obtained. Significant decreases after injection were observed in plasma concentrations of 17β estradiol, 17α-hydroxy progesterone, and progesterone in all treatments, while plasma testosterone concentrations increased significantly. This study revealed that a combined sGnRHa and dopamine antagonist can improve the effectiveness of artificial reproduction of Kutum. Since the combination of sGnRHa and domperidone is purer than CPE and has the most effective in inducing ovulation, it is preferred for inducing spawning in this species.

**Keywords:** Kutum, spawning induction, common carp pituitary, human chorionic gonadotropin, GnRH analogues, domperidone, sex steroids

B. Falahatkar [✉],  
Fisheries Department, Faculty of Natural Resources  
University of Guilan  
Sowmeh Sara, P.O. Box 1144, Guilan, Iran  
Tel./Fax: +98-182-322 3599; +98-182-322 3600  
e-mail: falahatkar@guilan.ac.ir

S. Poursaeid  
Young Researchers Club and Elite, Rasht Branch  
Islamic Azad University, Rasht, Iran

H.E. Langroudi  
Fisheries Department, Faculty of Natural Resources  
Islamic Azad University, Lahijan Branch, Lahijan, Guilan, Iran

I. Efatpanah, B. Meknatkhah, M. Rahmati  
Dr. Yousefpour Fish Hatchery Center, Siahkal, Guilan, Iran

## Introduction

The Kutum, *Rutilus frisii kutum* (Kamensky), a member of the cyprinid family, is an endemic species to the Caspian Sea. This species is distributed

from the estuary of the Volga River up to Astrakhan Bay, but the main population is confined to the south-western part of the sea (Abdoli 1999). This species is anadromous, inhabits the sea in small schools, and migrates to shallow river waters for spawning from March to April. A synchronous group pattern of ovarian development has been reported in this fish (Sharyati 1993), and, therefore, eggs are released once after ovulation. Kutum is considered one of the most valuable commercial species in the Caspian Sea region so that it comprises more than 70% of the total catch in the southern part of this sea. Stocks of valuable species are threatened by several factors including heavy fishing pressure, increasing water pollution, and habitat alteration and destruction.

In order to rehabilitate the stock of this important species, brood fish are caught in the sea and estuaries for controlled reproduction, and over 200 million Kutum fingerlings (1-2 g) are produced and released by the Iranian Fisheries Organization into the Caspian Sea annually. However, most of the broodstock caught for this aim are not fully mature, so it is necessary to transfer the unripe fish to hatcheries for hormonal induction. Most studies on this species have focused on reproduction status in the wild, especially during spawning migrations (Farabi et al. 2007, Shafiei Sabet et al. 2009, Heidari et al. 2010), while less research has been done on hormonal stimulation for breeding programs (Paykan Heyrati and Dorafshan 2006, Paykan Heyrati et al. 2007, Ahmadnezhad et al. 2012). Conventionally, ovulation is induced in captive Kutum using common carp pituitary extract (CPE) or a synthetic analogue of GnRH (sGnRH<sub>a</sub>). However, the use of CPE possess some problems such as high costs, unpredictable results because of great variability in pituitary luteinizing hormone (LH) content, the negative impact on the physiology of the treated fish, and the risk of pathogen transmission from donor fishes to the recipient broodstock (Drori et al. 1994, Zohar and Mylonas 2001). In addition to these problems, some Kutum brood did not respond to CPE injections. Alternative methods for inducing spawning in many teleosts include applications of human chorionic gonadotropin (hCG) and hypothalamic hormones with

or without dopamine antagonists (DA) (Zohar 1989, Haddy and Pankhurst 2000, Zohar and Mylonas 2001, Levavi-Sivan et al. 2004, Kagawa et al. 2009, Kiewek-Martinez et al. 2010). Different forms of gonadotropin releasing hormone agonists (GnRH<sub>a</sub>) stimulate the secretion of endogenous gonadotropins in teleost fish (Van Der Kraak et al. 1998). In addition, the ability of externally applied GnRH<sub>a</sub> to increase LH secretion in teleosts is also believed to be under the strong endogenous inhibitory impact of dopamine, and, consequently, the fish fail to ovulate (Yaron 1995). Such an inhibitory impact has been well documented in goldfish, *Carassius auratus* (L.) (Peter et al. 1991); common carp, *Cyprinus carpio* L. (Billard 1990); European eel, *Anguilla anguilla* (L.) (Dufour et al. 1988); and catfish, *Clarias gariepinus* (Burchell) (De Leeuw et al. 1986). Spawning is usually induced in these species only after using a combination of GnRH<sub>a</sub> and DAs. The importance of DA inhibitor regulation varies among teleost species from weak to strong. Therefore, it is necessary to examine the response of each species to DA under local conditions.

Many studies have shown that different reproduction parameters including latency time, working fecundity, number of eggs, the fertilizability of eggs, and sex steroids are affected by different hormonal manipulations (Szabó et al. 2002, Mylonas et al. 2003, Arabaci et al. 2004, Aizen et al. 2005, Barrero et al. 2008). Previous studies revealed that hormonal manipulations produced differential effects on sex steroid hormone levels (Haddy and Pankhurst 2000, Aizen et al. 2005, Barrero et al. 2008, Pham et al. 2010). Barrero et al. (2008) reported short-term elevations of testosterone (T) and 17 $\beta$  estradiol (E<sub>2</sub>) in adult catfish, *Ictalurus punctatus* (Rafinesque) in responding to hormonal treatments (CPE and LHRH<sub>a</sub>). Pham et al. (2010) reported that different exogenous hormones (LHRH<sub>a</sub>, CPE and hCG) decreased plasma T levels, but increased plasma concentrations of E<sub>2</sub> and progesterone (P) in female Waigieu seaperch, *Psammoperca waigiensis* (Cuvier).

No information is available regarding the best kind and dosage of hormone for the artificial reproduction of Kutum. No data is available regarding

hormonal changes during final maturation and spawning after hormonal manipulation. Therefore, the objective of the current study was to determine ovulatory response of Kutum to various agents (CPE, hCG, sGnRHa and LHRHa<sub>2</sub> alone or in combination with hCG, and the combination of sGnRHa with DA which is used commonly for induction of spawning), and also to examine the endocrine response of the fish to different treatments by measuring plasma concentrations of T, E<sub>2</sub>, 17 $\alpha$ -hydroxy progesterone (17-P), and P.

## Materials and methods

### Fish stocks and fish maintenance

The experiments were conducted at the Dr. Yousefpour Fish Hatchery Center in Siahkal, Guilan, Iran in April 2009. The Kutum broodstocks were caught in shallow, inshore habitats at water depths of 0-10 m on the Anzali coast of Guilan, Iran using a commercial beach seine during the spawning migration in April. The fish were then transported to the hatchery by truck in containers equipped with aeration systems. At the hatchery, 40 three-year-old female fish with mean body weights of 965.8 g  $\pm$  54.7 (mean  $\pm$  S.E) were selected based on the softness of their abdomens. Before injection, these fish were weighed individually, marked by placing tags on the dorsal fin, and divided randomly into eight experimental groups.

The tagged fish were held in an outdoor circular fiberglass tank (diameter 2.33 m and depth 45 cm, volume 1.92 m<sup>3</sup>) under a natural photoperiod of 14L:10D. The tanks were supplied with flow-through water at 20.2 l min<sup>-1</sup> throughout the experiment. Mean water temperature, dissolved oxygen, and oxygen saturation were 13.7°C  $\pm$  1.3, 9.3 mg l<sup>-1</sup>  $\pm$  0.8, and 89.7%  $\pm$  6.8, respectively. No significant differences were observed in the age or fish body weights among the experimental groups.

### Hormones and drugs

CPE was obtained from our hatchery-matured common carp, dried, and stored until use. Liquid Ovaprim (1.0 mL), containing 20  $\mu$ g of (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRHa and 10 mg of domperidone, was obtained from Biomeda MTC Animal Health (Ontario, Canada). Human chorionic gonadotropin (hCG, Corion; LG Life Sciences, India) was purchased from a local medical store. Luteinizing hormone releasing hormone analog (LHRHa<sub>2</sub>) was purchased from Ningbo Sansheng Pharmaceutical Co. (China). (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRHa (sGnRHa) was obtained from Bachem Chemicals (Switzerland).

### Experimental design

The effect of handling stress on the broodstock was minimized by anesthetizing the fish with clove powder (200 mg l<sup>-1</sup>). Groups of five fish were injected with the following agents: CPE (2 mg kg<sup>-1</sup> body weight (BW) as the positive control according to hatchery experience); hCG (100 IU kg<sup>-1</sup> BW); sGnRHa (10  $\mu$ g kg<sup>-1</sup> BW); LHRHa<sub>2</sub> (2  $\mu$ g kg<sup>-1</sup> BW); Ovaprim (comprising 20  $\mu$ g sGnRHa+10 mg Domperidone; 0.2 ml kg<sup>-1</sup> BW); LHRHa<sub>2</sub>+hCG (2  $\mu$ g kg<sup>-1</sup> BW+100 IU kg<sup>-1</sup> BW); sGnRHa+hCG (10  $\mu$ g kg<sup>-1</sup> BW+100 IU kg<sup>-1</sup> BW); saline solution (0.9% NaCl as the negative control). The hormones and dosages used in this study were selected according to previous studies on this species, similar teleosts (Zohar and Mylonas 2001, Szabó et al. 2002, Dorafshan and Paykan Heyrati 2006, Paykan Heyrati et al. 2007) and preliminary experiments in Iranian government hatcheries. All agents, with the exception of Ovaprim that was obtained in solvent form, were dissolved in a carrier (0.9% NaCl) and injected once into the base of the pectoral fin at a volume of 1 ml kg<sup>-1</sup> BW.

After injection, the fish were examined for signs of maturation and ovulation exhibited as abdominal swelling and determined by applying slight manual pressure to the abdomen 12 h post-injection and

subsequently every 2 h for 72 h. When ovulation occurred, the eggs were obtained from the fish by hand-stripping and artificially fertilized with milt collected from two non-injected males using the dry method. Sperm quality and quantity was evaluated under a light microscope before the milt was added to the egg batches; this ensured that the sperm used in the study had motility exceeding 80% and had the proper density. The sperm:egg ratio was approximately 1:100 by volume for fertilization. The fertilized eggs of the individual broodstock females were rinsed with fresh water for 45 min to eliminate egg stickiness, and then they were transferred to Zuger jars. In order to determine working fecundity, the eggs stripped from each brood female were weighed separately to the nearest 0.01 g. The fertilization rate (%) was calculated by counting the live and dead eggs under a dissecting microscope 48 h after fertilization when the eggs were at the gastrulation stage. Spawning success was calculated using the number of females that ovulated after injection divided by the total number of females injected. The latency period, which is the time between injection and ovulation, was also determined according to Drori et al. (1994).

Blood was drawn from the females before hormonal manipulations and immediately after ovulation to evaluate whether the hormone treatment affected sex steroid levels. Blood samples were also taken 72 h after injection from the fish that did not reach the spawning stage. Blood samples of approximately 2-ml were drawn from the caudal vasculature of anesthetized fish using a 5-ml heparinized syringe equipped with a 25-G needle. The plasma was separated by centrifugation for 10 min at  $1500 \times g$  and stored at  $-70^{\circ}\text{C}$  until the sex steroid analysis.

### Samples and data analysis

Sex steroid concentrations (T,  $E_2$ , 17-P, P) were determined with specific radioimmunoassay (RIA) using kits by Immunotech (Marseille, France) according to Kubokawa et al. (1999) with some slight modification. Inter-assay variations for T,  $E_2$ , 17-P, and P were 14.8, 12.1, 6.5, and 11.8%, respectively.

Specificity tests were performed according to our previous studies on this species. All samples were measured in duplicate.

Spawning success was analyzed with the Chi-square test. The normality of the data and the homogeneity of variance were checked with Kolmogorov-Smirnov and Levene's tests, respectively. The effects of the various agents on spawning parameters and sex steroids were analyzed with analysis of variance (ANOVA), and significant differences among means were evaluated with Tukey's post hoc test. The paired sample t-test was used to detect differences in sex steroid circulating levels before injection and after ovulation in each group. Statistical analysis was performed with SPSS (Version 15, SPSS Chicago, IL, USA), and differences of  $P < 0.05$  were considered to be statistically significant. The data are presented as mean  $\pm$  standard error (SE).

### Results

Chi-square test indicated significant difference in spawning success among various agents ( $P < 0.001$ ), and the results of spawning responses to the various agents are presented in Table 1. No fish ovulated in the group receiving only the saline solution (negative control). In  $2 \text{ mg kg}^{-1}$  BW CPE-treated fish, which was the positive control group, 4 of 5 fish ovulated (80%). In the other treatments; no fish ovulated in the group receiving  $100 \text{ IU kg}^{-1}$  BW hCG. Females treated with sGnRHa-alone ( $10 \mu\text{g kg}^{-1}$  BW), LHRHa<sub>2</sub>-alone ( $2 \mu\text{g kg}^{-1}$  BW), and sGnRHa+hCG ( $10 \mu\text{g kg}^{-1}$  BW+ $100 \text{ IU kg}^{-1}$  BW) showed low spawning percentages (20%). Treatment with a combination of LHRHa<sub>2</sub>+hCG yielded only 60% spawning, but working fecundity and hatching rates were the highest. The combination of LHRHa<sub>2</sub>+hCG was more efficient in inducing ovulation than the combination sGnRHa+hCG treatment (20%). Injecting the female brood fish with  $0.2 \text{ ml Ovaprim kg}^{-1}$  BW resulted in the highest spawning percentage ( $P < 0.05$ ).

**Table 1**

Effect of various hormonal treatments on spawning parameters of Kutum, *Rutilus frisii kutum* (n =5 for each treatment, mean  $\pm$  SE). CPE – carp pituitary extract; sGnRH<sub>a</sub> – (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRH<sub>a</sub>; LHRHa<sub>2</sub> – luteinizing hormone releasing hormone analog; hCG – human chorionic gonadotropin; Ovaprim – 20  $\mu$ g sGnRH<sub>a</sub>+10 mg domperidone

| Treatment                                 | Dosage   | Female body weight (g) | Spawning success (%) | Latency period (h) | Number of eggs g <sup>-1</sup> | Working fecundity ( $\times 10^3$ ) | Fertilization rate (%) |
|---|--|------------------------|----------------------|--------------------|--------------------------------|-------------------------------------|------------------------|
| Saline (0.9% NaCl)                        | -  | 878 $\pm$ 137.1        | 0                    | nd                 | nd                             | nd                                  | nd                     |
| CPE                                       | 2 mg kg <sup>-1</sup>                                | 878 $\pm$ 104.5        | 80                   | 19.2 $\pm$ 0.3     | 312.9 $\pm$ 10.3               | 33.3 $\pm$ 5.7                      | 78 $\pm$ 3.7           |
| sGnRH <sub>a</sub>                        | 10 $\mu$ g kg <sup>-1</sup>                          | 998 $\pm$ 194          | 20                   | 22.3               | 242.9                          | 63.1                                | 81.8 $\pm$ 2.6         |
| LHRHa <sub>2</sub>                        | 2 $\mu$ g kg <sup>-1</sup>                           | 940 $\pm$ 179          | 20                   | 22.1               | 275                            | 11                                  | 79.3 $\pm$ 4.6         |
| hCG                                       | 100 IU kg <sup>-1</sup>                              | 972 $\pm$ 172.9        | 0                    | nd                 | nd                             | nd                                  | nd                     |
| Ovaprim (sGnRH <sub>a</sub> +Domperidone) | 0.2 ml kg <sup>-1</sup>                              | 914 $\pm$ 153.6        | 100*                 | 21.2 $\pm$ 0.9     | 260.8 $\pm$ 18.8               | 32.3 $\pm$ 8.1                      | 76.4 $\pm$ 3.1         |
| sGnRH <sub>a</sub> +hCG                   | 10 $\mu$ g kg <sup>-1</sup> +100 IU kg <sup>-1</sup> | 1206 $\pm$ 170         | 20                   | 22.6               | 251.2                          | 27.6                                | 80.3 $\pm$ 4.7         |
| LHRHa <sub>2</sub> +hCG                   | 2 $\mu$ g kg <sup>-1</sup> +100 IU kg <sup>-1</sup>  | 940 $\pm$ 179          | 60                   | 21.2 $\pm$ 1       | 276.7 $\pm$ 7.3                | 65.8 $\pm$ 16.7                     | 84.1 $\pm$ 4.1         |

nd – values not determined because of a lack of ovulation, \*Significantly greater in comparison to all other treatments ( $P < 0.05$ , Chi-square test)

The number of eggs per each gram of egg weight and working fecundity were not significantly different among the groups ( $P > 0.05$ ). Spawning occurred 19-22 h after the injection. No significant difference was observed in latency period among fish treated with either of the hormones; however, the shortest latency period was observed in CPE-treated females

(19.2 h  $\pm$  0.3), followed by the group receiving combination of Ovaprim (21.2 h  $\pm$  0.9). The percentage of fertilized eggs ranged from 76 to 84%. There were no significant differences in fertilization rates among the groups ( $P > 0.05$ ).

The concentration of plasma T elevated significantly after hormonal induction in all groups (t-test,

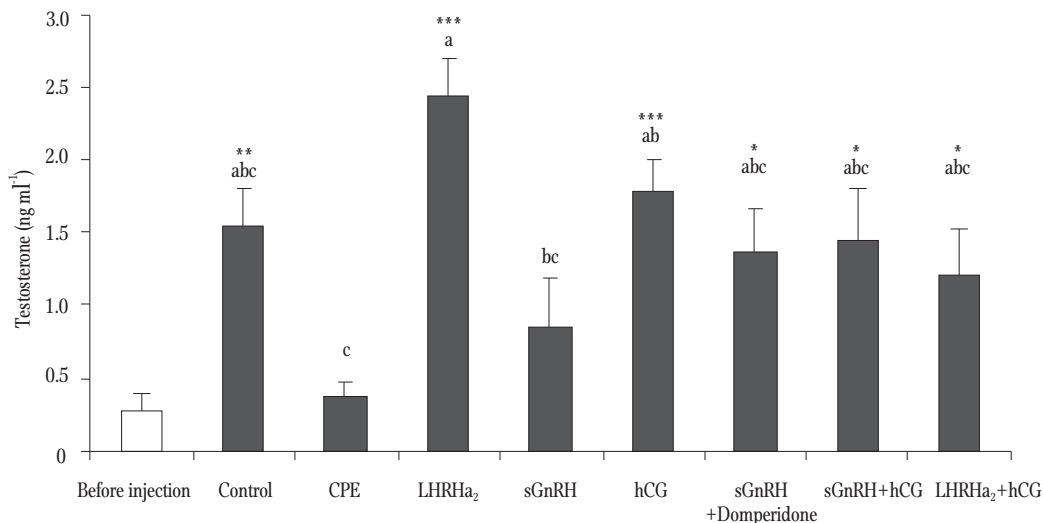


Figure 1. Effect of different treatments on plasma testosterone levels in female Kutum, *Rutilus frisii kutum*. N = 8 fish for hormone determinations before injection. Means with different letter subscript notation are significantly different ( $P < 0.05$ ). Statistically significant differences before injection are marked with asterisks (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ). Groups with different letter indexes differ significantly statistically. CPE – carp pituitary extract; sGnRH<sub>a</sub> – (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRH<sub>a</sub>; LHRHa<sub>2</sub> – luteinizing hormone releasing hormone analog; hCG – human chorionic gonadotropin; Ovaprim – 20  $\mu$ g sGnRH<sub>a</sub>+10 mg domperidone.



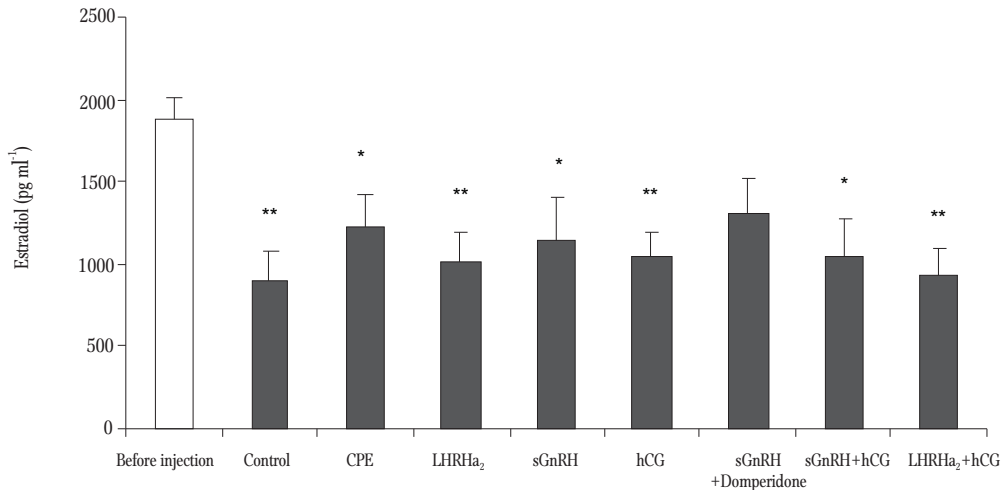


Figure 2. Effect of different treatments on plasma estradiol levels in female Kutum, *Rutilus frisii kutum*. N = 8 fish for hormone determinations before injection. Statistically significant differences before injection are marked with asterisks (\* P < 0.05; \*\* P < 0.01). CPE – carp pituitary extract; sGnRHa – (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRHa; LHRHa<sub>2</sub> – luteinizing hormone releasing hormone analog; hCG – human chorionic gonadotropin; Ovaprim – 20 µg sGnRHa+10 mg domperidone.

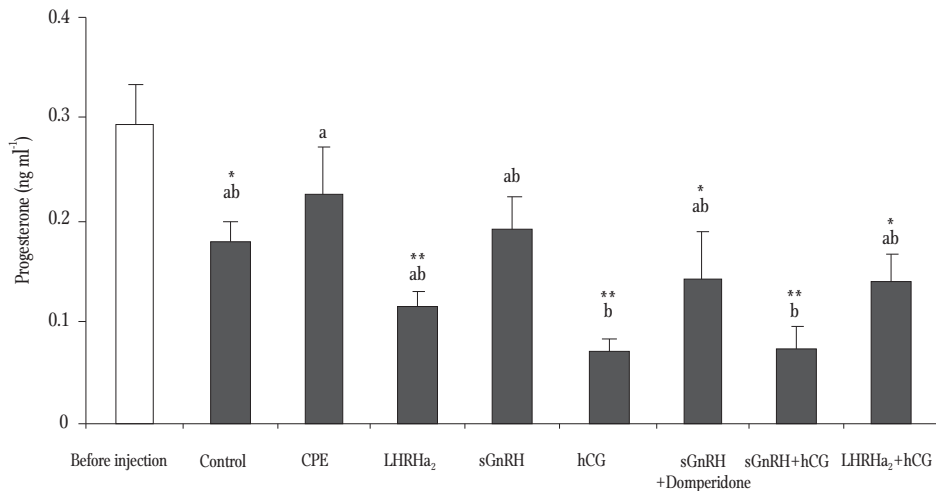


Figure 3. Effect of different treatments on plasma progesterone levels in female Kutum, *Rutilus frisii kutum*. N = 8 fish for hormone determinations before injection. Means with different letter subscript notation are significantly different (P < 0.05). Statistically significant differences before injection are marked with asterisks (\* P < 0.05; \*\* P < 0.01). CPE – carp pituitary extract; sGnRHa – (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRHa; LHRHa<sub>2</sub> – luteinizing hormone releasing hormone analog; hCG – human chorionic gonadotropin; Ovaprim – 20 µg sGnRHa+10 mg domperidone.

P < 0.05) except in sGnRHa-alone and CPE (t-test, P > 0.05; Fig. 1). The mean plasma T levels were significantly different among hormonal treatments (ANOVA, P = 0.001; Fig. 1). The levels of E<sub>2</sub> decreased significantly after injection in all treatments (t-test, P < 0.05), and no significant differences were observed in the concentration of plasma E<sub>2</sub> among treatments (ANOVA, P = 0.837; Fig. 2). Following injection, plasma P levels were significantly lower than those before injection in all groups (t-test, P < 0.05), but the mean P level did not

decrease significantly in fish treated with sGnRHa-alone and CPE (t-test, P > 0.05; Fig. 3). Concentrations of plasma P were significantly lower in female fish treated with hCG and sGnRHa+hCG-treated than those that received CPE (ANOVA, P = 0.006; Fig. 3). Similarly, a significant decrease was observed in 17-P levels after hormonal manipulation (t-test, P < 0.05), but no significant differences were observed among fish treated with various agents (ANOVA, P = 0.611; Fig. 4).

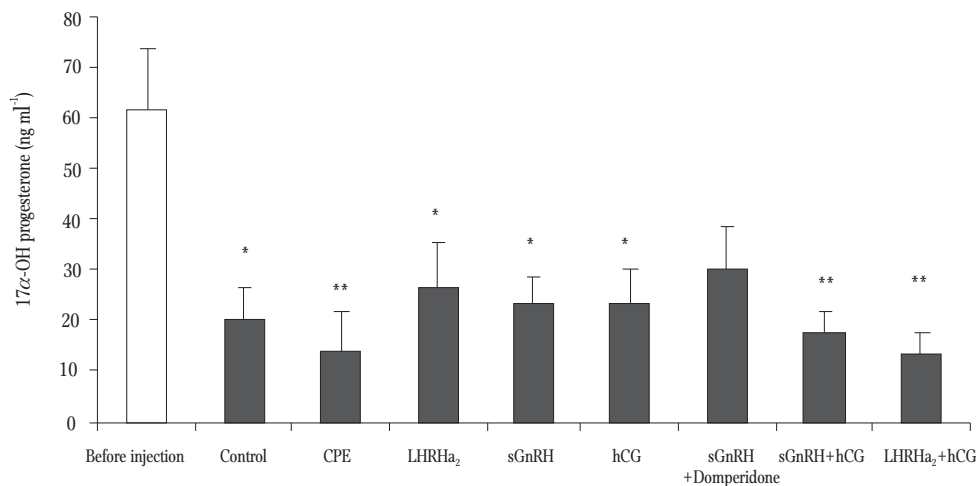


Figure 4. Effects of different treatments on plasma 17 $\alpha$ -OH progesterone levels in female Kutum, *Rutilus frisii kutum*. N = 8 fish for hormone determinations before injection. Statistically significant differences before injection are marked with asterisks (\* P < 0.05; \*\* P < 0.01). CPE – carp pituitary extract; sGnRH<sub>a</sub> – (D-Arg<sup>6</sup>, Pro<sup>9</sup>-Net)-salmon GnRH<sub>a</sub>; LHRHa<sub>2</sub> – luteinizing hormone releasing hormone analog; hCG – human chorionic gonadotropin; Ovaprim – 20  $\mu$ g sGnRH<sub>a</sub>+10 mg domperidone.

## Discussion

No fish ovulated in the control group, indicating that hormonal induction is necessary for Kutum broods in captivity condition. The results of this study demonstrate that a single injection of sGnRH<sub>a</sub> combined with domperidone as the DA is a successful hormonal treatment to induce Kutum spawning. Inducing spawning with a superactive analog of GnRH together with DA has been documented in most teleostean fish including goldfish (Peter et al. 1991); common carp (Billard 1990); European eel (Dufour et al. 1988); catfish (De Leeuw et al. 1986); American shad, *Alosa sapidissima* (Wilson) (Mylonas et al. 1995); hybrid tilapia *Oreochromis niloticus*  $\times$  *Oreochromis aureus* (Levavi-Sivan et al. 1995); grey mullet, *Mugil cephalus* L. (Aizen et al. 2005); Senegal sole, *Solea senegalensis* Kaup (Agulleiro et al. 2006); pond loach *Misgurnus anguillicaudatus* (Cantor) (Wang et al. 2009); asp, *Aspius aspius* (L.) (Targońska et al. 2010; 2011); African catfish (Sharaf 2012); and European grayling, *Thymallus thymallus* (L.) (Szmyt et al. 2012).

The current study revealed that CPE had lower potency in spawning success (80%) compared to the sGnRH<sub>a</sub>+domperidone treatment (100%). The main difference between the CPE and sGnRH<sub>a</sub> treatments

is that sGnRH<sub>a</sub> stimulates a higher level of hypothalamus-pituitary-gonad axis (HPG) than does CPE. This could also be attributed to the greater effectiveness, purity, and exact dosage of synthetic hormones (Yaron and Levavi-Sivan 2006). The application of 2 mg kg<sup>-1</sup> BW CPE is a conventional method for inducing spawning in Kutum based on local hatchery experience. Nevertheless, the current study demonstrated that the dose applied is not effective in inducing complete spawning in Kutum. Studies on other cyprinids demonstrated that higher doses of CPE (3-6 mg kg<sup>-1</sup> BW) administered in two injections increase the success of artificial spawning (Billard 1990, Szabo et al. 2002, Arabaci et al. 2004).

In this study, the dosage of 100 IU of hCG kg<sup>-1</sup> BW had no stimulatory effect in broodstock spawning. In many teleosts, spawning and oocyte maturation were successfully induced with a single injection of a much higher dose than 100 IU hCG kg<sup>-1</sup> BW (Haddy and Pankhurst 2000, García-Alonso and Vizziano 2004, Denson et al. 2007). Therefore, further experiments are required to determine the effect of higher doses of hCG for inducing ovulation in Kutum.

Injections of both LHRHa<sub>2</sub> and sGnRH<sub>a</sub> alone were not effective in inducing spawning in Kutum. The low ovulation in broods treated with sGnRH<sub>a</sub> alone may be due to inhibitory effect of dopamine on

LH secretion. On the other hand, the ineffectiveness of LHRHa<sub>2</sub> and sGnRHa after a single treatment could be related to the short half-life of these agents and/or rapid degradation activity by endopeptidases located in the pituitary, liver, and kidney of the injected fish (Zohar et al. 1990, Zohar and Mylonas 2001). In contrast, the application of LHRHa<sub>2</sub> combined with hCG resulted in higher ovulation rates, which were probably caused by the synchronic effects of LHRHa<sub>2</sub> and hCG on the pituitary gland and gonads, respectively, as has previously been described by Zohar and Mylonas (2001). Therefore, this combination of hormone not only induced a surge of gonadotropin release from the pituitary, but also acted directly at the level of the gonad. Thereby, the failure of hCG-treated fish to spawn might have been caused by lowered LH secretion or a lack thereof.

Considering that spawning was induced by other hormonal treatments in this study, it seems that the inhibitory effects of dopamine on GtH release in Kutum is less predominant compared to other cyprinids such as Chinese loach, *Paramisgurnus dabryanus* Dabry de Thiersant (Lin et al. 1989); common carp (Drori et al. 1994); nase, *Chondrostoma nasus* (L.) (Szabo et al. 2002); koi (Arabaci et al. 2004); and asp (Targońska et al. 2010).

The mean latency period recorded in the current study after hormonal treatment was higher than that reported for asp (Lin et al. 1991), ornamental common carp (Arabaci et al. 2004), and tench, *Tinca tinca* (L.) (Kujawa et al. 2011), but it was lower than in previous studies on Kutum (Dorafshan and Paykan Heyrati 2006, Paykan Heyrati et al. 2007). These discrepancies are probably related to species differences, physiological conditions, various hormonal treatments, and water temperature. However, the CPE-treated fish have a shorter latency time than the groups injected with the combination of sGnRHa+domperidone or LHRHa<sub>2</sub>+hCG. Similar results were obtained not only with other cyprinids (Billard 1990, Brzuska 2005, Kucharczyk et al. 2005, Paykan Heyrati et al. 2007, Krejszeff et al. 2008, Targońska et al. 2011), but also with percids (Kucharczyk et al. 1996, 1998, Szczerbowski et al.

2009). One explanation for the shorter latency time in the CPE-treated group could be linked with the direct activity of the CPE at the level of the gonads which elicited a quicker response, while GnRH analogues act at a higher level of the HPG axis (Donaldson and Hunter 1983).

Fertilization rates and working fecundity were not significantly different among the treatments, suggesting that the induction of ovulation with various agents did not have any adverse effect on egg viability. Similar results were reported in common carp (Drori et al. 1994, Kulikovskiy et al. 1996).

In the current study, significant increases in plasma T concentrations were observed in all treatments groups except that of CPE. A similar increase in T levels by maturing oocytes has also been demonstrated in the goldfish (Kobayashi et al. 1987), milkfish, *Chanos chanos* (Forsskål) (Tamaru and Lee 1987), and striped trumpeter, *Latris lineata* (Forster) (Pankhurst 1997). The precise role of this androgen is unclear in females, and further studies are needed to determine the relationship between this steroid and the reproductive cycle of female fish. High levels of plasma T in Kutum can be explained by the decrease of aromatase activity and/or its role as a pheromone. However, further studies are required to examine the pheromonal function of this androgen. The concentration of plasma E<sub>2</sub> declined after hormone injection. Similar results has been observed in other teleosts in where the highest of E<sub>2</sub> levels were measured before ovulation and then decreased during spawning (Kubokawa et al. 1999, Mylonas and Zohar 2001, Rinchard et al. 2001). The low plasma concentrations of E<sub>2</sub> following hormonal treatment and associated with high T values suggest that vitellogenesis has been completed and the females were ready to spawn. On the other hand, the low concentrations of E<sub>2</sub> exert negative feedback on the pituitary and result in an ovulatory gonadotropin peak (Richard et al. 2001). Furthermore, the low concentrations of P and 17-P after injection suggest that these two steroids were probably consumed in the thecal layer, and then were converted to a maturation-inducing steroid in the underneath granulosa by 20 $\beta$ -hydroxysteroid dehydrogenase (Kanamori et al.



1988). Differences in the levels of sex steroids among the experimental groups could be related to differences in gonadal stages.

In conclusion, the current results indicated that the use of the proper agent in a precise dose could induce Kutum to ovulate, while applying the wrong agents for inducing spawning resulted in ovarian atresia and reproduction failure. The results also showed changes in steroid concentrations before injection and after spawning and among the treatments in T and P levels, which could be caused by the functions of different inducing hormones on the HPG axis. Since the combination of sGnRHa with domperidone is cheaper and more available than CPE and can produce a higher number of ovulating females, this treatment is suggested for inducing ovulation in Kutum broodstocks.

**Acknowledgments.** We would like to thank K. Eslamloo and S.R. Akhavan for their kind help us during the sampling. Part of this study was supported by the Islamic Azad University through a grant made to H. Ershad Langroudi.

**Authors contributions.** BF designed and performed the experiment; SP analyzed the data, HEL supported the project financially and supplied the fish; IE, BM, and MR performed the experiment; BF and SP wrote the paper.

## References

- Abdoli A. 1999 – The Inland Water Fishes of Iran – Natural and Wild Life Museum of Iran, Tehran, Iran: 198-200.
- Agulleiro M.J., Anguis V., Cañavate J.P., Martínez-Rodríguez G., Mylonas C.C., Cerdà J. 2006 – Induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using different administration methods for gonadotropin-releasing hormone agonist – *Aquaculture* 257: 511-524.
- Ahmadnezhad M., Oryan Sh., Hosseinzadeh Sahafi H., Khara H., Sattari M. 2012 – Effects of LHRH-A<sub>2</sub> and chlorpromazine (dopamine antagonists) on inducing spawning in Caspian Kutum, *Rutilus frisii kutum*, from the southwest of the Caspian Sea – *Caspian J. Env. Sci.* 10: 33-42.
- Arabaci M., Çağırğan H., Sari M. 2004 – Induction of ovulation in ornamental common carp (Koi, *Cyprinus carpio* L.) using LHRHa ([D-Ser (tBu)<sup>6</sup>, Pro<sup>9</sup>-Net]-LHRH) combined with haloperidol and carp pituitary extract – *Aquac. Res.* 35: 10-14.
- Aizen J., Meiria I., Tzchoria I., Levavi-Sivan B., Rosenfeld H. 2005 – Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition – *Gen. Comp. Endocrinol.* 142: 212-221.
- Barrero M., Small B.C., D'abramo L.R., Waldbieser G.C., Hanson L.A., Kelly A.M. 2008 – Effect of carp pituitary extract and luteinizing hormone releasing analog hormone on reproductive indices and spawning of 3-year-old channel catfish – *N. Am. J. Aquacult.* 70: 138-146.
- Billard R. 1990 – The major carps and other cyprinids – In: *World Animal Sciences CIIX, Production of Aquatic Animals (Fishes)* (Ed.) C.E. Nash, Elsevier Science Publication: 21-55.
- Brzuska E. 2005 – Artificial spawning of carp (*Cyprinus carpio* L.), differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate, Ovopel or Dagin – *Aquac. Res.* 36: 1015-1025.
- De Leeuw R., Goos H.J.T.H., Van Oordt P.G.W.J. 1986 – The dopaminergic inhibition of the gonadotropin releasing hormone-induced gonadotropin release: an in vitro study with fragments and cell suspensions from pituitaries of African catfish, *Clarias gariepinus* (Burchell) – *Gen. Comp. Endocrinol.* 63: 171-177.
- Denson M.R., Jenkins W.E., Berlinsky D.L., Smith T.I.J. 2007 – A comparison of human chorionic gonadotropin and luteinizing hormone releasing hormone analogue for ovulation induction in black sea bass *Centropristis striata* (Linnaeus, 1758) – *Aquac. Res.* 38: 918-925.
- Donaldson E.M., Hunter G.A. 1983 – Induced final maturation, ovulation and spermiation in cultured fish – In: *Fish Physiology, Vol 9. Part B. Reproduction* (Eds) W.S. Hoar, D.J. Randall, E. M. Donaldson, Academic Press, Orlando, Florida: 351-403.
- Dorafshan S., Paykan Heyrati F. 2006 – Spawning induction in Kutum *Rutilus frisii kutum* (Kamenskii, 1901) using carp pituitary extract or GnRH analogue combined with metoclopramide – *Aquac. Res.* 37: 751-755.
- Drori S., Ofir M., Levavi-Sivan B., Yaron Z. 1994 – Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with methoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature – *Aquaculture* 119: 393-407.
- Dufour S., Lopez E., Le Menn F., Le Belle N., Baloche S., Fontaine Y.A. 1988 – Stimulation of gonadotropin release and of ovarian development, by the administration of a gonadoliberin agonist and of dopamine

- antagonists, in female silver eel pretreated with estradiol – Gen. Comp. Endocrinol. 70: 20-30.
- Farabi S.M.V., Khoshbavar Rostami H., Ghaneei Tehrani M., Ghiasi M., Azari A., Behrouzi S., Mosavi H., Firozkandian S., Habibi F., Zahedi Tabarestani A., Mallaei, H., Mahdavi Amiri A., Aghlmandi F., Binaei M. 2007 – The investigation of status brood stocks and releasing fingerlings of *Rutilus frisii kutum* (Kaminski, 1901) in the south of Caspian Sea (Mazandaran province, 2004) – Paj. Sz. 74: 156-166.
- Garcia-Alonso J., Vizziano D. 2004 – Induction of oocyte maturation in the whitch croaker *Micropogonias furnieri* (Pisces: Sciaenidae) by human chorionic gonadotropin – Braz. J. Biol. 64: 73-80.
- Haddy J.A., Pankhurst N.W. 2000 – The efficacy of exogenous hormones in stimulating changes in plasma steroids and ovulation in wild black bream *Acanthopagrus butcheri* is improved by treatment at capture – Aquaculture 191: 351-366.
- Heidari B., Roozati S.A., Yavari. L. 2010 – Changes in plasma levels of steroid hormones during oocyte development of Caspian Kutum (*Rutilus frisii kutum*, Kamensky, 1901) – Anim. Reprod. 7: 373-381.
- Kagawa H., Kasuga Y., Adachi J., Nishi A., Hashimoto H., Imaizumi H., Kaji S. 2009 – Effects of continuous administration of human chorionic gonadotropin, salmon pituitary extract, and gonadotropin-releasing hormone using osmotic pumps on induction of sexual maturation in male Japanese eel, *Anguilla japonica* – Aquaculture 296: 117-122.
- Kanamori A., Adachi S., Nagahama Y. 1988 – Developmental changes in steroidogenic response of ovarian follicles of amago salmon (*Oncorhynchus rhodurus*) to chum salmon gonadotropin during oogenesis – Gen. Comp. Endocrinol. 72: 13-24.
- Kiewek-Martinez M., Gracia-Lopez V., Carrillo-Estevez M. 2010 – Comparison of the effects of HCG and LHRHa on the induction of ovulation of wild and captive leopard grouper, *Mycteroperca rosacea* – J. World Aquacult. Soc. 41: 733-745.
- Kobayashi M., Aida K., Hanyu I. 1987 – Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish – Gen. Comp. Endocrinol. 67: 24-32.
- Krejszeff S., Kucharczyk D., Kupren K., Targońska K., Mamcarz A., Kujawa R., Kaczkowski Z., Ratajski S. 2008 – Reproduction of chub, *Leuciscus cephalus* L., under controlled conditions – Aquac. Res. 39: 907-912.
- Kubokawa K., Watanabe T., Yoshioka M., Iwata M. 1999 – Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season – Aquaculture 172: 335-349.
- Kucharczyk D., Kujawa R., Mamcarz A., Skrzypczak A., Wyszomirska E. 1996 – Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG – Aquac. Res. 27: 847-852.
- Kucharczyk D., Kujawa R., Mamcarz A., Skrzypczak A., Wyszomirska E. 1998 – Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide – Aquac. Res. 29: 131-136.
- Kucharczyk D., Kujawa R., Mamcarz A., Targońska-Dietrich K., Wyszomirska E., Glogowski J., Babiak I., Szabó T. 2005 – Induced spawning in bream (*Abramis brama* L.) using pellets containing GnRH – Czech J. Anim. Sci. 50: 89-95.
- Kujawa R., Kucharczyk D., Mamcarz A., Zarski D., Targońska K. 2011 – Artificial spawning of common tench *Tinca tinca* (Linnaeus, 1758), obtained from wild and domestic stocks – Aquacult. Int. 19: 513-521.
- Kulikovsky Z., Martin F.J.B., Yaron Z. 1996 – A comparison of two spawning inducing agent for common carp – Isr. J. Aquac. Bamidgeh. 48: 108-111.
- Levavi-Sivan B., Ophir M., Yaron Z. 1995 – Possible sites of dopaminergic inhibition of gonadotropin release from the pituitary of a teleost fish, tilapia – Mol. Cell. Endocrinol. 109: 87-95.
- Levavi-Sivan B., Vaiman R., Sachs O., Tzchori I. 2004 – Spawning induction and hormonal levels during final oocyte maturation in the silver perch (*Bidyanus bidyanus*) – Aquaculture 229: 419-433.
- Lin H.R., Peng C., Van Der Kraak G., Peter R.E. 1989 – Dopamine inhibits gonadotropins secretion in the Chinese loach – Fish. Physiol. Biochem. 6: 285-288.
- Lin H.R., Zhou X.J., Van Der Kraak G., Peter R.E. 1991 – Effects of gonadotropin-releasing hormone agonists and dopamine antagonists on gonadotropin secretion and ovulation in Chinese loach, *Paramisgurnus dabryanus* – Aquaculture 95: 139-147.
- Mylonas C., Zohar Y. 2001 – Endocrine regulation and artificial induction of oocyte maturation and spermiation in basses of the genus *Morone* – Aquaculture 202: 205-220.
- Mylonas C., Zohar Y., Richardson B.M., Minkinen S.P. 1995 – Induced spawning of wild American Shad *Alosa sapidissima* using sustained administration of gonadotropin-releasing hormone analog (GnRHa) – J. World Aquacult. Soc. 26: 240-251.
- Pankhurst N.W. 1997 – In vitro steroid production by isolated ovarian follicles of the striped trumpeter *Latris lineata* (Latrididae) – J. Fish Biol. 51: 669-685.
- Paykan Heyrati F., Mostafavi H., Toloe H., Dorafshan S. 2007 – Induced spawning of Kutum, *Rutilus frisii kutum* (Kamenskii, 1901) using (D-Ala<sup>6</sup>, Pro<sup>9</sup>-NEt) GnRHa combined with domperidone – Aquaculture 265: 288-293.

- Peter R.E., Trudeau V.L., Slolely B.D. 1991 – Brain regulation of reproduction in teleosts – Bull. Inst. Zool. Acad. Sin. Monogr. 16: 89-118.
- Pham H.Q., Nguyen A.T., Nguyen M.D., Arukwe A. 2010 – Sex steroid levels, oocyte maturation and spawning performance in Waigieu seaperch (*Psammoperca waigiensis*) exposed to thyroxin, human chorionic gonadotropin, luteinizing hormone releasing hormone and carp pituitary extract – Comp. Biochem. Physiol. 155(A): 223-230.
- Rinchar J., Dabrowski K., Ottobre J. 2001 – Sex steroids in plasma of lake whitefish *Coregonus clupeaformis* during spawning in Lake Erie – Comp. Biochem. Physiol. 129(C): 65-74.
- Shafiei Sabet S., Imanpoor M.R., Aminian Fatideh B., Gorgin S. 2009 – Study on sexual maturity and levels of gonad steroid hormones in female kutum *Rutilus frisii kutum* (Kamenskii, 1901) during spawning season from river Sefid-Rood of the southern Caspian sea – J. Cell Anim. Biol. 3: 208-215.
- Sharaf S.M. 2012 – Effect of GnRH<sub>a</sub>, pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish *Clarias gariepinus* – Theriogenology 77: 1709-1716.
- Sharyati A. 1993 – Fishes of the Caspian Sea Region – Iranian Fisheries Company, Iran: 77-79.
- Szabó T., Medgyasszay C., Horvath L. 2002 – Ovulation induction in nase (*Chondrosoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone – Aquaculture 203: 389-395.
- Szczerbowski A., Kucharczyk D., Mamcarz A., Łuczyński M.J., Targońska K., Kujawa R. 2009 – Artificial off-season spawning of Eurasian perch *Perca fluviatilis* L – Arch. Pol. Fish. 17: 95-98.
- Szmyt M., Dobosz S., Kucharczyk D., Grudniewska J., Lejk A.M. 2012 – Impact of selected hormonal agents on the effectiveness of controlled reproduction of cultivated female European grayling – Arch. Pol. Fish. 20: 289-297.
- Tamaru C.S., Lee C.S. 1987 – Testosterone and estradiol-17 $\beta$  profile of female milkfish, *Chanos chanos* undergoing chronic LHRHa and 17 $\alpha$ -methyltestosterone therapy – In: Reproductive Physiology of Fish (Eds) D.R. Idler, L.W. Crim, J.M. Walsh, Memorial University of Newfoundland, 218 p.
- Targońska K., Kucharczyk D., Kujawa R., Mamcarz A., Żarski D. 2010 – Controlled reproduction of asp, *Aspius aspius* (L.) using luteinizing hormone releasing hormone (LHRH) analogues with dopamine inhibitors – Aquaculture 306: 407-410.
- Targońska K., Kucharczyk D., Żarski D., Mamcarz A., Falahatkar B. 2011 – Optimization of artificial reproduction of asp, *Aspius aspius* (L.) under controlled conditions – Pol. J. Nat. Sci. 26: 151-157.
- Van Der Kraak G., Chang J.P., Janz D.M. 1998 – Reproduction – In: The Physiology of Fishes (Ed.) D.H. Evans, CRC Press, Boca Raton: 465-488.
- Wang Y., Hu M., Wang W., Liu X., Cheung S.G., Shin P.K.S., Song L. 2009 – Effects of GnRH<sub>a</sub> (D-Ala<sub>6</sub>, Pro<sub>9</sub>-NET) combined with domperidone on ovulation induction in wild loach *Misgurnus anguillicaudatus* – Aquaculture 291: 136-139.
- Yaron Z. 1995 – Endocrine control of gametogenesis and spawning induction in the carp – Aquaculture 129: 49-73.
- Yaron Z., Levavi-Sivan B. 2006 – Reproduction – In: The physiology of Fishes Vol 3 (Eds) D.H. Evans, J.B. Claiborne, CRC Press, Boca Raton: 343-388.
- Zohar Y. 1989 – Fish reproduction: Its physiology and artificial manipulation – In: Fish Culture in Warm Water Systems: Problems and Trends (Eds) M. Shilo, S. Sarig, CRC Press, Boca Raton, Florida: 65-119.
- Zohar Y., Goren A., Fridkin M., Elhanati E., Koch Y. 1990 – Degradation of gonadotropin-releasing hormones in the gilthead seabream *Sparus aurata*: II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver – Gen. Comp. Endocrinol. 79: 306-331.
- Zohar Y., Mylonas C.C. 2001 – Endocrine manipulations of spawning in cultured fish: from hormones to genes – Aquaculture 197: 99-13.