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The concentration of NaCl Fertilization Solution in Climbing Perch Hatchery (*Anabas testudineus Bloch*)

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Abstract
Climbing perch (*Anabas testudineus Bloch*) is one of Indonesia's endemic fish that has high economic value in the Riau area, especially in Kampar Regency. The community's need for this fish is still obtained solely from catches in public waters, especially from the waters of the Kampar River. The problem faced in the artificial spawning of Climbing perch is the low value of egg fertilization by sperm caused by a lack of spermatozoa fluid. The solution to increase the motility of spermatozoa and the value of fertilization is to add NaCl fertilization solution at the time of fertilization of eggs by spermatozoa. This research was conducted at the Laboratory of Fish Hatchery and Breeding, Faculty of Fisheries and Marine Sciences, Riau University from May to August 2021. The design used in this study was a Completely Randomized Design (CRD) with one treatment and three replications. The treatment used in this study was the addition of different concentrations of NaCl fertilization solution, consisting of 0.3%, 0.5%, 0.7%, and 0.9%. The results obtained that the best treatment was in the treatment of 0.7% NaCl concentration of fertilization solution with 91.9% fertilization value, 92.7% hatching value, 82.8% survival rate, 1.6% abnormal larval value, spermatozoa motility 4 (very good) duration of motile spermatozoa was 56.3 minutes, absolute weight growth for 40 days was 0.8877 g, absolute length growth was 4.1850 cm, the specific growth rate was 16.9142 %, and survival was 71.7778%.

Keywords: Absolute weight growth, absolute length growth, specific growth, and survival

Introduction

Climbing perch (*Anabas testudineus* Bloch) is one of Indonesia's endemic fish that has high economic value and includes native Indonesian fish species that live in swamps, rivers, lakes, and puddles (Syulfia *et al.*, 2015). In the Riau area, especially in Kampar Regency, Climbing perch is one of the economical fish that is very popular with the local community. The community's need for this fish is still obtained from catches in public waters, especially from the waters of the Kampar River which is one of the four largest rivers in Riau Province. The survival of the Climbing perch is feared to be threatened with extinction due to continuous fishing from nature. One way that is very appropriate to do so that the preservation of Climbing perch from nature can be maintained and the community's needs for these fish can be met is to find the right hatchery technology through artificial spawning. This is because 8 species of fish from 31 species of economically important fish in the waters of the Kampar River have previously been found with hatchery and cultivation technology, these types of fish are, jamis (*Ompok hypopthalmus*) (Putra, Sukendi and Yurisman, 2011) [12], motan (*Thynnichtys thynoides* Blkr) (Sukendi, Putra and Yurisman, 2011a) [13], tambakan (*Helostoma temmincki* CV) (Sukendi, Putra and Yurisman, 2011b) [14b], sepat siam (*Trichogaster pectoralis* Regan) (Sukendi, Putra and Yurisman, 2012), pearl sepat (*Trichogaster leeri* Blkr) (Sukendi, Putra and Yurisman, 2013), ingir-ingir (*Mystus negriceps* CV) (Sukendi, Putra and Nur'Asiah, 2015) [15].
Climbing perch hatchery can be done by artificial spawning. The problem faced in the hatchery of Climbing perch is the low

rate of fertilization of eggs by sperm because it is caused by a lack of spermatozoa fluid so that the motility of spermatozoa is reduced. Sperm motility and sperm density determine the ability to fertilize spermatozoa (Krol *et al*., 2006; Khodadadi *et al*., 2016) [6, 5]. One of the efforts that can be done to increase the motility of spermatozoa and the value of fertilization in artificial spawning is to add a fertilization solution at the time of fertilization of eggs by spermatozoa. The use of an appropriate fertilization solution will increase the motility of spermatozoa and increase the fertilization rate. The diluent used so far is physiological NaCl solution. However, the use of physiological NaCl solution is not optimal for fish hatchery because it still produces low fertilization values.

Material and Method

This research was carried out at the Fish Breeding and Hatchery Laboratory, Faculty of Fisheries and Marine Affairs, Riau University from May to August 2021. The Climbing perch used came from the selection of catches carried out by fishermen in the Kampar River with criteria for gonad maturity (Gonad Maturity Level). IV). The number of female fish spawned was 10 males and 5 females. To stimulate ovulation, female and male parents were injected using ovaprim stimulant with a dose of 1 ml/kg body weight for females and 0.5 ml/kg body weight for males. The design used was a completely randomized design (CRD) with one treatment and three replications. The treatment used in this study was the addition of different concentrations of NaCl fertilization solution, consisting of 0.3%, 0.5%, 0.7%, and 0.9%.

The variables measured in this study are : 1) the fertilization value was determined using the formula: the number of fertilized eggs/the total number of eggs x 100%; 2) the hatching value was determined using the formula: number of hatched eggs/number of fertilized eggs x 100%; 3) survival was determined using the formula: number of larvae at the end of the study / number of larvae at the end of the study the beginning of the study x 100%; 4) Number of abnormal larvae was determined using the formula : the number of abnormal larvae/number of hatching larvae x 100%; 5) Spermatozoa motility was obtained based on the categories stated by Kurniawan *et al*., (2013) [7] as shown that: score 4 (very good) with characteristic Big waves, many, dark, thick, and fast-moving, score 3 (good) with characteristic The waves are small, thin, infrequent, less clear, and moving slowly, score 2 (fairly good) with characteristic No visible waves but only progressive active individual movements, score 1 (poor) with characteristic Little move, not even individual movement; 6) Motility duration (seconds) by observing the movement of spermatozoa until they stop moving; 7) absolute weight growth was determined using the formula: larval weight at the end of the study - larval weight at the beginning of the study; 8) the specific growth rate (SGR) was determined using the formula: Ln weight of larvae at the end of the study - Ln weight of larvae at the beginning of the study / time x 100%.

The growth and survival data obtained were tabulated and statistical tests were carried out using the SPSS 16 application. The statistical tests carried out were homogeneity of variances and one-way analysis of variance (ANOVA) tests. If the results of the ANOVA test showed a significant difference ($P < 0.05$), further tests were carried out using the SNK test to determine the differences between treatments. The water quality data obtained are tabulated and described.

Results and Discussion

Fertilization Value

The best fertilization value of Climbing perch eggs was obtained in the treatment of 0.7 NaCl concentration of 91.9% while the lowest fertilization value was obtained at the treatment of 0.3 NaCl concentration of 79.3% as presented in Figure 1. Based on the results of the analysis of variance (ANOVA) It is known that the treatment of NaCl concentration of fertilization solution had a very significant effect ($P<0.01$) on the fertilization value of Climbing perch eggs. The best treatment of 0.7 NaCl concentration obtained in this study was thought to be caused by the different quality of spermatozoa in each treatment.

The more active the spermatozoa, the higher the ability of the spermatozoa to fertilize the egg. This is following the opinion of Adipu *et al*., (2011) [1] which states that in conditions of active and agile sperm movement, sperm have the ability and energy to penetrate the egg microfil hole. Nainggolan *et al*., (2015) [10] stated that fertilization in fish will occur if it is supported by good quality spermatozoa.

Hatching Value

The best hatching value of Climbing perch eggs was obtained in the treatment of 0.7 NaCl concentration of 92.7% while the lowest hatching degree value was obtained in the treatment of 0.3 NaCl concentration of 68.7%. As presented in Figure 2. Based on the results of the analysis of variance (ANOVA) it was found that the NaCl concentration treatment of the fertilization solution had a very significant effect ($P<0.01$) on the hatching value of Climbing perch eggs. The best hatching value of Climbing perch eggs in the treatment of 0.7 NaCl concentration was due to the observation that the fertilization value was also obtained in that treatment so that with the high fertilization value, the hatching value will also be higher. The hatching value of fish eggs is influenced by internal factors, one of which is the quality of less motile spermatozoa, causing delays in embryonic development (Masrial and Efrizal, 1997) [8]. The same thing was stated by Arfah *et al*., (2015) [2], stated that the embryo will be able to develop well if the quality of the spermatozoa is good.

3-day old larvae survival

The best survival value for 3-day-old Climbing perch larvae was obtained in the treatment with NaCl concentration of 0.7 of 82.8%, while the lowest survival value of 3-day-old Climbing perch larvae was obtained in the treatment of 0.3 NaCl concentration of 75.7%. As presented in Figure 3. Based on the results of the analysis of variance (ANOVA) it was found that the NaCl concentration treatment of fertilization solution had no significant effect ($P>0.05$) on the survival value of 3-day old Climbing perch larvae. Mulyani *et al*., (2014) [9], stated that the survival of fish is influenced by internal and external factors. Internal factors come from the fish itself. Spermatozoa quality is a determinant of internal factors that affect embryonic development and is also thought to affect the survival of larvae in the early hatching phase. This can be seen from the survival data for 3-day old larvae that are directly proportional to the value of spermatozoa motility. Where the higher the value of spermatozoa motility, the higher the survival value of the larvae.

Abnormal larvae

The highest number of abnormal larvae was obtained in the treatment with a NaCl concentration of 0.3 by 2% while the lowest number of abnormal larvae of Climbing perch was obtained in the treatment with a NaCl concentration of 0.7 at 1.6% as presented in Figure 4. Based on the results of the analysis of variance (ANOVA) it is known that the treatment of NaCl concentration of fertilization solution had no significant effect (P>0.05) on the percentage value of abnormal Climbing perch larvae. The smallest percentage value of abnormal larvae was obtained at the NaCl concentration treatment of 0.7 of 1.6% while the largest percentage value of abnormal larvae was obtained at the treatment of 0.3% NaCl concentration of 2.0%.

Spermatozoa motility

The best sperm motility value of Climbing perch was obtained in the treatment of 0.7 NaCl concentration and 0.9 NaCl concentration treatment with a motility value of 4 (very good) while the lowest sperm motility value of Climbing perch has obtained in the treatment of 0.3 NaCl concentration with a value of 0.3 motility 2 (good enough), as presented in Figure 5. Based on the results of the analysis of variance (ANOVA) it was found that the NaCl concentration treatment of fertilization solution had a very significant effect (P<0.01) on the motility value of Climbing perch spermatozoa. The results of this statistical test indicate that the concentration of NaCl in the diluent solution is very important in the fertilization process. Diluent solution determines the level of motility of spermatozoa. The level of motility of spermatozoa determines the success of fertilization and hatching of eggs. The higher the motility value of the spermatozoa, the higher the probability of the sperm fertilizing the egg. This is following the results of the value of the degree of fertilization obtained from this study which is directly proportional to the value of the motility of spermatozoa. Sperm cells in fish are usually active because they are triggered by the osmolality of the aquatic environment and when sperm cells are in the sperm sac, the osmolality of the seminal plasma at a certain level can keep sperm from being active (Irawan, 2014) [4].

Spermatozoa duration

The best motile duration of Climbing perch spermatozoa was obtained in the treatment of 0.7 NaCl concentration with a motile duration of 56.3 minutes while the lowest motile duration of Climbing perch spermatozoa was obtained at 0.3 NaCl concentration treatment with a motile duration of 11.3 minutes, as presented in Fig. Figure 6. Based on the results of the analysis of variance (ANOVA) it was found that the NaCl concentration treatment of the fertilization solution had a very significant effect (P<0.01) on the duration of motile spermatozoa in Climbing perch. With the largest value of spermatozoa motile duration obtained in the NaCl concentration of 0.7, the treatment showed the best treatment, because the longer the motile motile duration, the better the fish spawning process. This is related to the ability of spermatozoa to survive and fertilize eggs.

Absolute weight growth at 40 days

The best absolute weight growth was found in the NaCl concentration treatment of 0.7 with an absolute weight growth of 0.8877 g, followed by the NaCl concentration treatment of 0.8737 g, the treatment with 0.5 NaCl concentration of 0.8721 g, and the treatment of 0.3 NaCl concentration of 0.8634 g, such as presented in Figure 7. Based on the analysis of variance (ANOVA) it was found that the NaCl concentration treatment of fertilization solution had no effect (P>0.05) on the absolute weight growth value of Climbing perch larvae. The results of the Student-Newman-Keuls follow-up test showed that the treatment with NaCl concentration 0.3 was not significantly different from the treatment at NaCl concentration 0.5, not significantly different from the treatment at NaCl concentration 0.7, and not significantly different from the treatment with NaCl concentration 0.9. Giving different concentrations of NaCl fertilization solution at the time of spawning of Climbing perch did not affect the weight growth of the Climbing perch larvae. Growth is influenced by the feed given to the Climbing perch larvae according to the amount of feed needed.

Specific growth rate

The best specific growth rate of Climbing perch was obtained in the treatment of 0.7 NaCl concentration with a specific growth rate of 16.9142 %/day followed by the treatment of 0.9 NaCl concentration of 16.8715 %/day, the treatment of 0.5 NaCl concentration of 16.8715 %/day, treatment with 0.3% NaCl concentration of 16.8431%/day, as shown in Figure 8. Based on the analysis of variance (ANOVA) showed that the use of different sperm diluents did not significantly affect the specific growth rate of Climbing perch larvae (P > 0.05). Giving sperm diluent to spawning Climbing perch does not affect the growth of Climbing perch larvae. Growth occurs because the natural feed consumed by the larvae is following the needs and the amount of feed available.

The viability of seeds aged 40 days

The highest survival rate of 40-day-old Climbing perch was obtained in the treatment with NaCl concentration of 0.7 with a survival rate of 71.7778%, NaCl concentration of 0.9 of 71.3364%, NaCl concentration of 0.5 of 68.5648%, and NaCl concentration of 0,3 of 67.6019% as presented in Figure 9. Based on the analysis of variance (ANOVA) showed the use of different sperm diluents did not significantly affect the survival rate of Climbing perch larvae (P>0.05). The survival of the Climbing perch larvae depends on the feed given. The amount of feed that is following the needs of the fish can increase the survival of the larvae. According to Prasetya *et al* (2016), a higher amount of natural feed provides an opportunity for the availability of feed for larvae to seed size. The ability of larvae to get food will be influenced by the availability of the amount and type of natural feed given.

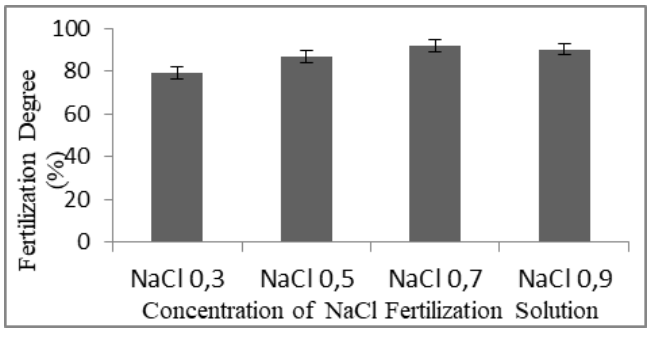


Fig 1: Fertilization value of Climbing perch eggs with NaCl concentration treatment Fertilization Solution

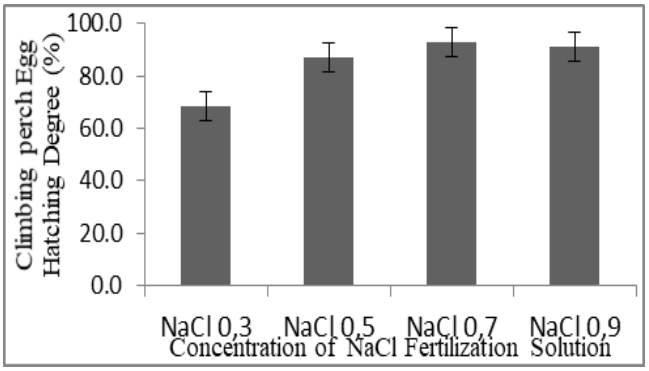


Fig 2: The hatching value of Climbing perch eggs with the treatment of NaCl concentration of Fertilization Solution

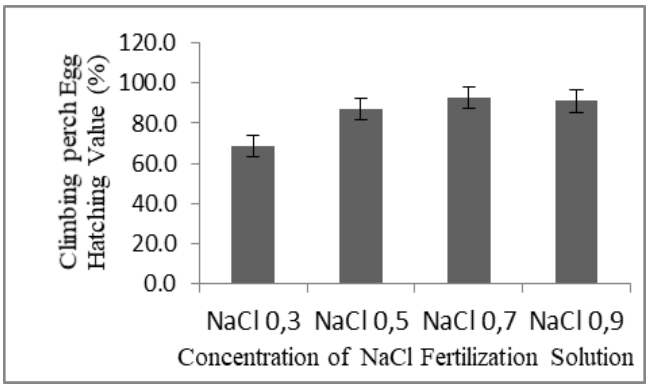


Fig 3: The hatching value of Climbing perch eggs with the treatment of NaCl concentration of Fertilization Solution

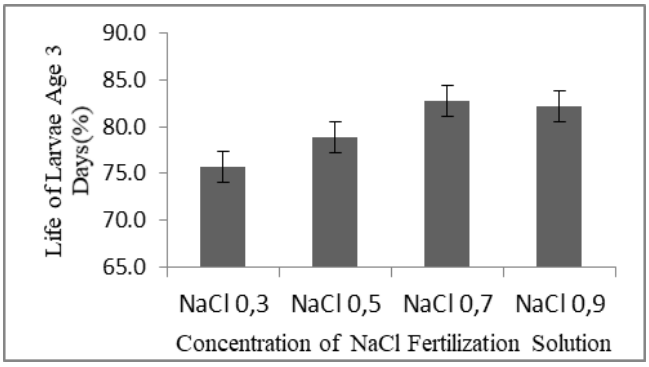


Fig 4: The survival of the Climbing perch with the treatment of the concentration of NaCl Fertilization Solution

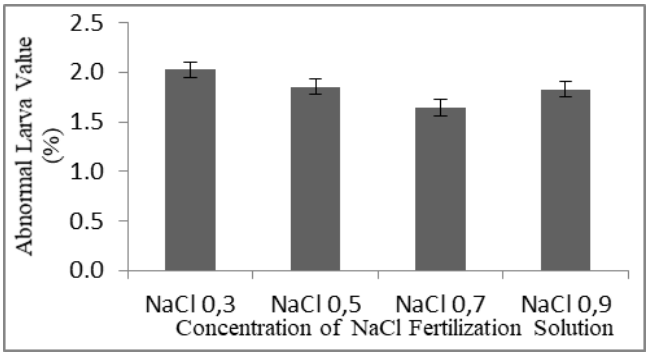


Fig 5: The value of abnormal larvae of Climbing perch with treatment with NaCl concentration of Fertilization Solution

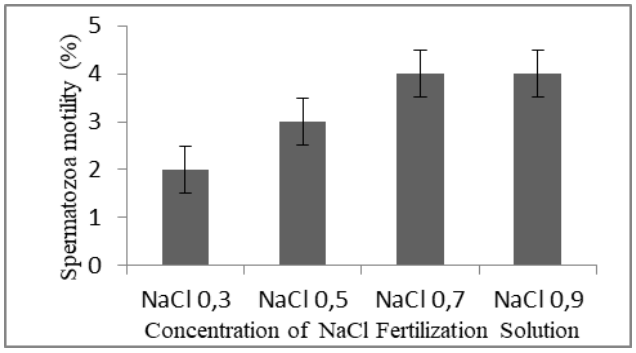


Fig 6: Motility of spermatozoa of Climbing perch with treatment with NaCl concentration of Fertilization Solution

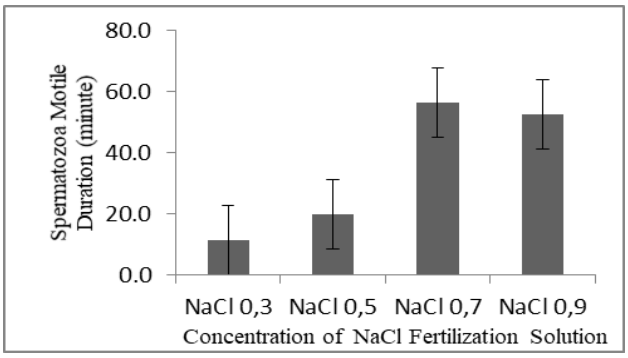


Fig 7: Duration of motile spermatozoa of Climbing perch with treatment with NaCl concentration of Fertilization Solution

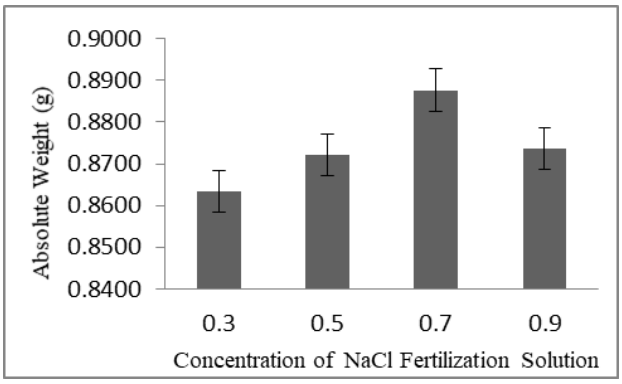


Fig 8: Growth of absolute weight of Climbing perch with NaCl concentration treatment Fertilization Solution

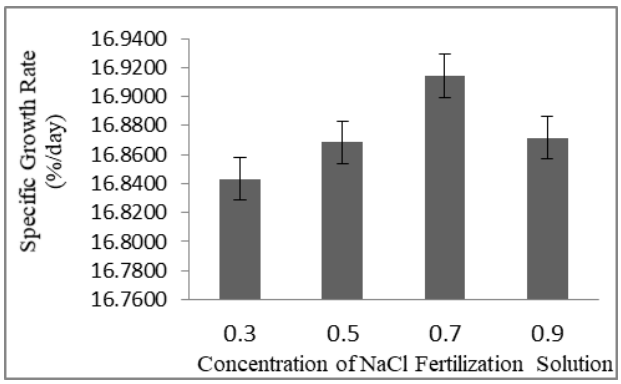


Fig 9: The specific growth rate of Climbing perch with NaCl concentration treatment Fertilization Solution

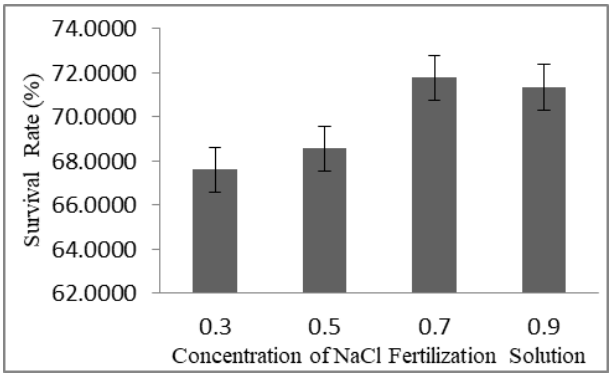
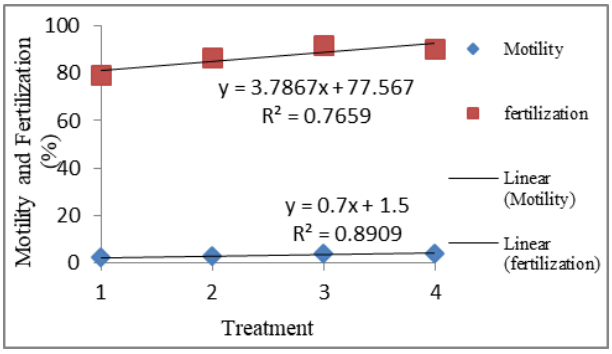
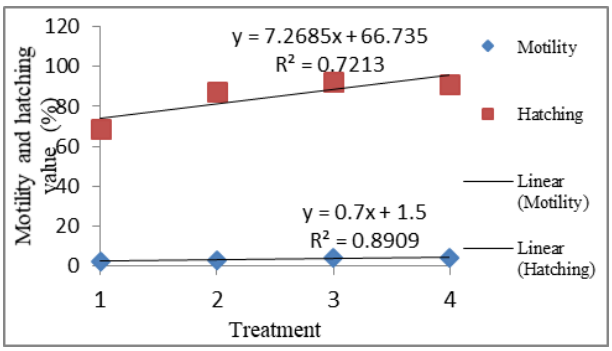
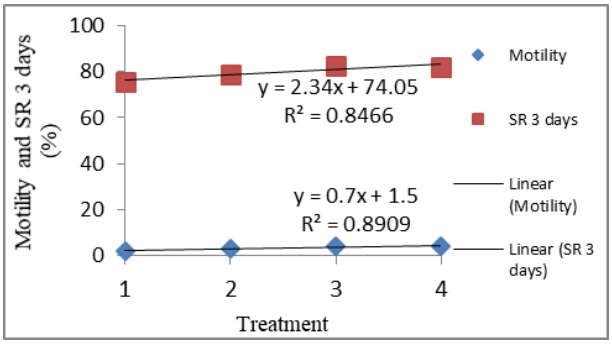


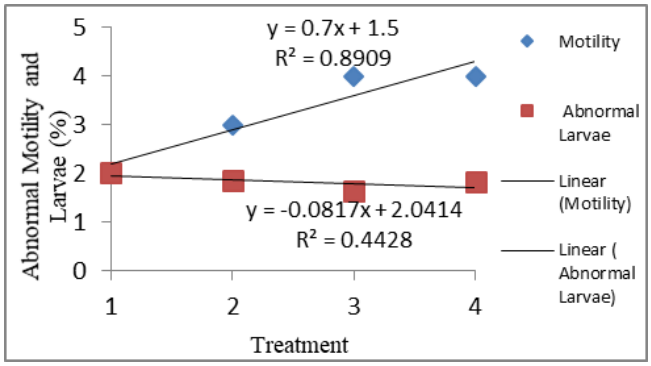
Fig 10: The 40-day life span of Climbing perch treated with NaCl concentration of Fertilization Solution

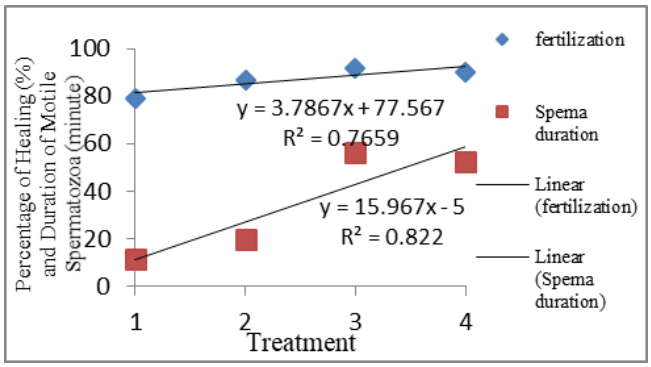
The relationship between spermatozoa quality parameters and spawning success parameters is presented in Figure 11. The histogram of the relationship between parameters shows that the spermatozoa quality parameters are positively related to the spawning success parameters. This indicates that the quality of spermatozoa determines the success of spawning Climbing perch. The better the spermatozoa, the better the spawning results obtained. The diluent solution in this case affects the quality of the spermatozoa. The concentration of 0.7% NaCl in the diluent solution resulted in the best spermatozoa quality and the best spawning results.

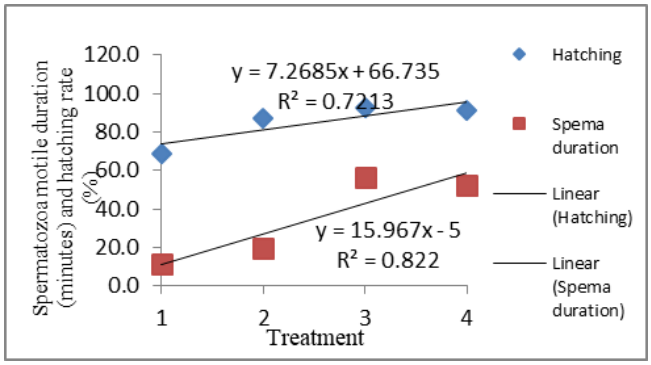


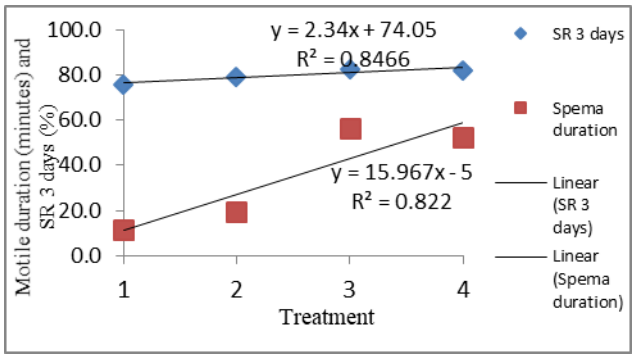












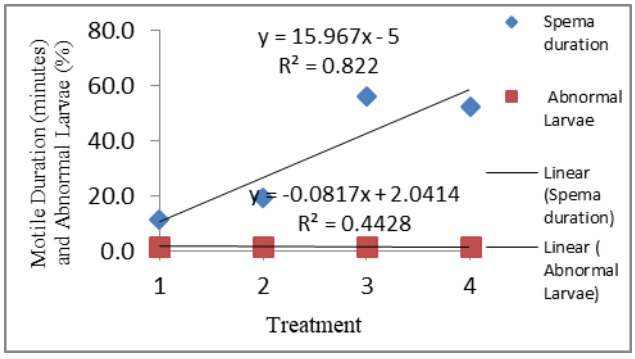


Fig 11: Relationship of Spermatozoa Quality Parameters with Spawning Parameters of Climbing perch

Conclusion

Based on the results obtained, it can be concluded that the quality of Climbing perch seeds in artificial spawning can be improved by increasing the concentration of NaCl fertilization at the time of fertilization. The best NaCl concentration in NaCl of 0.7%, resulting in fertilization value 91.9%, hatching value 92.7%, survival rate 82.8%, abnormal larval value 1.6%, spermatozoa motility 4 (very good) motile duration spermatozoa 56.3 minutes, absolute weight growth for 40 days of maintenance 0.8877 g, absolute length growth 4.1850 cm, specific growth rate 16.9142 %, and survival rate 71.7778%.

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References

1. Adipu Y, Sinjal H, Watung J. The ratio of Sperm Dilution to Fertilized Spermatozoa Motility and Hatchability of Catfish (*Clarias* sp). UNSRAT. 2011; VII.
2. Arfah H, Hasan F, Setiawan M. Provision of various types of honey with different dilution ratios on sperm quality of *Pangasianodon hypopthalmus*. Indonesian Journal of Aquaculture. 2015; 14(2):164-170.
3. Fitri PM, Aryani N, Nuraini. Effect of Stocking Density and Amount of Feed on Growth and Survival of Snakehead Fish (*Channa striata*) larvae, Student Online Journal. 2021; 8(1):1-13.
4. Irawan H. Effect of pH on Extender on Storage and Motility of Carp (*Cyprinus carpio*) Sperm Cells. Maritime Dynamics. 2014; 3(2):30-39.
5. Khodadadi M, Arab A, Amir Jaferian. A Preliminary Study on Sperm Morphology, Motility, and Composition of Seminal Plasma of Shirbot, *Barbus grypus*. Turkish Journal of Fisheries and Aquatic Sciences. 2016; 16:947-951
6. Krol J, Glogowski J, Demska-Zakes K, Hliwa P. Quality of semen and histological analysis of testes in Eurasian perch *Perca luviatilis* L. during a spawning period. Czech Journal of Animal Science. 2006; 51:220–226.
7. Kurniawan IY., Basuki F, dan Susilowati T. Addition of Coconut Water and Glycerol to Sperm Storage on Spermatozoa Motility and Fertility of Common Carp (*Cyprinus carpio*). Journal of Aquaqulture Management and Technology. 2013; 2(1):51-56.
8. Masrial and Efrizal. Effect of Semen Dilution Ratio on Sperm Fertility and Hatching Eggs of Carp (*Cyprinus carpio* L.). Fisheries Journal Crisp. 1997; 6:1-9.
9. Mulyani Y., Yulisman S, and Fitrani M. Growth and Feed Efficiency of Tilapia (*O. niloticus*) Periodically Fasted. Journal of Indonesian Swamp Aquaculture. 2014; 2(1):1-12.
10. Nainggolan R, Monijung RD, and Mingkid W. Addition of Honey in Sperm Dilution for Spermatozoa Motility, Fertilization, and Hatchability of Tilapia Fish Eggs (*Oreochromis niloticus*). Manado. Journal of Aquaculture. 2015; 3(1):131-140.
11. Prasetya J, Muslim M, Fitriani M. Spawning of Climbing perch (*Anabas testudineus* bloch) stimulated by hypophyseal extract of Climbing perch with different weight ratios of donor and recipient fish. Indonesian Journal of Swamp Aquaculture. 2016; 3(2)36–47.
12. Putra RM, Sukendi and Yurisman. Effect of combination injection of ovaprim and prostaglandin F2 2 on semen volume and spermatozoa quality of marmalade fish (*Ompok hypopthalmus*) Terubuk Fisheries Periodic. 2011; 39(2):67-76.
13. Sukendi, Putra RM, and Yurisman. Effect of combination injection of ovaprim and prostaglandin F2 on ovulation stimulation and egg quality of motan fish (*Thynnicthys thynnoides* Blkr). XXXIX Worst Fisheries Period. 2011[a]; 2:95–103.
14. Sukendi, Putra RM, and Yurisman. Fish hatchery technology (*Helostoma temmincki* CV) using a combination injection of ovaprim and prostaglandin F2 (PG F2). Professor's Research Report. Riau University. 2011b.
15. Sukendi, Putra RM and Nur'Asiah. Increased ovulation stimulation and egg quality and semen volume of senggaringan fish (*Mystus nigriceps* CV) for artificial spawning needs in seed production. Faculty of Fisheries and Marine Science, University of Riau. 2015.
16. Syulfia R, Son I and Rusliadi. Growth and Survival of Climbing perch (*Anabas testudineus*) With Different Stocking Density. Online Journal of Fisheries Students. 2015.