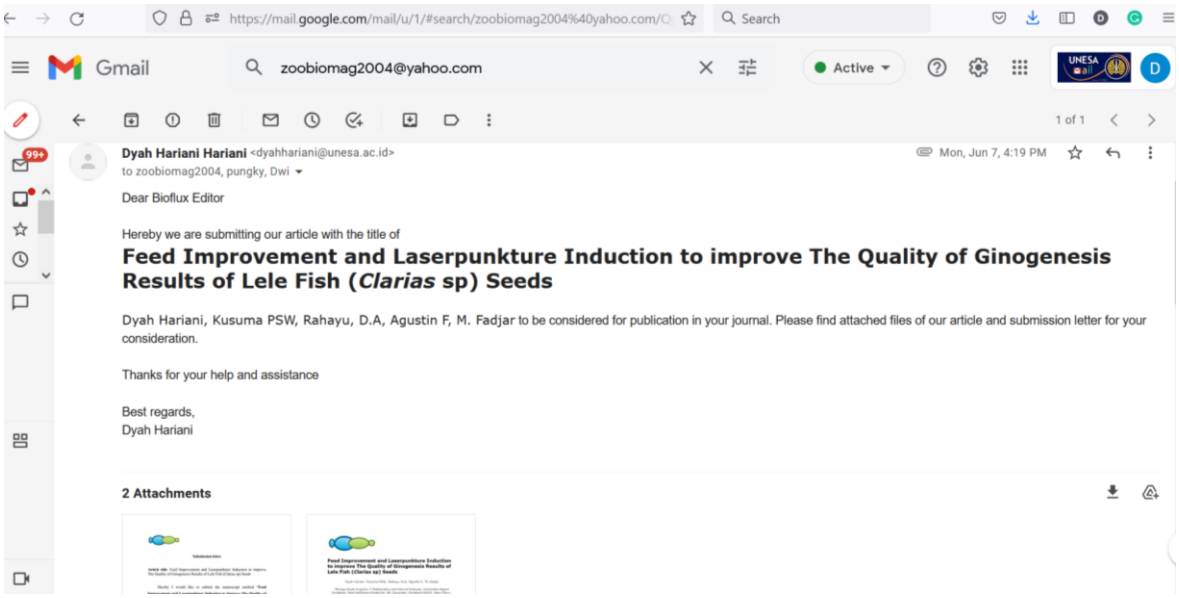
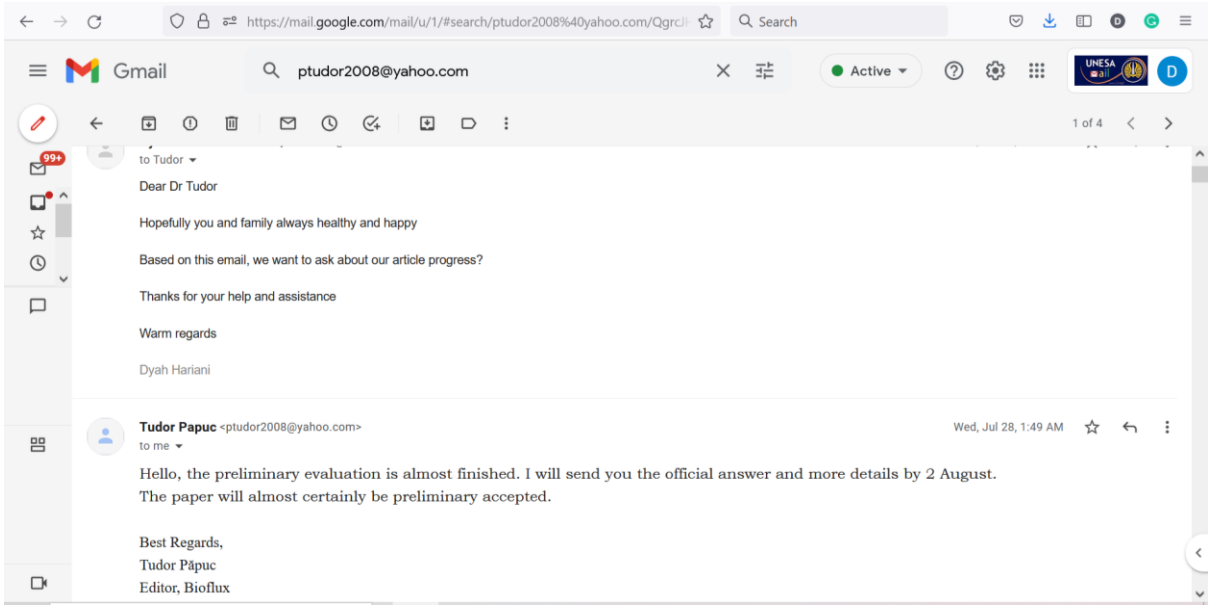

https://mail.google.com/mail/u/1/#search/zoobiomag2004%40yahoo.com/Q
Search
Gmail
zoobiomag2004@yahoo.com
Active
1 of 1
Dyah Hariani Hariani <dyahhariani@unesa.ac.id>
to zoobiomag2004, pungky, Dwi
Mon, Jun 7, 4:19 PM
Dear Bioflux Editor
Hereby we are submitting our article with the title of
Feed Improvement and Laserpunkture Induction to improve The Quality of Ginogenesis Results of Lele Fish (Clarias sp) Seeds
Dyah Hariani, Kusuma PSW, Rahayu, D.A, Agustin F, M. Fadjar to be considered for publication in your journal. Please find attached files of our article and submission letter for your consideration.
Thanks for your help and assistance
Best regards,
Dyah Hariani
2 Attachments


https://mail.google.com/mail/u/1/#search/ptudor2008%40yahoo.com/QgrcJ
Search
Gmail
ptudor2008@yahoo.com
Active
1 of 4
to Tudor
Dear Dr Tudor
Hopefully you and family always healthy and happy
Based on this email, we want to ask about our article progress?
Thanks for your help and assistance
Warm regards
Dyah Hariani
Tudor Papuc <ptudor2008@yahoo.com>
to me
Wed, Jul 28, 1:49 AM
Hello, the preliminary evaluation is almost finished. I will send you the official answer and more details by 2 August.
The paper will almost certainly be preliminary accepted.
Best Regards,
Tudor Păpuc
Editor, Bioflux

https://mail.google.com/mail/u/1/#search/ptudor2008%40yahoo.com/QgrcJ

Search

Gmail ptudor2008@yahoo.com Active

1 of 4

Tudor Papuc
to me

Fri, Aug 27, 5:10 AM

Hello, I am back with the paper with comments. What you need to do is this:

1. Read all the paper carefully, because the English was corrected and the text was formatted.

2. Read carefully all the comments first (before starting the corrections, because some comments might relate to each other) and try to correct as best as you can. **Please work on this version of the manuscript. Please mark your changes (highlight with yellow, or use track changes; you can also leave the comments), so I can check them**. If you cannot correct, do not wish to do so, or have your own explanations, please write the reason as a reply to the comment or as a new comment.

3. If you have anything to add/change to the text not based on comments, please do so, but mark the changes like in point 2.

4. Try to respect the formatting when making changes.

5. After you make the corrections, please check again, to make sure everything is in order.

6. Send me back the corrected version of the manuscript.

I will check it, give it a final form, and send you the final version for a last check before publication.

I want to highlight 2 problems in the paper where you need to pay more attention when making corrections:
1. The figures show different numbers from what you say in the text.
2. In the Discussion part, there are many paragraphs which show information that was already presented.
So please be careful with these 2 problems, because they really need to be solved. If not, it will be hard to publish the paper by 3 September.

https://mail.google.com/mail/u/1/#search/ptudor2008%40yahoo.com/QgrcJ

Search

Gmail ptudor2008@yahoo.com Active

1 of 4

Dyah Hariani Hariani <dyahhariani@unesa.ac.id>
to Tudor

Mon, Aug 30, 4:33 PM (11 days ago)

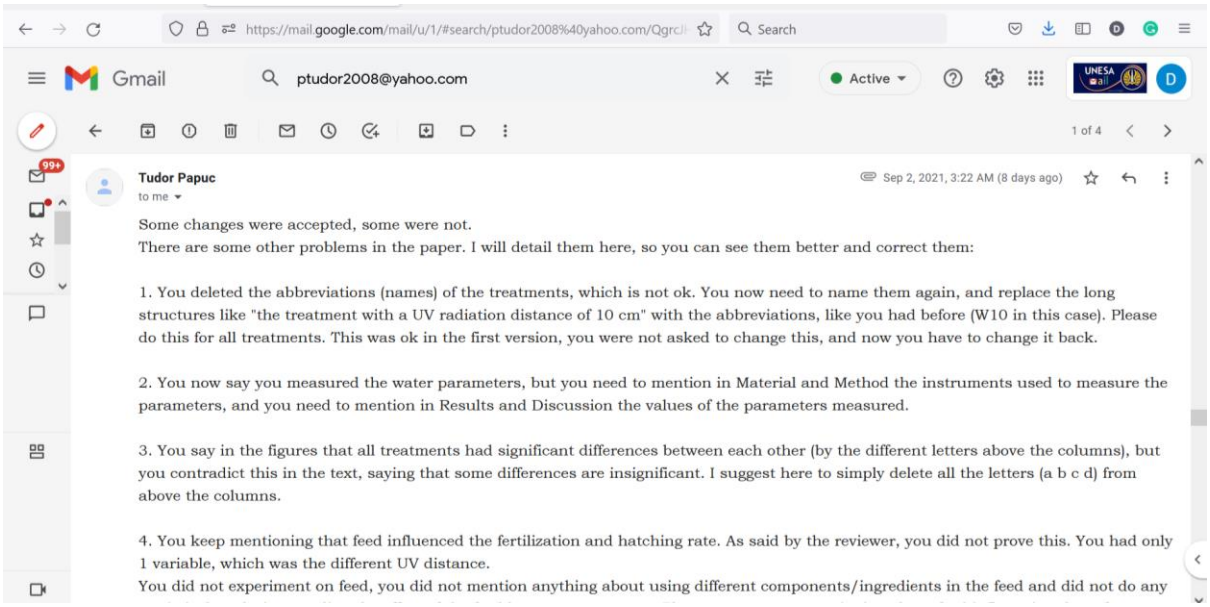
Dear Dr. Tudor

Thanks for your revise for our paper

im already revise based on reviewer comments

Thanks for your help and assistance

Best Regrads,
Dyah Hariani



Tudor Papuc Sep 2, 2021, 3:22 AM (8 days ago)
to me

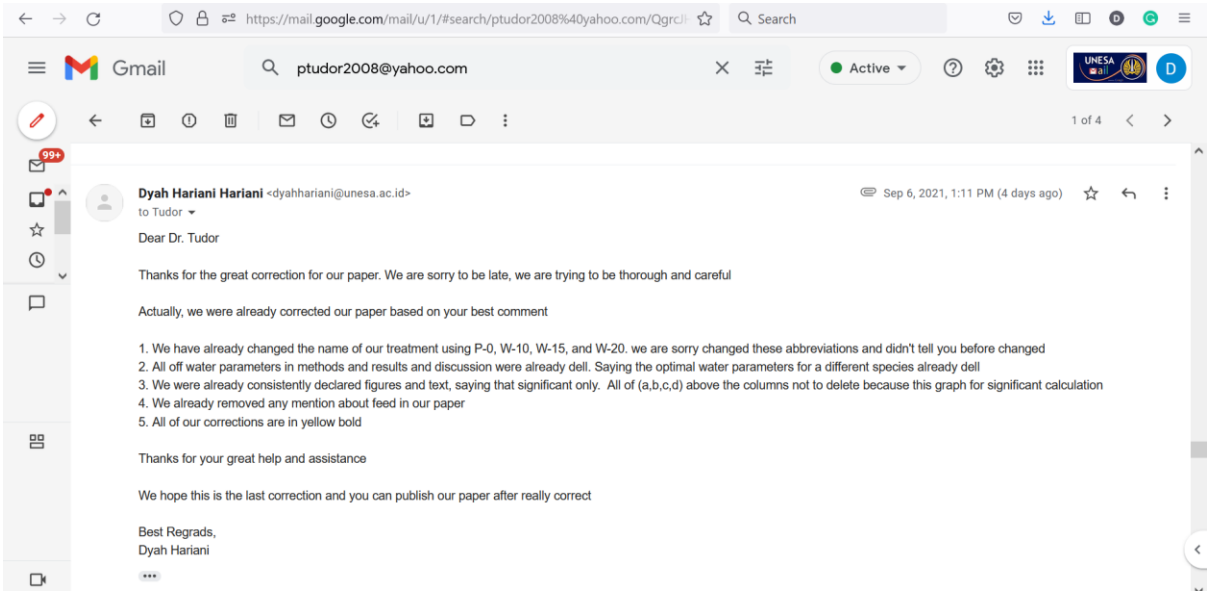
Some changes were accepted, some were not.
There are some other problems in the paper. I will detail them here, so you can see them better and correct them:

1. You deleted the abbreviations (names) of the treatments, which is not ok. You now need to name them again, and replace the long structures like "the treatment with a UV radiation distance of 10 cm" with the abbreviations, like you had before (W10 in this case). Please do this for all treatments. This was ok in the first version, you were not asked to change this, and now you have to change it back.

2. You now say you measured the water parameters, but you need to mention in Material and Method the instruments used to measure the parameters, and you need to mention in Results and Discussion the values of the parameters measured.

3. You say in the figures that all treatments had significant differences between each other (by the different letters above the columns), but you contradict this in the text, saying that some differences are insignificant. I suggest here to simply delete all the letters (a b c d) from above the columns.

4. You keep mentioning that feed influenced the fertilization and hatching rate. As said by the reviewer, you did not prove this. You had only 1 variable, which was the different UV distance.
You did not experiment on feed, you did not mention anything about using different components/ingredients in the feed and did not do any



Dyah Hariani Hariani <dyahhariani@unesa.ac.id> Sep 6, 2021, 1:11 PM (4 days ago)
to Tudor

Dear Dr. Tudor

Thanks for the great correction for our paper. We are sorry to be late, we are trying to be thorough and careful

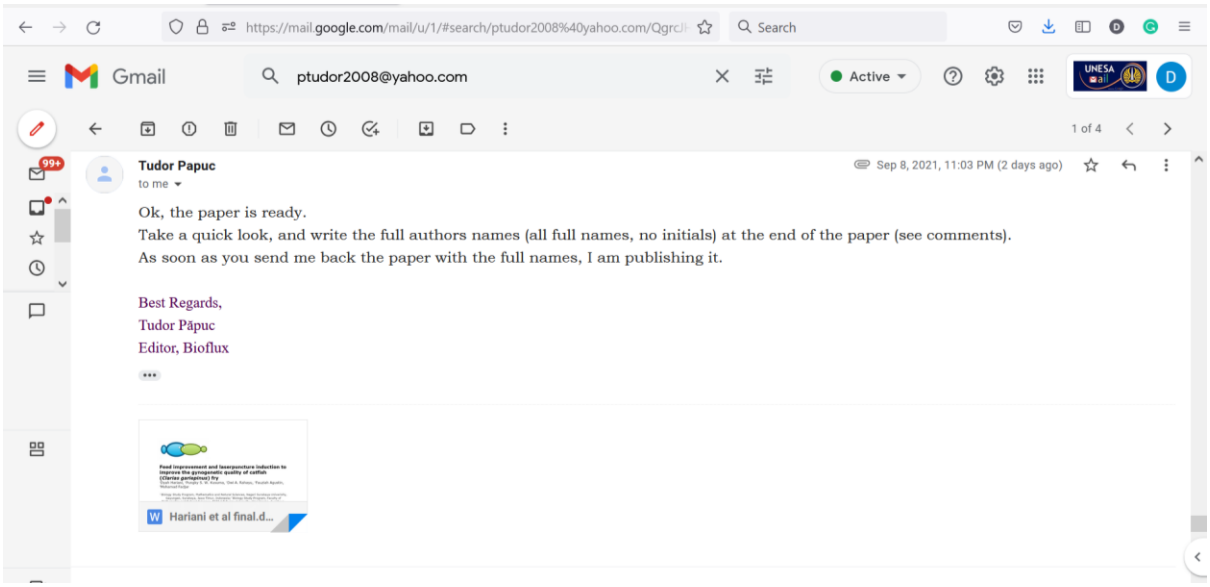
Actually, we were already corrected our paper based on your best comment

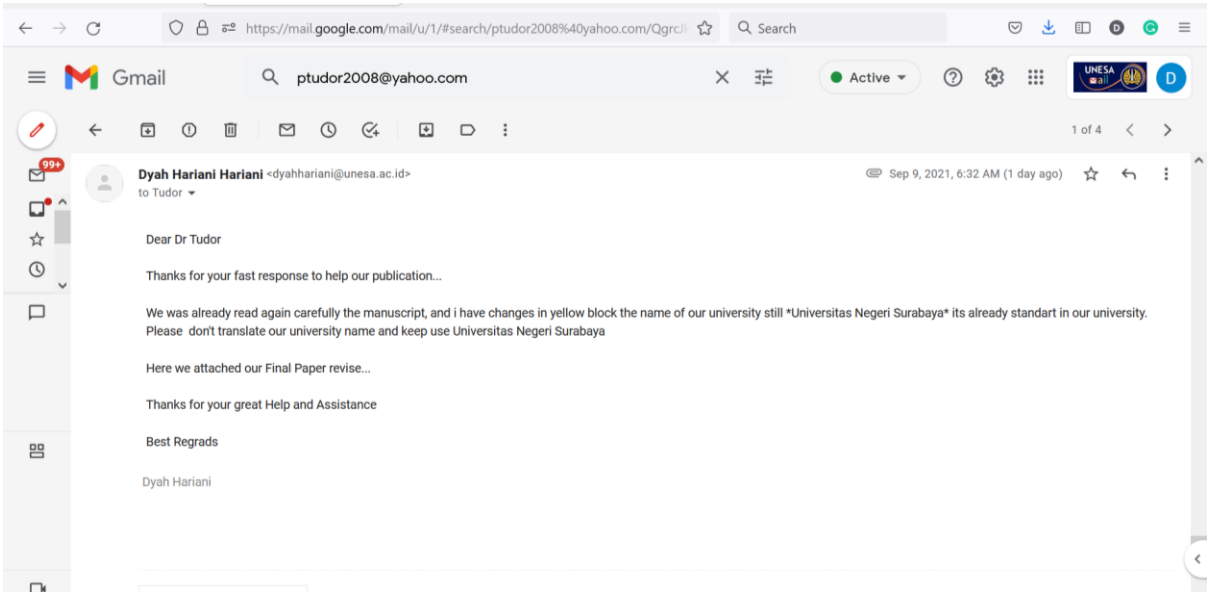
1. We have already changed the name of our treatment using P-0, W-10, W-15, and W-20. we are sorry changed these abbreviations and didn't tell you before changed
2. All off water parameters in methods and results and discussion were already dell. Saying the optimal water parameters for a different species already dell
3. We were already consistently declared figures and text, saying that significant only. All of (a,b,c,d) above the columns not to delete because this graph for significant calculation
4. We already removed any mention about feed in our paper
5. All of our corrections are in yellow bold

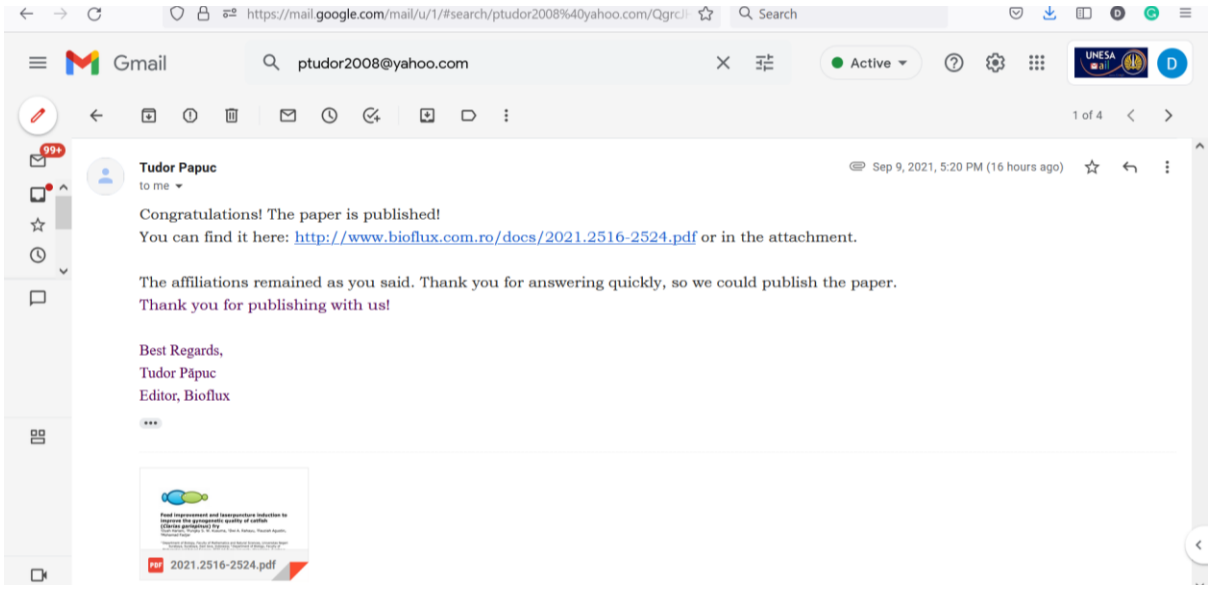
Thanks for your great help and assistance

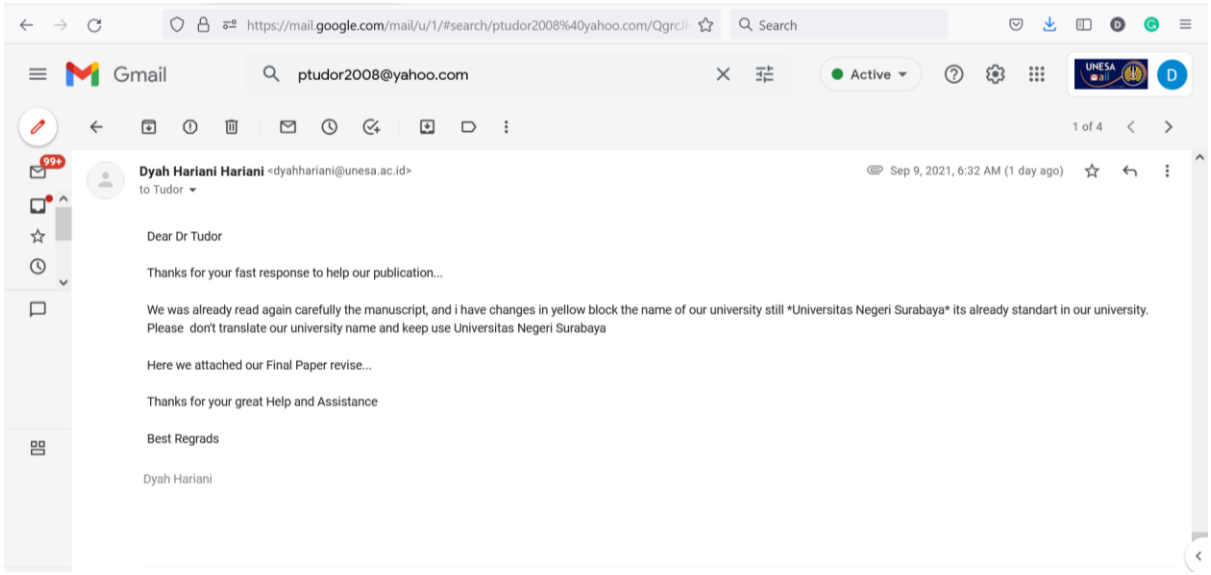
We hope this is the last correction and you can publish our paper after really correct

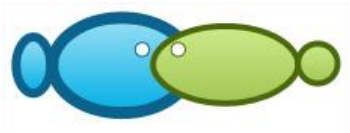
Best Regrads,
Dyah Hariani


https://mail.google.com/mail/u/1/#search/ptudor2008%40yahoo.com/QgrcJ
Search
Gmail
ptudor2008@yahoo.com
Active
1 of 4
Tudor Papuc
to me
Sep 8, 2021, 11:03 PM (2 days ago)
Ok, the paper is ready.
Take a quick look, and write the full authors names (all full names, no initials) at the end of the paper (see comments).
As soon as you send me back the paper with the full names, I am publishing it.
Best Regards,
Tudor Păpuc
Editor, Bioflux
Hariani et al final.d...


https://mail.google.com/mail/u/1/#search/ptudor2008%40yahoo.com/QgrcJ
Search
Gmail
ptudor2008@yahoo.com
Active
1 of 4
Dyah Hariani Hariani <dyahhariani@unesa.ac.id>
to Tudor
Sep 9, 2021, 6:32 AM (1 day ago)
Dear Dr Tudor
Thanks for your fast response to help our publication...
We was already read again carefully the manuscript, and i have changes in yellow block the name of our university still *Universitas Negeri Surabaya* its already standart in our university.
Please don't translate our university name and keep use Universitas Negeri Surabaya
Here we attached our Final Paper revise...
Thanks for your great Help and Assistance
Best Regrads
Dyah Hariani


Gmail
ptudor2008@yahoo.com
Active
1 of 4
Tudor Papuc
to me
Sep 9, 2021, 5:20 PM (16 hours ago)
Congratulations! The paper is published!
You can find it here: http://www.bioflux.com.ro/docs/2021.2516-2524.pdf or in the attachment.
The affiliations remained as you said. Thank you for answering quickly, so we could publish the paper.
Thank you for publishing with us!
Best Regards,
Tudor Păpuc
Editor, Bioflux
2021.2516-2524.pdf


Gmail
ptudor2008@yahoo.com
Active
1 of 4
Dyah Hariani Hariani <dyahhariani@unesa.ac.id>
to Tudor
Sep 9, 2021, 6:32 AM (1 day ago)
Dear Dr Tudor
Thanks for your fast response to help our publication...
We was already read again carefully the manuscript, and i have changes in yellow block the name of our university still *Universitas Negeri Surabaya* its already standart in our university. Please don't translate our university name and keep use Universitas Negeri Surabaya
Here we attached our Final Paper revise...
Thanks for your great Help and Assistance
Best Regrads
Dyah Hariani



Feed improvement and laserpuncture induction to improve the gynogenetic quality of catfish (*Clarias* sp.) fry

[1]Dyah Hariani, [2]Kusuma PSW, [1]Rahayu DA, [2]Agustin F, [3]M. Fadjar

[1]Biology Study Program, Mathematics and Natural Sciences, Negeri Surabaya Univerisity, Gayungan, Surabaya, Jawa Timur, Indonesia; [2] Biology Study Program, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana Univresity, Wonokromo, Surabaya, Jawa Timur, Indonesia; [3] Aquaculture Department, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia. Corresponding author: D. Hariani, dyahhariani@unesa.ac.id

Abstract. An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturation of reproductive catfish. However, it is relatively unclear whether broodstock semen, when irradiated with ultraviolet (UV), will produce the same results in egg fertilization by gynogenesis techniques. Gynogenesis is a parthenogenesis reproduction technique where embryonic development occurs by obtaining genetic material only from females, without male contribution. This study aimed to determine whether the distance of UV radiation on catfish broodstock semen enhanced by feed improvement and laserpuncture induction will successfully fertilize eggs. The distance between the UV source and semen was divided into 4 treatments namely: control, distance of 10 cm, 15 cm, and 20 cm, with 6 replications. Furthermore, the data collection technique involved a completely randomized design (CRD). The parameters observed included the Hatching Rate (HR), Survival Rate (SR), and Specific Growth Rate Length (SGRL) of the larvae, until the fry were 6 weeks old. The results showed that 20 cm UV radiation distance on semen had a significant effect ($p<0.05$) on the increasing percentage of HR values (63%), SR values (54%), and SGRL (83%) compared to other treatments.

Key Words: *Feed improvement, laserpuncture induction, UV radiation, gynogenesis*

Commented [u1]: please write the full first name, then the initials of the middle names, then the full family name

Commented [u2]: add the scientific name in brackets

Commented [u3]: add scientific name in brackets

Commented [u4]: key words need to be different words from words used in the title; please change these key words

Introduction. Catfish (*Clarias* sp.) is one of the cultivated commodities with high economic value. The market share for this commodity tends to have an increasing trend. Therefore, the demand for catfish is also increasing. One of the efforts to obtain quality fry involves reproducing only high quality catfish. This requires support by improving the male and female broodstock feeds, thereby improving the quality of eggs, sperm and, subsequently, fry. The quality feed contains protein and lipids which are the essential components of egg yolks, as they play a vital role during embryogenesis. Quality feeds are necessary to support the survival of embryos and larvae (Izquierdo et al 2001; Çek & Yilmaz 2009; Sink et al 2010). Research by Coldebella et al (2011) & Ghaedi et al (2019) showed that a low protein content (10-20%) in broodstock feed was proven to significantly reduce the survival of larvae, and that it also produced a low fertilization rate and a higher percentage of larval abnormalities.

Commented [u5]: please delete; all feed has protein and lipids, not only the quality one

The presence of probiotics in feed improves the quality, as it increases the digestibility value, growth and survival rate of fish (Crab et al 2012; Iribarren et al 2012; Krishna et al 2015; Chowdhury & Roy 2020; Lins Rodigues et al 2020). In addition, the bacteria present in probiotics also increase the nutritional value of feed by synthesizing vitamins, proteins and essential fatty acids, like amylase, lipase, and proteases (Irianto & Austin 2002; LeBlanc et al 2011; Ray et al 2012; Oktavianawati et al 2016).

In general, genetic engineering can be carried out to improve the quality of fry. This involves the manipulation of chromosomes in the fertilization process, such as gynogenesis. According to Durhan (2004) and Arai (2001), gynogenesis is carried out in two important stages: deactivating the genetic material of male gametes through

ultraviolet light (UV) radiation, and restraining the second polar body in meiosis II or restraining the first cell division at mitosis I. This is done by administering a hot shock of 38°C for 3 minutes after fertilization (Volckaert et al 1994; Laczynska et al 2020). When a fertilized egg develops, it produces a diploid female gynogenetic embryo.

UV radiation at wavelengths below 254 nm is proven to be strongly absorbed by certain biological substances, especially nucleic acids, proteins and coenzymes. Furthermore, UV light at a wavelength of 254 nm has been demonstrated to sufficiently damage the function of pyrimidine DNA, which represents the genetic material of male fish sperm. Although the pyrimidine function of sperm DNA is inactivated by UV radiation, it does not inactivate the ability of sperm to move and fertilize eggs (Zan-Bar et al 2005; Mekkawy et al 2010).

An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturatio of broodstock catfish (Kusuma et al 2015; Dyah & Pungky 2019). Kusuma et al (2015) confirm that the use of helium-neon laserpuncture technology on the reproductive acupoint precisely at 2/3 ventral parts of the body through induction for 15 seconds is optimal for the maturation of catfish gonads. Hariani et al (2020) stated that helium-neon low-power laserpuncture technique at the reproductive acupoint of 15 seconds every two weeks is optimal for the maturation of catfish gonads.

This research focused on improving feed and laserpuncture induction as a reproductive biostimulator to accelerate the maturation male and female catfish gonads. In addition, the semen was irradiated with ultraviolet light (UV) at a certain distance to determine whether it still possesses the ability to fertilize eggs until they hatch.

Material and Method. This research was conducted at the Physiology Laboratory, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana Surabaya University. The data collection method was carried out using a completely randomized design (CRD) with 4 treatments: control, UV radiation distance of 10, 15 and 20 cm, with six replications. Five female catfish 900-1500 g and 5 males of 1140-1750 g were used. The fish were obtained from catfish farmers in Pare, Kediri, East Java.

The acclimation of catfish broodstock was carried out separately for 2 weeks in concrete ponds (2x2x0.9 m). Broodstocks were fed with commercial floating pellets (PF-128), with a crude protein content of 38% and probiotics (Probio-7, Tamasindo Veterinary product) with the following composition: *Saccharomyces cerevisiae, Aspergillus oryzae, Lactobacillus acidophilus, Bacillus subtilis, Rhodopseudomonas, Actinomycetes* and *Nitrobacter*, with a density for each bacteria of >1x1011 CFU L^{-1}. Feed was administered twice daily, in the morning and evening, 5% of the body weight, until gonadal maturation.

Eggs and semen collection from the broodstock. Before collecting eggs and semen, the broodstock was treated with laserpuncture at the reproductive acupoint of precisely 2/3 of the ventral part of their body for 15 seconds, with an induction frequency of once every 2 weeks (Hariani et al 2018a). After laserpuncture induction, the catfish were returned to the spawning pond until spawning symptoms were observed. The females showing spawning symptoms were stripped to obtain eggs. The eggs were collected in a dry plastic basin and placed in an icebox at 4°C. Meanwhile, males were dissected to remove the gonads, which were pressed to obtain semen. 1 mL of semen was measured with a pipette and diluted with 9 mL of NaCl physiological/ringer. The semen was then poured into a 1 mm thick petri dish, irradiated with a 15-watt germ lamp (Philips™, Holland) with a wavelength of 260 nm for 2 min, from a distance of: 0 cm (P-0), 10 cm (W-10), 15 cm (W-15), 20 cm (W-20).

Both semen that was irradiated with UV light and not irradiated (control) was mixed with the eggs and stirred evenly using chicken feathers. The fertilized eggs were spread on a tea filter, placed in an incubation container at 28°C for 3 min, then shocked at 38°C for 2 min. This was done to prevent the second polar body's jump and ensure that the number of chromosomes remained 2N (diploid). After the temperature shock, the fertilized eggs were incubated in a pond at 28°C until they hatching.

Commented [u6]: please add a reference for this statement

Commented [u7]: please add a reference for this statement

Commented [u8]: please add the scientific name in brackets

Commented [u9]: this is missing from References; please add it there too

Commented [u10]: please add the species scientific name

Commented [u11]: from what species?

The counting of fertilized and unfertilized eggs was carried out approximately 8 h after the fertilization process in the incubation pond. The fertilized eggs were cloudy white, while unfertilized eggs were clear.

The next step involved calculating the percentage of hatched eggs, or hatching rate (HR), and the survival rate (SR) of catfish larvae until the 5th day of life. Furthermore, the specific growth rate length (SGRL) of gynogenesis results was measured after the 5th day every 2 weeks for 6 weeks using the following equation (Vesal et al 2016):

Hatching Rate (%): HR = a/(a+b) x 100

Survival Rate (%): SR = (Final fish number/Initial fish number) x 100

Specific Growth Rate Length (%/day) = SGRL

SGRL = [(final body length-initial body length)/days of experiment] x100

Where: a - number of eggs hatched; b - number of unhatched eggs.

Data analysis. The data was calculated using SPSS and analyzed using one-way Analysis of Variance (ANOVA) at a significance level of $p<0.05$. The analysis was used to determine the effect of UV radiation distance on the percentage of HR, SR and SGRL of catfish. If a difference between treatments was observed, it was followed by the LSD test. The percentages of HR, SR, and SGRL of larvae before the variance analysis were transformed to arcsin.

Results and Discussion

Hatching rate of catfish eggs. The mean HR values of catfish eggs with and without UV irradiation are presented in Figure 1.

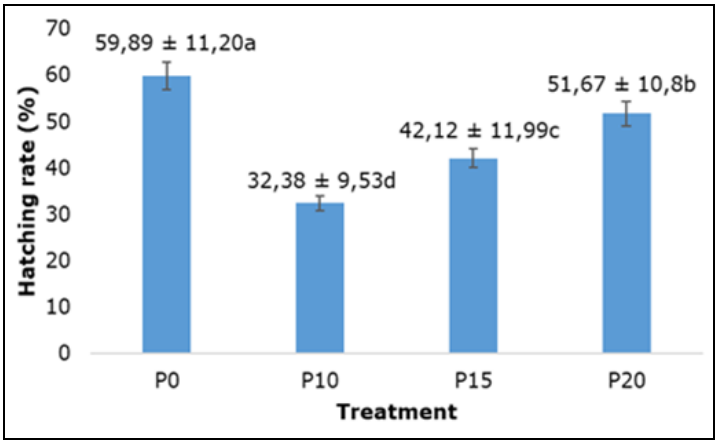


Figure 1. Hatching rate of catfish (*Clarias* sp.) eggs in the 4 treatments. Different letters above columns show.

Commented [u12]: please mention here what the letters above the columns are

The HR of fertilized catfish eggs without UV radiation (P0) was 71%. This was significantly higher ($p<0.05$) than that of P10 at 42%. Furthermore, the HR of P10 was insignificantly lower ($p>0.05$) than that of P15 at 54%. P10 was also insignificantly lower ($p>0.05$) than the HR of P20, at 63%.

Commented [u13]: - you need to check again your numbers, because they are different from numbers used in the figure; please correct where necessary

Survival rate of catfish larvae. The average SR of catfish larvae with and without UV irradiation of male catfish semen is presented in Figure 2.

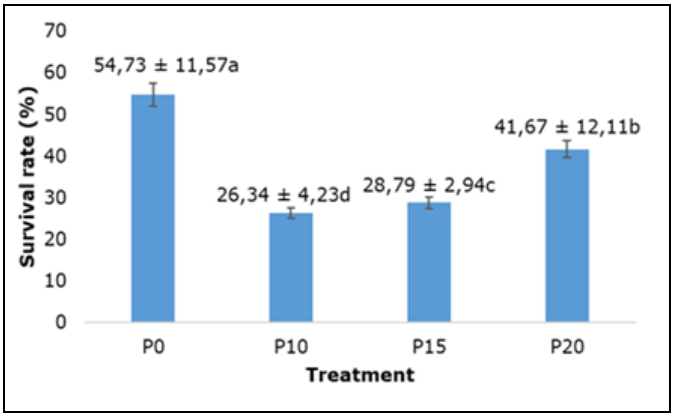


Figure 2. Survival rate of catfish (*Clarias* sp.) larvae in the 4 treatments. Different letters above columns show .

Commented [u14]: please mention here what the letters above the columns are

The SR of catfish larvae in P0 was 66%. This was significantly higher ($p<0.05$) compared to the SR of P10, at 31%. Furthermore, the SR of P10 was insignificantly lower ($p>0.05$) than the SR of P15, at 32%. The SR of P15 was also insignificantly lower ($p>0.05$) compared to that of P20, at 54%.

Commented [u15]: - you need to check again your numbers, because they are different from numbers used in the figure; please correct where necessary

Average growth of catfish body weight. The average growth values of catfish fry up to the age of 6 weeks is presented in Figure 3.

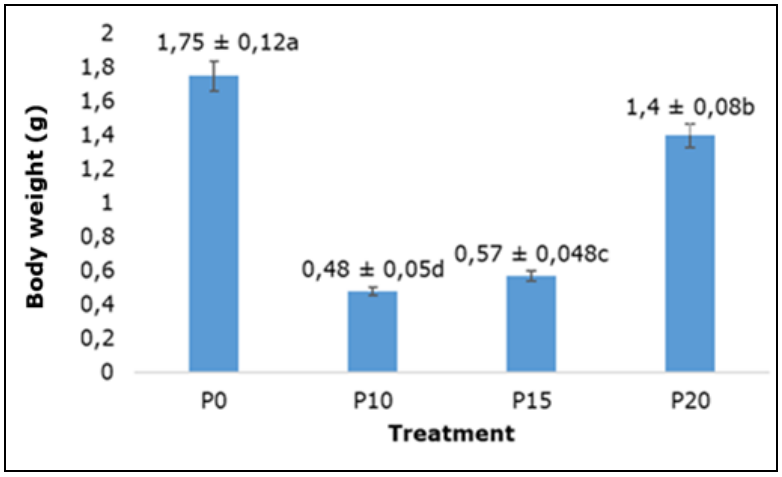


Figure 3. Growth of average body weight of catfish (*Clarias* sp.) fry.

Commented [u16]: please mention here what the letters above the columns are

The average growth of body weight up to the age of 6 weeks in P0 was 1.8 g. This was significantly higher ($p<0.05$) than the average growth value in P10, at 0.5 g. Furthermore, P10 was insignificantly lower ($p>0.05$) than the average growth value in

P15, at 0.6 g. The weight growth in P15 was insignificantly lower ($p>0.05$) than the average growth value of catfish fry in P20, at 1.5 g.

Average growth of catfish fry body length. The average growth of catfish body length within 6 weeks of fertilization is presented in Figure 4.

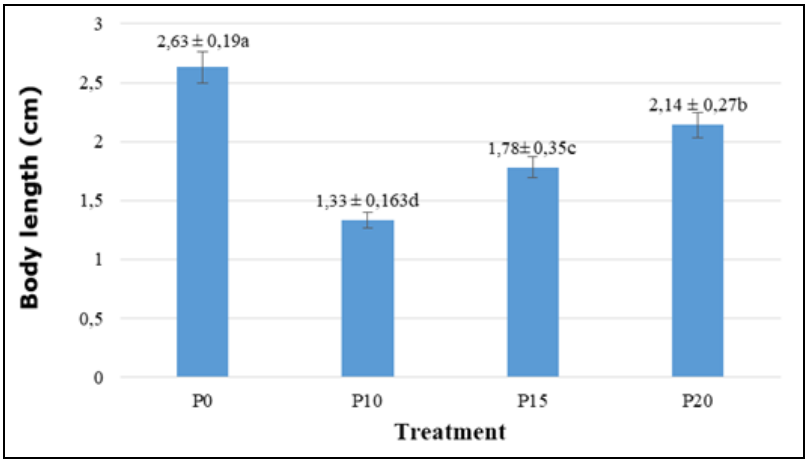


Figure 4. The average growth of catfish (*Clarias* sp.) body length.

The average growth of catfish fry length until the 6th week was 2.8 cm in P0. This was significantly higher ($p<0.05$) than the average value of catfish seed growth length. In P10, the length was 1.5 cm, insignificantly lower ($p>0.05$) than in P15, of 2.1 cm. The average growth length of P15 was insignificantly lower ($p>0.05$) than in P20, which was 2.3 cm.

Daily specific growth rate length of catfish fry. The daily specific growth rate length (SGRL) of catfish fry of 6 weeks of age is presented in Figure 5.

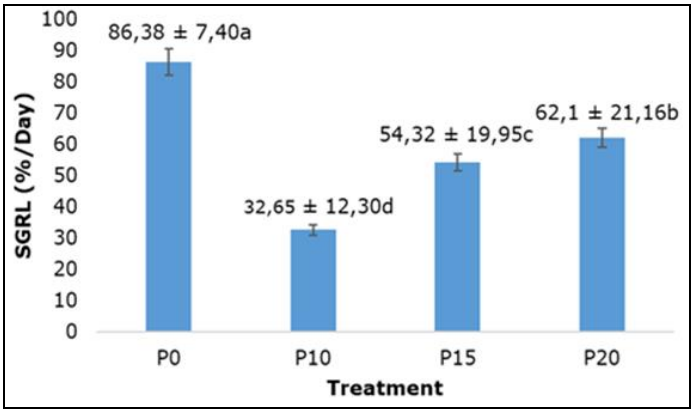


Figure 5. The specific growth rate length (SGRL) of catfish (Clarias *sp.*) fry.

Commented [u17]: please mention here what the letters above the columns are

Commented [u18]:

Commented [u19]: from what treatment?

Commented [u20]: please check again the numbers; they do not match the figure

Commented [u21]: please mention here what the letters above the columns are

The percentage daily specific growth rate length of catfish seeds up to 6 weeks of age indicated that the eggs fertilized with semen without UV radiation P0 were 94% significantly greater ($P<0.05$) than the other treatments. Semen-fertilized catfish seeds of UV radiation distance P10 was insignificantly lower ($P>0.05$) than the average percentage value of P15 at 76%. Furthermore, P15 was insignificantly ($P> 0.05$) lower than the average value of UV radiation distance P20 at 83%.

The effect of catfish semen UV radiation distance on hatching rate. The results showed that the effect of UV radiation distance on catfish semen on the HR of eggs in P20 was significant ($p<0.05$), compared to P10 and P15. Therefore, HR was influenced by the distance of UV radiation, while the success of egg fertilization was significantly influenced by the quality of feed administered to female and male parent catfish before the eggs and semen were obtained. In addition to the quality of the feed given, the water quality handling factor during the study was well controlled in order to support HR. This factor enhanced the successful development of seeds that resistant to environmental changes. Bobe (2015) and Baroiller et al (2009) stated that the success of HR is determined by external factors (temperature, dissolved oxygen, pH, sanitation and light intensity), as well as internal factors, including the quality of the eggs and spermatozoa.

P20 showed a high percentage of HR compared to P10 and P15, because a closer UV radiation to semen had a negative effect by inhibiting the rate of sperm movement. Therefore, P20 was optimal for destroying the genetic material of spermatozoa without affecting motility. This was evidenced by an increasing enzymatic activity that enabled the movement of spermatozoa to fertilize more eggs. The results of this study were supported by Godwin (2001), who discovered that UV radiation at a wavelength of 254-260 nm determines whether or without UV rays will penetrate biological materials, especially nucleic acids, proteins and coenzymes. UV radiation at a wavelength of 254-260 nm causes damage to DNA, somatic and genetic cells. In addition, at a wavelength of 254-260 nm, UV light radiation causes chromosomal changes to occur in the cell cycle, especially in the metaphase of meiosis. Cells that divide relatively often have a greater chance of being damaged by UV radiation at a wavelength of 254-260 nm. Furthermore, the success of gynogenesis was also determined by the temperature shock sometime after the egg was fertilized. This temperature shock enables the stimulation of the diploid zygote. Therefore, it should be carried out at the right time to increase the diploidization of gynogenetic seeds, namely during meiosis II and mitosis I (Galbusera et al 2000, Gheyas et al 2001; Tiwary et al 2004).

In addition to the inactivation of semen DNA due to UV radiation distance, its properties to fertilize eggs were no affected, because, in the acrosome part of the spermatozoa, there are hydrolytic enzymes (acrosin and hyaluronidase) that function in egg fertilization. Therefore, there were fewer homozygous larvae in P10, compared to P15 and P20. Due to the proximity of UV radiation to the semen, the temperature shock produced a decrease in enzyme activity, because an increase in temperature will either denature the enzyme or damage the egg cytoplasmic proteins. This enzyme is called chorions, and it consists of pseudoceratine, which works to reduce chorion hardness (Rustidja 2004). Meanwhile, in the control group, the HR was significantly different ($p<0.05$) compared to treatments P10, P15 and P20, but the control produced heterozygotic male and female larvae. In P10, P15, and P20, only homozygous female larvae were observed. Another factor that caused a decrease in the HR was the frequency of spawning, which was too high, and affected the quality of spermatozoa and eggs produced (Bobe 2015)

Effect of UV light exposure distance on the survival rate of catfish larvae. The effect of UV light exposure distance on SR showed a significant value ($p<0.05$) between treatments. The percentage of larvae SR in P0 was 66%, which decreased in P10 by 31%, and experienced an increase in (P15) and (P20) by 32% and 54%, respectively. The control group P0 obtained medium to high heterozygous catfish larvae in the treatment groups P10, while in P15 and P20, a high number of female homozygous catfish larvae were obtained.

Commented [u22]: the values are different in the figure; please correct where necessary

Commented [u23]: you did not study or prove this; please delete

Commented [u24]: you did not present anywhere water parameters (not in Material and Method, and not in Results); please delete

Commented [u25]: this is missing from the References; please add it there too

Commented [u26]: not understandable; please rephrase

Commented [u27]: is this correctly written? it should not have the same name with the egg membrane

Commented [u28]: remember to check you numbers in the figures, because the numbers are different

Commented [u29]: not understandable; please rephrase

The high and low SR values could have occurred due to the quality of the broodstock. The increase in SR percentage is also closely related to the quality of feed administered before the reproduction. The results of Kusuma & Hariani (2019) showed that providing quality feed to catfish before spawning helps accelerate the gonadal maturity. This is because quality feed supports the production of egg yolk and fertile spermatozoa. Egg yolk is highly useful during embryogenesis, and function as an initial nutrient reserve for larvae before exogenous feeding.

Eggs in P10 had a lower percentage of hatching compared to P15 and P20. Therefore, this significantly affected the SR of larvae. Temperature shock also played an important role in gynogenesis because it held the second polar body in meiosis II or withstood the division of the first cell at the time of mitosis I. This produces homozygous female individuals (Dunhan 2004).

The results of gynogenesis showed that, as a whole, homozygous females were obtained, whereas the control group produced heterozygous individuals. However, the high level of homozygotism of gynogenesis reduced the ability of larvae to survive. To support their ability to survive, the quality of water needs to be improved.

The water quality parameters, temperature, pH, and dissolved oxygen were in optimal conditions. Furthermore, the temperature of water incubation during the study ranged between 26-28ºC, while pH was 7 and dissolved oxygen 8 ppm. Dissolved oxygen is required in the process of embryo metabolism within the egg. This is in line with he observations of Brauhn (1971), who states that the optimal temperature for catfish spawning ranges from 24-30°C. Chatakondi (2020) mentions that the optimal temperature for broodfish is 26.6°C. Temperatures optimum for fertilization and hatching African catfish *Heterobranchus longifilis* was reported to be 25–29°C (Nguenga *et al*.,2004). Other factors that influenced the egg hatching process include pH and dissolved oxygen. The optimal pH range for hatching is 6.7-7.5 (Marimuthu et al 2019).

The effect of UV exposure distance of semen on weight and length growth of catfish fry. The average growth in body weight of gynogenetic catfish seeds in the P20 UV radiation distance treatment on semen was significantly greater than other treatments P10 and P15. The increase in weight of catfish seeds is shown in Figures 3. The treatment of P20 UV radiation distance on semen, the growth weight of catfish seeds was 1.5 g greater compared to treatment P10 of 0.5 g and P15 of 0.6 g showed that after six weeks of acclimation, while for the average growth in body length of gynogenetic catfish seeds in the P20 UV radiation distance treatment on semen was significantly greater than other treatments P10 and P15. The increase in body length of catfish seeds is shown in Figures 4. The treatment of P20 UV radiation distance on semen, the growth body length of catfish seeds was 2.3 cm greater compared to treatment P10 of 1.5 cm and P15 of 2.1 cm. The growth of the weight and length of the seed resulting from gynogenesis is due to the treatment of feed that has been fermented probiotic and laserpuncture-induced before the mother catfish is colonized there is an increase in the percentage value of female homozygotes in the treatment of UV P20 irradiation distance. This suggests that probiotic feed can help increase the growth of catfish fry. Hariani et al (2018b) stated that fish growth is closely related to the availability of protein in feed, because protein is a source of energy for fish and protein is a much-needed nutrient for growth. The long growth of fish bodies in addition to being influenced by the genetics of each individual is also the intake of proteins to support the growth obtained from feed. To help the utilization of proteins contained in feed needed the help of proteolytic microorganisms that can break down proteins into polypeptides, oligopeptides and amino acids that can be directly utilized can be directly utilized by the body of the fish to help its growth.

These Figures 5 showed that after six weeks of acclimatization, the treatment of P20 UV radiation distance on semen, the growth was 83% greater compared to treatment P10 of 45% and P15 of 76%. Meanwhile, the percentage of daily specific growth rate length (SGRL) of catfish seeds treated with P20 UV radiation distance on semen showed a significant difference (P <0.05) where the eggs fertilized with semen became seeds after 6 weeks at 83%. The SGRL in P20 proved to be optimal. The

Commented [u30]: you say next that water quality parameters were in optimal condition; so water quality was not the cause of low SR; please rephrase and correct

Commented [u31]: please delete; this is not your species

Commented [u32]: this information was already presented; please delete

Commented [u33]: too long and not understandable; please rephrase and make shorter sentences

Commented [u34]: fat and saccharides are the main 2 sources of energy for fish; protein is mainly used in the structure of tissues (muscles, organs); if the fish uses protein as an energy source, it is a starving fish, without any fat or sugar reserves; please correct here

Commented [u35]: not understandable; please rephrase

Commented [u36]: not understandable; please rephrase with shorter sentences

Commented [u37]: please remove; these results were already presented

successful growth of fry weight and length is strongly influenced by the quality of feed administered to the reproductive catfish males and females. Improving the quality of feed by adding probiotics during acclimation before spawning can increase the vitellin content of eggs and also the quality of the spermatozoa.

Vitellin plays an important role in embryogenesis and food reserves before the larvae can find their own food. The feed quality also plays a role in gametogenesis to produce fertile spermatozoa in male catfish. Therefore, when the parent is spawned this will increase the number of fertilized eggs, which will then hatch. The larvae will survive until the fifth day and then develop into seeds, which are then maintained until the sixth week. This research showed that the best average growth value of catfish fry after six weeks of acclimatization was in the P20 UV radiation distance treatment on parent semen. P20 produced greater weight and length of catfish seeds compared to P10 and P15 treatments as well as the specific daily growth rate (SGRL). Furthermore, the provision of probiotics in feed and laserpuncture induction before the parent is spawned proved optimal for the growth, development, oogenesis, and spermatogenesis stages of female and male catfish. The addition of probiotics enhanced the feed, and could have improved the digestibility and balance of the microorganism community in the digestive tract (Wang 2007). Furthermore, the addition of probiotics to feed in this study also had a significant effect ($p<0.05$) on HR, SR, weight and length increase of catfish fry. It was also able to provide a higher SGRL. These results are believed to be due to the activity of bacteria like the *Bacillus* group and/or *Lactobacillus* sp. from the administered probiotics. Wang (2007) stated that *Lactobacillus* sp. plays a role in balancing the microbe composition of the digestive tract. This increases digestibility by converting carbohydrates into lactic acid, which in turn, lowers pH. Furthermore, this decrease in pH stimulates the production of endogenous enzymes in the digestive tract, increasing nutrient absorption, feed consumption, growth, and the inhibition of pathogenic organisms in the fish body. The *Lactobacillus* sp. bacteria is known to be one of the fermentation microbes present in probiotics. Therefore, when these probiotics are added to the feed, they improve its quality. The digestibility of the feed is then enhanced, which in turn increases the weight and length of the catfish fry. According to Irianto & Austin (2002), the work of the first probiotic bacteria involves the suppression of the bacterial population through competition, either by producing antimicrobial compounds, nutritional competition, or the strategic place of attachment on the intestinal wall. The second bacteria play a role in changing the bacterial metabolism by increasing or decreasing enzyme activity and increasing antibody levels in the fish's body.

This research shows that to produce pure female catfish, it is necessary to prepare the male broodstock before obtaining semen. This involves the improvement of feed and the induction of laserpuncture to enable the quick maturation of gonads. The superiority of the gynogenesis results signifies that when a pure female catfish is mated with an immature male, a third offspring of the same type as the female catfish is produced.

Commented [u38]: please delete; already said

Commented [u39]: already said; please delete

Commented [u40]: this is missing from the References; please add it there too

Commented [u41]: please move this to the start of the conclusions

Commented [u42]: you did not prove this; you did not use immature males; please delete

Conclusions. The improvements in feed and laserpuncture induction in catfish broodstock increased the HR ($p<0.05$) (63%). In addition, the SR value (54%) and the SGRL (83%) of catfish fry were also significantly increased in the first 6 weeks of life.

Acknowledgements. The authors express their gratitude to Mrs. Dra. Sulistyowati, M.Si, at the Faculty of Mathematics and Natural Sciences laboratory, Universitas PGRI Adi Buana, Surabaya, for granting permission to use the physiology laboratory facilities for this research.

References

Arai K., 2001 Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. Aquaculture 197(1-4):205-228.

Baroiller J. F., D'Cotta H., Saillant E., 2009 Environmental effects on fish sex determination and differentiation. Sexual Development 3(2-3):118-135.

Bobe J., 2015 Egg quality in fish: Present and future challenges. Animal Frontiers 5(1):66-72.
Brauhn J. L., 1971 Fall spawning of Channel catfish. The Progressive Fish-Culturist 33(3):150-152.
Çek Ş., Yilmaz E., 2009 The effect of varying dietary energy on gonad development at first sexual maturity of the Sharptooth catfish (*Clarias gariepinus* Burchell, 1822). Aquaculture International 17:553-563.
Chatakondi N. G., 2020 Effects of incubation temperature and parental male species on hatching success and progeny performance of channel catfish and hybrid catfish. Journal of the Southeastern Association of Fish and Wildlife Agencies 7:78-83.
Chowdhury M. A., Roy N. C., 2020 Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. Aquaculture Reports 18:100504.
Coldebella I. J., Neto J. R., Mallmann C. A., Veiverberg C. A., Bergamin G. T., Pedron F. A., Ferreira D., Barcellos L. J. G., 2011 The effects of different protein levels in the diet on reproductive indexes of Rhamdia quelen females. Aquaculture 312(1-4):137-144.
Crab R., Defoirdt T., Bossier P., Verstraete W., 2012 Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture 356-357:351-356.
Dunham R. A., 2004 Aquaculture and fisheries biotechnology. Genetic approaches. Cabi Plubishing, USA.
Galbusera P., Volckaert F. A. M., Ollevier F., 2000 Gynogenesis in the African catfish *Clarias gariepinus* (Burchell, 1822) III. Induction of endomitosis and the presence of residual genetic variation. Aquaculture 185(1-2):25-42.
Ghaedi A., Hosseinzadeh H., Hashim R., 2019 Effect of different protein levels on reproductive performance of snakehead murrel *Channa striatus* (Bloch 1793). Iranian Journal of Fisheries Sciences 18(4):812-829.
Gheyas A. A., Mollah M. F. A., Islam M. S., Hussain M. G., 2001 Cold-shock induction of diploid gynogenesis in stinging catfish, *Heteropneustes fossilis*. Journal of Applied Aquaculture 11(4):27-40.
Godwin C. J., 2001 Genetic manipulation studies in the Indian catfish *Heteropneustes fossilis*. Doctoral Thesis, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India, 140 p.
Hariani D., Purnama E. R., Purnomo T., 2018a Molecular dynamics of androgen-binding protein (ABP) due to laser-structure induction effect on increasing testosterone levels and the Gonado Somatic Index (GSI) value of male catfish (*Clarias* sp). PDUPT Final Research Report, 1st year, Institute for Research and Community Service, State University of Surabaya, Surabaya, Indonesia.
Hariani D., Rahayu D. A., Kusuma P. S. W., 2018b Production performance with probiotic use in feed with addition of spices as an environmentally friendly catfish cultivation engineering. International Conference on Science and Technology, pp. 645-651.
Hariani D., Kusuma P. S. W., 2019 Combination of feed protein level and laserpuncture induction of broodstock catfish (*Clarias* sp.) to increase estrogen, vitellogenin, and egg quality. EurAsian Journal of BioSciences 13(2):769-779.
Hariani D., Purnama E. R., Purnama T., Fadjar M., 2020 Effect of laserpuncture induction to increase GSI and HSI male catfish broodstocks. Journal of Fisheries and Marine Research 4(1):71-77.
Irianto A., Austin B., 2002 Probiotics in aquaculture. Journal of Fish Diseases 25:333-342.
Iribarren D., Dagá P., Moreira M. T., Feijoo G., 2012 Potential environmental effects of probiotics used in aquaculture. Aquaculture International 20(4):779-789.
Izquierdo M. S., Fernández-Palacios H., Tacon A. G. J., 2001 Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1-4):25-42.
Krishna P. V., Gopi G., Panchakshari V., Prabhavathi K., 2015 Effects of probiotics on the survival and growth of *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and P*angasius hypophthalmus* under polyculture system. International Journal of Advanced Research 3(10):625-632.

Commented [u43]: please add the total number of pages of the book

Commented [u44]: please mention the number of pages of the document; if it is in Indonesian, please mention at the end of the reference

Commented [u45]: this is not cited in the text; either cite it somewhere, or delete the reference

Kusuma P. S. W., Hariani D., 2019 Biological study of increasing vitellogenin level and gonado somatic index by laserpuncture exposure at any protein level of dietary on catfish broodstock (*Clarias* sp.). EurAsian Journal of BioSciences 13(1):177-183.
Kusuma P. S. W., Ngadiani N., Hariani D., 2015 Utilization of laserpuncture induction as spawning stimulation in catfish (*Clarias* spp.) crossbreeding toward egg quality. The Egyptian Journal of Aquatic Research 41(4):353-358.
Laczynska B., Demska-Zakes K., Siddique M. A. M., Fopp-Bayat D., 2020 Meiotic gynogenesis affects the individual and gonadal development in sterlet *Acipenser ruthenus*. Aquaculture Research 51(9):3623-3630.
LeBlanc J. G., Laiño J. E., Juarez del Valle M., Vannini V., van Sinderen D., Taranto M. P., Font de Valdez G., Savoy de Giori G., Sesma F., 2011 B-group vitamin production by lactic acid bacteria - current knowledge and potential applications. Journal of Applied Microbiology 111(6):1297-1309.
Marimuthu K., Palaniandya H., Muchlisin Z. A., 2019 Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae). Aceh Journal of Animal Science 4(2):80-88.
Mekkawy I. A. A., Mahmoud U. M., Osman A. G., Sayed A. E. D. H., 2010 Effects of ultraviolet A on the activity of two metabolic enzymes, DNA damage and lipid peroxidation during early developmental stages of the African catfish, *Clarias gariepinus* (Burchell, 1822). Fish Physiology and Biochemistry 36:605-626.
Nguenga, D., Teugels, G.G., Legendre, M., Ollevier, F., 2004. Effects of storage and incubation temperature on the viability of eggs, embryos and larvae in two strains of an African catfish, Heterobranchus longifilis (Siluriformes, Clariidae). Aquac. Res. 35, 1358–1369. https://doi.org/10.1111/j.1365-2109.2004.01161.x
Oktavianawati I., Andinata D., Isnaeni A. N., Hermiastuti M., Rahmawati N., Handayani W., Winata I. N. A., 2016 Effects of feeding diets containing *Azolla pinnata* and probiotic on the growth and nutritional content of patin fish (*Pangasius djambal*). Agriculture and Agricultural Science Procedia 9:403-410.
Ray A. K., Ghosh K., Ringø E., 2012 Enzyme-producing bacteria isolated from fish gut: A review. Aquaculture Nutrition 18(5):465-492.
Lins Rodigues M., Damasceno D. Z., Gomes R. L. M., dos Santos Sosa B., Moro E. B., Boscolo W. R., Bittencourt F., Signor A., 2020 Probiotic effects (*Bacillus cereus* and *Bacillus subtilis*) on growth and physiological parameters of silver catfish (*Rhamdia quelen*). Aquaculture Nutrition 27(2):454-467.
Rustidja, 2004 [Spawning artificial tropical fishes] Bahtera Press, Malang, Indonesia. [In Indonesian].
Sink T. D., Lochmann R. T., Camilo Pohlenz C., Buentello A., Gatlin D., 2010 Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. Aquaculture 298(3-4):251-259.
Tiwary B. K., Kirubagaran R., Ray A. K., 2004 The biology of triploid fish. Reviews in Fish Biololgy and Fisheries 14:391-402.
Vesal S. E., Mooraki N., Vosooghi A. R., 2016 Effect of dietary fish oil on growth responses of severum (*Heros severus*). AACL Bioflux 9(1):81-90.
Volckaert F. A. M., Galbusera P. H. A., Hellemans B. A. S., Van den Haute C., Vanstaen D., Ollevier F., 1994 Gynogenesis in the African catfish (*Clarias gariepinus*). I. Induction of meiogynogenesis with thermal and pressure shocks. Aquaculture 128(3-4):221-233.
Zan-Bar T., Bartoov B., Segal R., Yehuda R., Lavi R., Lubart R., Avtalion R. R., 2005 Influence of visible light and ultraviolet irradiation on motility and fertility of mammalian and fish sperm. Photomedicine and Laser Surgery 23(6):549-555.

Received:. Accepted:. Published online:.

Authors:

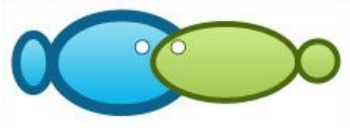
Commented [u46]: this corresponds to a text section that should be deleted, so please delete this too

Commented [u47]: please add the total number of pages of the book

Dyah Hariani, Biology Study Program, F Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Wiyata No. 48, Gayungan, Surabaya 60231, Jawa Timur, Indonesia.
Kusuma PSW, Biology Study Program, Fac. of Mathematics and Natural Sciences, Universitas PGRI Adi Buana, Jalan Ngagel Dadi IIIB/37 Wonokromo, Surabaya 60245, Jawa Timur, Indonesia
Rahayu, D.A, Biology Study Program, F Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Wiyata No. 48, Gayungan, Surabaya 60231, Jawa Timur, Indonesia.
Agustin, F, Biology Study Program, Fac. of Mathematics and Natural Sciences, Universitas PGRI Adi Buana, Jalan Ngagel Dadi IIIB/37 Wonokromo, Surabaya 60245, Jawa Timur, Indonesia
M.Fajar, Aquaculture Dept., Fac. Of Fisheries and Marine Science, University of Brawijaya, Jl. Veteran, Malang 65145, Indonesia

Commented [u48]: please add the email address for all authors

How to cite this article:



Feed improvement and laserpuncture induction to improve the gynogenetic quality of catfish (*Clarias gariepinus*) fry

[1]Dyah Hariani, [2] Pungky S W Kusuma, [1] Dwi A Rahayu, [2] Fauziah Agustin, [3] Mohamad Fadjar

[1]Biology Study Program, Mathematics and Natural Sciences, Negeri Surabaya Univerisity, Gayungan, Surabaya, Jawa Timur, Indonesia; [2] Biology Study Program, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana Univresity, Wonokromo, Surabaya, Jawa Timur, Indonesia; [3] Aquaculture Department, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia. Corresponding author: D. Hariani, dyahhariani@unesa.ac.id

Abstract. An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturation of reproductive catfish (*Clarias gariepinus*) However, it is relatively unclear whether broodstock semen, when irradiated with ultraviolet (UV), will produce the same results in egg fertilization by gynogenesis techniques. Gynogenesis is a parthenogenesis reproduction technique where embryonic development occurs by obtaining genetic material only from females, without male contribution. This study aimed to determine whether the distance of UV radiation on catfish (*Clarias gariepinus*) broodstock semen enhanced by feed improvement and laserpuncture induction will successfully fertilize eggs. The distance between the UV source and semen was divided into 4 treatments namely: control, distance of 10 cm, 15 cm, and 20 cm, with 6 replications. Furthermore, the data collection technique involved a completely randomized design (CRD). The parameters observed included the Hatching Rate (HR), Survival Rate (SR), and Specific Growth Rate Length (SGRL) of the larvae, until the fry were 6 weeks old. The results showed that 20 cm UV radiation distance on semen had a significant effect ($p<0.05$) on the increasing percentage of HR values (63%), SR values (54%), and SGRL (83%) compared to other treatments.

Key Words: *UV radiation distance on semen, Hatching Rate, Survival Rate, Specific Growth Rate Length*

Commented [u1]: please write the full first name, then the initials of the middle names, then the full family name

Commented [u2]: add the scientific name in brackets

Commented [u3]: add scientific name in brackets

Introduction. Catfish (*Clarias* sp.) is one of the cultivated commodities with high economic value. The market share for this commodity tends to have an increasing trend. Therefore, the demand for catfish is also increasing. One of the efforts to obtain quality fry involves reproducing only high quality catfish. This requires support by improving the male and female broodstock feeds, thereby improving the quality of eggs, sperm and, subsequently, fry. Quality feeds are necessary to support the survival of embryos and larvae (Izquierdo et al 2001; Çek & Yilmaz 2009)

Commented [u4]: please delete; all feed has protein and lipids, not only the quality one

The presence of probiotics in feed improves the quality, as it increases the digestibility value, growth and survival rate of fish (Crab et al 2012; Iribarren et al 2012; Krishna et al 2015; Chowdhury & Roy 2020; Rodigues et al 2020). In addition, the bacteria present in probiotics also increase the nutritional value of feed by synthesizing vitamins, proteins and essential fatty acids, like amylase, lipase, and proteases (Irianto & Austin 2002; LeBlanc et al 2011; Ray et al 2012; Oktavianawati et al 2016).

In general, genetic engineering can be carried out to improve the quality of fry. This involves the manipulation of chromosomes in the fertilization process, such as gynogenesis. According to Durhan (2004) and Arai (2001), gynogenesis is carried out in two important stages: deactivating the genetic material of male gametes through ultraviolet light (UV) radiation, and restraining the second polar body in meiosis II or restraining the first cell division at mitosis I. This is done by administering a hot shock of 38°C for 3 minutes after fertilization (Volckaert et al 1994; Laczynska et al 2020). When a fertilized egg develops, it produces a diploid female gynogenetic embryo.

UV radiation at wavelengths below 254 nm is proven to be strongly absorbed by certain biological substances, especially nucleic acids, proteins and coenzymes. Furthermore, UV light at a wavelength of 254 nm has been demonstrated to sufficiently damage the function of pyrimidine DNA, which represents the genetic material of male fish sperm (Ijiri & Egami, 1980; Valcarcel et al.,1994; Saber et al., 2017). Although the pyrimidine function of sperm DNA is inactivated by UV radiation, it does not inactivate the ability of sperm to move and fertilize eggs (Zan-Bar et al 2005; Mekkawy et al 2010).

An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturatio of broodstock catfish (*Clarias gariepinus*)(Kusuma et al 2015; Hariani & Kusuma, 2019). Kusuma et al (2015) confirm that the use of helium-neon laserpuncture technology on the reproductive acupoint precisely at 2/3 ventral parts of the body through induction for 15 seconds is optimal for the maturation of catfish gonads. Hariani et al (2020) stated that helium-neon low-power laserpuncture technique at the reproductive acupoint of 15 seconds every two weeks is optimal for the maturation of catfish (*Clarias gariepinus*) gonads.

This research focused on improving feed and laserpuncture induction as a reproductive biostimulator to accelerate the maturation male and female catfish gonads. In addition, the semen was irradiated with ultraviolet light (UV) at a certain distance to determine whether it still possesses the ability to fertilize eggs until they hatch.

Material and Method. This research was conducted at the Physiology Laboratory, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana Surabaya University. The data collection method was carried out using a completely randomized design (CRD) with 4 treatments: control, UV radiation distance of 10, 15 and 20 cm, with six replications. Five female catfish (*Clarias gariepinus*)(900-1500 g and 5 males of 1140-1750 g were used. The fish were obtained from catfish farmers in Pare, Kediri, East Java.

The acclimation of catfish broodstock was carried out separately for 2 weeks in concrete ponds (2x2x0.9 m). Broodstocks were fed with commercial floating pellets (PF-128), with a crude protein content of 38% and probiotics (Probio-7, Tamasindo Veterinary product) with the following composition: *Saccharomyces cerevisiae, Aspergillus oryzae, Lactobacillus acidophilus, Bacillus subtilis, Rhodopseudomonas, Actinomycetes* and *Nitrobacter*, with a density for each bacteria of >1x1011 CFU L^{-1}. Feed was administered twice daily, in the morning and evening, 5% of the body weight, until gonadal maturation.

Eggs and semen collection from the broodstock. Before collecting eggs and semen, the broodstock was treated with laserpuncture at the reproductive acupoint of precisely 2/3 of the ventral part of their body for 15 seconds, with an induction frequency of once every 2 weeks (Hariani et al 2018a). After laserpuncture induction, the catfish were returned to the spawning pond until spawning symptoms were observed. The females showing spawning symptoms were stripped to obtain eggs. The eggs were collected in a dry plastic basin and placed in an icebox at 4°C. Meanwhile, males were dissected to remove the gonads, which were pressed to obtain semen. 1 mL of semen was measured with a pipette and diluted with 9 mL of NaCl physiological/ringer. The semen was then poured into a 1 mm thick petri dish, irradiated with a 15-watt germ lamp (Philips™, Holland) with a wavelength of 260 nm for 2 min, from a distance of UV radiation: 0 cm 10 cm,15 cm, and 20 cm.

Both semen that was irradiated with UV light and not irradiated (control) was mixed with the eggs and stirred evenly using chicken feathers. The fertilized eggs were spread on a tea filter, placed in an incubation container at 28°C for 3 min, then shocked at 38°C for 2 min. This was done to prevent the second polar body's extruded and ensure that the number of chromosomes remained 2N (diploid). After the temperature shock, the fertilized eggs were incubated in a pond at 28°C until they hatching.

The counting of fertilized and unfertilized eggs was carried out approximately 8 h after the fertilization process in the incubation pond. The fertilized eggs were cloudy white, while unfertilized eggs were clear.

The next step involved calculating the percentage of hatched eggs, or hatching rate (HR), and the survival rate (SR) of catfish larvae until the 5th day of life. Furthermore, the

Commented [u5]: please add a reference for this statement

Commented [u6]: please add a reference for this statement

Commented [DAR7]: Already added

Commented [u8]: please add the scientific name in brackets

Commented [u9]: this is missing from References; please add it there too

Commented [u10]: please add the species scientific name

Commented [u11]: from what species?

specific growth rate length (SGRL) of gynogenesis results was measured after the 5th day every 2 weeks for 6 weeks using the following equation (Vesal et al 2016):

Hatching Rate (%): HR = a/(a+b) x 100

Survival Rate (%): SR = (Final fish number/Initial fish number) x 100

Specific Growth Rate Length (%/day) = SGRL

SGRL = [(final body length-initial body length)/days of experiment] x100

Where: a - number of eggs hatched; b - number of unhatched eggs.

Parameters of water medium measured: incubation water temperature, pH and dissolved oxygen.

Commented [DAR12]: Already added

Data analysis. The data was calculated using SPSS and analyzed using one-way Analysis of Variance (ANOVA) at a significance level of $p<0.05$. The analysis was used to determine the effect of UV radiation distance on the percentage of HR, SR and SGRL of catfish. If a difference between treatments was observed, it was followed by the LSD test. The percentages of HR, SR, and SGRL of larvae before the variance analysis were transformed to arcsin.

Results and Discussion

Hatching rate of catfish eggs. The mean HR values of catfish eggs with and without UV irradiation are presented in Figure 1.

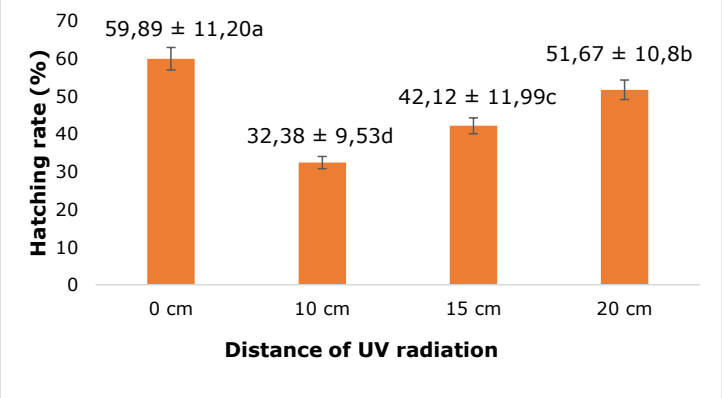


Figure 1. The effect of distance UV irradiation to hatching rate of catfish (*Clarias gariepinus*) egg. Number followed by different letter/s (a,b, etc) indicated significantly different

The HR of fertilized catfish eggs without UV radiation (0 cm) was 59,89 ± 11,20 %. This was significantly higher ($p<0.05$) than distance of UV radiation 10 cm at 32,38 ± 9,53%. Furthermore, the HR of distance of UV radiation 10 was non significantly lower ($p>0.05$) than that of 15 cm distance of UV radiation at 42,12 ± 11,99%. Distance of UV radiation 10 cm was also non significantly lower ($p>0.05$) than the HR of 20 cm distance of UV radiation 20 cm, at 51,67 ± 10,8 %.

Commented [u13]: - you need to check again your numbers, because they are different from numbers used in the figure; please correct where necessary

Commented [DAR14R13]: Already revise

Survival rate of catfish larvae. The average SR of catfish larvae with and without UV irradiation of male catfish semen is presented in Figure 2.

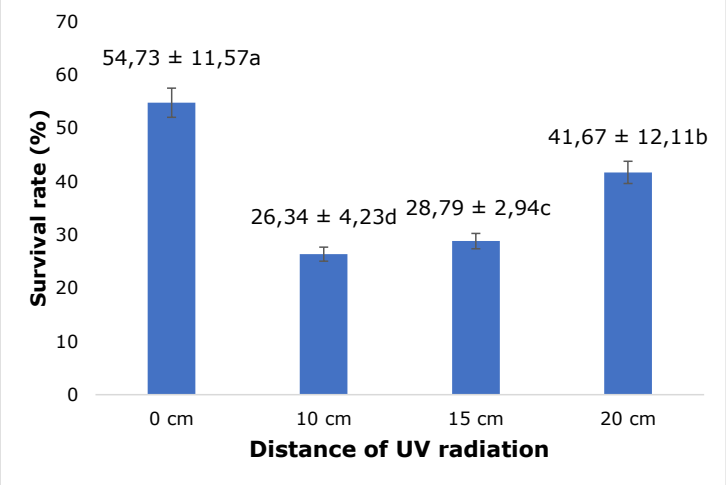


Figure 2 The effect of distance UV irradiation to survival rate (*Clarias gariepinus*) larvae. Different letters above columns show. Number followed by different letter/s (a,b, etc) indicated significantly different

The SR of catfish larvae in distance of UV radiation 0 cm was 54,73 ± 11,57%. This was significantly higher ($p<0.05$) compared to the SR of P distance of UV radiation 10 cm, at 26,34 ± 4,23%. Furthermore, the SR of distance of UV radiation 10 cm was 28,79 ± 2,94 % non-significantly lower ($p>0.05$) than the SR of 15 cm distance of UV radiation, at. The SR of 15 cm distance of UV radiation was also non-significantly lower ($p>0.05$) compared to that of 20 cm distance of UV radiation, at 41,67 ± 12,11.

Average growth of catfish body weight. T
The average growth values of catfish fry up to the age of 6 weeks is presented in Figure 3.

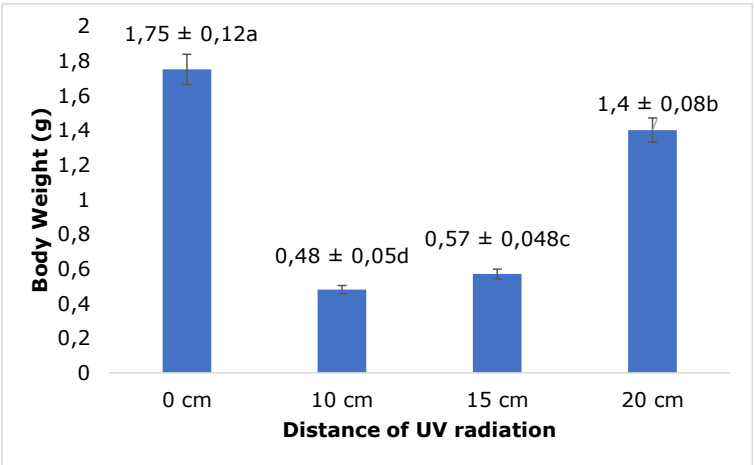


Figure 3. The effect of distance UV irradiation to growth of average body weight of catfish (*Clarias gariepinus*) fry. Number followed by different letter/s (a,b, etc) indicated significantly different

The average growth of body weight up to the age of 6 weeks in distance UV irradiation 0 cm was 1,75 ± 0,12 g. This was significantly higher ($p<0.05$) than the average growth value in distance UV irradiation 10 cm, at 0,48 ± 0,05 g. Furthermore, distance UV irradiation 10 cm was non-significantly lower ($p>0.05$) than the average growth value in distance UV irradiation 15 cm, at 0,57 ± 0,048 g. The weight growth in distance UV irradiation 15 cm was non-significantly lower ($p>0.05$) than the average growth value of catfish fry in distance UV irradiation 20 cm, at 1,4 ± 0,08 g.

Average growth of catfish fry body length. The average growth of catfish body length within 6 weeks of fertilization is presented in Figure 4.

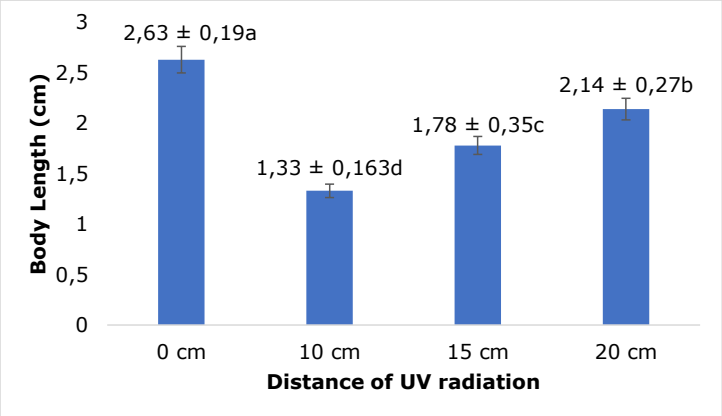


Figure 4. The effect of distance UV irradiation to the average growth of catfish (*Clarias gariepinus*) body length fry. Number followed by different letter/s (a,b, etc) indicated significantly different

The average growth of catfish fry length until the 6th week after treatment using distance UV irradiation was 2,63 ± 0,19 cm in distance UV irradiation 0 cm. This was significantly higher ($p<0.05$) than the average value of catfish fry growth length. In distance UV irradiation 10 cm, the length was 1,33 ± 0,163 cm, non-significantly lower ($p>0.05$) than in distance UV irradiation 15 cm of 1,78 ± 0,35 cm. The average growth length of distance UV irradiation 15 cm was non-significantly lower ($p>0.05$) than in distance UV irradiation 20 cm, which was 2,14 ± 0,27 cm.

Daily specific growth rate length of catfish fry. The daily specific growth rate length (SGRL) of catfish fry of 6 weeks of age is presented in Figure 5.

Commented [u15]: from what treatment?

Commented [DAR16R15]: distance UV irradiation

Commented [DAR17]: Already revise

Commented [u18]: please check again the numbers; they do not match the figure

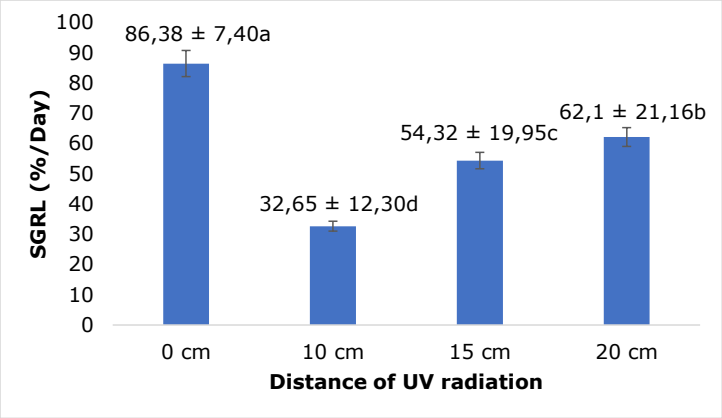


Figure 5. The effect of distance UV irradiation to of growth of average body weight of catfish (*Clarias gariepinus*) fry. Number followed by different letter/s (a,b, etc) indicated significantly different

The percentage daily specific growth rate length of catfish seeds up to 6 weeks of age indicated that the eggs fertilized with semen without UV radiation (distance UV irradiation 0 cm were 86,38 ± 7,40% significantly greater (P<0.05) than the other treatments. Semen-fertilized catfish seeds of UV radiation distance 10 cm was 32,65 ± 12,30 non-significantly lower (P>0.05) than the average percentage value of distance UV irradiation 15 cm at 54,32 ± 19,95%. Furthermore, 15 cm was non-significantly (P>0.05) lower than the average value of UV radiation distance 20 cm at 62,1 ± 21,16 %.

The effect of catfish semen UV radiation distance on hatching rate. The results showed that the effect of UV radiation distance on catfish semen on the HR of eggs in 20 cm was significant (p<0.05), compared to distance UV irradiation 10 cm and and 15 cm. Therefore, HR was influenced by the distance of UV radiation, while the success of egg fertilization was significantly influenced by the quality of feed administered to female and male parent catfish before the eggs and semen were obtained. In addition to the quality of the feed given, the water quality handling factor during the study was well controlled in order to support HR. This factor enhanced the successful development of fry that resistant to environmental changes. Bobe (2015) and Baroiller et al (2009) stated that the success of HR is determined by external factors (temperature, dissolved oxygen, pH, sanitation and light intensity), as well as internal factors, including the quality of the eggs and spermatozoa.

Distance of UV irradiation 20 cm showed a high percentage of HR compared to 10 cm and 15 cm, because a closer UV radiation to semen had a negative effect by inhibiting the rate of sperm movement. Therefore, distance of UV irradiation 20 cm was optimal for destroying the genetic material of spermatozoa without affecting motility. This was evidenced by an increasing enzymatic activity that enabled the movement of spermatozoa to fertilize more eggs. The results of this study were supported by Godwin (2001), who discovered that UV radiation at a wavelength of 254-260 nm to determines whether or without UV rays will penetrate biological materials, especially nucleic acids, proteins and coenzymes. UV radiation at a wavelength of 254-260 nm causes damage to DNA, somatic and genetic cells. In addition, at a wavelength of 254-260 nm, UV light radiation causes chromosomal changes to occur in the cell cycle, especially in the metaphase of meiosis. Cells that divide relatively often have a greater chance of being damaged by UV radiation at a wavelength of 254-260 nm. Furthermore, the success of gynogenesis was also determined by the temperature shock sometime after the egg was fertilized. This

Commented [u19]: the values are different in the figure; please correct where necessary

already revise

Commented [u20]: you did not study or prove this; please delete

Commented [DAR21R20]: we are treatment this fertilization rate, hatching rate

Commented [u22]: you did not present anywhere water parameters (not in Material and Method, and not in Results); please delete

Commented [DAR23R22]: already added in method

Commented [u24]: this is missing from the References; please add it there too

Commented [DAR25]: already added in references

Commented [u26]: not understandable; please rephrase

temperature shock enables the stimulation of the diploid zygote. Therefore, it should be carried out at the right time to increase the diploidization of gynogenetic seeds, namely during meiosis II and mitosis I (Galbusera et al 2000, Gheyas et al 2001; Tiwary et al 2004).

In addition to the inactivation of semen DNA due to UV radiation distance, its properties to fertilize eggs were no affected, because, in the acrosome part of the spermatozoa, there are hydrolytic enzymes (acrosin and hyaluronidase) that function in egg fertilization. Therefore, there were fewer homozygous larvae in 10 cm, compared to 15 cm and 20 cm. Due to the proximity of UV radiation to the semen, the temperature shock produced a decrease in enzyme activity, because an increase in temperature will either denature the enzyme or damage the egg cytoplasmic proteins. This enzyme is called egg membrane, and it consists of pseudoceratine, which works to reduce chorion hardness (Rustidja, 2004). Meanwhile, in the control group, the HR was significantly different ($p<0.05$) compared to treatments 0 cm, 15 cm and 20 cm, but the control produced heterozygotic male and female larvae. In 0 cm, 15 cm, and 20 cm, only homozygous female larvae were observed. Another factor that caused a decrease in the HR was the frequency of spawning, which was too high, and affected the quality of spermatozoa and eggs produced (Bobe, 2015)

Effect of UV light exposure distance on the survival rate of catfish larvae. The effect of UV light exposure distance on SR showed a significant value ($p<0.05$) between treatments. The percentage of larvae SR in cm was 54,73 ± 11,57%, which decreased in 10 cm by 26,34 ± 4,23 %, and experienced an increase in 15 cm and 20 cm by 32% and 54%, respectively. The control group 0 cm obtained medium to high heterozygous catfish larvae in the treatment groups 10 cm, while in 15 cm and 20 cm, a high number of female homozygous catfish larvae were obtained.

The high and low SR values could have occurred due to the quality of the broodstock. The increase in SR percentage is also closely related to the quality of feed administered before the reproduction. The results of Kusuma & Hariani (2019) showed that providing quality feed to catfish before spawning helps accelerate the gonadal maturity. This is because quality feed supports the production of egg yolk and fertile spermatozoa. Egg yolk is highly useful during embryogenesis, and function as an initial nutrient reserve for larvae before exogenous feeding.

Eggs in 10 cm had a lower percentage of hatching compared to 15 cm and 20 cm. Therefore, this significantly affected the SR of larvae. Temperature shock also played an important role in gynogenesis because it held the second polar body in meiosis II or withstood the division of the first cell at the time of mitosis I. This produces homozygous female individuals (Dunhan 2004).

The results of gynogenesis showed that, as a whole, homozygous females were obtained, whereas the control group produced heterozygous individuals. However, the high level of homozygotism of gynogenesis reduced the ability of larvae to survive. To support their ability to survive, the quality of water needs to be improved.

The water quality parameters, temperature, pH, and dissolved oxygen were in optimal conditions. Furthermore, the temperature of water incubation during the study ranged between 26-28ºC, while pH was 7 and dissolved oxygen 8 ppm. Dissolved oxygen is required in the process of embryo metabolism within the egg. This is in line with he observations of Brauhn (1971), who states that the optimal temperature for catfish spawning ranges from 24-30°C. Chatakondi (2020) mentions that the optimal temperature for broodfish is 26.6°C. Temperatures optimum for fertilization and hatching African catfish *Heterobranchus longifilis* was reported to be 25–29°C (Nguenga *et al*.,2004). Other factors that influenced the egg hatching process include pH and dissolved oxygen. The optimal pH range for hatching is 6.7-7.5 (Marimuthu et al 2019).

The effect of UV exposure distance of semen on weight and length growth of catfish fry. The growth of the weight and length of the seed resulting from gynogenesis is due to the treatment of feed that has been fermented probiotic and laserpuncture-induced before the mother catfish is colonized there is an increase in the percentage value

Commented [DAR27]: already added in references

Commented [DAR28]: already added in references

Commented [u29]: remember to check you numbers in the figures, because the numbers are different

Commented [u30]: you say next that water quality parameters were in optimal condition; so water quality was not the cause of low SR; please rephrase and correct

Commented [u31]: please delete; this is not your species

of female homozygotes in the treatment of UV 20 cm irradiation distance. This suggests that probiotic feed can help increase the growth of catfish fry. Hariani et al (2018b) stated that fish growth is closely related to the availability of protein in feed, because nutrient in feed is a source of energy for fish and protein is a much-needed nutrient for growth. The long growth of fish bodies in addition to influenced by the genetics of each individual is also the intake of proteins to support the growth obtained from feed.

The SGRL in 20 cm proved to be optimal. The successful growth of fry weight and length is strongly influenced by the quality of feed administered to the reproductive catfish males and females. Improving the quality of feed by adding probiotics during acclimation before spawning can increase the vitellin content of eggs and also the quality of the spermatozoa.

The addition of probiotics enhanced the feed, and could have improved the digestibility and balance of the microorganism community in the digestive tract (Wang 2007). Furthermore, the addition of probiotics to feed in this study also had a significant effect ($p<0.05$) on HR, SR, weight and length increase of catfish fry. It was also able to provide a higher SGRL. These results are believed to be due to the activity of bacteria like the *Bacillus* group and/or *Lactobacillus* sp. from the administered probiotics. Wang (2007) stated that *Lactobacillus* sp. plays a role in balancing the microbe composition of the digestive tract. This increases digestibility by converting carbohydrates into lactic acid, which in turn, lowers pH. Furthermore, this decrease in pH stimulates the production of endogenous enzymes in the digestive tract, increasing nutrient absorption, feed consumption, growth, and the inhibition of pathogenic organisms in the fish body. The *Lactobacillus* sp. bacteria is known to be one of the fermentation microbes present in probiotics. Therefore, when these probiotics are added to the feed, they improve its quality. The digestibility of the feed is then enhanced, which in turn increases the weight and length of the catfish fry. According to Irianto & Austin (2002), the work of the first probiotic bacteria involves the suppression of the bacterial population through competition, either by producing antimicrobial compounds, nutritional competition, or the strategic place of attachment on the intestinal wall. The second bacteria play a role in changing the bacterial metabolism by increasing or decreasing enzyme activity and increasing antibody levels in the fish's body.

The finding of this results indicates that to produce quality catfish (*Clarias gariepinus*) fry, it is necessary to prepare male and female brooders before the semen and eggs are taken through proper selection of broodstock, in addition to feed improvement and laserpunctur induction need to be carried out to accelerate the gonadal maturation process. The superiority of gynogenesis from the preparation of catfish broodstock before spawning is that many eggs are fertilized and hatch into larvae and larvae that develop into seeds resistant to controlled water media.

Conclusions. The improvements in feed and laserpuncture induction in catfish broodstock increased the HR ($p<0.05$) 59,89 ± 11,20 %. In addition, the SR value (54%) 41,67 ± 12,11 % and the SGRL 62,1 ± 21,16%. of catfish fry were also significantly increased in the first 6 weeks of life.

Acknowledgements. The authors express their gratitude to Mrs. Dra. Sulistyowati, M.Si, at the Faculty of Mathematics and Natural Sciences laboratory, Universitas PGRI Adi Buana, Surabaya, for granting permission to use the physiology laboratory facilities for this research.

References

Arai K., 2001 Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. Aquaculture 197(1-4):205-228.

Baroiller J. F., D'Cotta H., Saillant E., 2009 Environmental effects on fish sex determination and differentiation. Sexual Development 3(2-3):118-135.

Bobe J., 2015 Egg quality in fish: Present and future challenges. Animal Frontiers 5(1):66-72.

Commented [u32]: too long and not understandable; please rephrase and make shorter sentences

Commented [u33]: fat and saccharides are the main 2 sources of energy for fish; protein is mainly used in the structure of tissues (muscles, organs); if the fish uses protein as an energy source, it is a starving fish, without any fat or sugar reserves; please correct here

Commented [DAR34R33]: already revise

Commented [u35]: not understandable; please rephrase

Commented [DAR36R35]: already revise

Commented [u37]: this is missing from the References; please add it there too

Commented [DAR38]: already added

Commented [u39]: - you need to check again your numbers, because they are different from numbers used in the figure; please correct where necessary

Commented [u40]: the values are different in the figure; please correct where necessary

Brauhn J. L., 1971 Fall spawning of Channel catfish. The Progressive Fish-Culturist 33(3):150-152.
Çek Ş., Yilmaz E., 2009 The effect of varying dietary energy on gonad development at first sexual maturity of the Sharptooth catfish (*Clarias gariepinus* Burchell, 1822). Aquaculture International 17:553-563.
Chatakondi N. G., 2020 Effects of incubation temperature and parental male species on hatching success and progeny performance of channel catfish and hybrid catfish. Journal of the Southeastern Association of Fish and Wildlife Agencies 7:78-83.
Chowdhury M. A., Roy N. C., 2020 Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. Aquaculture Reports 18:100504.
Coldebella I. J., Neto J. R., Mallmann C. A., Veiverberg C. A., Bergamin G. T., Pedron F. A., Ferreira D., Barcellos L. J. G., 2011 The effects of different protein levels in the diet on reproductive indexes of Rhamdia quelen females. Aquaculture 312(1-4):137-144.
Crab R., Defoirdt T., Bossier P., Verstraete W., 2012 Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture 356-357:351-356.
Dunham R. A., 2004 Aquaculture and fisheries biotechnology. Genetic approaches. Cabi Plubishing, USA. 504 p.
Galbusera P., Volckaert F. A. M., Ollevier F., 2000 Gynogenesis in the African catfish *Clarias gariepinus* (Burchell, 1822) III. Induction of endomitosis and the presence of residual genetic variation. Aquaculture 185(1-2):25-42.
Ghaedi A., Hosseinzadeh H., Hashim R., 2019 Effect of different protein levels on reproductive performance of snakehead murrel *Channa striatus* (Bloch 1793). Iranian Journal of Fisheries Sciences 18(4):812-829.
Gheyas A. A., Mollah M. F. A., Islam M. S., Hussain M. G., 2001 Cold-shock induction of diploid gynogenesis in stinging catfish, *Heteropneustes fossilis*. Journal of Applied Aquaculture 11(4):27-40.
Godwin C. J., 2001 Genetic manipulation studies in the Indian catfish *Heteropneustes fossilis*. Doctoral Thesis, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India, 140 p.
Hariani D., Purnama E. R., Purnomo T., 2018a Molecular dynamics of androgen-binding protein (ABP) due to laser-structure induction effect on increasing testosterone levels and the Gonado Somatic Index (GSI) value of male catfish (*Clarias* sp). PDUPT Final Research Report, 1st year, Institute for Research and Community Service, State University of Surabaya, Surabaya, Indonesia. 120 p.
Hariani D., Rahayu D. A., Kusuma P. S. W., 2018b Production performance with probiotic use in feed with addition of spices as an environmentally friendly catfish cultivation engineering. International Conference on Science and Technology, pp. 645-651.
Hariani, D & Kusuma, P. S.W., 2019.Combination of feed protein level and laserpuncture induction of broodstock catfish (*Clarias* sp.) to increase estrogen, vitellogenin, and egg quality. Eurasia J Biosci 13, 769-779.
Hariani D., Purnama E. R., Purnama T., Fadjar M., 2020 Effect of laserpuncture induction to increase GSI and HSI male catfish broodstocks. Journal of Fisheries and Marine Research 4(1):71-77.
Hariani D., Purnama E. R., Purnama T., Fadjar M., 2020 Effect of laserpuncture induction to increase GSI and HSI male catfish broodstocks. Journal of Fisheries and Marine Research 4(1):71-77.
Ijiri, K. and Egami, N. 1980. Hertwig effect causes by UVirradiation of sperm of *Oryzias latipes* (Teleost) and its photoreactivation. Mutation Research 69: 241–248.
Irianto A., Austin B., 2002 Probiotics in aquaculture. Journal of Fish Diseases 25:333-342.
Iribarren D., Dagá P., Moreira M. T., Feijoo G., 2012 Potential environmental effects of probiotics used in aquaculture. Aquaculture International 20(4):779-789.
Izquierdo M. S., Fernández-Palacios H., Tacon A. G. J., 2001 Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1-4):25-42.
Krishna P. V., Gopi G., Panchakshari V., Prabhavathi K., 2015 Effects of probiotics on the survival and growth of *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and P*angasius*

Commented [u41]: please add the total number of pages of the book

Commented [u42]: please mention the number of pages of the document; if it is in Indonesian, please mention at the end of the reference

hypophthalmus under polyculture system. International Journal of Advanced Research 3(10):625-632.
Kusuma P. S. W., Hariani D., 2019 Biological study of increasing vitellogenin level and gonado somatic index by laserpuncture exposure at any protein level of dietary on catfish broodstock (*Clarias* sp.). EurAsian Journal of BioSciences 13(1):177-183.
Kusuma P. S. W., Ngadiani N., Hariani D., 2015 Utilization of laserpuncture induction as spawning stimulation in catfish (*Clarias* spp.) crossbreeding toward egg quality. The Egyptian Journal of Aquatic Research 41(4):353-358.
Laczynska B., Demska-Zakes K., Siddique M. A. M., Fopp-Bayat D., 2020 Meiotic gynogenesis affects the individual and gonadal development in sterlet *Acipenser ruthenus*. Aquaculture Research 51(9):3623-3630.
LeBlanc J. G., Laiño J. E., Juarez del Valle M., Vannini V., van Sinderen D., Taranto M. P., Font de Valdez G., Savoy de Giori G., Sesma F., 2011 B-group vitamin production by lactic acid bacteria - current knowledge and potential applications. Journal of Applied Microbiology 111(6):1297-1309.
Marimuthu K., Palaniandya H., Muchlisin Z. A., 2019 Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae). Aceh Journal of Animal Science 4(2):80-88.
Mekkawy I. A. A., Mahmoud U. M., Osman A. G., Sayed A. E. D. H., 2010 Effects of ultraviolet A on the activity of two metabolic enzymes, DNA damage and lipid peroxidation during early developmental stages of the African catfish, *Clarias gariepinus* (Burchell, 1822). Fish Physiology and Biochemistry 36:605-626.
Oktavianawati I., Andinata D., Isnaeni A. N., Hermiastuti M., Rahmawati N., Handayani W., Winata I. N. A., 2016 Effects of feeding diets containing *Azolla pinnata* and probiotic on the growth and nutritional content of patin fish (*Pangasius djambal*). Agriculture and Agricultural Science Procedia 9:403-410.
Ray A. K., Ghosh K., Ringø E., 2012 Enzyme-producing bacteria isolated from fish gut: A review. Aquaculture Nutrition 18(5):465-492.
Rodigues, L, M., Damasceno D. Z., Gomes R. L. M., dos Santos Sosa B., Moro E. B., Boscolo W. R., Bittencourt F., Signor A., 2020 Probiotic effects (*Bacillus cereus* and *Bacillus subtilis*) on growth and physiological parameters of silver catfish (*Rhamdia quelen*). Aquaculture Nutrition 27(2):454-467.
Rustidja, 2004. Spawning artificial tropical fishes] Bahtera Press, Malang, Indonesia. [In Indonesian. 115 p.
Saber,M, H., Pourkazemi,M., Noveiri, S.B & Shahhosseini, G.R., 2017. The influence of gamma and UV irradiation on the motility percent in heterologous sturgeon sperm, International Conference on the Future of Sturgeon Aquaculture, 170-178 p.
Sink T. D., Lochmann R. T., Camilo Pohlenz C., Buentello A., Gatlin D., 2010 Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. Aquaculture 298(3-4):251-259.
Tiwary B. K., Kirubagaran R., Ray A. K., 2004 The biology of triploid fish. Reviews in Fish Biololgy and Fisheries 14:391-402.
Valcarcel, A., Guerrero, G. and Maggese, C. 1994. Hertwig effect caused by UV irradiation of sperm of the catfish Rhamdia sapo (Pisces, Pimelodidae) and its photoreactivation. Aquaculture 128: 21–28.
Vesal S. E., Mooraki N., Vosooghi A. R., 2016 Effect of dietary fish oil on growth responses of severum (*Heros severus*). AACL Bioflux 9(1):81-90.
Volckaert F. A. M., Galbusera P. H. A., Hellemans B. A. S., Van den Haute C., Vanstaen D., Ollevier F., 1994 Gynogenesis in the African catfish (*Clarias gariepinus*). I. Induction of meiogynogenesis with thermal and pressure shocks. Aquaculture 128(3-4):221-233.
Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp Penaeus vannamei. Aquaculture 269, 259–264.
Zan-Bar T., Bartoov B., Segal R., Yehuda R., Lavi R., Lubart R., Avtalion R. R., 2005 Influence of visible light and ultraviolet irradiation on motility and fertility of mammalian and fish sperm. Photomedicine and Laser Surgery 23(6):549-555.

Commented [u43]: please add the total number of pages of the book

Received:. Accepted:. Published online:.

Authors:
Dyah Hariani, Biology Study Program, F Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Wiyata No. 48, Gayungan, Surabaya 60231, Jawa Timur, Indonesia. Email: dyahhariani@unesa.ac.id
Kusuma PSW, Biology Study Program, Fac. of Mathematics and Natural Sciences, Universitas PGRI Adi Buana, Jalan Ngagel Dadi IIIB/37 Wonokromo, Surabaya 60245, Jawa Timur, Indonesia. Email: pungky@unipasby.ac.id
Rahayu, D.A, Biology Study Program, F Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Wiyata No. 48, Gayungan, Surabaya 60231, Jawa Timur, Indonesia. Email: dwirahayu@unesa.ac.id
Agustin, F, Biology Study Program, Fac. of Mathematics and Natural Sciences, Universitas PGRI Adi Buana, Jalan Ngagel Dadi IIIB/37 Wonokromo, Surabaya 60245, Jawa Timur, Indonesia. Email: fladysta@gmail.com
M.Fajar, Aquaculture Dept., Fac. Of Fisheries and Marine Science, University of Brawijaya, Jl. Veteran, Malang 65145, Indonesia. Email: f4dj4r_02@yahoo.com

Commented [u44]: please add the email address for all authors

How to cite this article: