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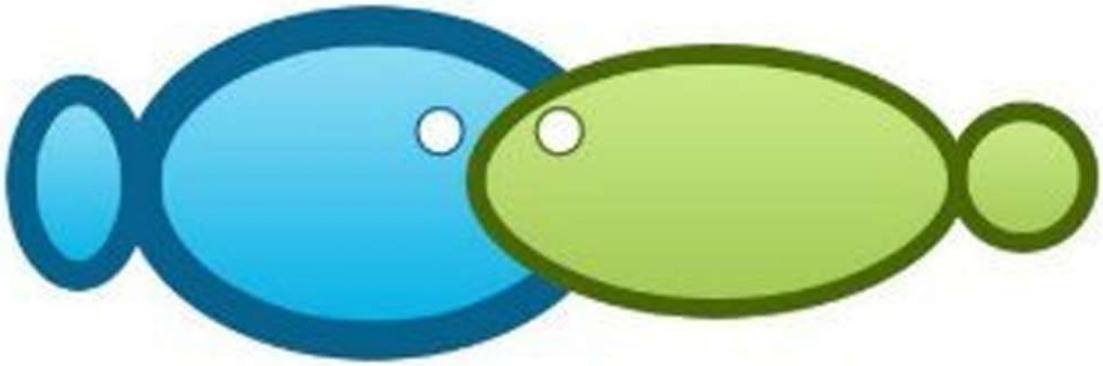

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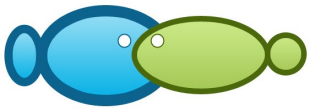

Contents

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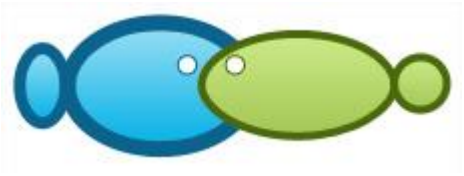

The effect of immersion time in tannin solution towards the adhesiveness and hatching degree of the eggs of common carp (*Cyprinus carpio*)

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Abstract. Tannin solution can eliminate the adhesion of carp eggs due to its ability to precipitate glycoproteins in the egg layer. However, this solution may adversely affect the egg in case of prolonged immersion, as tannin may harden the egg, thus making the embryo difficult to hatch. This study aims to determine the optimal immersion time for carp (*Cyprinus carpio*) eggs in tannin solution and its effect towards adhesion and hatching degree. The experimental study was conducted by means of a Completely Randomized Design (CRD), consisting of three treatment groups and six replications. Samples were divided in control group (egg without immersion) and treatment groups (egg immersed in tannin solution for 120 and 180 seconds). The acquired data was analysed by ANOVA testing to find differences between groups, then using Duncan's multiple distances to find the best performed treatment. There was no significant difference for carp egg adhesion ($p>0.05$), yet the carp egg hatching degree was significantly different among groups ($p<0.05$). The highest recorded mean of the hatching rate was for the control group, 74.66%. The duration of egg immersion in tanning solution did not eliminate the egg adhesion, but reduced the hatching degree.
Key Words: adhesion, carp, hatching, tannin.

Introduction. The common carp (*Cyprinus carpio*) is a first rate commodity, which has been the focus of the fish farming production target during 2010-2014. The production of common carp has a satisfying trend, with an average production increase of 7.09% from 2010 to 2013 (KKP 2014). Carp hatchery businesses can be done naturally, semi-artificially, and artificially. Natural seeding has some shortcomings, like the limited number of obtained seeds, as it depends on the spawning season in nature and other factors. This can be surmounted by artificial seeding. However, one hindering factor in the artificial hatchery is the nature of carp eggs that have adhesive characteristics after entering the water, making them easy to clot and difficult to spread in the water.

Artificially bred carp have eggs that easily attach to leaves, plant roots and other materials after the chorion hardening process. Carp eggs are classified as an adhesive egg type (Effendie 1997). The adhesive nature is caused by glycoproteins in the layer of carp egg shells (Woynárovich & Horváth 1980). The main nutrients in the chorion membrane are proteins and carbohydrates derived from the monosaccharide group, such as glucose, fructose and galactose (Mansour et al 2009). The vitelline membrane of carp eggs consists of 4 layers. The first layer consists of two parts and has a thickness of 0.44 μm, with fine filaments and branching. The upper and lower parts that can be distinguished through the chemical composition. The upper part is rich in protein accompanied by acid phosphatase (AcPase) activity while the lower part has a higher carbohydrate content, without acid phosphatase (AcPase) activity (Kudo 1982). The egg adhesiveness may hamper its spreading in egg-incubating ponds during artificial hatchery processes (Bokor et al 2013). Moreover, egg clusters may reduce the oxygen diffusion, thus the eggs are difficult to hatch (El-Gamal & El-Greisy 2008) and become easier to break (Walker et al 2010).

The adhesive properties of carp eggs can be lessened by tannin (Woynárovich & Horváth 1980), as it has the ability to bind and precipitate glycoproteins. Tannin phenolic

compounds have a high molecular weight containing hydroxyl (OH) and other groups such as carboxyl (COOH) (Ashok & Upadhyaya 2012). A number of tannin phenolic groups can form hydrogen bonds with proteins (Haslam 1989). Hydrogen bonds form between positive atoms of hydrogen (H) with atoms F, O, N (Moore & Stanitski 2014). The phenolic groups contained in tannins are an exquisite hydrogen donor and may form strong hydrogen bonds with carboxyl groups contained in proteins. The hydrogen and protein bonds may reduce the egg adhesiveness and open the pores of the egg for breathing, thus increasing the degree of fertilization and hatching. Nonetheless, tannins may harden the eggshells and thus impede the embryo to hatch (Bokor et al 2013), particularly at high concentrations (Kareem et al 2017).

The optimum dose of tannin solution for soaking carp eggs is 0.5 g/L. Soaking the carp eggs in tannin solutions for 5-120 seconds has been proven to not cause harm (Bokor et al 2013). However, another study on pikeperch revealed a different result (Demska-Zakęś et al 2005). The minimum immersing time of pikeperch eggs in tannin solution was 120 seconds. After 30 seconds, regardless the dose (1-1.5 g/L), the adhesion of eggs would not be eliminated. Based on these, this study aims to determine the optimal immersion time of carp eggs in tannin solution and its effect regarding egg adhesion and hatching degree.

Material and Method. This study was conducted at the Freshwater Cultivation Installation Punten (IBAT) in April-May 2015. The tools used in this study were fiber tubs (360x7x23 cm^3), large filters with diameters of 15 cm, small filters with diameters of 10 cm, rock and aeration hoses, basins, chicken feathers, brood tubs, a pH Pen type PH-009, a water quality multiparameter type YSI 556 MPS (Multiprobe System), a heater, styrofoam, a hand-counter and a stopwatch. The materials used in this study were a pair of carp fish strain Punten, tannin powder, distilled water, Ringer's lactate solution and physiological NaCl.

This study was carried out in several stages. First was the preparation of the research media, then the procurement of carp broodstock, making tannin solution by mixing 0.5 g of tannin powder into 1 L of aquades (distillated water) (Bokor et al 2013), egg stripping and fertilization, soaking eggs in tannin solution and counting and incubating eggs. The preparation of research media begins with the preparation of the egg incubation media. Carp broodstock (strain Punten) with ripe gonad and ready to be spawned has been procured, one female and one male. After preparing the tannin solution, the egg and milt stripping and egg fertilization took place.

Milt was collected by a 1 mL syringe and diluted using physiological NaCl as much as 9 ml. Diluted milt was placed in a container where the eggs were placed beforehand. Both were stirred with feathers for one minute and Ringer's lactate solution was added (as much as 30 mL) while stirring for one minute, totaling two minutes. Fertilized eggs were placed in a container with water for the control treatment and a container with tannin solution for soaking the eggs for 120 seconds and 180 seconds. Eggs that have been soaked in a tannin solution were rinsed with fresh water, using a spray bottle, after which the filters were put into a fiber container for calculation. Water quality examination included temperature, oxygen and pH monitoring. Water quality measurements were carried out from the beginning of the study, prior to and during egg incubation until the eggs hatched into larvae. Water quality measurements were carried out at 08.00 and 16.00 (GMT+7).

This study used a Completely Randomized Design (CRD), consisting of three treatments and six replications resulting in 18 experimental units. Samples were divided in: A – control, without immersion in tannin solution); B - soaking the eggs in tannin solution for 120 seconds; C - soaking the eggs in tannin solution for 180 seconds.

The independent variable was egg immersion time in tannin solution, while the dependent variables were adhesion, the degree of fertilization and the degree of hatching. Observation of the adhesion of eggs was carried out by looking directly at the condition of the eggs and calculating the number of eggs that clustered. The method for calculating the adhesion of eggs is as follows:

Egg adhesiveness (%) = (Number of attached eggs/Total number of eggs)x100

Observation of the degree of fertilization of eggs was done 3 hours after fertilization. The method of calculation used the following formula (Effendie 1997):

Degree of fertilization (%) = (Number of fertilized eggs/Total number of eggs)x100

Observation of the degree of hatching can be done starting 24 hours after the fertilization process. The degree of hatching can be calculated after the carp eggs hatch using the following formula (Effendie 1997):

Degree of hatched eggs (%) = (Number of hatched eggs/Total number of eggs)x100

The observed parameters were categorized into main and supporting parameters. The main parameters were the adhesive power of carp eggs and the degree of hatching. The supporting parameters were the degree of fertilization of carp eggs and water quality parameters.

Results and Discussion

The adhesion of carp eggs. Data regarding the adhesion of carp eggs is used to determine the effect of tannin solutions in removing the stickiness of carp eggs with different immersion times. The egg adhesion counting was carried out 3 hours after the eggs were fertilized. The mean values of carp egg adhesion are presented in Table 1.

Table 1

The mean value of the adhesive power of carp eggs

Treatment	*Mean Egg Adhesiveness (%) ± SD*
Control	52.20±2.44
120 seconds	49.43[a]±3.30
180 seconds	48.87[a]±2.79

Description: superscript shows no significant differences (p>0.05).

The ANOVA test and the Duncan Multiple Distance Test revealed no significant differences (p>0.05) among the three groups. The adhesion of eggs is presented in Figure 1.

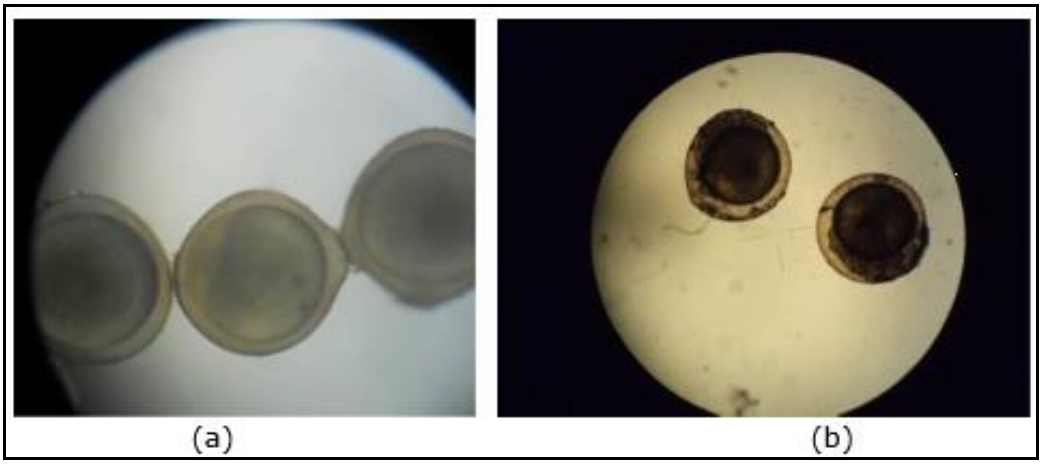

Figure 1. Observation of the adhesiveness with microscope; (a) - showing the egg cluster; (b) - the eggs do not stick together.

Eggs are considered adhesive when two or more eggs are sticking together in one place. In Figure 1 (a) it can be seen that the eggs stick together with other eggs, while in figure 1 (b) it can be seen that the eggs are not sticking together.

The degree of hacking of carp eggs. The hatching degree of eggs was determined based on eggs hatched into larvae. The mean values of the hatching degree of carp are presented in Table 2.

Table 2

The mean value of the hatching degree of carp eggs

Treatment	*Mean Degree of Hatching (%) ± SD*
Control	74.66^{a}±3.44
120 seconds	28.76^{b}±1.86
180 seconds	31.63^{b}±2.54

Description: superscript shows no significant differences ($p>0.05$).

Based on the ANOVA test and Duncan's Multiple Distance Test, it was found that there were significant differences between eggs that were not immersed in tannin solution and eggs soaked in tannin solution ($p<0.05$). The mean value of adhesion and the hatching degree of carp eggs is presented in Figure 2.

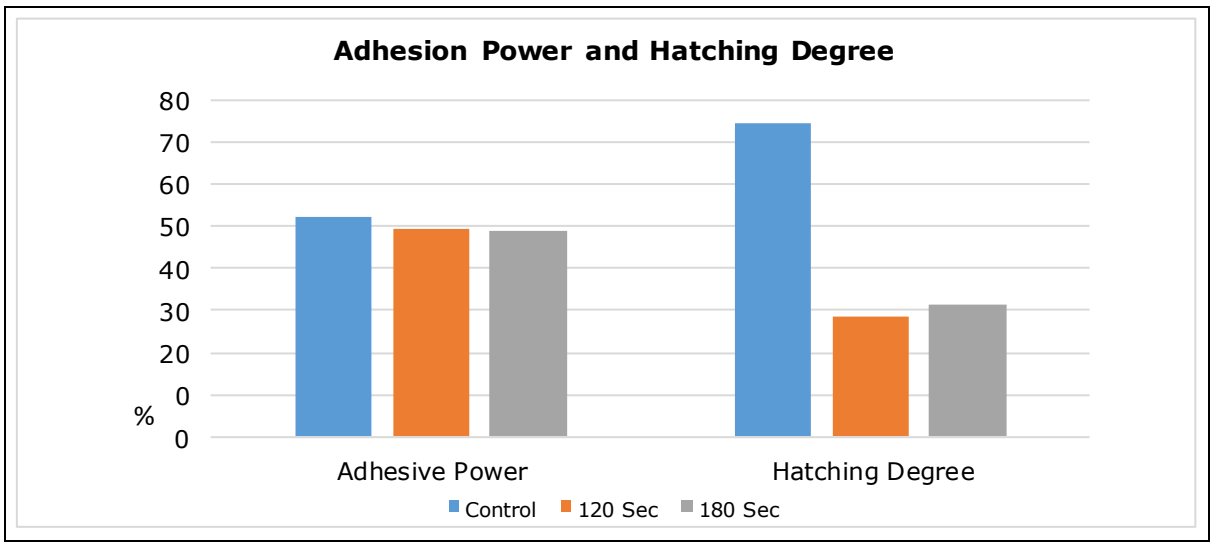

Figure 2. Mean adhesive power and degree of hatching of carp eggs.

Based on Figure 2, it can be seen that the carp eggs not immersed in tannin solution have a remarkably higher value of adhesive power compared to eggs soaked in tannin solution. Carp eggs soaked in tannin have a significantly lower hatching rate compared to eggs that are not immersed in tannin solution.

The degree of carp egg fertilization. The degree of fertilization was calculated 3 hours after the fertilization process. The fertilized egg presents a clear colour while the unfertilized egg is cloudy white. The average value of fertilization of carp eggs can be observed in Table 3. Carp eggs have different degrees of fertilization in response to the different treatments.

Table 3

The mean value of the degree of fertilization of carp eggs

Treatment	*The mean degree of fertilization (%) ± SD*
Control	91.38±2,18
120 seconds	92.45±1,56
180 seconds	90.19±2.33

Water Quality. Data on water quality parameters before and during egg incubation is presented in Table 4. The water quality parameters before and during egg incubation undergoes changes in temperature, dissolved oxygen, and pH that are not significant.

Table 4

Water quality before and during egg incubation

Parameter	*Before Egg Incubation*		*During Egg Incubation*	
Time **(hour)**	08.00	16.00	08.00	16.00
Temperature (°C)	24.7	25.1	25.1-26.1	26.3-27
Dissolved Oxygen (mg/L)	5.4	5.6	5.2-5.8	5.5-5.9
pH	8.3	8.2	8.2-8.3	8.2-8.3

The results of this study show that tannin does not eliminate the adhesive nature of carp eggs, probably due to weak bonds between tannins and proteins that are influenced by the tannin structure. Some types of tannins include tannic acid, gallic acid and catechins (Fajriati 2006). The polyphenol model size determines its ability to bind with protein (Haslam 1989). The difference in structure and size of tannin polyphenols may affect the adhesiveness of carp eggs, considering that the larger the molecule, the greater the ability to form a bond with proteins, because of a larger number of atoms to form a bond (Haslam 1989).

The egg-hatching rate of the control group has a higher value compared to the one of the eggs in the treatment group. This shows that the tannin solution affects the degree of carp egg hatching. Eggs immersed in tannin solution for 120 seconds and 180 seconds did not show any differences. This suggests that the difference in immersion duration between the two time intervals does not affect the degree of carp eggs hatching rate. The low hatching rate of carp eggs soaked in tannin solution for 120 seconds and 180 seconds when compared to the one of eggs that were not immersed in tannin solution showed the influence of tannin on the carp egg hatching degree, lowering it.

Mizuno had conducted an experiment regarding the hatching degree of willow leaf fish (*Spirinchus lanceolatus*) eggs that were immersed in a tannic acid solution (Mizuno et al 2004). The percentage of the tannin dose used was 0.15% for 10 seconds and the eggs had a lower hatching value when compared to eggs not immersed in a tannic acid solution. Fertilized eggs cannot survive and hatch because the tannin interferes with the egg development process. In addition, the same thing happens to pikeperch (*Sander lucioperca*) fish eggs. Eggs immersed in a tannic acid solution with the highest concentration of 1.5 g/L for 120 seconds produced the lowest hatching rate of 35.3%. Tannic acid is astringent and has the ability to bind toxic substances and cause the chorion layer to become hard and inhibits the ability of tench eggs to hatch (Demska-Zakęś et al 2005). In a different study, eggs immersed in tannin with a dose of 0.1% for 60-90 seconds and 0.15% for 30-90 seconds presented embryos that were unable to break the chorion layer and the embryos died due to fatigue. Some larvae are only able to break out some parts of their bodies, while others remain inside the chorion layer. The larva dies since it is unable to free itself from the chorion layer (Kujawa et al 2010).

This study revealed a high degree of fertilization. The carp fish eggs rate of fertilization incubated at temperatures of 26-29°C only ranged from 42.78 to 55%. This shows that the treated eggs are fertilized eggs (Rahaman et al 2012). Water quality during the study steadily met the criteria for carp egg incubation. The temperature for

incubation ranged from 25.5°C to 27°C with a dissolved oxygen content from 5.2 to 5.9 mg/L and pH from 8.2 to 8.3. The optimal temperature interval for carp hatching is 22-24°C, but some authors observed better results in higher temperatures, suggesting an optimum at 27°C (El-Gamal 2009). Carp can survive in low dissolved oxygen conditions, which ranges from 0.3 to 0.5 mg/L, while dissolved oxygen below 0.8 mg/L may harm the embryo and lead to death (Mohan & Choudhary 2010). The compulsory dissolved oxygen interval in carp hatchery activities is 5-12 mg/L and the optimal pH value is around 7-8 (Billard 1999).

The results of this study indicate that tannin solution is still dangerous as a removal material to disperse carp eggs clusters, since it takes more than 180 seconds of immersion to eliminate egg adhesive properties, which, unfortunately, may reduce the carp egg-hatching rate. A method for pikeperch eggs adhesiveness removal by tannic acid had better results than urea or cow milk, yet the obtained results were not entirely satisfactory (Demska-Zakęś et al 2005). The use of tannins has spread among fish farmers in Hungary, but, in some cases, tannins are not recommended for use due to negative effects (Bokor et al 2013).

Conclusions. Based on the results in this study, it can be concluded that the immersion duration of eggs in tannin solutions was not able to eliminate the adhesive properties of eggs. Moreover, it reduced the hatching rate of carp eggs. In addition, there is no optimum time of soaking eggs in tannin solutions. The purity of tannins needs to be reconsidered for future studies, because the tannin structure influences the bond between tannins and proteins in removing the adhesion of carp eggs.

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