Aquacult Int (2011) 19:513–521
DOI 10.1007/s10499-010-9366-z

Artificial spawning of common tench *Tinca tinca* (Linnaeus, 1758), obtained from wild and domestic stocks

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Received: 26 February 2010 / Accepted: 4 August 2010 / Published online: 24 August 2010

Abstract The study encompasses three reproduction seasons. Tench spawners caught during the spawning season originated from carp ponds (domestic stock) and a lake (wild stock). Fish were reproduced under controlled conditions after hormonal stimulation with GnRHa-containing pellets combined with metoclopramide (Ovopel) or carp pituitary homogenate (CPH). As a result of hormonal stimulation, eggs were obtained from a larger number of females originating from the lake (71.7%) than those originating from the pond (58.3%), although no other statistical differences were found. A similar relationship was recorded for the spermatozoa motilities (range from 72 to 76%). The obtained results indicate that both investigated reservoirs are suitable for tench broodstock management due to the fact that synchronization of ovulation among different stocks is easy to achieve. For this purpose, among the tested spawning agents, Ovopel could be recommended as being slightly more effective.

Keywords Artificial spawning · Fish culture · Tench · Domestic and wild population

Introduction

Cyprinid aquaculture is focused mainly on carps such as common *Cyprinus carpio* (L.), crucian *Carassius carassius* (L.), bighead *Hypophthalmichthys nobilis* (Richardson) or grass *Ctenopharyngodon idella* (Valenciennes). Effective production of these species has become possible mainly by the artificial reproduction under controlled conditions (Horvath et al. 1997; Brzuska 2005; Kucharczyk et al. 2008). Properly developed techniques of propagation and controlled rearing have allowed rationalization of commercial production. Simultaneously, in recent years interest in common tench, *Tinca tinca* (L.), culturing has increased significantly (Gela et al. 2006; Wang et al. 2006). This fish species has been recently treated as the most promising species in freshwater aquaculture. Common tench has been reared for many years as a supplementary fish species in carp ponds, causing

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better use of forage and, as a consequence, better productivity per unit of area (Regadera 1995; Steffens 1995).

Tench production has been focused on two main aspects: production of commercial fish in fish farms and production of material for restocking natural waters. The market demand for tench stocking material is increasing from year to year (Mamcarz and Skrzypczak 2006; Skrzypczak and Mamcarz 2006). On the other hand, older stocking material is often recommended for the stocking of lakes where, despite appropriate nutritional conditions, there are no natural spawning grounds to ensure an appropriate level of reproduction of that species. There is much published data available concerning the artificial reproduction of common tench under controlled conditions, but it mainly describes the reproduction of cultured stocks (e.g. Kouril et al. 1986; Linhart and Billard 1995; Gela et al. 2003; Rodina et al. 2004; Mamcarz et al. 2006). It could be caused by many difficulties with capturing the fish from natural reservoirs. Among others, it is necessary to provide a proper method of catching, which has a major and direct impact on the spawners' condition. Another important aspect in the reproduction of cyprinids under controlled conditions is the right (not too early (Krejszeff et al. 2008) or not too late (Targońska et al. 2010)) time of catching because the moment of carrying out the controlled reproduction procedures can be a major case of spawning failure. It is very often caused by unfavorable thermal conditions, which could be found in both ponds and open waters (Targońska et al. 2010). The sustainable culture procedures could become dependent on natural populations due to proper gene management (Harada et al. 1998; Vrijenhoek 1998). However, synchronization of ovulation of wild and cultured stocks is difficult (Kucharczyk et al. 1997b, c, 2005; Krejszeff et al. 2009). Therefore, investigations regarding this aspect could have an important effect on practical implementations. Support with hormonal injections of carp or tench pituitary homogenates (Gela et al. 2006; Kucharczyk et al. 2007) as well as analogs of GnRH (Linhart et al. 1995; Linhart and Billard 1995; Kouřil 1998; Kujawa et al. 2009) plays the dominating role in artificial tench reproduction. Successful reproduction of wild stocks of tench under controlled conditions is determined first of all by the stage of maturity of spawners and, which is linked to it, the time and place of obtaining them from natural waters. But there is lack of studies comparing between the artificial reproduction of wild and cultured tench spawners under controlled conditions. For this purpose, long-term studies aiming at checking the influence of spawners' origin and stimulators used on the effects of tench reproduction under controlled conditions are required.

Materials and methods

Spawners' origin and manipulations

Tench spawners were obtained during three consecutive spawning seasons (in mid-June) from Lake Maróz, a mesotrophic lake with a total area of 332 ha, maximal depth of 41 m and average depth of 11.9 m) and from earthen ponds (total area 2.5 ha, average depth 1.5 m), both located in northern Poland. The fish were collected during their natural spawning season. The fish from the lake and the pond were obtained using commercial fishing traps when the water temperature reached 19°C. The spawners were transported to the hatchery of the Department of Lake and River Fisheries in Olsztyn in plastic bags with oxygen and anesthetic medium (2-phenoxyethanol at dose 0.15 cm^3 dm^{-3}). In total, 180 selected individuals (120 females and 60 males) with an average weight of 0.3–1.4 kg were obtained during 3 years. For each year, 20 females (weights ranging from 0.61 to 0.93 kg)

and 10 males (weights ranging from 0.43 to 0.56 kg) were selected from both locations. The mean weights of the fish originating from the lake and the pond did not differ statistically (*t*-test, $P < 0.05$). The research was conducted during the three following seasons.

After transport, the fish were treated with a preventive bath in a solution of NaCl (0.5%) and were then placed in tanks of 1,000 dm^3 capacity with aeration and thermal control (Kujawa et al. 1999). The maximum load of spawners was 25 kg m^{-3}. The dissolved oxygen level ranged between 6.0 and 8.4 ppm. The photoperiod was 16 h of light and 8 h of darkness per day (16L:8D). The fish were acclimated for 2 days at a water temperature of 22°C. The oocytes samples (minimum 50–70 oocytes in each sample) from females were collected in vivo with a catheter shortly before first injection. Sampled oocytes were placed in Serra's solution for clarification of the cytoplasm (prepared with 70% ethanol, 40% formaldehyde and acetic acid in proportions of 6:3:1, respectively). After 5 min, the position of the oocyte nucleus was determined using a 4-stage scale. The oocyte samples ($n = 40$) were photographed using a stereoscopic microscope (Leica MZ 12.5, Germany). The oocyte diameter measurements were taken, and the percentages of different oocyte sizes were calculated. All measurements (±0.01 mm) were taken using ProgRes® Capture Pro 2.5 (Jenoptik, Germany) software.

Hormonal stimulation and spawning procedure

After acclimation, the fish were randomly divided (among stocks) into two groups for which different hormonal stimulation was provided. The first group was treated with Ovopel (mammalian GnRH analog [D-Ala^6, Pro^9Net-mGnRH] with metoclopramide—dopamine antagonist) and the second one with CPH (carp pituitary homogenate). The first injection of Ovopel was given with 0.1 and the second with 1 pellet kg^{-1} of spawner's bodyweight. In case of carp pituitary, CPH injections at 0.4 and 3.6 mg kg^{-1} of spawner bodyweight were administered. The males (in groups and stock) were treated with the same doses of hormonal preparations as the females. The time between injections was 12 h. The injections were administered intraperitoneally under the ventral fin. After the resolving injection, the temperature of water was increased from 22 to 24°C. The fish were checked 9 h after the resolving injection and then every 2 h. During all manipulations, the fish were anaesthetized with 2-phenoxyethanol at 0.5 ml dm^3 of water. The same stimulation schedule and remaining procedure were applied during each year of the experiment. During this procedure, latency time and the number of ovulated females were recorded.

Gamete management

Eggs were obtained, placed into dry plastic vessels and fertilized with pooled semen obtained from a minimum of 6 males from the same stock as females. Sperm was collected in the plastic syringes calibrated at 0.1 cm^3. Only those sperm that showed a motility of more than 80% of spermatozoa was used for egg fertilization. The sperm motility was assessed subjectively under a light microscope (500×) in a 0.5% NaCl solution (Glogowski et al. 1997). Two egg samples (250–300 eggs each) from each female were mixed with 0.05 ml of pooled milt sample. The eggs were incubated at 24°C on a Petri dish at a water temperature of 24°C, which was found to be optimal for tench embryonic

development (Kokurewicz 1970). The survival of embryos at the eyed-egg-stage (2nd day of incubation) was assessed.

Statistical analysis

Statistical differences between groups regarding ovulation rate and embryos survival were analyzed using a *t*-test ($P < 0.05$). Regression analysis was performed to determine the correlation between latency time and percentage of ovulation.

Results

Oocytes taken from females before applying hormonal induction were different both in size and developmental stages. The oocytes were classified into 3 groups, i.e. small, medium and large. The diameter intervals of each group were 0.18–0.22, 0.48–0.57, and 0.95–1.01 mm, respectively. But in all females, minimum 15% of oocytes were "large" size. There was no relationship between the percentage of the largest oocyte diameter and female ovulation and percentage of obtained egg weight. The mass of eggs was between 4.2 and 15.6% of female BW. There were no differences between tench populations or yearly fluctuations (Table 1).

Better effects expressed by percentage of ovulation were obtained in both stocks by the application of Ovopel (average 72%) than CPH (average 58%). In all cases, the percentage of ovulation was higher in the wild stock than in the cultured one (Table 2). It is noteworthy that the results obtained in the second year of the study were the worst regardless of fish origin. No differences were found (*t*-test, $P > 0.05$) among populations or hormonal treatments.

It was found that the origin of the females had no influence on synchronization of ovulation. The females after application of CPH ovulated earlier (14.0 + 3.2 h) than after Ovopel (16.7 + 2.9 h) (Fig. 1). The ovulation peak of females stimulated by CPH appeared 12 h after the resolving injection, whereas in groups stimulated by Ovopel, it was 15 h. Generally, there were no differences in the latency time between wild (15.7 + 3.2 h) and cultured fish (15.2 + 3.5 h) (Fig. 2). About 80% of females ovulated between 12 and 18 h after the resolving injection.

Table 1 Percentage of obtained eggs in relation to females BW recorded during three consecutive spawning seasons of tench collected from different stocks and treated with different spawning agents

	Lake		Pond	
	Ovopel	CPH	Ovopel	CPH
Season I	8.3 ± 1.4	8.6 ± 1.5	8.2 ± 1.3	8.1 + 1.6
Season II	7.9 ± 1.6	7.8 ± 1.3	7.7 ± 1.5	8.0 + 1.2
Season III	8.4 ± 1.2	8.6 ± 1.1	8.7 ± 2.0	8.4 + 1.5
Mean for treatment	8.2 ± 0.2	8.3 ± 0.3	8.2 + 0.5	8.2 + 0.2
Mean for stock	8.3 ± 0.3		8.2 ± 0.3	

For details see the material and methods section. No statistical differences were found using *t*-test ($P > 0.05$)

Table 2 Ovulation rate (%) recorded during three consecutive spawning seasons of tench collected from different stocks and treated with different spawning agents

	Lake		Pond	
	Ovopel	CPH	Ovopel	CPH
Season I	90	80	70	50
Season II	60	50	50	40
Season III	80	70	80	60
Mean for treatment	76.7	66.7	66.7	50.0
Mean for stock*	71.7		58.3	

For details see the material and methods section. No statistical differences were found using *t*-test ($P > 0.05$)

* Mean value for each stock within whole 3 years study

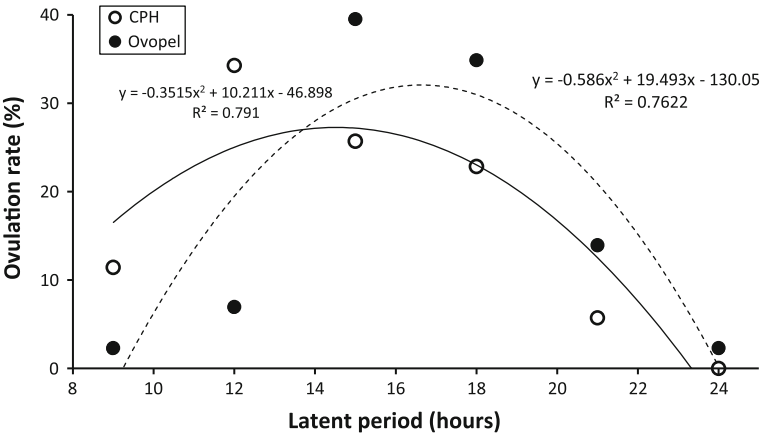

Fig. 1 Correlation between latency time and ovulation rate in common tench in relation to different spawning agent used

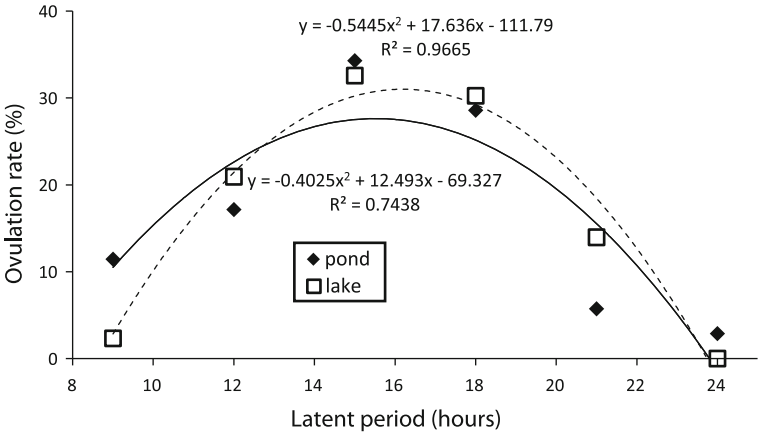

Fig. 2 Correlation between latency time and ovulation rate in common tench in relation to fish origin (pond or lake)

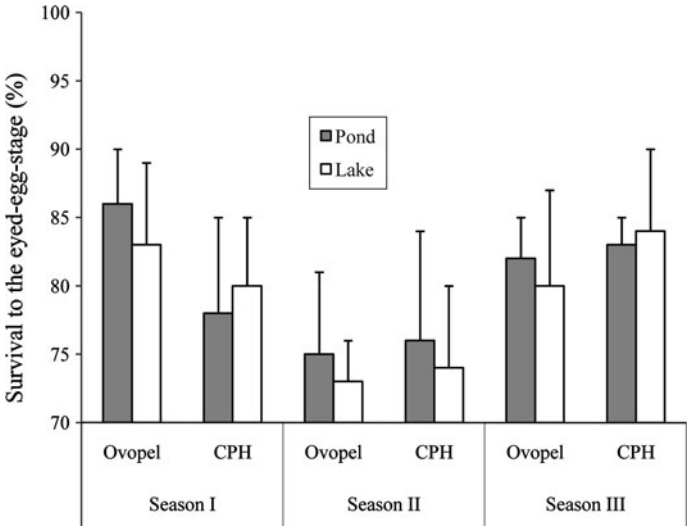

Fig. 3 Survival of common tench embryos to the eyed-egg-stage. Eggs were obtained after artificial spawning with the application of different spawning agents (Ovopel and CPH), and fish from different stocks were used

There was no influence of fish origin or applied hormonal treatment on embryo survival (t-test, $P > 0.05$) (Fig. 3). In each season, there were no differences in this parameter, although during season II, the recorded value was lower than in other seasons.

There were no differences in spermiation percentages between wild and cultured stock or between different hormonal stimulations. The spermatozoa motility was similar and ranged between 72 and 76%.

Discussion

Artificial reproduction of cyprinids is possible mainly after hormonal stimulation (Kucharczyk et al. 1997a, 2005; Brzuska 2005; Szabo et al. 2002; Linhart et al. 2006; Krejszeff et al. 2008, 2009; Podhorec and Kouril 2009; Yaron et al. 2009; Żarski et al. 2009). The application of different spawning agents and doses resulted in different ovulation rates and gametes' quality (Glogowski et al. 1997, 1999; Babiak et al. 1998; Kucharczyk et al. 2005, 2007; Cejko et al. 2010), which was usually caused by different interactions on the fish organism. The largest differences were noted when fish from different stocks were reproduced (Krejszeff et al. 2009) as well as different spawn forms of one species (Krejszeff et al. 2010), while multi-batch spawners are much more difficult to reproduce (Kucharczyk et al. 1997c). Tench has reproduction periods 2–3 times during the season, on average (Horoszewicz 1983; Alas and Solak 2004; Benzer et al. 2007), and this performance of tench spawning is the cause of numerous difficulties in obtaining gametes under controlled conditions, where one of the most important aspects is the time of hormonal injection after fish collection (Kucharczyk et al. 2007).

The studied fish originated from different environments with different thermal characteristics during the year. During the winter, the water temperature in ponds is usually lower than in the lake. Additionally, faster warming of water is usually observed in the ponds

during the spring and summer. Rapid and frequent changes in water temperature during spring and summer under natural conditions may influence the number of spawning portions during the spawning season (Horoszewicz 1981, 1983; Alas and Solak 2004; Benzer et al. 2007). The thermal regime during winter and the rate of thermal gradation during the pre-spawning period also have a strong effect on gametogenesis (Horoszewicz 1981; Linhart et al. 2006). During this study, the spawners were collected from different environments, but at the same time, during each year and from the same thermal conditions. Thus, the observed differences (however, not statistically confirmed) in effects of artificial reproduction between 3 years of the study among both stocks might result from the different thermal characteristics of respective reservoirs. During seasons with larger, rapid changes in water temperature, the individual variability of reproductive rhythms usually increases. It has an important effect on the spawning success of tench, where thermal fluctuations are the cause of reproduction failure in numerous cases (Horoszewicz 1983; Alas and Solak 2004; Benzer et al. 2007). During season II of the experiment, the lowest percentages of ovulated females and embryos survival rates were observed (without statistical differences). This might result from the adverse effect of temperature prior to spawning. Similarly, the negative influence of thermal fluctuation shortly before spawning on spawning effectiveness, expressed as the percentage of ovulated females and embryo survival rate, was recorded for cyprinids such as ide *Leuciscus idus* (L.) and asp *Aspius aspius* (L.) by Targońska et al. (2010 and unpublished) and also for burbot *Lota lota* (L.) by Żarski et al. (2010) .

Differences in the latency time of tench were observed in the case of different spawning agents, but not in the case of fish origin. CPH usually involves a shorter latency time than Ovopel, and this was noted not only in the case of cyprinids (Brzuska 2005; Kucharczyk et al. 2005; Krejszeff et al. 2008) but also for percids (Kucharczyk et al. 1996, 1998; Szczerbowski et al. 2009). Domesticated fish were reported to be able to spawn under controlled conditions without hormonal stimulation, and the latency time was much shorter (Krejszeff et al. 2010). The data obtained for tench were the opposite. In the present study, no differences were noted for spermatozoa motility or embryo survival for either stocks of tench. There were also no differences between groups treated with different hormonal preparations among the populations. The survival rate was very high and similar to those reported by other authors (Kucharczyk et al. 2007; Kujawa et al. 2009). Thus, it could be assumed that environmental differences in an earthen pond and lake are insignificant, and these reservoirs are both suitable for tench management. Generally, the obtained results of artificial reproduction of wild and domesticated tench under controlled conditions showed many similarities. It suggests that the method developed for cultured stock might be used successfully for wild fish, and there is a high possibility to synchronize the ovulation in fish originating from different stocks. For this purpose, among the tested agents, Ovopel is recommended due to both its reproductive and economic effectiveness (Hakuć-Błażowska et al. 2009, 2010).

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