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Effects of stress on spawners reproductive performance in female Grass Carp (*Ctenopharyngodon idella)*

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ABSTRACT

The present study was conducted to evaluate the effect of some stress factor on spawners reproductive performance in Grass Carp(*Ctenopharyngodon idella)* females. A total number of sixty-three grass carp females was injected with 3mg / kgm carp pituitary extract (CPE). All females were divided according to total body might. The first experiment (36 females with an average body weight of 2.7-2.8 kg) were divided into four groups varying in depth of water column and pesticides ,whereas the second experiment (27 females with an average body weight of 3.5-3.7 kg) were divided into three groups each of them expose to different oxygen level. In the first experiment , Latency time 9-9.5 hours, the mass of eggs and egg weight index percentage (EWIP) was significantly higher in group 3 (were water depth high (1m) and low (50 cm) water column directly were 2 times in the month without pesticide) than in group 1, group 2 and group 4 respectively, however the mean fertilization rate and hatching rate was insignificantly in the three groups(3,1 and 2), however was significantly higher in group 4. In the second experiment, Latency time 8.5-10 hours, the mass of eggs and EWIP was significantly higher in group 2than in group 1 and in group 3, respectively. On the other hand, the mean fertilization rate and Hatching rate was insignificantly in the between the group 1and group 2, while the third group are not where it can fertilize this could be due to lack of oxygen .From the previous study it will be concluded that the water depth were high(1m) and low (50cm) before breeding season directly 2 times in the month without spraying pesticide. Also , at the breeding season the oxygen level did not less than 4.5 mg/l.

Keywords: stress, water depth, pesticide(Trichlorofon), oxygen, reproductive Performance.

INTRODUCTION

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances (Rottmann, *et al*., 1991). Many fish spawn in environments that are nearly impossible to stimulate in a hatchery, therefore, hormone-induced spawning is the only reliable method to induce reproduction in these fishes. Depending on the stage of an animal's development, stress can have different reproductive consequences. In mammals, the high cost of reproduction in terms of energy, time, and risk may have led to natural selection of physiological

mechanisms that terminate reproduction when them likelihood of progeny survival is low (Wasser and Barash, 1983). One suggested is that stress is a cause of some forms of infertility(Wasser, *et al.,* 1993). Fishes also mobilize considerable energy resources for reproduction, and therefore they may have developed similar physiological mechanisms that modulate reproductive success. In a sequence of several stressful events, discrete influences of each event may contribute to a total response of the animal that compromises its performance capacity (Schreck,1996). In addition, the extent and dynamics of a stress responsen might be strongly influenced by the stage of development of the animal and the severity and duration of the stressor, among other factors (Barton, 1988 and Schreck and Li , 1990). For example, primary and secondary stress response factors may show different patterns among immature, maturing, and mature fish exposed to the same generalized stress (Pickering and Christie ,1981 and Schreck ,1996). Larnaea spp, Play very important and diverse roles in freshwater aquaculture operations, including some that are beneficial and other that are extremely adverse and may result in complete production losses(Wojciech, et al., 2004)

Chakroff,(1976) reported that fish begin to be stressed when the dissolved oxygen level falls below 4mg/litre .

Broodstocks with bruise used for artificial fish propagation when compared with control shown a reduction on the number of eggs released % ,hatchability, high deformed larvae and mortality(Adebayo, 2006).

Brett (1983) reported two types of stress found in fish as internal stress

caused as a result of physiological changes such as breading and sexual maturation and external stress which include bad handling , poor feeding and sudden changes in water quality. Internal stressors cannot be controlled, the culturist should focus attention in reducing the external stress factors for soaring yield and productivity(Brett,1983) . The poor response of fish to artificial propagation and high mortality is due to poor incubation condition and stressing of broodstocks during or after hormone administration .It is therefore imperative to carry out more research on the effects of handling and environmental stress factors as it affects of the reproductive performance, growth and survival of the fish especially at their very first stage of development. The aim of the work to explain the effect of stress broodstock on off spring.

MATERIALS AND METHODS

Spawning experiments were conducted during April 2010 on 2-3 years old grass carp ***(****Ctenopharyngodon idella*) , females, , at Fish hatchery belongs to the Central Laboratory of Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia Governorate, Egypt. Thirty sex adult females were used in the first experiment and twenty seven females were used in the second experiment . Female fish weighing 2,7-2,8 and 3.5-3.7 kg body weight in first experiment and second experiment respectively, were selected from earthen ponds for ripeness. The water quality was measured (Dewis and Freiles 1970) twice daily (Table1).

Experiment 1: The first experiment a total number of 36 females were selected, weighed and accommodated in 12 concrete ponds, (3 females each). The second step, a total number of 36 female apparently healthy of grass carp(*Ctenopharyngodon idella*) , this selection was based on the softness of their abdomens as pointed out by Brzuska, (1999),divided into four equal groups. The first group (G1) water depth were maintained at 1m for two months before breeding season directly without spraying

Table1: ***Physico-chemical characteristics of earthen water ponds of Grass carp (Ctenopharyngodon idella) during climatic period before the experiment .***

Items	Mean	Items	Mean
Temperature (c)	**26-28 ^{o}C**	**Nitrate (mg/l)**	**0.01**
PH	**8.7**	**Nitrite (mg/l)**	**0.02**
Oxygen (mg/l)	**7.1**	**Salinity (mg/l)**	**0.3**

pesticide Lernaea , Salinity was calculated by relation (1000 micromos = 0.7g salinity according to Dewis and Freiles, (1970). The second group (G2) were water depth were maintained at 1m for two months before breeding season directly to spray private pesticide used(Trichlorofon 80%) . , the third group(G3) water depth (1m) and lower to (50 cm) water column directly were 2 times in the month without spraying pesticide Lernaea and the four group (G4) were water depth high (1m) and low (50 cm) directly were 2 times in the month to spray private pesticide used(Trichlorofon 80%) in the treatment of hatcheries Lernaea before breeding season directly. All the work were repeated as triplicate . At breeding season, selection of brood fishes from the segregated brooder ponds they were randomly distributed in 12 indoor fiberglass tanks with running water of 27 ± 1.0°C for 24 hours as an adaptation period.

Experiment 2: The second experimental a total number of 27 females were selected, weighed and accommodated in 9 indoor fiberglass tanks, (3 females each). After selection of brood fishes from the segregated brooder ponds they were randomly distributed in 9 indoor fiberglass tanks with running water of 27.0 ± 1.0°C for 24 hours as an adaptation period. A total number of 27 female apparently healthy of grass carp(*Ctenopharyngodon idella*)divided into three equal groups (by control of source oxygen), the first group (G1) were oxygen level 7.0 mg/l, the second group(G2) were oxygen level 4.5 mg/l and the third group(G3) were oxygen level 2 mg/l respectively. All experimental fish were injected by carp pituitary extract (CPE) 3mg / kg in two doses as ovulation stimulators.

Prior to injection, fish were anesthetized according to Bowser (2001) in 1 cm Quinaldine / 40 L Water (2–4 methyl chinolin) bath, individually, and then weighed in a hand net using a spring balance. The breeders were then ready to receive injection.

Spawning technique were adapted for left in tanks and collection eggs, eggs were collected in buckets and incubated in fiberglass at which supplied with continuous water at temperature of 26-28 ^{o}C (Brzuska *et al*., 1999).

Ovulation ratio and EWIP were estimated according to Szabo (2003)

Ovulation ratio = number of ovulated females /number injected.

Egg weight index percentage (EWIP) = (weight of stripped egg mass/BW before stripping) x100

For eggs of both experiments , the percentage of fertilization was estimated after 12 hrs of incubation according to Gheyas, *et al*. (2001) as follows:

Fertilization rate = (Number of fertilized eggs / Total number of eggs) x 100

Hatching rate = (Number of hatched eggs (larvae) /Total number of eggs) x100

Then after 24 hrs of incubation hatching percentage was estimated.

Statistical analysis

All statistics were carried out using statistical analysis systems (SAS, 2004).

RESULTS AND DISCUSSION

Experimental 1

Artificial spawning of grass carp was successfully carried out through the use of hypophysation 3mg /kg to females and males were successfully used to induce spawning in grass carp.

Latency time(time between the secondary injection and ovulation) recorded in this study was found to be between 9-9.5 hours at temperature rang 26-28c, 86.6% female ovulated in G3 , G1 and G2. While, in G4 did not ovulated females . These findings is in agreement with the Brzuska (2003).

The results of (Table 3) showed that the mass of eggs (380 ± 25.5 g) and EWIP % (13.7± 0.6) was significantly higher in group 3 than in group 1were mass of eggs (330 ± 57.0) and EWIP %11.6 ± 3.6 and mass of eggs were (190 ± 34.0) EWIP % (6.8± 1.2) in group 2 , however in group 4 mass of eggs and EWIP % were zero, respectively.

On the other hand, the mean fertilization rate and hatching rate was insignificantly in the three groups . Broodstock that has been treated with insecticide did not release eggs at all, and this is due to the extreme stress of the pesticide despite a raising and lowering the water column to stimulate for fish spawning, but it did not occur. Broodstock with bruise and bad health should not used for seed propagation rather a healthy fish free from injuries and disease should be used to ensure good results. Ayinla,(1991) reported that spawners with bruise and poor health when used for induced breeding have adverse effect on the reproductive performance of the fish as a result of injuries which exposed the spawners to easy infection by bacteria and other microorganisms.

Table2: ***Statistical characteristics of the investigation traits of Grass carp (Ctenopharyngodon idella).***

Investigation	G1	G2	G3	G4
Latency time (hours)	**9.5**	**9.2**	**9**	**0.0**
Body weight before spawning (g)	**2830 ± 266.1 [a]**	**2790 ± 1321 a**	**2760 ± 156.4 [a]**	**2750 ± 145.8 [a]**
Body weight after spawning (g)	**2500 ± 742.0 [b]**	**2600 ±211.1 b**	**2380 ± 334.5 [b]**	**2790± 523.1 [a]**
Mass of eggs (g)	**330 ± 57.0 [b]**	**190.0 ± 34.0 c**	**380 ± 25.5 [a]**	**0.0**
EWIP (%)	**11.6 ± 3.6 [b]**	**6.8 ± 1.2 c**	**13.7 ± 0.6 [a]**	**0.0**
Fertilization rate (%)	**78 ± 6.5 [a]**	**78.0± 2.0 a**	**79 ± 2.7 [a]`**	**0.0**
Hatching rate (%)	**74 ± 6.5 [a]**	**77.0 ± 1.4 a**	**75 ± 2.7 [a]**	**0.0**

Experimental 2

Artificial spawning of grass carp was successfully carried out through the use of hypophysation 3mg /kg to females and males were successfully used to induce spawning in grass carp.

Latency time(time between the primary injection and ovulation) recorded in this study was found to be between 8.5 and 10 hours at temperature rang 23-24c, 85.1 % female ovulated in G2. While, in G1 and G3were ovulated females 20,10%,respectively (Table 3). These findings is in agreement with the Brzuska (2003).

The results of (Table 3) showed that the mass of eggs (304 ± 55.2) and EWIP % (8.5 ± 0.7) was significantly higher in group 2than in group 1 (50.2 ± 23.1 &1.3 ± 0.7,) and in group 3 (20 ± 13.8 & 0.5 ± 0.6), respectively.

These findings is in agreement with the \Brzuska (2004) and Akar (2006) showed that synchronization of ovulation silver carp was observed in all the females after the injection by Aquaspawn.

On the other hand, (Table 3) the mean fertilization rate and Hatching rate was insignificantly in the between the group 1and group 2,while the third group are not where it can fertilize this

Table3: Statistical characteristics of the investigation traits of Grass carp (Ctenopharyngodon idella).

Investigation	G1	G2	G3
Latency time (hours)	**10**	**8.5**	**9.0**
Body weight before spawning (g)	**3780 ± 421.2 [a]**	**3569 ± 230.2 [a]**	**3548 ± 160.5 [a]**
Body weight after spawning (g)	**2729.8 ± 558.6 [b]**	**3265 ± 523.1 [a]**	**3528 ± 423.2 [a]**
Mass of eggs (g)	**50.2 ± 23.1 [b]**	**304 ± 55.2 [a]**	**20 ± 13.8 [b]**
EWIP (%)	**1.3 ± 0.7 [b]**	**8.5 ± 0.7 [a]**	**0.5 ± 0.6 [b]**
Fertilization rate (%)	**10 ± 4.2 [a]**	**78 ± 4.5 [a]**	**0.0**
Hatching rate (%)	**6 ± 1.2 [b]**	**75 ± 4.5 [a]**	**0.0**

could be due to lack of oxygen. Chakroff, (1976) reported that fish begin to be stressed when the dissolved oxygen level falls below 4 mg/ litter.

CONCLUSION

It could be concluded that the water depth were high(1m) and low (50cm) before breeding season directly 2 times in the month without spraying pesticide Lernaea(trichlorofon).Also , at the breeding season the oxygen level did not less than 4.5 mg/l.

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تأثير اجهاد الامهات علي الكفاءه التناسليه لأناث مبروك الحشائش

عادل محمد عبدالحميد عكر

المعمل المركزي لبحوث الثروه السمكيه- العباسه – ابوحماد – شرقيه

استهدفت هذه الدراسه تقييم اجهاد الامهات علي الكفاءه التناسليه في اناث مبروك الحشائش . استخدم لهذا البحث عدد 63 انثي مبروك حشائش تم حقنها بالغده النخاميه ثم كل الاناث قسمت الي تجربتين

التجربه الاولي : عدد الامهات 36 من الاناث متوسط الوزن ٢,٧ – ٢,٨ كجم تم تقسيم هذه الاسماك الي 4مجموعات تبعا لعمود الماء قبل موسم التفريخ:

المجموعه الاولي عمود الماء1 م ثابت لمدة شهرين، المجموعة الثانية عمود الماء ١م ثابت ايضا لمدة شهرين ولكن مع رش مبيد الليرنيا , والمجموعه الثالثه عمود الماء فيها متغير من واحد متر الي نصف متر خلال الشهرين دون رش مبيد، المجموعه الرابعه عمود الماء فيها متغير خلال الشهرين من ١م الي نصف متر لكن مع رش مبيد الليرنيا.

وكانت النتائج كما يلي: الفتره بين الحقن وانطلاق البيض كانت تقريبا من ٩- ٩,٥ ساعه في كل المجاميع ، كمية البيض ودليل المنا سل كان ذو معنويه مرتفعه في المجموعه الثالثه عن باقي المجاميع، بينما نسبة الاخصاب والفقس كانت الاختلافات غير معنويه

التجربه الثانية :عدد الامهات ٢٧ من الاناث متوسط الوزن ٣,٥ – ٣,٧ كجم تم تقسيم هذه الاسماك الي ثلاثة مجاموعات متساويه وذلك تبعا الي نسبة الاكسجين المجموعه الآولي كانت نسبة الاكسيجين ٧جم/ لتر , المجموعه الثانيه كانت نسبة الاكسجين ٤,٥ جم/لتر والمجموعه الثالثه كانت نسبة الاكسجين ٢حم/لتر.

وكانت النتائج كما يلي: الفتره بين الحقن وانطلاق البيض كانت تقريبا من ٨,٥- ١٠ ساعه في كل المجموعات ,كمية البيض ودليل المناسل كان ذو معنويه مرتفعه في المجموعه الثانيه عند مقارنتها مع باقي المجموعات , بينما نسبة الاخصاب والفقس كانت الاختلافات غيرمعنويه عند مقارنة المجموعة الاولي والثانيه بينما المجموعه الثالثه لا يوجد بها اخصاب ربما يرجع ذلك لنقص الاكسجين

مما سبق يتضح لنا ان ارتفاع وانخفاض عمود المياه قبل موسم التفريخ مهم وذو فعاليه كبيره في تحسين الكفاءه التناسليه لاسماك مبروك الحشائش ولكن دون استخدام اي مبيدات قد تحدث اجهاد لاناث مبروك الحشائش . كذلك عند دخول الامهات (اناث وذكور) للتزاوج في حوض التفريخ المعد لذلك بعد حقن الامهات يفضل الايقل مستوي الاكسجين داخل الحوض او التنك عن ٤,٥مج/لتر.