Influence of age of wild ide Leuciscus idus (L.) female on spawning effectiveness under controlled conditions

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Abstract

The aim of this study was to determine the effect of age of female ide on spawning success under controlled conditions. All ide breeders were collected from one wild population. Data concerning reproductive parameters were collected during induced spawning under hatchery conditions. The age of mature females ranged from 4 (average weight 504 g) to 9 years (average weight 2540 g) and the youngest collected females (3+) (average weight 330 g) were immature. The females from group 4+ produced smaller oocytes (1.28 mm diameter after hydration) than older fish (1.44-1.57). There were no differences in oocyte sizes in females 6+ and older. The highest relative fecundity (mean 44,621 eggs kg⁻¹) was noted for fish aged from 5 to 7. Eggs obtained from the oldest females had the lowest biological quality: the highest percentage of morphological abnormalities in hatched larvae (5.6%). In addition, in the group of the oldest females, only 67% of fish produced eggs.

Introduction

Spawning effectiveness in fish is affected by many factors. These include the species and the population origin, fish condition, size, age as well as a number of environmental factors including photo-thermal conditions (Brooks et al., 1997; Anguis and Canavate, 2005; Bromage et al., 2001). Proper manipulations of thermal regime, photoperiod (Hilder and Pankhurst, 2003; Kucharczyk et al., 2008a; Targóńska et al., 2010) and hormonal agents (Brzuska, 2006; Yaron et al., 2009) were the main factors determining successful reproduction in captivity. In artificial reproduction of the cyprinids, the type of hormones and its combination affected the latency time, ovulation rate and gamete quality (Yaron 1995; Kucharczyk et al., 2005; Brzuska et al., 2006; Szabo 2003; Krejszeff et al., 2008; Zarski et al., 2009; Cejko et al., 2010; Targóńska et al., 2010). Only domesticated stock of cyprinids was reported to be able to spawn after stimulation of photo-thermal conditions (Krejszeff et al., 2009).

Research into the effect of age on the reproductive parameters of fish has been limited (Brooks et al., 1997). There are scarce reports, mainly comparing the females which spawned for the first time with those a year older (Bromage and Cumaranatunga, 1988). It has been shown in recent studies of aquarium fish – Buenos Aires tetra, Hypseleobrycon anisitsi (Eigenmann, 1907) and neon tetra, Paracheirodon innesi (Myers, 1936), that viable offspring cannot be obtained from females which have spawned 6-7 times after they reached maturity (Kucharczyk et al., 2008b; 2010). This indicates that there is a relationship between the number of spawns and the biological quality of eggs and possibility of fertilizing. However, there have been no comprehensive reports about the effect of female age on spawning.

Ide, Leuciscus idus (L.), is one of the most popular sport fish in eastern and central Europe (Krejszeff et al., 2009). The production of ide fry for restocking purposes is an important source of income for fish farms and open water managers (Wojda, 2004). One of the bottlenecks in riphilic cyprinid fry production is controlled reproduction (Kucharczyk et al., 2008b) where hormonal preparations and a thermal regime were reported to be very important factors affecting successful spawning (Zarski et al., 2009; Cejko et al., 2010). Among the many preparations tested in ide controlled reproduction, the best results were achieved after applying preparations containing GnRH analogues and the dopamine inhibitors: Ovopel and Ovaprim (Krejszeff et al., 2009; Żarskiarski et al., 2009; Cejko et al., 2010). In addition, an elevated water temperature was found to be a limiting factor for embryo viability, which was also reported for asp Aspius aspius (L.) (Targófska et al., 2010). There is no other data concerning other factors affecting ide reproduction effectiveness.

The aim of this study was to examine how ide female age affects spawning effectiveness, defined as the percentage of ovulations, as well as the number and quality of gametes obtained.
Results

Before the hormonal stimulation was started, it was found (based on recognition of the oocyte samples) that female fish aged 3+ were sexually immature (oocytes at stage 1). Oocytes taken from these females were much smaller (0.34 mm) than in older fish (0.76 mm). In the latter, oocytes were at maturity stage 2. It is noteworthy, that it was possible to extract the oocytes from immature females. Oocytes from two individuals from the oldest (9+) group could not be extracted by a catheter. Thus, it could be suggested that these females did not produce oocytes. After reproductive procedures (hormonal and photo-thermal stimulation), the oocytes were not stripped and it was also not possible to extract oocytes with a catheter. When the oocyte maturity stage was checked after the experiment was completed, they had the same size (0.34 mm in diameter) and maturity stage 1. Ovulation took place 36 hours after the resolving injection (Table 1). All the fish in age classes 4+ to 8+ ovulated. A slight progression of the oocyte maturation was observed in the control group. The oocytes were at stage 2 – 23. No statistical differences were found in embryo survival to the eyed-egg stage in eggs obtained from females aged between 5+ and 8+ (average about 70%) (Table 1). Lower egg quality was obtained from fish aged 4+ and 9+. Eggs obtained from the oldest fish were of the lowest quality, where a 50.1% embryo survival to the eyed-egg stage was recorded. Survival of the eggs obtained from the youngest spawning females was significantly better (59.6%). Similar relationships were found after analyzing the percentage of deformed larvae. The highest deformation rate was found in the group with 9-year-old females (5.6%). Significantly better results (P<0.05) were recorded in the 3+ group (2.3% deformed larvae). However, in the remaining groups, the

Table 1. Spawning effects (mean ±SD) of female ide in various age groups. There were 10 females in each group, except the group with age 9+ females, where there were 6 fish.

| Age of female ide | Weight before spawn, g | Percentage of ovulations, % | Latency time, hours | Embryo survival to the eyed-egg stadium, % | Percentage of individuals hatched with body deformities, % |
|-------------------|------------------------|----------------------------|--------------------|--------------------------------------------|-------------------------------------------------------|
| Control group     | -                      | 0                          | -                  | -                                          | -                                                      |
| 3                 | 330±32                 | -                          | -                  | -                                          | -                                                      |
| 4                 | 504±40                 | 100                        | 36                 | 59.6±3.60                                  | 2.3±0.50                                              |
| 5                 | 889±49                 | 100                        | 36                 | 69.8±3.80                                  | 0.1±0.00                                              |
| 6                 | 1236±69                | 100                        | 36                 | 70.1±5.10                                  | 0.0*                                                   |
| 7                 | 1658±86                | 100                        | 36                 | 69.9±4.30                                  | 0.0*                                                   |
| 8                 | 2030±68                | 100                        | 36                 | 70.3±3.90                                  | 0.2±0.14                                              |
| 9                 | 2540±89                | 67*                        | 36                 | 50.1±3.50                                  | 5.6±0.7*                                               |

*Thirty three % of females of the 5+ age group did not produce oocytes which could ovulate in 2009. **Data with the same letter index in a column are not statistically different.
deformation rate did not exceed 0.01% (P>0.05) (Table 1).

Relative fecundity, expressed as the number of eggs per kg of female body weight before ovulation, showed an increasing tendency between the fourth and the seventh year of life (from 23,584 to 48,789 eggs kg⁻¹) (Figure 1). Subsequently, the fecundity was seen to decrease to 30,698 eggs kg⁻¹ in the older females. Moreover, a positive correlation was found between the female size and the diameter of swollen oocytes after hydration. The value ranged from 1.2 mm in the fourth to 1.6 mm in the ninth year of life (Figure 2).

Discussion

A significant amount of recent research has been devoted to various aspects of fish ageing. However, there have been no reports on the direct effect of fish age on gamete quality and spawning effects. In order to determine this, studies were started on species with short life cycles, such as turquoise killfish, Nothobranchius furzeri, Buenos Aires tetra and neon tetra (Kucharczyk et al., 2007; Terzibasi et al., 2004). It was found in the latter two species that viable offspring after hydration. The results obtained indicate that using too young fish for reproduction is not a guarantee of breeding success either. As the examples of Buenos Aires tetra (Kucharczyk et al., 2008b) and neon tetra (Kucharczyk et al., 2010) have shown, fish which spawn for the first time produce fewer oocytes of slightly lower quality than during the next few spawns. Similar results, associated with a distinct decrease in the reproductive capacity of fish with a short life span, have been reported by Patnaik et al., (1994) and Gerhard (2007). However, published data on the effect of age on the reproductive outcome in fish with a longer life-span were only fragmentary reports. It has been shown in the European sea bass (Dicentrarchus labrax) and rainbow trout (Bromage et al., 1992; Brooks et al., 1997) that the quality and amount of eggs obtained from fish which spawn for the first time was lower than in fish which spawn for the second time. However, no differences have been found in the outcome of the first two reproductions in 1-year old tench (Rodriguez et al., 2004). It was found in the present study that the quality of oocytes and fecundity of ide which spawn for the first time is also lower in older fish (aged 5-8 years).

The results obtained indicate that ide females from the lake Mosag, where fish for this experiment were caught, reached sexual maturity at year 4 of their lives. The high ovulation rate in groups aged from 4+ to 8+ was close to the data reported for the species by e.g., Kucharczyk et al., (2008a), Krejzeff et al., (2009) and Żar ski et al., (2009). Only in the oldest fish in this study (age group 8+) was the ovulation rate found to have decreased. It resulted from the fact that 33% of females did not produce oocytes. These fish may have been too old to reproduce or an annual break in reproduction might have taken place, which has been observed in some species. For example, it has been found that not all burbot, Lota lota (L.), females were ready for reproduction during their spawning season and it was supposed that some of them reproduce every two or more years (Pulliainen and Korhonen 1993). However, such observation for ide has never been reported and, in the present study, it was strictly related with the age. It should be pointed out that it is the first report on a reproduction failure of the ide during the spawning season where eggs were not sampled with a catheter (Kucharczyk et al., 2008c; Krejzeff et al., 2009; Żar ski et al., 2009). This was probably related to the fact that such old fish (9+) with such high weights had not previously been caught for spawning (Kucharczyk et al., 2008c). However, it has to be stated that this phenomenon should be studied more precisely.

Latency time after hormonal stimulation was related to inter-populational differences (Kujawa et al., 2011), degree of domestication (Krejzeff et al., 2009) and type or dose of hormonal treatment (Żar ski et al., 2009; Targonka et al., 2010). Differences in latency resulting from the size and age of females were reported for Eurasian perch, Perca fluviatilis L., where ovulation in older (bigger) fish took place later than in younger ones (Kucharczyk et al., 2001). It created the necessity for separation of the spawners into different sizes or age classes to avoid handling stress in females.

![Figure 1. Relationship between relative fecundity (eggs per body weight, BW) and ide female age.](image1)

![Figure 2. Relationship between diameter of swollen eggs and ide female age.](image2)
which ovulated later. The age of the ide females has not been found to affect latency time, which was the same in all the age groups. Thus, during the reproduction of ide, spawners of different ages could be kept together.

According to Brooks et al. (1997), oocyte size might be positively correlated with oocyte quality and offspring survival. Higher egg size usually increased the quality. In the halibut, Hippoglossus hippoglossus, larvae quality was strictly related to oocyte size (Evans et al., 1996; Mazorra et al., 2003). A similar relationship with respect to the oocyte diameter has been found in the carp (Kucharczyk et al., 2008a). In the present study, no clear relationship between egg size and quality was found. Low quality was recorded for both the smallest and largest eggs, obtained from the youngest and oldest females. Thus, it might suggest that the age of females is a more important factor than egg diameter, which directly affects egg and larval viability.

Conclusions

The current study indicates that the age of fish is a very important factor affecting the effectiveness of reproduction, but only where too young or too old fish were used. Females fish aged from 4+ to 8+ were comparably useful spawners for commercial purposes. It provides farmers with a great opportunity to use such widely-aged broodstock without negative effects on production effectiveness. The culture program should include the highest quality gene pool as possible, especially for restocking purposes and managing the wild populations. Thus, eliminating the oldest fish from a spawning schedule because of the slightly lower biological quality of eggs is not justified as it could reduce the genetic pool of future generations (Wildt et al., 1993), excluding specimens in which eggs are not possible to sample with a catheter.

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