Influence of thermal conditions on successful ide (Leuciscus idus L.) artificial reproduction during the spawning season

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Abstract

Two forms of ide Leuciscus idus (L.) spawns: wild-coloured and ornamental: yellow-coloured were kept at three various temperature regimes shortly before spawning at optimal temperature regimes (group 1), under natural temperature conditions (group 2) and in rapidly increasing temperature (group 3). The quality and quantity of collected semen, ovulation rate and survival rate of embryos to the eyed-egg-stage were recorded. The quantity of semen from group 3 where the temperature increased over the thermal spawning optimum was the worst (46 and 51% motility of spermatozoa for the wild-coloured and yellow form, respectively). The quantity of collected semen also was the lowest in the same groups (1.1 and 1.0 cm³ kg⁻¹ for the wild-coloured and yellow form, respectively). Increasing the temperature and its synchronization (Targońska et al., 2010; 2011), which is an essential condition for further effective activities, including controlled rearing. The synchronization of ovulation under controlled conditions in many fish species is possible, mainly through hormonal treatment. Many fish were artificially reproduced using hormonal stimulation. In freshwater cyprinid reproduction, many kinds of spawning agents were applied, i.e. common carp Cyprinus carpio (L.) pituitary homogenate, hCG or GnRH analogues combined with dopamine antagonist (Krejzseff et al., 2009).

Ide Leuciscus idus (L.) has a huge economic value in Poland, as well as many other European countries, because its production is very high in comparison to other rheophilic cyprinids and it is produced as a market and sport fish. In addition, the ornamental form of this species (golden orfe) is also cultured. For example, between 2000 and 2002, the production of ide summer fry for restocking was about 4,700,000 specimens. One-year-old fish production (2004) was about 27,000 kg, which is 69% and 91% of the total production of riverine cyprinids for 2000 and 2002, respectively, including barbel, Barbus barbus (L.), asp Aspius aspius (L.), chub Leuciscus cephalus (L.), nase, Chondrostoma nasus (L.) and vimba bream, Vimba vimba (L.). Ide production was based on artificial reproduction, in which many different hormonal stimulants were tested. Various differences in the spawning results of wild and domesticated stock have been described (Krejzseff et al., 2009).

Temperature plays an important role throughout a fish’s life, including cyprinids. This is based on many studies into the effect of temperature on fish (Kucharczyk et al., 1998; Davies and Bromage, 2002; Hilder and Pankhurst, 2003; Angius and Canavate, 2005; Daufresne and Boët, 2007; Kupren et al., 2011). This is clearly visible during the most important phase of fish development: e.g. embryonic (Kucharczyk et al., 1997; Kupren et al., 2010; 2011) or larval development (Kucharczyk et al., 1998; Kupren et al., 2010; 2011). The effect of temperature is species-dependent, which is caused by its thermal preferences. In addition, temperature is one of the most important factors during finale gametes’ maturation and spawning. Ide is a species which usually spawn at a temperature of about 10 - 14°C during early spring (Mann, 1996; Kupren et al., 2011) when water temperatures are rising. Due to this, ide spawners should be kept at this temperature regime before controlled reproduction. In a typical protocol for applying hormonal stimulation of this species, the temperature increased slightly from 10-11°C to 12°C after the primary injection and up to 13.5-14.5°C after the resolving
injection (Kucharczyk et al., 2008; Krejczef et al., 2009; Żarski et al., 2009). However, the temperature effect on spawning success in ide has not yet been considered. Personal observations, conducted on fish farms over several spawning seasons indicate that high and rapid changes in temperatures affected spawning effectiveness in rheophilic cyprinids, i.e. ide, dace (Leuciscus leuciscus) and chub, defined as gamete quality and the percentage of ovulation and spermatization. A similar situation was also noted for asp (Targóńska et al., 2010) and burbot (Lota lota) (Żarski et al., 2010). These changes were usually caused by a rapid change of weather during spawning and incubation periods which influenced the water temperature of rivers. These rivers are very often the main source of water for commercial hatcheries where spawners are kept before spawning at this time. In the present paper, the influence of different temperature regimes (including short-term increases shortly before hormonal induction of spawning) was studied on wild and cultured ide.

## Materials and methods

Ide spawners (wild form) were obtained from lakes and rivers in Warmia (north-eastern Poland) a few months before the natural spawning period. The collected fish were kept in a pond on the Janowo Fish Farm near Szczytno (north-eastern Poland). The golden form (cultured) of ide (orfe) were collected from the Olesnica Fish Farm near Pila (south-western Poland). Both forms were kept under natural photo-thermal conditions during the winter. At the beginning of the spawning season, fish from both stocks were selected according to the following criteria: the belly of the females had to be fully distended and bulging, soft and resilient to touch; males had to be slightly spermiating (Kucharczyk et al., 2009). However, the size of the wild spawners ranged from 0.8 to 1.2 kg, whereas the cultured fish size was much smaller (0.2-0.3 kg). All fish were individually marked (using fly tags), weighed and oocytes were taken from females using a catheter. The oocytes were sampled in vivo and placed in Serra’s solution for five minutes.

After clarification of the cytoplasm, the position of the germinal vesicle was determined according to a four-stage scale described by Brzuska (1979) for common carp.

Following the clarification of the cytoplasm, the position of the germinal vesicle was determined in oocytes according to a four-degree scale:

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

For further experiments, only females with oocytes at stage 2 or 3 were taken for the spawning experiment. The number of males and females in all groups was 10. There were no statistical differences between the weights of fish from the different origin groups: wild (average weight 982±86 g) and cultured yellow (average weight 223±29 g) fish. The selected males and females from each group were kept in a hatchery in separate 1000 dm³ tanks with controlled temperature and photoperiod. The maximum fish load was 25 kg m⁻³. Fish from both stocks were randomly divided into three (wild fish) or two groups (cultured fish). All fish were kept in the same type of tanks. In each group, a different thermal regime before spawning was tested:

**Group 1**: wild and cultured fish, were kept in a model temperature regime for ide artificial reproduction (hatchery of Department of Lake and River Fisheries – wild and yellow form of ide) (Kucharczyk et al., 2008). The temperature was gradually increased from 10 to 14°C (Figure 1).

**Group 2**: wild form only, which were kept indoors under natural temperature conditions (hatchery Fish Farm Janowo); water temperature was variable and oscillated between 10 to 14°C (Figure 1).

**Group 3**: fish (both forms) were kept initially to each sample to the gametes for their activation. After 3 min, the water was changed. Eggs were incubated at 15°C, which was the best temperature for ide embryonic development (Kucharczyk et al., 2008). Oocyte samples from non-ovulated females were taken with a
catheter after the experiment and their maturity stage was recorded.

Survival of the embryos was checked at the eyed-egg-stage (7th day of incubation). Statistical differences between groups (incubation success, semen characteristics) were analyzed with an analysis of variance (ANOVA) and post-hoc Tukey’s test (P<0.05) for wild-coloured ide and a t-test for orfe (P<0.05). Normal distribution of the data was checked using a Shapiro-Wilk test. Statistical analysis was done using Statistica 8.0 (StatSoft) software.

**Results**

In the present study, differences in percentage of spermiation, ovulation, semen quantity and gamete quality were observed (Tables 1 and 2) in relation to temperature. The percentage of spermiation in males from group 3 was the lowest, as well as the sperm volume and spermatozoa motility in both cases of wild and cultured fish. The results obtained in group 3 were statistically different from other groups.

The obtained results of female reproduction also showed the impact of temperature on successful reproduction. The percentage of ovulated females was different in all groups (Tables 1 and 2). The highest was observed in group 1, where eggs were stripped from all females. In ide, there were no differences in the quality of eggs in groups 1 and 2, expressed as embryo survival. The lowest percentage of ovulated females was noted in group 3 (70% and 60% for wild- and yellow-coloured form respectively). In the same group in the wild-coloured form, over 40% of the obtained eggs (for 3 from 7 females) were of very low quality; the eggs were over-matured and they were not used for the fertilization process. The percentage of ovulation and quality of eggs, as well as the embryo survival from the eggs obtained (and fertilized) from the rest of the females in this group was the lowest. However, the latency time was the shortest in this group (32.36 h), but was close to group 1. Oocytes from non-ovulated females were in stages 3 and 4 for fish from group 2 and during resorption in fish from group 3.

**Discussion**

The results obtained in this study confirmed the influence of temperature on final oocyte maturation and spawning effectiveness in fish. Because other environmental parameters, such as photoperiod, dissolved oxygen level or pH might mask the influence of temperature on successful spawning, all measured environmental factors were similar (as well as the maturation stage of spawners) in order to demonstrate the decisive effect of temperature. Reproductive processes in fish (defined in aquaculture as the percentage of spermiated and ovulated spawners, gametes quality and quantity) is controlled by environmental factors, among which the photoperiod and water temperature are the most important (Stacey, 1984). In many teleost fish, the photoperiod is the most important factor in the reproductive cycle (Bromage et al., 2001) and the temperature is mainly important in the final gamete maturation, ovulation and spawning (Hilder and Pankhurst, 2003; Angius and Canavate, 2005; Targońska et al., 2010; Źarski et al., 2010). In rhipheic cyprinid fish, such as asp, dace, ide or chub, shortly before spawning under controlled conditions, the temperature usually increases slightly (Kucharczyk et al., 2008; Krejszeff et al., 2009). This suggests an important role of temperature in final gamete maturation. Temperature not only strongly influences the process of reproduction in fish (Bromage et al., 2001), but also the gamete quality (Hilder and Pankhurst, 2003; Kupren et al., 2010, 2011).

During artificial reproduction of ide, similar results as in the case of group 3 were noted in out-of-season reproduction of ide (Kucharczyk et al., 2008). The obtained results suggest that only four days of increasing temperature before reproduction might have a strong influence on spawning effectiveness. In spiny damselfish, Acanthochromis polyacanthus (Bleeker) only three days of rising temperature was enough to affect spawning results (Hilder and Pankhurst, 2003). A similar situation was noted for a domesticated strain of ide (Krejszeff et al., 2009). Generally in cyprinids, females without hormonal stimulation failed to ovulate and their oocytes did not mature. A similar observation was made for asp, common bream, chub, dace, nase, ide, common carp and many other fish species (Krejszeff et al., 2009; Targońska et al., 2010). This indicates that stimulation with environmental conditions alone, without hormonal stimulation, is not enough to cause final gamete maturation and ovulation under controlled conditions. In the present study, the results of the reproduction of ide males were similar to those obtained earlier during the spawning season (Kucharczyk et al., 2008), although males kept under natural temperature conditions produced a smaller volume of semen (group 2). In

| Parameter                              | Group 1                     | Group 2                     | Group 3                     |
|----------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Percentage of spermiation, %           | 100                         | 100                         | 60                          |
| Spermatozoa motility, %                | 72±3′                       | 70±4′                       | 46±8′                       |
| Sperm volume, cm³ kg⁻¹                 | 3.5±0.3′                    | 2.8±0.2′                    | 1.1±0.3′                    |
| Percentage of ovulation                | 100                         | 80                          | 70                          |
| Percentage of over-matured eggs⁵       | 0                           | 0                           | 43                          |
| Range of the latency time, h           | 36                          | 44-50                       | 32-36                       |
| Embryo survival to the eyed-egg-stage⁶ | 70.3±3.5′                   | 68.2±5.6′                   | 48.9±7.8′                   |
| Oocytes maturity in non-ovulated females, stages | -                           | 3-4                         | Resorption                  |

⁵Females produced over-matured eggs 100% all ovulated female in each group; ⁶data marked in row with the same letter did not differ statistically (Tukey test). In group 3, for calculating embryo survival, the over-matured eggs were excluded.

| Parameter                              | Group 1                     | Group 3                     |
|----------------------------------------|-----------------------------|-----------------------------|
| Percentage of spermiation, %           | 100                         | 70                          |
| Spermatozoa motility, %                | 80±4′                       | 51±6′                       |
| Sperm volume, cm³ kg⁻¹                 | 4.1±0.4′                    | 1.0±0.2′                    |
| Percentage of ovulation                | 100                         | 60                          |
| Range of the latency time, h           | 36                          | 32-36                       |
| Embryo survival to the eyed-egg-stage⁶ | 77.7±3.3′                   | 47.8±6.3′                   |
| Oocytes maturity in non-ovulated females, stages | -                           | Resorption                  |

⁶Data marked in row with the same letter did not differ statistically (t-test). In group 3, for calculating embryo survival, the over-matured eggs were excluded.
addition, there were no differences between either forms of ide (wild or cultured), although in other cases some differences during artificial reproduction between wild and domesticated strain were recorded (Krejzseff et al., 2009). Temperature-dependent reproduction effectiveness of ide females was confirmed by the data in this study. The data are quite similar to those obtained earlier (Kucharczyk et al., 2008) for both in- and out-of-season ide spawning. In captivity, the strong influence of temperature on artificial reproduction was also noted for spiny dace (Hilder and Pankhurst, 2003) and Senegal sole, *Solea senegalensis* (Angius and Canavate, 2005). In the case of the latter species, keeping the fish under natural temperature conditions caused a longer reproduction period. In the present study, keeping the fish under natural conditions only influenced elongation of the latency time and asynchronous ovulation, but did not decrease the biological quality of eggs. A similar observation was made by Targóńska et al. (unpublished) for dace. In the present work, temperature fluctuations up to the highest tested level occurred at decreased levels of all measured parameters. The same tendency, with a much stronger decrease in the percentage of ovulations and embryo survival, was noted for asp after a sudden increase in temperature during the spawning season (Targóńska et al., 2010).

**Conclusions**

The present study shows that the temperature regime in ide artificial reproduction should be kept as optimal (Kucharczyk et al., 2008), especially for artificial reproduction of wild populations (Zarski et al., 2009). A decrease in temperature to the level of 12°C or below caused only by longer latency time along with an increase in temperature to about 16°C decreased the number of ovulated females and quality of gametes. Rapid changes in water temperature or an excessively high pre-spawning temperature probably cause overly rapid gamete maturation and adversely affects their quality. The temperature shortly before reproduction should not exceed the thermal optimum for each species. For example, it was about 13°C for asp (Targóńska et al., 2010) and about 16°C for ide (Kucharczyk et al., 2008; present paper). It creates the necessity for much more attentive observations on the influence of temperature on the reproductive performance of various fish species during controlled reproduction.

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