Induced artificial androgenesis in common tench, Tinca tinca (L.), using common carp and common bream eggs

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Abstract

This study presents artificial induction using tench eggs, Tinca tinca (L.), of androgenetic origin. The oocytes taken from common bream, Abramis brama (L.) and common carp, Cyprinus carpio L. were genetically inactivated using UV irradiation and then inseminated using tench spermatozoa. Androgenetic origin (haploid or diploid embryos) was checked using a recessive colour (blond) and morphological markers. The percentage of hatched embryos in all experimental groups was much lower than in the control groups. All haploid embryos showed morphological abnormalities, which were recorded as haploid syndrome (stunted body, poorly formed retina, etc.). The optimal dose of UV irradiation of common bream and common carp eggs was 3456 J m⁻². At this dose, almost 100% of haploid embryos were produced at a hatching rate of over 6%. Lower UV-ray doses affected abnormal embryo development. The highest yield of tench androgenesis (about 2%) was noted when eggs were exposed to thermal shock 30 min after egg activation.

Materials and methods

Bercsenyi et al., 1998; Kucharczyk et al., 2008b, 2008c). The common tench Tinca tinca (L.) is a species which has a huge commercial value in many European countries, such as the Czech Republic, Hungary, Italy, Spain and Poland and other continents (Wang et al., 2006; Celada et al., 2009; Kujawa et al., 2011). For developing a culture method, different aspects of artificial reproduction (Kujawa et al. 2011; Targóńska et al., 2012), gamete management (Mamcarz et al., 2006), hatchery techniques (Kujawa et al., 2010) and larval and juvenile rearing (Mamcarz et al., 2011; Nowosad et al., 2013) are studied. Since some of the common tench wild and cultured stocks may be extinct, many aspects of biotechnology should be studied, e.g. cryopreservation of sperm (Rodina et al., 2007) or genome manipulation like androgenesis.

The aim of this study is to determine the possibility of obtaining androgenotes from common tench, using the eggs of common carp and bream Abramis brama (L.).
Experimental groups of eggs (common carp and common bream) after exposition to the different time of UV irradiation: E1, E3, E6, E8, E9, E10, E12 or E14 min (dose of UV irradiation ranged from 384 to 5376 J m\(^{-2}\)) were inseminated with 0.05 mL of yellow-coloured trench sperm. After irradiation, eggs from additional control groups were fertilised (control of treatment in ovarian fluid: groups D2). The eggs were incubated in a laboratory recirculating system at 21°C. All experimental groups were analysed in triplicate. During androgenesis, experimental groups were fertilised with 0.05 mL of yellow trench form sperm after exposition of the oocytes to a 9 min UV irradiation (an UV irradiation dose of 3456 J m\(^{-2}\)). Inseminated eggs were exposed to thermal shock from 20 to 60 min after egg activation (40°C; 2 min duration). This constituted E20, E30, E40, E50 and E60 groups, respectively. Eggs treated with UV irradiation, but not exposed to the thermal shock, were fertilised using the yellow form of trench sperm (control of irradiation quality, group I). After the experiment, eggs from other control groups were inseminated (control of treatment in ovarian fluid: groups D2). The whole procedure during both experiments was carried out in darkness to avoid genetic photo-reactivation (Kaastrup and Horlyck, 1987). Before the application of thermal shock, the eggs were kept at 21°C. After the experiment, the eggs were incubated in a laboratory recirculating system at 21-22°C, which was found to be the optimum incubation temperature for trench, carp and bream (Kucharczyk et al., 1997a, 1997b, 1998, 2005). All experimental groups were analysed in triplicate.

The success of androgenetic development in embryos in present study was determined in few ways: i) haploid syndrome (poorly formed retina, stunted body, etc.); ii) colour marker (wild-dark colour or yellow); and iii) morphological marker: the differences between embryos of pure species and their hybrids (Kucharczyk, 2002; Manczarz et al., 2006).

The differences in hatching success and in the survival of trench embryos were analysed using ANOVA and tested by post-hoc Duncan’s multiple range test (P<0.05). All of the values expressed as percentages were arcsine transformed prior to statistical analysis.

**Results and discussion**

The optimum irradiation dose of eggs for both fish species (bream and carp) was 3456 J m\(^{-2}\) (9 min exposure) (Figure 1A and 1B). In these groups, the highest percentage of live embryos at the eyed-egg-stage, as well as the highest hatching rate was observed. All hatched embryos from these groups were yellow-coloured and showed morphological abnormalities, which were recorded as haploid syndrome, i.e. stunted body, poorly-formed retina. The survival of newly-hatched (5 h after hatching) larvae in all experimental groups was statistically lower than in the control groups (groups K and D) (Figure 1A and 1B). Such results were in contrast with embryos survival to the eyed-egg-stage, where there were no significant differences between treated and control groups (P>0.05). All hatched larvae in these groups irradiated from 6 (dose 1152 J m\(^{-2}\)) to 14 min (dose 5376 J m\(^{-2}\)) were yellow, whereas all specimens from control groups were wild (dark) coloured. In groups where the lowest UV doses were applied (1 and 3 min of treatment), dark diploid, aneuploid, as well as a few blond haploid embryos were recognised. Many abnormal dark-coloured embryos, morphologically similar to the typical haploids, were probably aneuploids.

The survival of newly-hatched larvae (5 h after hatching) in all experimental groups involving androgenesis, was significantly lower than in the control groups. In the latter groups, the survival was high, except in the control groups where common carp eggs were used (Figure 2A and 2B). Yellow-coloured haploid embryos showed morphological abnormalities. The highest yield of androgenesis (P<0.05) was noted when eggs were exposed to shock 30 min after egg activation. The high level of survival to the eyed-egg-stage in control (D1 and D2) and treated groups suggests that stirring and keeping the eggs in artificial ovarian fluid is not harmful to embryonic development. Diploid androgenotes of trench were morphologically different than embryos from the control groups (K). Haploid larvae were viable only for the next 3 to 4 days after hatching.

During the experimental period, it was observed that one of the negative effects on common tench production is the unpredictable variation of environmental conditions. This situation has prompted a search for ways to increase the tench population or at least maintain the population in Polish lakes. Tench is economically important for Polish inland fisheries and the gradual decline in production has caused serious difficulties, particularly in increasing interspecies competition, rural and industrial origin pollution and overexploitation. One solution to the problem is to apply modern genome...
engineering. The aquaculture of many more species was dynamically powered to the developing genome engineering (Krasznai and Marian, 1986; Goryczko et al., 1991; Kucharczyk et al., 2008b, 2008c; Ocalewicz et al., 2010) and the results of the studies are used not only for commercial purposes but are also of scientific interest. The low hatching rate of tench embryos from genetically inactivated oocytes (0-2%) has been observed in many other fish species. Similar data to the present work have been reported by other authors: i.e. Scheerer et al. (1986) and Babiak et al. (2002a, 2002b) for rainbow trout (Oncorhynchus mykiss Walbaum 1792), May et al. (1988) for brook trout (Salvelinus fontinalis Mitchill 1814), Arai et al. (1992) for loach (Misgurnus anguillicaudatus Cantor 1842), Bongers et al. (1994) for common carp, Kucharczyk (2002) for common bream, Lin and Dabrowski (1998) for northern pike and Kucharczyk (2001) and Kucharczyk et al. (2008b, 2008c) for ide (Leuciscus idus L.) and dace (Leuciscus leuciscus L.). Such low survival rates of androgenotes were probably connected with the synergic effect of a few sublethal manipulations such as irradiation of oocytes and applied temperature shock as well as increased inbreeding.

The high level of survival to the eyed-egg-stage in control (D1 and D2) and treated groups suggests that stirring is not harmful to eggs. Similar observations have been made by Bongers et al. (1994). The obtained results show that UV treatment successfully inactivated the nuclear DNA in common carp and common bream eggs. Androgenetic origin (haploid or diploid embryos) was checked using a recessive colour marker (blond) and a morphological marker. Data published by Mamcarz et al. (2006) showed that newly-hatched hybrids between tench and carp or bream are morphologically different from genetically pure tench specimens. The low survival of hatched diploid embryos in the case of common carp – tench hybrids was also previously reported by Mamcarz et al. (2006). The applied dose of UV irradiation was 3456 J m−2, at which almost 100% haploid embryos were produced at a hatching rate of over 2%. These doses were higher than those described as the optimum UV oocyte treatment for common carp (2500 J m−2) by Bongers et al. (1994) or for northern pike (660 to 1320 Jm−2) by Lin and Dabrowski (1998) and were similar to those reported by Kucharczyk (2002) for common bream and Kucharczyk (2001) for ide (2700 to 3500 J m−2). For doubling chromosomal material during the androgenesis process, heat shocks were usually applied and, in some cases, very intensively (up to 42°C) (Arai et al., 1995; Grunina et al., 1995; Bercsenyi et al., 1998; Pandian and Koteeswaran, 1998; Kucharczyk, 2002). The survival of larvae of androgenetic origin is variable (usually very low) and depended on many different factors. The data published by Scheerer et al. (1986), Bongers et al. (1994, 1995) and by Pandian and Koteeswaran (1998), Rothbard et al. (1999), Babiak et al. (2002a, 2002b) showed that if androgenesis is involved in inbred lines, the survival was much higher than in wild ones. The obtained results in the present work showed that the survival of androgenetic-origin larvae was between 0 and 2%. However, the results of the present research were not different from those obtained by Kucharczyk (2002).

![Figure 1](image_url)

Figure 1. Inactivated oocyte genome in common bream (A) and carp (B). The haploid hatched groups marked with the same letter do not differ statistically (post-hoc Duncan test, α=0.05).
for pure tench androgenesis. Grunina et al. (1995) and Bercsenyi et al. (1998) suggest that if eggs from other fish species than spermatozoa were used, this usually increased embryo survival. However, the obtained results in the present work did not differ from the data obtained by Kucharczyk (2002) for pure tench androgenesis.

Conclusions

The study found that androgenetic tench production using eggs from common carp and bream is possible. However, the yield of androgenesis is not satisfactorily high, although it is enough for the stock producing of androgenetic tench.

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Figure 2. Artificial androgenesis induction in tench using common bream oocytes (A) and common carp oocytes (B). The diploid hatched groups (E20’-E60’) marked with the same letter do not differ statistically (post-hoc Duncan test, α<0.05).
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