Effects on the reproduction of C. gariepinus after ovulation stimulation with carp pituitary homogenate (CPH), Ovopel or Dagin were investigated. After the application of Ovopel all the females spawned while after CPH or Dagin treatment spawning was reduced to 83.3%. No statistically significant effect of the stimulator was found on the weight of eggs expressed in g and in % of female B.W. however, the highest mean values of these parameters were found after Dagin. A statistically significant effect of the stimulator was noted for the percentage of fertilization and living embryos after 24h and 28h incubation. The highest percentage of live embryos after a 28h incubation of eggs was found after the treatment with Dagin and the lowest after Ovopel; the difference between the means of these stimulators was statistically significant. The females ovulated 12h after Ovopel treatment yielded eggs of a higher weight and of significantly higher quality compared with those obtained 3h later. The relative effectiveness of reproduction, expressed as the number of live embryos per kg of female B.W., was highest after Dagin treatment.
Badano wyniki rozrodu suma afrykańskiego C. gariepinus po stymulowaniu owulacji homogenatem przysadki karpia (CPH), Ovopelem lub Daginem. Po podaniu Ovopelu ikrę oddały wszystkie samice, a po zastosowaniu CPH lub Daginu 83.3% ryb. Nie odnotowano statystycznie istotnego wpływu stymulatora na masę pozyskanej ikry, wyrażonej zarówno w gramach, jak i w procencie masy ciała samic, ale najwyższe wartości tych parametrów wykazano po podaniu Daginu. Statystycznie istotny wpływ stymulatora owulacji odnotowano dla procentu zapłodnienia i żywych zarodków po 24 i 28 godz. inkubacji jaj. Najwyższy procent żywych zarodków po 28 godz. inkubacji jaj wykazano po podaniu Daginu, a najsłabszy po użyciu Ovopelu; różnica między średnimi dla tych stymulatorów była statystycznie istotna. Samice, które owulowały 12 godz. po podaniu Ovopelu, oddały ikrę w większej masie i istotnie lepszej jakości w porównaniu z ikrą pozyskaną od ryb, które owulowały 3 godziny później. Relatywna efektywność rozrodu, wyrażona jako liczba żywych zarodków na 1 kg masy ciała samic, była najwyższa po podaniu Daginu.

Introduction

The African catfish Clarias gariepinus is a species well established in European aquaculture (HUISMAN and RICHTER 1987, KUCZYŃSKI et al. 1999). In optimal conditions the growth of this fish is rapid and the production cycle can be shortened to seven months. The flesh of this species has high nutritional quality and good taste. In the temperate climatic zone in summer months, C. gariepinus can be stocked in fishing ponds as a species interesting for anglers. C. gariepinus is a model species for reproduction endocrinological investigations in Teleosts (VAN OORDT and GOOS 1987, RESINK et al. 1989, VAN WEERD et al. 1990). The above traits and the fact that C. gariepinus can mature and reproduce in captivity (HUET 1972, HOGENDOORN and VISMANS 1980) encourage the investigation of improvements to the reproduction biotechnology of this interesting fish species.

In carrying out the reproduction of fish in controlled conditions the basic aim is to obtain the highest possible yield of the best quality eggs and, hence, to produce the highest possible numbers of good quality hatches. To this end, attempts are made to find ovulation stimulators which ensure the best effects of the controlled reproduction. Also it is obvious that suitable maternal and paternal material should be used to obtain satisfactory results in stimulated fish propagation. In the case of the African catfish C. gariepinus different preparations were used to induce the maturation of oocytes and ovulation. Among these materials were acetone-dried powdered carp pituitary (HOGENDOORN and VISMANS 1980, ADAMEK 1993, DE GRAAF et al. 1995), crude pituitary extract of Clarias albopunctatus and frog Rana elegans (Inyang and Hettiarchchi 1994), 17α-hydroxy-progesterone (RICHTER et al. 1985, RICHTER et al. 1987), 11-desoxycorticosterone-acetate (RICHTER and VAN DEN HURK 1982), human chorionic gonadotropin-hCG (EDING et al. 1982; Inyang and Hettiarchchi 1994), hCG + Oxitocin (Hecht et al. 1982), Des Gly\(^{10}\)[D-Ala\(^6\)]LHRH-
ethylamide with pimozide (DE LEEUW et al. 1985, RICHTER et al. 1987, RESINK et al. 1989) or in combination with different drugs with anti-dopamine and anti-serotonin properties (GOOS et al. 1987, KOUŘI et al. 1992). A list of the hormonal glycoproteins used to induce spawning in C. gariepinus was given by HAYLOR (1993).

The extended studies on the effects of controlled reproduction of C. gariepinus which started in 1996 at the Golysz Institute of Ichthybiology and Aquaculture (Polish Academy of Sciences) included numerous experiments using different ovulation stimulators of natural and synthetic origin. The natural spawning agents with which the fish were treated, were carp Cyprinus carpio pituitary homogenate (see BRZUSKA 2005a), bream Abramis brama pituitary homogenate (Brzuska et al. 1998b) and human chorionic gonadotropin hCG (BRZUSKA et al. 1998c, 1999, 2000). The synthetic spawning agents were preparations of: desGly^10,[D-Ala^6]-LHRH Ethylamide applied with dopaminergic inhibitor pimozide (BRZUSKA et al. 1998c, 1999) and [Tle^6,ProN^9]mGnRH (Lecirelin) applied with dopaminergic inhibitor metoclopramide (BRZUSKA et al. 2004). The complex ovulation inducing preparations contained GnRH-a and a dopamine receptor blocker at the pituitary level used in aquaculture. The preparation Aquaspawn (Republic of South Africa) was also tested on the females of C. gariepinus at Golysz Institute (BRZUSKA 2003) as well as Ovopel produced in Hungary (HORVÁTH et al. 1997) (BRZUSKA 2001b, 2002a,b, 2004, 2005a, BRZUSKA et al. 1998a, 2000).

The results of the successive experiment presented in this paper illustrate the progress in the studies on this interesting fish species carried out at Golysz Institute. An experimental agent for inducing spawning in fish named Dagin (DRORI et al. 1994, KULIKOWSKY et al. 1996, “Dagin” – Instructions for use) was used in the present study. It has been successfully used for ovulation stimulation in different fish species e.g. in carp Cyprinus carpio (KOUŘI et al. 2003a, BRZUSKA 1999, 2005b, 2006), in grass carp Ctenopharyngodon idella (KOUŘI et al. 2003a) in tench Tinca tinca (KOUŘI et al. 2003b) and in the European catfish Silurus glanis (BRZUSKA, unpubl. data). However, an attempt at ovulation stimulation with Dagin in pike Esox lucius failed (SZABÓ 2003).

Dagin combines a superactive salmon GnRH analogue [(D-Arg^6,Pro^9NEt)3sGnRH] and the dopamine receptor antagonist, metoclopramide. Each dose, calculated per kg body weight of fish, contains 10 μg of the analogue and 20 mg metoclopramide (KULIKOVSKY et al. 1996). A diagram showing latency dependence on water temperature has been elaborated for Dagin, facilitating the proper timing of ovulation control (YARON et al. 2002). A study on the use of this preparation in C. gariepinus – a species not used previously in the tests with this preparation – was undertaken owing to the clear advantages of Dagin. Dagin is made of fully synthetic components and is therefore free of any pathogen which may reside in pituitaries of the
donor fish used in hypophysation. Ovulation induction by Dagin requires only a single injection and hence reduced stress to the females and less work compared with carp pituitary homogenate treatment. An important factor in hatchery conditions is that the preparation of this stimulator for injection is very simple since there is no need to pound it in a mortar, as is the case with carp pituitary or Ovopel. Neither is it necessary to weigh Dagin in order to calculate its proper dose. It is only necessary to dissolve the content of the dry matter in the vials (provided with the number of doses per body weight of the spawners given on the label) in a saline solution or water and inject it into the fish.

The aim of the present investigation was to compare the effectiveness of reproduction of African catfish *C. gariepinus* after the Dagin treatment with propagation results after CPH or Ovopel which are the most frequently used ovulation stimulators in this fish species. The investigation also concerned the dependence between the reproduction effects after ovulation stimulation with different spawning agents and the latent period.

**Material and Methods**

The experiment was carried out at the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences in Gołysz. It included 18 females of the African catfish *C. gariepinus* of body weight varying from 1.50 kg to 2.53 kg. The fish, selected from a larger population of spawners, were divided into three groups of six (Table 1). The external signs of maturity (large and soft abdomen) were taken into consideration in selecting the females. They were placed in nine 3 m³ volume tanks with two females in each tank. Thus, the fish from each group were in three tanks. During the experiment the water temperature was maintained in the range of 24–25°C. After a 24h adaptation period the ovulation was stimulated with CPH in group I; with Ovopel in group II; and with Dagin in group III. The doses of the applied agents for spawning induction and the application method are given in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of females</th>
<th>Substances</th>
<th>Dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Carp pituitary</td>
<td>4 mg (i.p.)</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Ovopel</td>
<td>1 pellet (i.p.)</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Dagin</td>
<td>1 standard dose</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* dose per kg body weight; i.p. intraperitoneally

1 pellet of Ovopel contains 18–20 μg of D-Ala⁶,Pro⁹NEt-m GnRH and 18–20 mg of metoclopramide (HORVÁTH et al. 1997)
The control of ovulation began 10h after stimulation with the above three preparations and was continued every hour during the next five hours. The fish were checked for ovulation by gentle pressing of the abdomen.

Eggs yielded by stripping the females were weighed and fertilized from each fish separately with pooled milt taken from the macerated testes of three killed males (INYANG and HETTIARACHCHI 1994). The incubation of the eggs from each female was carried out in separate Weiss glasses 7L in volume. After a 12h incubation the percentage of egg fertilization and after 24h and 28h incubation the percentage of live embryos were calculated as follows: the mean percentage of fertilization and the mean percentage of live embryos were calculated for each fish separately from three samples of 100 eggs taken on a Petri dish. After the hatching of larvae the correctness of their development was observed and the percentage of deformed individuals was calculated.

The obtained data (descriptive statistics are given in Table 2) were subjected to an analysis of variance using the least-squares method (HARVEY 1987)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Descriptive statistics</th>
<th>n</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of females [kg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>6</td>
<td>2.16</td>
<td>1.50</td>
<td>2.53</td>
<td>0.40</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>1.99</td>
<td>1.60</td>
<td>2.50</td>
<td>0.34</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>2.12</td>
<td>1.80</td>
<td>2.30</td>
<td>0.19</td>
</tr>
<tr>
<td>Weight of eggs [g]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>260.40</td>
<td>182.00</td>
<td>307.00</td>
<td>48.48</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>216.67</td>
<td>103.00</td>
<td>334.00</td>
<td>79.58</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>265.60</td>
<td>175.00</td>
<td>394.00</td>
<td>86.23</td>
</tr>
<tr>
<td>Weight of eggs [percentage of female body weight]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>11.97</td>
<td>11.46</td>
<td>12.53</td>
<td>0.40</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>10.76</td>
<td>6.44</td>
<td>13.36</td>
<td>2.51</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>12.44</td>
<td>9.05</td>
<td>17.90</td>
<td>3.57</td>
</tr>
<tr>
<td>Fertilized eggs after 12-h incubation [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>98.00</td>
<td>97.00</td>
<td>99.00</td>
<td>0.71</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>95.83</td>
<td>94.00</td>
<td>98.00</td>
<td>1.83</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>97.60</td>
<td>94.00</td>
<td>99.00</td>
<td>2.07</td>
</tr>
<tr>
<td>Live embryos after 24-h incubation [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>97.20</td>
<td>97.00</td>
<td>98.00</td>
<td>0.44</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>93.00</td>
<td>87.00</td>
<td>97.00</td>
<td>3.41</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>96.00</td>
<td>93.00</td>
<td>98.00</td>
<td>2.12</td>
</tr>
<tr>
<td>Live embryos after 28-h incubation [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>91.00</td>
<td>90.00</td>
<td>93.00</td>
<td>1.41</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>85.17</td>
<td>64.00</td>
<td>93.00</td>
<td>10.85</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>94.80</td>
<td>91.00</td>
<td>98.00</td>
<td>2.77</td>
</tr>
</tbody>
</table>
whose main classification factor was the ovulation stimulator. The investigated parameters were: the weight of the eggs expressed in grams and in the percentage of female body weight, the fertilization percentage after a 12h incubation and the percentage of live embryos after a 24h and 28h incubation of the eggs. Analysis of variance was carried out according to the following linear model:

\[ Y_{ij} = \alpha + g_i + bW_{ij} + e_{ij} \]

where:
\( \alpha \) – the theoretical general mean (with the assumption that \( W_{ij} = 0 \));
\( g_i \) – the effect of treatment (spawning agent) \( i (i = 1...3) \);
\( b \) – the regression on female body weight;
\( W_{ij} \) – the body weight of the female \( j \);
\( e_{ij} \) – the random error connected with the observation \( j \).

Since the ovulation did not occur at the same time in all the females treated with Ovopel, the question arose whether the latency significantly affected the results of reproduction. An analysis of variance was carried out to answer this question, the main classification factor being the ovulation time. The following linear model was used:

\[ Y_{ij} = \alpha + c_i + bW_{ij} + e_{ij} \]

where:
\( \alpha \) – the theoretical general mean with the assumption that \( W_{ij} = 0 \);
\( c_i \) – the effect of time \( i \) on the ovulation \( (i = 1...2) \);
\( b \) – the regression on female body weight;
\( W_{ij} \) – the body weight of a female;
\( e_{ij} \) – the random error associated with the observation \( j \).

In the present investigation we also attempted to resolve the problem of significant differences in reproduction effects in the case of females which ovulated after the same latent period but after the application of different preparations. Therefore, two separate analyses of variance for the ovulation time of 12h and 15h were carried out, using the least-squares method according to the following linear model:

\[ Y_{ij} = \alpha + g_i + bW_{ij} + e_{ij} \]

where:
\( \alpha \) – the theoretical general mean with the assumption that \( W_{ij} = 0 \);
\( g_i \) – the effect of ovulation stimulator \( i (i = 1...2) \);
\( b \) – the regression on female body weight;
\( W_{ij} \) – the body weight of a female;
\( e_{ij} \) – the random error associated with the observation \( j \).
The significance of the effect of treatment on the investigated parameters was verified with the F-test while Duncan’s multiple range test was used for analyzing the significance of differences between the means of the three investigated groups (Table 3). The estimated constants and the means of the least squares for the investigated parameters within the three groups are given in Table 3. The least-squares means, characterizing the effect of propagation associated with the time of ovulation, are given in Table 4. Phenotypic correlations between all the parameters were calculated separately for each group.

Relative effectiveness of the reproduction (expressed as the number of live embryos after 28 h incubation per kg female B.W.) for each ovulation stimulator was calculated as:

\[ RER = \frac{ab}{100} \]

where:
\( a \) – number of eggs in the weight of eggs obtained per 1 kg female body weight;
\( b \) – mean percentage of live embryos after 28 h incubation of eggs

The calculation was carried out on the assumption that the mean weight of one *C. gariepinus* egg is 1.43 mg (VIVEEN et al. 1986).

**Results**

**Percentage of females ovulating after hormonal stimulation**

After the application of Ovopel, eggs were obtained from all the females while after CPH and Dagin from 83.3% of fish treated with these preparations.

**Ovulation time**

In the group of females treated with CPH in five females ovulation occurred 12h after its application. One female which did not spawn at that time was controlled 13, 14 and 15h after the CPH treatment. At the first two controls no information was obtained; after 15h the release of single degenerated eggs was found, showing the disturbed maturation process. Therefore, no further controls were carried out.

In the case of Dagin five females yielded eggs 15h after the injection. At that time in one fish which did not give eggs the release of single degenerated eggs
Table 3

Constants (LSC) and least-squares means (LSM) for investigated reproduction parameters and results of Duncan’s test

<table>
<thead>
<tr>
<th>Classification factor</th>
<th>Weight of eggs [g]</th>
<th>Weight of eggs (% of female body weight)</th>
<th>Percentage of fertilized eggs after 12 h incubation</th>
<th>Percentage of living embryos after 24 h incubation</th>
<th>28 h incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSC</td>
<td>LSM</td>
<td>SE</td>
<td>α = 246.31</td>
<td>α = 172.47</td>
</tr>
<tr>
<td>Ovulation stimulator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPH (group I)</td>
<td>-0.57</td>
<td>245.74^a</td>
<td>22.64</td>
<td>0.02</td>
<td>1.11</td>
</tr>
<tr>
<td>Ovopel (group II)</td>
<td>-10.96</td>
<td>235.35^b</td>
<td>20.92</td>
<td>-0.64</td>
<td>1.03</td>
</tr>
<tr>
<td>Dagin (group III)</td>
<td>11.57</td>
<td>257.94^a</td>
<td>22.43</td>
<td>0.61</td>
<td>1.10</td>
</tr>
<tr>
<td>Regression/female body weight</td>
<td>172.47</td>
<td>172.47</td>
<td>42.64</td>
<td>2.83</td>
<td>2.09</td>
</tr>
</tbody>
</table>

Group means designated by the same letters do not differ significantly from each other. Mean values marked with different letters are significantly different: with capital letters at P ≤ 0.01 and with small letters at P ≤ 0.05.

α – the theoretical general mean; SE – standard error of least-squares means; CPH – carp pituitary homogenate.
in abundant ovarian fluid was observed and hence further controls were
stopped.

In the group of females treated with Ovopel two terms of ovulation were
noted; 50% of fish yielded eggs 12h after the injection of this preparation and
after a further three hours the remaining 50% of females.

**Effect of the ovulation stimulator on the weight and quality
of obtained eggs**

The values of the least squares means for weight of eggs expressed both in
grams and in percentage of female body weight show that the highest weight of
eggs was found in the group of females treated with Dagin and the lowest after
Ovopel stimulation (257.94 g, 12.32% and 235.35 g, 11.07%, respectively; Table 3).
However, the ovulation stimulator did not significantly determine these traits
(Table 3). This main classification factor significantly affected investigated
traits characterizing the quality of eggs ($P \leq 0.05$; $P \leq 0.01$; $P \leq 0.05$) (Table 3).
After the incubation of 12h, 24h and also 28h the poorest quality characterized
eggs yielded by females treated with Ovopel (95.89%, 92.70% and 84.22%,
respectively; Table 3). The difference between the mean of this group and the
mean of the groups after CPH as well as after the Dagin treatment was
statistically significant for the percentage of live embryos both after the 24h
and 28h incubation (Table 3). The highest percentage of live embryos after
a 28h incubation of eggs was found for fish stimulated with Dagin (95.19%)
however, it was not statistically significantly higher than the respective value
noted for hypophysesd females (91.74%) – Table 3.

**Ovulation time and the weight and quality of eggs**

No statistically significant difference was found between the means deter-
mining the weight of eggs – both expressed in grams and in the percentage of
female body weight – obtained 12h and 15h after the application of Ovopel
(Table 4). However, in the case of 12h latency, the mean values for these traits
were higher by 28.35g and 1.86% (Table 4). The latent period significantly
affected the percentage of fertilization and the percentage of live embryos after
24h and 28h incubation of eggs ($P \leq 0.05$; $P \leq 0.05$; $P \leq 0.05$; Table 4) while the
means for these traits showed higher values for 12h latency compared with 15h
latency (97.4%, 94.4%, 89.6% and 94.3%, 91.6%, 80.7%, respectively; Table 4).

Within the latency of 12 h the effect of the ovulation stimulator was only
statistically significant ($P < 0.05$) with respect to the percentage of live embryos
Table 4

Constants (LSC) and least-squares means (LSM) characterizing the effects of propagation associated with the time of ovulation

<table>
<thead>
<tr>
<th>Classification factor</th>
<th>Weight of eggs [g]</th>
<th>Weight of eggs [% of female body weight]</th>
<th>Percentage of fertilized eggs after 12 h incubation</th>
<th>Percentage of living embryos after 24 h incubation</th>
<th>Percentage of living embryos after 28 h incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>LSC</td>
<td>LSM</td>
<td>SE</td>
<td>F</td>
</tr>
<tr>
<td>Ovopel</td>
<td>216.67</td>
<td>14.18</td>
<td>230.84</td>
<td>19.93</td>
<td>-</td>
</tr>
<tr>
<td>Time of ovulation 12h</td>
<td>-14.18</td>
<td>202.49</td>
<td>19.93</td>
<td>-</td>
<td>-0.98</td>
</tr>
<tr>
<td>Time of ovulation 15h</td>
<td>238.68</td>
<td>8.26</td>
<td>246.97</td>
<td>9.99</td>
<td>-</td>
</tr>
<tr>
<td>Carp pituitary</td>
<td>-8.26</td>
<td>230.42</td>
<td>13.21</td>
<td>-</td>
<td>-0.41</td>
</tr>
<tr>
<td>Ovopel</td>
<td>247.14</td>
<td>-13.46</td>
<td>233.68</td>
<td>38.36</td>
<td>-</td>
</tr>
<tr>
<td>Time of ovulation 15h</td>
<td>13.46</td>
<td>260.59</td>
<td>29.67</td>
<td>-</td>
<td>0.83</td>
</tr>
</tbody>
</table>

SE – standard error of least-squares means; α – the theoretical general mean * P ≤ 0.05; ** P ≤ 0.01
after 24h incubation of eggs. The least-squares mean for this trait was higher after the CPH treatment compared with the mean calculated for females treated with Ovopel (97.44% and 94.60%, respectively; Table 4).

Within the 15h latency the effect of the ovulation stimulator was statistically significant with respect to three investigated traits determining the quality of eggs ($P \leq 0.05; P \leq 0.05; P \leq 0.01$; Table 4). The least-squares means for the percentages of egg fertilization and live embryos after a 24h and 28h incubation showed higher values in the group of fish stimulated with Dagin compared with the respective values calculated for fish injected with Ovopel (97.56%, 96.04%, 95.11% and 94.39%, 90.94%, 78.16%, respectively; Table 4).

### Dependences between the investigated parameters

The coefficient of correlation between the body weight of females and the weight of obtained eggs showed positive high values for fish after a CPH treatment and for females treated with Ovopel (+0.98 and +0.93, respectively) while its value for fish treated with Dagin was markedly lower (+0.52). The weight of spawning females was positively correlated with the percentage of live embryos after a 28h incubation of eggs both in the group of fish stimulated with CPH (+0.62) and in the group treated with Dagin (+0.71) while after the application of Ovopel the correlation coefficient between these parameters showed a negative value (-0.80). Similarly, in the group of fish stimulated with Ovopel, the coefficient of correlation between the weight of eggs expressed in grams and the percentage of live embryos after a 28h incubation of eggs only showed negative values. The index of correlation between the fertilization percentage and the percentage of live embryos after a 24h incubation of eggs, as well as between the percentage of fertilization and the percentage of live embryos after a 28h incubation showed high positive values only for fish injected with Dagin (+0.91 and +0.90, respectively). The percentage of live embryos after a 24h incubation of eggs and the percentage of live embryos after 28h was positively correlated both in the group of females stimulated with Ovopel and in the group of fish after the application of Dagin; the values of the respective coefficients were high (+0.93 and +0.92). The correlation between these parameters was negative (-0.40) for hypophysed fish.
Occurrence of deformed larvae

The occurrence of larvae with body deformations was found in three investigated groups. The mean percentage of deformed larvae was low in all the investigated groups treated with different preparations: in group I it was 6.20%; in group II – 7.06%; and in group III – 5.63%.

Relative effectiveness of reproduction after ovulation stimulation with CPH, Ovopel or Dagin

The lowest relative effectiveness of reproduction was found in the group of fish treated with Ovopel. The number of live embryos per kg female body weight after the application of this preparation was 65,551. The highest relative effectiveness of reproduction was found in the group of fish treated with Dagin. The number of live embryos per kg body weight of females was 83,324. In the group of fish treated with CPH the number of live embryos per kg female body weight was 76,580. A statistically significant \( P \leq 0.01 \) difference was noted only between values of RER for the Dagin and Ovopel treatment.

Discussion

The results obtained in the present experiment show that the application of Dagin resulted in satisfactory effects on reproduction. After using this preparation a high percentage of females ovulated, the weight of eggs was high and their quality after a 28h incubation exceeded the quality of eggs yielded by hypophysed females and by fish treated with Ovopel. It is particularly worth stressing that after the Dagin treatment the very high quality of eggs, expressed by the fertilization percentage after a 12h incubation, was maintained during the rest of the incubation. In this group the mean percentage of live embryos after a 28h incubation of eggs was lower only by 2.4% compared with the mean fertilization percentage. In the group of fish treated with CPH the respective values were approximately 6%. In the investigation carried out at the Gołysz Institute attention was paid to the fact that after some spawning agents applied to \( C. \) gariepinus the quality of eggs decreased during their incubation. A decrease in the quality of \( C. \) gariepinus eggs during incubation was observed after the application of desGly\(^{10}\)[D-Ala\(^8\)]-LHRH Ethylamide (50 \( \mu \)gkg\(^{-1}\) body weight of females) (BRZUSKA et al. 1999), of [D-Tle\(^6\),ProN-HEt\(^9\)]m GnRH (15 \( \mu \)gkg\(^{-1}\)) (BRZUSKA et al. 2004) and of the complex preparation-Aquaspawn (0.5 mLkg\(^{-1}\)) (BRZUSKA 2003).
The results presented here show that the most pronounced decrease in the quality of eggs (in the period between 12 and 28h of incubation) occurred in the group of females treated with Ovopel. A difference of 11.7% was noted between the mean percentage of fertilization and the mean percentage of live embryos after 28h incubation of eggs. The results of three previous experiments with Ovopel applied to C. gariepinus showed distinctly that in these experiments no serious decreases in quality occurred during the period of egg incubation (BRZUSKA 2004). An analysis of data obtained in an investigation conducted on a different species of the African catfish, i.e., Heterobranchus longifilis showed that, in the course of incubation, the quality of eggs yielded by females ovulating 14h after an Ovopel application deteriorated to a higher degree in comparison with decreases in the quality of eggs yielded by fish two hours earlier (BRZUSKA and ADAMEK 2008).

The striking fact is that after the application of Dagin all the females of C. gariepinus spawned at the same time. A synchronized ovulation induced in all the females by this spawning agent is an important positive trait determining its value for hatchery practice. The lack of ovulation synchronization in spawners hinders and disorganizes the work order in a hatchery at the time of a controlled fish reproduction.

The results of an investigation of the common carp Cyprinus carpio and grass carp Ctenopharyngodon idella (KOURI et al. 2003a) clearly showed that, in these two species, the application of Dagin induced the synchronization of ovulation in all the treated females. In the common carp the time of stripping was 14h 30 min (water temperature 24°C) and in grass carp 16h (the water temperature 22.5°C). In tench, Tinca tinca treated with Dagin the latency period was much longer – up to 30h 30min (water temperature 22.5°C) and was the same in all the females which yielded eggs (KOURI et al. 2003b). The effects of experiments with the common carp carried out at the Institute of Ichthyobiology and Aquaculture at Golysz showed that this preparation did not synchronize ovulation in females from two Hungarian breeding strains: strain W (BRZUSKA 2005b) and strain 7 (BRZUSKA 2006). However, the ovulation synchronization after the Dagin treatment was recorded in common carp females of the Polish strain 6 (BRZUSKA 2005b). The time interval between the injection of Dagin and the initial egg release in the females of strain 6 (at 21.5°C) was 14h and was above one hour shorter as compared with the respective time interval reported by DRORI et al. (1994) and by YARON et al. (2002). The application of Dagin induced the ovulation in all the females of the European catfish Silurus glanis at the same time, i.e., 24h after the injection of this spawning agent (BRZUSKA, unpubl. data).

Synchronized ovulation also occurred in individuals treated with CPH while after the application of Ovopel two different terms of egg release were
recorded. We stress that a higher weight of eggs was obtained from females ovulating 12h after the treatment with Ovopel. The percentage of live embryos both after a 24h and 28h incubation developing in these eggs was significantly higher compared with the percentage of live embryos developing in eggs obtained from females ovulating three hours later. These results may suggest that females which ovulated earlier were characterized by a higher physiological readiness for ovulation induction using a preparation based on the stimulation of the endogenous gonadotropin from the pituitary of fish treated with a synthetic hypothalamic hormone. In previous experiments carried out with females of this fish species stimulated with Ovopel the synchronization of ovulation was noted in all the investigated fish both after the application of Ovopel at one dose (1 pellet kg⁻¹) or at two doses (1/5 + 1 pellet kg⁻¹) (BRZUSKA et al. 2000, BRZUSKA et al. 1998b, BRZUSKA 2002a,b, 2004). The lack of ovulation synchronization after an Ovopel treatment was found in the European catfish Silurus glanis (BRZUSKA 2001a) and in the African catfish Heterobranchus longifilis (BRZUSKA and ADAMEK 2008).

A very important piece of information obtained in the investigation of C. gariepinus is that after the application of Dagin the percentage of deformed larvae was not higher in comparison with the hypophysed group or that treated with Ovopel. The high percentage (>20%) of larvae hatched with body deformations was found in this fish species whose ovulation was stimulated with desGly¹⁰[D-Ala⁶]-LHRH (applied with pimozide) irrespective of the LHRH – a dose of 50 μg kg⁻¹ or 20 μg kg⁻¹ (BRZUSKA et al. 1998c). It is also important that eggs obtained from all the fish after an ovulation stimulation with Dagin were of a very good quality.

In summary, the results of controlled reproduction after the application of the tested preparations were satisfactory. In spite of the fact that the results of reproduction of C. gariepinus after ovulation stimulation with Dagin can be only regarded as preliminary, the best effects were found after the treatment with this preparation. In the group of fish treated with Dagin synchronization of ovulation was noted in five females in which the ovulation occurred. The highest mean weight of obtained eggs was found after the application of this stimulator, however, it did not significantly differ from the mean weight of eggs obtained from hypophysed females or those treated with Ovopel. The highest mean percentage of live embryos after 28h incubation of eggs was obtained after the application of Dagin, however, it differed significantly from the mean percentage of live embryos in the group of fish treated with Ovopel. The stimulation of ovulation with Dagin resulted in a higher relative effectiveness of propagation (the number of live embryos per kg female body weight) than the stimulation with carp pituitary homogenate or Ovopel.
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