Method of evaluation of wild common tench, *Tinca tinca* (L.), female suitability for artificial reproduction during the spawning season

Katarzyna Targońska, Tomasz Perkowski, Daniel Źarski, Sławomir Krejszeff, Andrzej Mamcarz, Roman Kujawa, Dariusz Kucharczyk

Department of Lake and River Fisheries, University of Warmia and Mazury, Olsztyn, Poland

Abstract

The aim of this study was to evaluate the suitability of females for artificial reproduction of common tench based on an analysis of the size and percentage share of oocytes sampled *in vivo*. The females were collected from natural waters (lakes) during the spawning season. After catching, the selected fish were transported to the hatchery, where the fish were kept under controlled conditions and oocytes were collected from all females. It was found that sampled oocytes were different in size and developmental stages. Oocytes were classified into 3 groups: small, medium and large. The diameter intervals for each group were 0.18-0.22 mm, 0.48-0.57 mm and 0.95-1.01 mm, respectively. Analysis of the percentage of the largest oocytes showed that their numbers decreased in time from over 40% on Day 1 to slightly over 10% on Day 10. A positive correlation was found between the percentage of the largest oocytes in the cathetered egg sample and the weight index of the stripped eggs during artificial reproduction. The percentage of the largest oocytes (diameter 0.95-1.01 mm) might be used as an indicator of the possibility of reproducing wild tench. No statistical differences were observed in latency time and embryo survival of females showing different quantities of the largest oocytes.

Introduction

The common tench, *Tinca tinca* (L.), is one of the freshwater cyprinid species that has had a huge impact on freshwater aquaculture (Wang et al., 2006; Celada et al., 2009; Kujawa et al., 2011). In recent years, the tench has become very important for both lake exploitation (Mamcarz and Skrzypczak, 2006) and pond aquaculture (Celada et al., 2009). This has led to the rapid development of new techniques regarding different aspects of tench artificial reproduction (e.g., Rodina et al., 2007; Mamcarz et al., 2006; Kouril et al., 2008) and the rearing of larvae and juveniles under controlled conditions (e.g., Wolnicki et al., 2006; Celada et al., 2009; Mamcarz et al., 2011). In most cases, the studies were carried out using cultured stocks of common tench (e.g., Svoboda et al., 2001; Rodriguez et al., 2004; Rodina et al., 2007; Kujawa et al., 2011) and they are in contrast with the published data concerning the different aspects of wild fish reproduction (Pimpicka et al., 1990; Alas and Solak, 2004; Benzer and Yilmaz, 2007; Kucharczyk et al., 2007).

Data on the reproduction of wild cyprinids under hatchery conditions are limited. One of the most important problems is collecting fish from natural waters (lakes and rivers) (Kujawa et al., 2006, 2011). For this reason, in many cases the research was carried out on a limited number of spawners (Kucharczyk et al., 1997a, b, c; Babiak et al., 1998; Babai et al., 1999; Targońska et al., 2008, 2009). Preparation of an efficient protocol of artificial reproduction requires testing many different parameters, including the type and doses of applied spawning agents (Kucharczyk et al., 2005; Cejko et al., 2008; Krejszeff et al., 2008, 2009), time of collection of spawners during the reproductive season (Krejszeff et al., 2008; Kucharczyk et al., 2008; Targońska et al., 2011), and the quality of gametes and their storage (Glogowski et al., 1997, 1999; Ciereszko et al., 1999; Lahnsteiner et al., 2003, 2004).

The success of tench reproduction under controlled conditions is determined, among other things, by the maturity stage of the spawners. This, in turn, is related to the moment of the spawning season and the location of the obtained spawners. One of the most important aspects in artificial reproduction of common tench is to identify the suitability of females for artificial reproduction. In many cyprinids, the position of the germinal vesicle is the most important factor (e.g., Brzuska, 1979; Kucharczyk et al., 1997a, 2005; Krejszeff et al., 2009; Targońska et al., 2010). In other fish (e.g., barbel *Barbus barbus*, goldfish *Carassius auratus*, chub *Leuciscus cephalus*, rudd *Scardinius erythrophtalmus*) that, like tench, are batch spawners, the method based on germinal vesicle position in oocytes was not successful (Kucharczyk et al., 1997c; Krejszeff et al., 2010; Targońska and Kucharczyk, 2011; Targońska et al., 2011). In multi-batch spawners, other indexes are applied, e.g., body weight index (BWI) and size or percentage of the largest oocytes (Pedersen, 2003; Kucharczyk et al., 2007).

The aim of this study was to evaluate the suitability of females for artificial reproduction of tench, collected from natural waters (lakes) during the spawning season, based on an analysis of the size and percentage share of oocytes sampled *in vivo*.

Materials and methods

Broodstock collection

Tench spawners were collected from wild stock in mid-June (spawning season) from Lake Sasek Wielki, in north-eastern Poland. The fish were caught after a 10-day period in which the water in the lake had cooled (to 14°C) at the moment when the water temperature started to rise again. This was to make sure that fish were not actually spawning and that it was not too soon (2-3 days) before female ovulation. During the period in which the water cools (which in north-eastern Poland is very often in mid-June) tench usually stop spawning. The collected fish were immediately transported to the hatchery. Fish were selected according to the following criteria: the belly of females had to be fully distended and bulging, soft and resilient to touch; males had to be...
slightly spermiating. The size of spawners ranged from 0.6 to 1.2 kg. The water temperature in the lake when the fish were caught was about 17°C. The selected males and females were kept in separate 1000 dm³ tanks in the hatchery with controlled temperature and photoperiod (16 h light, 8 h dark) (Kujawa et al., 1999). The water temperature in the hatchery unit was gradually increased from 18 to 20°C over one day. The maximum load of spawners was 25 kg m⁻³. The level of dissolved oxygen was a minimum of 6 ppm.

Checking oocyte maturation

All fish were individually marked using flowtags and weighed. The oocytes from females were collected in vivo with a catheter. 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 (Brzuska, 1979):

- stage 1 - germinal vesicle in the central position;
- stage 2 - early migration of germinal vesicle (less than half of radius);
- stage 3 - late migration of germinal vesicle (more than half of radius);
- stage 4 - periphery germinal vesicle or germinal vesicle breakdown (GVBD).

The oocyte samples were photographed using a stereoscopic microscope (Leica MZ 12.5, Germany). Diameters of oocytes were measured and the percentages of different sized oocytes were calculated. Oocytes were classified into 3 groups: small, medium and large. The diameter intervals for each group were 0.18-0.22 mm, 0.48-0.57 mm and 0.95-1.01 mm, respectively. All measurements (±0.01 mm) were taken with ProgRes® Capture Pro 2.5 (Jenoptik, Germany) analytical software. From the same females, randomly chosen from the selected fish (10 specimens), the oocytes were sampled on the day they were caught (Day 0) and later on Days 1, 2, 3, 5, 8 and 10, and the percentage of large oocytes was calculated. The remaining females (37 specimens) received a single injection of Ovopel (a mammalian analog of GnRH in a complex with metoclopramide) at a dose of 1 pellet kg⁻¹ (one Ovopel pellet contained approx. 18-20 µg of GnRHs and 8-10 mg of metoclopramide; Horvath et al., 1997) on Day 1. When ovulation occurred in hormonally-treated females, eggs from each female were collected manually. Each female’s body weight and total egg weight were recorded. Before manipulation, the fish were anesthetized with 2-phenoxethanol (0.5 cm³ dm⁻³).

Collection of gametes and incubation

Ripe gamete donors were anesthetized in 2-phenoxethanol solution (0.5 mL per litre). Milt was collected with plastic syringes and kept at 4°C. Females were checked every h between 8 and 16 h after injection. Eggs were stripped into a plastic vessel and fertilized using the dry method. Sperm were then stripped from males into dry plastic syringes directly before insemination. Sperm motility was evaluated immediately after collection. Only sperm showing over 80% motility of spermatozoa were used for egg fertilization. Sperm motility was identified subjectively under a light microscope (500 X). Three egg samples (250-300 eggs each) from each female were mixed with 0.05 mL of pooled milt sample. Eggs were incubated at 24°C on Petri dishes. The survival of embryos was recorded during the eyed-egg stage.

Statistical analysis

Statistical analysis of data was carried out using STATISTICA software for Windows version 8.0 PL (StatSoft) and MS Excel. All values expressed as percentages were arcsine transformed prior to statistical analysis. Regression analysis was performed to determine the correlation of the percentage of large oocytes and handling time of spawners. The same analysis was performed for the weight of obtained eggs and percentage of large oocytes. Statistical differences between groups were analyzed by an analysis of variance (ANOVA) and Tukey’s post-hoc test (criterion α=0.05).

Results

The oocytes obtained from tench females were not only at different stages as far as the position of the germinal vesicle was concerned, but they were also different in size. The germinal vesicle position in the largest oocytes was at stages from 1 to 4 and was observed in the majority of the females. In the medium and small oocytes, the germinal vesicle position was at stage 1, i.e., positioned centrally. The numbers of large (0.98±0.03), medium (0.53±0.04) and small (0.21±0.02) oocytes varied. Analysis of the percentage of the largest oocytes showed that with the passage of time their numbers decreased from an average of over 40% on Day 1 to slightly over 10% on Day 10 (Figure 1). Initially, the highest percentage of largest oocytes was observed on Day 1 and these percentages later decreased. Over time, the range of recorded values also increased. Induced reproduction of the females on the first day after collection from the natural environment showed that obtaining eggs is possible regardless of the percentage of the largest oocytes. A significant correlation, however, was found between the percentage of the largest oocytes and fecundity index: the weight of the obtained eggs (Figure 2). If the percentage of the largest oocytes was 10% or under, a very low weight of eggs-to-female body weight ratio was obtained. No statistical difference was seen between latency time and embryo survival of females showing different levels of the largest oocytes (Table 1).

Discussion

Results identified the optimal moment to start application of hormonal stimulation in the artificial reproduction of tench collected from natural waters. The oocytes collected from the females were different in size and at different developmental stages. A correlation was found between the percentage of the largest oocytes and the weight of the eggs obtained. The highest percentage of eggs was obtained from females that had the highest percentage of large oocytes.

Many factors influence the economic effectiveness of fish production. When stocking material or material for restocking natural waters, the type and dosage of hormonal preparation and time of their application, quality of gametes and larvae are of major importance (Kupren et al., 2008; Turkowski et al., 2008; Hakuć-Blażowska et al., 2009). The application of hormonal treatment at the appropriate moment of gamete maturity causes ovulation and spermiation of the fish and influences the quality and quantity of the obtained gametes (e.g., Yaron, 1995; Kucharczyk et al., 1997a, 2005; Krejczew et al., 2008). For female freshwater fish, the germinal vesicle position in the oocytes is considered one of the most practical indicators (Brzuska, 1979; Krejczew et al., 2009; Targófska et al., 2010). In percids (Kucharczyk et al., 1996, 1998; Szczersowski et al., 2009) and some cyprinids (Kucharczyk et al., 1997b, 2005; Krejczew et al., 2009; Targófska et al., 2010) stages 2/3 (Brzuska,
1979) were considered optimal for initiation of hormonal stimulation. However, in percids, this system was not very precise and a new system to recognize oocyte maturity stage was proposed by Żarski et al. (2011 a, b). That method, however, was unreliable in batch spawners as the oocytes were of different sizes and at different stages of development (Kucharczyk et al., 1997c, 2007; Krejszeff et al., 2010; Targórńska and Kucharczyk, 2011; Targórńska et al., 2011). Thus, in the case of batch spawners, such as tench, other methods that could be used successfully in fishery practice should be investigated. The percentage of the largest diameter oocytes applied in this study may be one such method.

The results obtained showed the number of the largest diameter oocytes decreased over time; this is consistent with results obtained by Kucharczyk et al. (2007) who found that, over time, the percentage of tench females in which ovulation occurred decreased after treatment with Ovopel. In the present study, it was shown that obtaining eggs is possible independently of the quantity of the largest oocytes. This is crucial from the perspective of fishery practices. Nevertheless, a very small quantity of eggs (equivalent to under 1% of the female’s bodyweight), obtained when the percentage of the largest oocytes was 10% or under, indicates the need to reject such fish during reproduction in commercial hatcheries. There is no point in keeping these fish longer at the hatchery because over time the numbers of the largest oocytes decrease; this, in practical terms, means the female will not ovulate and no eggs will be obtained. The diameters of the largest oocytes measured during this study were very similar to those reported by Alas and Solak (2004) for the tench population from the Kayabogazi Dam Lake in Turkey. During those studies on the yearly development cycle of gonads in tench, it was established that tench females are ready for spawning in May-July (Pimpicka, 1990; Alas and Solak, 2004). In this study, the high percentage level (over 30%) of the largest oocytes may be associated with the fact that tench females were reproducing for the first or second time during the season.

In the present study, no differences were seen in the percentage of ovulation, ovulation latency and embryo survival to the eyed-egg stage. This is probably due to the effect of preparing artificial reproduction under optimal temperature regimes in captivity (Kouril et al., 2008, Kujawa et al., 2011). Differences were only seen in the case of weight of stripped eggs in relation to female body weight (fecundity index). The highest fecundity index was recorded in females with the highest percentage of the largest oocytes in the ovary (>40%).

### Conclusions

No differences were seen in artificial spawning effectiveness, such as ovulation rate, latency time or survival to the eyed-egg stage, of wild tench females collected from one population at the same time during the spawning season. Differences were seen in the percentage of largest (diameter 0.95-1.01 mm) oocytes present. The presence of oocytes of this size decreased over time. There was a positive correlation between percentage of the largest oocytes and fecundity index of stripped females. Percentage of the largest oocytes in sampled oocytes could be used in commercial reproduction of wild tench in hatcheries.

### Table 1. Spawning effectiveness (±SD) of tench females with different percentages of large oocytes. In each group N=10.

| Percentage of largest oocytes (%) | 0-10%   | 10-20%  | 20-30%  | 30-40%  | >40%   |
|----------------------------------|---------|---------|---------|---------|--------|
| Ovulation rate, %                | 90      | 90      | 90      | 90      | 90     |
| Average bodyweight, kg           | 0.85±0.21 | 0.87±0.19 | 0.89±0.20 | 0.84±0.22 | 0.86±0.18 |
| Fecundity index: weight of eggs, | 0.96±0.08a | 1.97±0.14a | 3.05±0.15a | 4.23±0.21a | 5.89±0.28a |
| % of body female weight          |         |         |         |         |        |
| Latency time, hr                 | 16-19   | 16-18   | 16-18   | 16-18   | 16-17  |
| Embryo survival to the eyed-egg-stage | 87.3±3.4 | 88.2±3.4 | 87.9±3.6 | 88.2±4.2 | 89.3±4.3 |

*Means within a row with different superscripts differ (P<0.05).*
Ciereszko, A., Solak K., 2004. The Reproductive Biology of the Trench (Tinca tinca L., 1758) in Kayabogazi (Ktahya, Turkey) Dam Lake. Turk. J. Vet. Anim. Sci. 28:879-885.

Babiak, I., Glogowski, J., Kujawa R., Kucharczyk, D., Mamcarz A., 1998. Cryopreservation of asp, Aspius aspius (L.) sperm. Prog. Fish-Cult. 60:146-148.

Benzer, S.S., Gul, A., Yilmaz, M., 2007. The effect of inorganic and organic pollutants on sperm motility of some freshwater teleosts. J. Fish Biol. 65:1283-1297.

Kouril, J., Mraz, J., Hamackova, J., Barth, T., 2008. Hormonal induction of trench (Tinca tinca L.) ovulation with the same treatments over two consecutive reproductive seasons. Cybium 32(Suppl.2):61.

Krejzsef, S., Kucharczyk, D., Kuprem, K., Targańska, K., Mamarcz, A., Kujawa, R., Kaczkowski, Z., Rajtaš, S., 2008. Reproduction of chub, Leuciscus cephalus L. under controlled conditions. Aquacult. Res. 39:907-912.

Krejzsef, S., Targańska, K., Żarski, D., Kucharczyk, D., 2009. Domestication affects spawning of the ide (Leuciscus idus) – preliminary study. Aquaculture 295:145-147.

Horvath, L., Szabo, T., Burke, J., 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. Pol. Arch. Hydrobiol. 44: 221-226.

Kouřil, J., Mraz, J., Hamáčkova, J., Barth, T., 2008. Hormonal induction of trench (Tinca tinca L.) ovulation with the same treatments over two consecutive reproductive seasons. Cybium 32(Suppl.2):61.

Krejzsef, S., Kucharczyk, D., Kuprem, K., Targańska, K., Mamarcz, A., Kujawa, R., Kaczkowski, Z., Rajtaš, S., 2008. Reproduction of chub, Leuciscus cephalus L. under controlled conditions. Aquacult. Res. 39:907-912.

Krejzsef, S., Targańska, K., Żarski, D., Kucharczyk, D., 2009. Domestication affects spawning of the ide (Leuciscus idus) – preliminary study. Aquaculture 295:145-147.

Krejzsef, S., Targańska, K., Żarski, D., Kucharczyk, D., 2010. Comparison of artificial reproduction of two different spawn forms of the chub. Repr. Biol. 10:67-71.

Kucharczyk, D., Kujawa R., Lucznyszyn, M., Glogowski, J., Babiak, I., Wyszomska, E., 1997a. Induced spawning in bream, Abramis brama (L.), using carp pituitary extract and hCG. Aquacult. Res. 28:139-144.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomska, E., 1996. Induced spawning in perch, Perca fluviatilis L. using carp pituitary extract and hCG. Aquacult. Res. 27:847-852.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomska, E., 1998. Induced spawning in perch, Perca fluviatilis L. using FSH + LH with pimozide or metoclopramide. Aquaculture. Res. 29:131-136.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targańska, K., Krejzsef, S., Wyszomska, E., 2007. Artificial spawning of common tench (Tinca tinca L.) collected from wild populations. Pol. J. Nat. Sci. 22:37-45.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomska, E., 1998. Induced spawning in perch, Perca fluviatilis L. using FSH + LH with pimozide or metoclopramide. Aquaculture. Res. 29:131-136.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targańska, K., Krejzsef, S., Wyszomska, E., 2007. Artificial spawning of common tench (Tinca tinca L.) collected from wild populations. Pol. J. Nat. Sci. 22:37-45.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targańska, K., Krejzsef, S., Wyszomska, E., 2007. Artificial spawning of common tench (Tinca tinca L.) collected from wild populations. Pol. J. Nat. Sci. 22:37-45.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targańska, K., Krejzsef, S., Wyszomska, E., 2007. Artificial spawning of common tench (Tinca tinca L.) collected from wild populations. Pol. J. Nat. Sci. 22:37-45.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targańska, K., Krejzsef, S., Wyszomska, E., 2007. Artificial spawning of common tench (Tinca tinca L.) collected from wild populations. Pol. J. Nat. Sci. 22:37-45.
tench, Tinca tinca (L.) females in lake Drweckie. Acta Ichth. Piscat. 20:53-75.
Rodina, M., Gela, D., Kocour, M., Hadi Alavi, S.M., Hulak, M., LInhart, O., 2007. Cryopreservation of tench, Tinca tinca, sperm: Sperm motility and hatching success of embryos. Theriogenology 67:931-940.
Rodriguez, R., Celada, J.D., Saez-Royuela, M., Carral, J.M., Aguilera, A., Melendre, P.M., 2004. Artificial reproduction in 1-year-old tench (Tinca tinca L.). J. Appl. Ichthyol. 20:542-544.
Svoboda, M., Kouril, J., Hamaakova, J., Kalab, P., Savina, L., Svobodova, Z. Vykusova, B., 2001. Biochemical profile of blood plasma of tench (Tinca tinca L.) during pre- and post-spawning period. Acta Vet. Brno 70:259-268.
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.
Targońska, K., Kucharczyk, D., 2011. The application of hCG, CPH and Ovopel in successful artificial reproduction of Goldfish (Carassius auratus auratus) under controlled conditions. Reprod. Dom. Anim. 46:651-655.
Targońska, K., Kucharczyk, D., Kujawa, R., Mamcarz, A., Žarski, 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., Žarski, D., Cejko, B.I., Krejszeff, S., Kupren, K., Król, R., Dryl, K., Kowalski, R.K., Glogowski, J., 2011. Artificial reproduction of wild and cultured barbel (Barbus barbus, Cyprinidae) under controlled conditions. Acta Vet. Hung. 59:363-372.
Turkowski, K., Kucharczyk, D., Kupren, K., Hakuć-Blaźowska, A., Targońska, K., Žarski, D., Kwiatkowski, M., 2008. Economic aspects of the experimental rearing of asp, Aspius aspius (L.), ide, Leuciscus idus (L.), and dace, Leuciscus leuciscus (L.), under controlled conditions. Arch. Pol. Fish. 16:397-411.
Wang, J., Min, W., Guan M., Gong, L., Ren, J., Huang, Z., Zheng, H., Zhang, J., Liu, H., Han, Y., 2006. Tench farming in China: present status and future prospects. Aquacult. Int. 14:205-208.
Wolnicki, J., Myśzkowski, L., Korwin-Kossakowski, M., Kamiński, R., Stanny, A., 2006. Effects of different diets on juvenile tench, Tinca tinca (L.) reared under controlled conditions. Aquacult. Int. 14:89-98.
Yaron, Z., 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture 129:49-73.
Żarski, D., Bokor, Z., Kotrik, L., Urbanyi, B., Horváth, A., Targońska, K., Krejszeff, S., Palińska, K., Kucharczyk, D., 2011a. A new classification of pre-ovulatory oocyte maturation stage suitable for synchronization of ovulation in controlled reproduction of Eurasian perch Perca fluviatilis L. Reprod. Biol. 11:194-209.
Żarski, D., Kucharczyk, D., Targońska, K., Palińska, K., Kupren, K., Fontaine, P., Kestemont, P., 2011b. A new classification of pre-ovulatory oocyte maturation stages in pikeperch, Sander lucioperca (L.), and its application during artificial reproduction. Aquaculture Res. (In press).