Artificial reproduction of two different spawn-forms of the chub

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SUMMARY

The aim of this study was to compare, under controlled conditions, reproduction results of cultured and wild stock of the chub. Wild fish spawned only once a season whereas the cultured stock spawned at least two times. In the multiple-spawn stock, fewer fish spawned and the weight of produced oocytes was reduced compared to the single-spawn stock. Larvae obtained from the multi-spawn forms were smaller than those of the single-spawn stock. The occurrence of one species with two forms of spawning performance in the same area makes it difficult to develop an efficient method for controlling the reproduction. Reproductive Biology 2010 10 1: 67-74.

Key words: artificial spawning; ovulation; Leuciscus cephalus; cultured stock; wild population

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INTRODUCTION

Chub *Leuciscus cephalus* (L.) has recently become an important cultured species in Polish aquaculture primarily due to the increased interest from recreational fisheries. Data concerning reproductive biology, hatchery techniques and methods of artificial spawning of wild cyprinids including those of the chub are very limited [4, 7]. Development of controlled reproduction of wild cyprinids is an integral component of ongoing conservation efforts [16]. The artificial spawning of the chub requires the application of spawning agents. Among a few tested spawning agents such as carp pituitary extract (CPH), human chorionic gonadotropin (hCG) and Ovopel (GnRHa-containing pellets combined with dopamine inhibitor), the latter was the most effective. The tactics of chub reproduction are still not clear. Chub spawners do not spawn spontaneously in captivity [6], the breeders are very sensitive to being caught and stress of handling. During our eight-year experience with controlled reproduction of wild *L. cephalus* in captivity, all fish spawned only once per season. In 2008, we observed that one stock of chubs spawned more than once during the spawning season. Some data [5] show that different spawning forms of this species were recognized in the area of their distribution: single-batch spawners (one spawning per season) or multiple spawners (2-3 spawnings during two-three months). The aim of this study was to compare, under controlled conditions, the results of reproduction of two different spawning forms of the chub.

MATERIAL AND METHODS

Chub broodstock originated from two locations: cultured stock (*F*₂ generation from the Warta River) from Knieja Fish Farm (southern Poland) and wild population from the Warta and Odra Rivers (western Poland). During the entire time of the experiment, the cultured stock was kept in earthen ponds under ambient conditions. The wild stock was collected in October 2007 and kept in a similar pond under the same conditions until spring 2008. Spawner size (250-400 g) was similar in both stocks. Before spawning (May
2008), all fish were transported to the hatchery (16°C; 14L:10D) and placed separately according to sex and stock in 1000 dm³ tanks. Aeration, water temperature (±0.1°C) and daylight were controlled in each tank [14]. After five days of acclimation, the temperature was raised to 18°C. All breeders were weighed and marked with floy-tags. Before hormonal stimulation, oocyte maturity was determined according to a four-degree scale: stage 1 – germinal vesicle in 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). Oocytes (~50 from each female) were collected with a catheter and placed in Serra’s solution. After the exposure to Serra solution (5 min), the position of the nucleus was determined and oocytes were photographed. Before manipulations, the fish were anaesthetized in a 2-phenoxyethanol solution (0.05%).

The first experiment was performed on wild (n=39) and cultured (n=40) females. The treated fish received a single injection of GnRHa-containing pellet (Ovopel; 1 pellet/kg). One Ovopel pellet (~25 mg) contains a mammalian GnRH analogue (D-Ala⁶, Pro⁹Net-mGnRH, 18-20 µg) and dopamine antagonist, metoclopramide (8-10 mg). The control fish (n=10 females) were injected with saline (0.9% NaCl). In the second experiment, females previously stimulated by Ovopel were randomly divided into two groups: Ovopel-treated (1 pellet/kg; n=18 and n=22 for wild and cultured stock, respectively) or hCG-treated (500 IU/kg; n=18 and n=17 for wild and cultured stock, respectively). hCG was not used in the first experiment due to its ineffectiveness usually observed in single-spawn cyprinids. However, hCG was found to be an effective spawning agent in cyprinids that are multiple spawners such as rudd Scardinius erythrophthalmus (L.), goldfish Carassius auratus (L.) and common tench Tinca tinca (L.) [10, 12, Targońska – unpublished]. Therefore, the application of hCG in the second experiment allowed us to support a thesis of a multi-spawn performance of the chub. The control females (n=10) were injected with saline (0.9% NaCl). Injections were applied intraperitoneally under the ventral fin. Hormonal preparations were homogenized (Ovopel) or dissolved (hCG) in a sterile 0.9% NaCl. After the injection, the water temperature was raised from 18°C to 19.5°C and was
kept constant during spawning. Before the first and second reproductions, the fish were kept at 16°C. The between-spawning interval lasted 20 days and ended with the above-described thermal stimulation.

Gametes were stripped manually. Semen was collected into syringes and kept at 4°C. Eggs were collected into plastic containers by gently pressing the abdomen. The ovulation rate was recorded as well as female weight before ovulation and weight of obtained eggs. Pseudogonadosomatic index (PGSI) was calculated according to the formula: \(\frac{[\text{weight of eggs}] \times 100\%}{\text{female weight}}\). A small portion of eggs (approximately 100) was mixed with 0.05 cm\(^3\) of pooled milt, and gametes were activated by water addition. The diameter of oocytes was measured in each female. Egg incubation (each group from each population was incubated separately) was conducted in triplicates in a closed recirculation system. Eggs were incubated (19°C) in Petri dishes placed in 1 dm\(^3\) glass aquaria which, in turn, were placed in a 50 dm\(^3\) water bath with continuous water flow (7 dm\(^3\)/h). Each small aquarium had its overflow secured with a mesh (size 200 µm) which prevented the escape of freshly hatched larvae. Larvae were measured after hatching and when over 50% of them started exogenous feeding. Oocytes and larvae were photographed with Leica MZ 12.5 microscope (Germany). All measurements (±0.01 mm) were done with ProgRes® Capture Pro 2.5 (Jenoptik, Germany) software. Data were expressed as means±SD. Statistical differences between the groups were determined using t-test at a significance level of p<0.05.

RESULTS AND DISCUSSION

Significant progress in the propagation of fish from the genus *Leuciscus* has been made recently [4, 6, 17]. However, a reliable protocol for artificial reproduction is still needed. The recognition of the best time for applying hormonal induction in cyprinid artificial spawning is very important. In most single-batch spawning species the best method is examining the oocyte maturity stage [6, 7, 9, 11, 13, 17]. Oocytes of fish with more than one ovulation during spawning e.g. common tench, differ in size and maturity stage [12]. In such a case, the exact time for hormonal stimulation should be
established experimentally. In the present study, the cultured stock oocytes were heterogeneous with regards to maturity stage and size (diameter), whereas the wild fish oocytes were homogenous (stage 2-3) and large. Oocyte diameter in the wild stock of the chub was 1.11±0.1 mm, whereas in the cultured multi-spawn form three oocyte sizes were distinguished: large (1.08±0.11 mm), medium (0.61±0.09 mm) and small (0.25±0.06). The average ratio for large, medium and small eggs was 38:27:35, respectively. All small and medium oocytes were in stage 1, whereas large oocytes had germinal vesicle in all four stages.

The ovulation rate in Ovopel-treated wild stock (tab. 1) was similar to the previously reported [6]. The percentage of ovulation in the multiple-spawn form was twofold lower than that of the single-batch form. Ovulation

Table 1. Selected reproductive parameters (mean±SD) in the single-batch spawn and multiple-spawn chub

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single-batch spawn form (wild stock)</th>
<th>Multi-spawn form (cultured stock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulation rate (%)</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>PGSI (%)</td>
<td>12.8±2.3a</td>
<td>4.1±1.5b</td>
</tr>
<tr>
<td>Total length of larvae after hatching (mm)</td>
<td>6.7±0.2a</td>
<td>6.2±0.2b</td>
</tr>
<tr>
<td>Total length of larvae at the beginning of exogenous feeding (mm)</td>
<td>8.0±0.2a</td>
<td>7.7±0.3b</td>
</tr>
</tbody>
</table>

hCG: second spawning

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single-batch spawn form (wild stock)</th>
<th>Multi-spawn form (cultured stock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulation rate (%)</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>PGSI (%)</td>
<td>-</td>
<td>2.2±0.2</td>
</tr>
</tbody>
</table>

Ovopel: second spawning

<table>
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<tr>
<th>Parameters</th>
<th>Single-batch spawn form (wild stock)</th>
<th>Multi-spawn form (cultured stock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulation rate (%)</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>PGSI (%)</td>
<td>-</td>
<td>3.2 ± 0.2</td>
</tr>
</tbody>
</table>

Pseudogonadosomic index (PGSI): [(weight of eggs)×100%]/female weight
Different superscripts within a row mean significant (p<0.05) differences
was not demonstrated in the control groups. Despite fish being kept under favorable environmental conditions, no progress was observed in oocyte maturation expressed as germinal vesicle migration. This confirms the necessity of hormonal induction of ovulation in the chub in captivity. The same phenomenon was described in the single-batch spawn form of chub females stimulated with hCG [6] as well as in other cyprinids [7, 9, 11, 13, 17]. hCG is generally used for multiple-spawned marine fish species, e.g., snapper *Pagrus auratus* (L.) [2], mulloway *Argyrosomus hololepidotus* (Lacepède, 1801) [3] and sand whiting *Sillago ciliata* Cuvier (1829) [1]. In cyprinids, hCG alone is usually ineffective [9] with the exception of a few species [10, 12]. The influence of hCG on the oocyte maturation is manifested by moving the nucleus towards the cell perimeter. However, hCG did not affect the fish strongly enough to start spawning. A similar phenomenon was observed in bream females [11]. The effectiveness of hCG was proven in cyprinids with portional ovulation: i.e., tench, rudd [10, 12] or Eurasian perch *Perca fluviatilis* L. [8]. The females spawned during the second experiment were also found to spawn during the first one. PGSI was higher in the single-spawn form (wild) than in the multiple-spawn form (cultured).

The examined stocks did not differ in embryo survival to the eyed-egg stage. Larvae obtained from both stocks were different in size during hatching and at the start of exogenous feeding. Similar results were described for Eurasian perch originated from different stocks [15] and common carp reproduced during different periods of a season [13]. The size variation of fish larvae during hatching may be affected by stock origin or time of spawning. Larvae obtained from the single-spawn form (wild) were longer and more homogenous during hatching and at the start of the exogenous feeding than those from the multiple-spawn form (cultured). The differences in larval size was noted in the common carp spawned within- and out-of season [13].

The cultured forms of chub collected from the southern Poland are multi-spawners. The main restrictions for controlling reproduction of the chub in captivity are difficulties with hormonal stimulation as well as low numbers of ovulated females and obtained eggs. In future research, more spawning agents with wider dose range should be tested. Moreover, other hatchery techniques should be developed. The occurrence of one species
with two forms of spawning performance in the same area makes difficult to develop an efficient method for the control of reproduction.

REFERENCES


