JURNAL PERIKANAN DAN KELAUTAN
Volume 29 No. 3, October 2024: 375-380

e-issn : 2721-8902
p-issn : 0853-7607

Histopathology of Striped Catfish (*Pangasianodon hypophthalmus*) Fed with Moringa Leaf Addition and Maintained in Photoperiod Aquaponics

Histopatologi Ikan Jambal Siam (Pangasianodon hypophthalmus) yang Diberi Pakan dengan Penambahan Daun Kelor dan Dipelihara pada Fotoperiod Akuaponik

Zulmi Arnoli[1*], Windarti[1], Morina Riauwaty[1]
[1]Department of Marine Science, Postgraduate, Universitas Riau, Pekanbaru 28293 Indonesia
**email: zulmiarnoli04@gmail.com*

Received
30 August 2024

Accepted
05 October 2024

Abstract

Moringa is one of the plants with high nutrients and antioxidants and can improve fish's immune system. This study aimed to look at the structure of the skin and kidney tissue of striped catfish fed with feed containing Moringa leaf flour after being tested for *Aeromonas hydrophila* bacteria. This research was conducted from January to May 2024 at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau, and fish histology observations were made at the Aquatic Biology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. The method used in this study is an experimental method by applying a one-factor Completely Randomized Design (CRD) with five levels of treatment and three replicates, namely KN (without moringa leaves and *A. hydrophila* challenge test), KP (without moringa leaves and challenged with *A. hydrophila*), P1 (dose of 10 g/kg feed), P2 (dose of 15 g/kg feed), P3 (dose of 20 g/kg feed). The results showed that most organ tissues were still in a normal state. The best dose of moringa flour addition to feed is 10 g/kg feed (P1). Fish tissue structure in P1 has damage such as hypertrophy, hemorrhage, and lipofuscin pigment on fish skin. Fish survival during the study was 97.67%.

Keywords: Striped Catfish, Moringa Leaf, Histology

Abstrak

Kelor merupakan salah satu tanaman memiliki nutrisi dan antioksidan yang tinggi, serta mampu meningkatkan sistem imun ikan. Tujuan dari penelitian ini adalah untuk melihat struktur jaringan kulit dan ginjal striped catfish yang diberi pakan mengandung tepung daun kelor setelah diuji tantang bakteri *Aeromonas hydrophila*. Penelitian ini dilaksanakan pada bulan Januari - Mei 2024 di Laboratorium Bioteknologi Fakultas Perikanan dan Kelautan Universitas Riau, pengamatan histologi ikan dilakukan di Laboratorium Biologi Perairan Fakultas Perikanan dan Kelautan Universitas Riau. Metode yang digunakan dalam penelitian ini adalah metode eksperimen dengan menerapkan Rancangan Acak Lengkap (RAL) satu faktor dengan lima taraf perlauan dan 3 kali ulangan, yaitu KN (tanpa daun kelor dan tanpa uji tantang *A. hydrophila*), KP (tanpa daun kelor dan diuji tantang dengan *A. hydrophila*), P1 (dosis 10 g/kg pakan), P2 (dosis 15 g/kg pakan), P3 (dosis 20 g/kg pakan). Hasil penelitian menunjukkan sebagian jaringan organ masih dalam keadaan normal. Dosis terbaik penambahan tepung daun kelor pada pakan adalah 10 g/kg pakan (P1). Struktur jaringan ikan pada P1 memiliki kerusakan seperti

hypertropi, hemoragi serta terdapat pigmen lipofuscin pada kulit ikan. Kelulushidupan ikan selama penelitian adalah 97.67%.

Kata kunci: Ikan Jambal Siam, Daun Kelor, Histologi

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is a prevalent fish in Riau because it is the main ingredient for traditional dishes such as spicy sour and curry. Striped catfish is widely traded in fresh and smoked form. The high market demand has caused many people to cultivate striped catfish.

The striped catfish is a nocturnal fish that actively moves and searches for food at night (Windarti et al., 2021). The effectiveness of striped catfish cultivation can be increased by extending the active time to eat by keeping fish in the dark or manipulating photoperiods (Magwa et al., 2020). Behind the advantages, photoperiod manipulation has disadvantages, including a decrease in water quality. This is caused by the high amount of organic matter resulting from fish's remaining feed and metabolic waste. One of the technological innovations applied is aquaponic technology. Aquaponic technology is proven to successfully maintain water quality by utilizing organic matter absorbed by plant roots where the organic matter is a nutrient for plants.

With a good immune system, fish can survive the attack of pathogens. Increasing the immune system of fish can be done by using natural plants, such as Moringa leaves (*Moringa oleifera*). These leaves are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins (Leone et al., 2015). By giving moringa leaves, the immunity of cultured fish is expected to increase to avoid disease attacks. This study looks at the gills, kidneys, liver, and skin tissue structure and the survival of striped catfish fed with moringa leaves.

2. Material and Method

2.1. Time and Place

This research was conducted from January to May 2024. Fish rearing was conducted at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, and fish hematology observations were made at the Aquatic Biology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

2.2. Methods

The The research method used is an experimental method using a one-factor Completely Randomized Design (CRD) with 5 levels of treatment to reduce the error level, so that replication is carried out 3 times so that 15 experimental units are needed. The treatment in the study refers to Bbole et al. (2016), namely:

KN: Negative Control (without moringa leaves and *A. hydrophila* challenge test)
KP: Positive control (without moringa leaves and challenged with *A. hydrophila*)
P1: Dose of 10 g/kg feed and challenged with *A. hydrophila*.
P2: Dose of 15 g/kg feed and challenged with *A. hydrophila*.
P3: Dose of 20 g/kg feed and challenged with *A. hydrophila*.

2.3. Procedures

The feed used in this study was commercial pellets enriched with moringa flour. The procedure for making feed refers to (Windarti et al., 2023): Tapioca flour, as much as 20 g, is mixed with 100 mL of boiling water and stirred until thickened. Then, the mixture is mixed evenly on 1 kg of commercial pellets. Furthermore, the pellets are dried in the sun. After drying, the pellets are stored in a container and ready to be given to the fish. Fish were kept for 60 days and tested for 15 days. Feed was given three times a day to satiate them.

After being maintained for 60 days, a challenge test was carried out on day 62 by infecting the test fish with *A. hydrophila* bacteria at a bacterial density of 10^8 CFU/mL, which had increased resistance (virulence) to the test fish. The challenge test was conducted by injecting 0.1 mL/ fish intramuscularly into the caudal vein. The test fish were still fed during the challenge test period, and post-challenge maintenance was carried out for 14 days.

Renewable renal tissue structures were prepared according to Windarti & Simarmata (2015), and samples were fixed with 10% formalin for 24-48 hours and transferred to 4% formalin. After that, the dehydration process is carried out, and the samples that have been fixed are moved into a graded alcohol series starting from 70%, 80%, 90%, 96%, and absolute alcohol for 1 hour each. The immersion in absolute alcohol was done two times, 1 hour each. Next, the samples were put back into pure xylol solution twice for 1 hour. After that, the samples were placed into xylol: paraffin (1:1) solution for 1 hour (the process was done in a 60°C oven). Then, the samples were immersed in pure paraffin twice for 1 hour. The following process was embedded in paraffin using a mold (thick paper), which was allowed to harden at room temperature.

Then, the sample was cut with a microtome with a thickness of 5 µm. The paraffin ribbon was then placed in a water bath. Next, the paraffin tape was taken using a glass object and left to dry. An adhesive made of glycerin and albumin (1:1) was given to firmly attach the sample to the glass object. After that, the sample was dried in an oven dryer set at 45°C for at least 24 hours. Then, the samples were stained using Haematoxylin and Eosin (HE).

The staining procedure is as follows: first, the paraffin on the preparation must be removed by soaking the sample with xylol for 2 minutes. Then, rehydration is done by dipping the preparation into alcohol series down, from absolute to 35% each, for 2 minutes. After that, the sample was immersed in a hematoxylin solution for 4 minutes and washed with running water. Next, the sample was immersed in an eosin solution for 1.5 minutes and washed using running water.

The last process is the closing process. This process begins by dipping in an alcohol series up from 70%, 80%, 90%, 96%, and absolute for 20 seconds. Next, the preparation was put into a pure xylol solution for 2 minutes. After that, the sample was dripped with Stellan new and covered with cover glass. The closed preparations were then stored in an oven dryer for 2-3 days. Preparations are ready to be observed using an Olympus microscope.

2.4. Data Analysis

Data obtained from fish survival were analyzed using analysis of variance (ANOVA). If the treatment showed a significant difference where $p<0.05$, then Student Newman-Keuls further test was conducted to determine the difference between each treatment. Skin and kidney abnormality data obtained will be discussed descriptively.

3. Result and Discussion

3.1. Tissue Structure of Striped Catfish Skin

The skin organ is a vital organ for the fish body. Fish skin functions as a body protector from disease and infection, regulates fish body temperature, and helps fish move smoothly. In addition, the skin also produces mucus, which also serves to protect the fish body from pathogen attacks. The structure of the skin tissue can be seen in Figure 1.

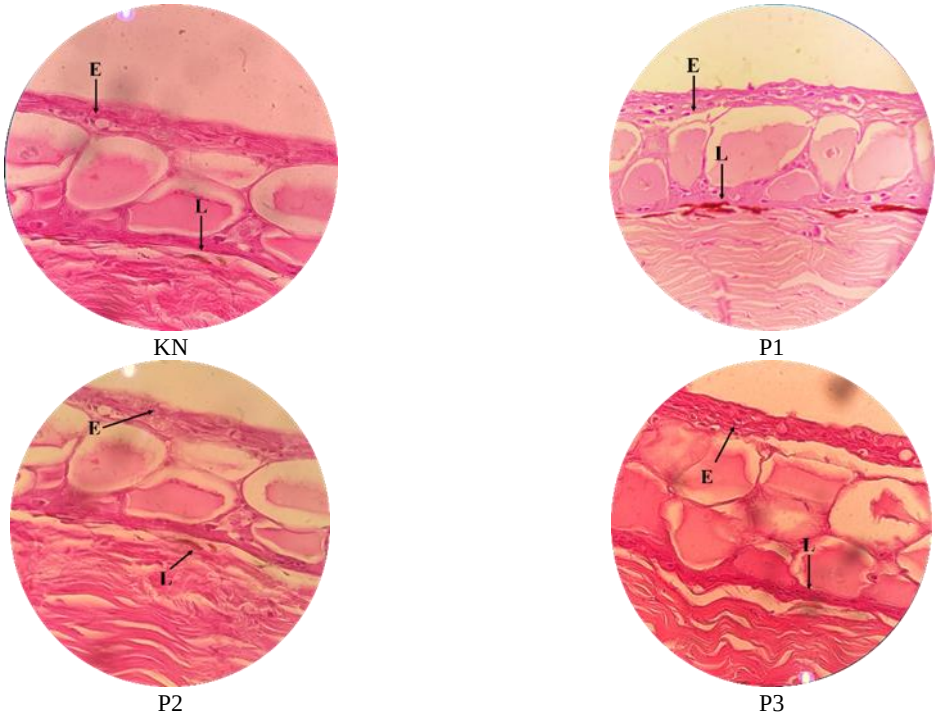

Figure 1. Striped Catfish Skin Tissue Structure *1000x
Description : E: Epidermis, L: Lipofuscin

In this study, the skin of fish infected with *A. hydrophila* bacteria was damaged on day two after infection and got worse on day 5. After day 8, the fish began to heal, and the wounds on the fish's skin gradually healed. In the P1 treatment, the fish skin experienced the fastest healing phase. This is because feeding moringa leaf flour affects the healing of fish skin tissue. Sjamsuhidajat (2010) states that the wound-healing process consists of three phases: inflammation, proliferation, and remodelling. Collagen fibers are formed in the proliferation phase, during which fibroblasia occurs. The inflammatory phase starts from the first day of the wound until day 5, while the proliferation phase starts at the end of the inflammatory phase and ends between the second and third weeks (Li et al., 2007).

Moringa leaves have ingredients that are instrumental in the inflammatory and proliferation phases. These ingredients are vitamin A, arginine, and saponins. Vitamin A plays a vital role in the inflammatory phase of the wound-healing process by increasing the number and activation of macrophages and monocytes (Stechmiller, 2010). Arginine is an amino acid that plays a vital role in wound healing. According to Campos et al. (2008), one of the roles of arginine in wound healing is to stimulate the immune system during the inflammatory phase.

On day 14, the skin condition of the striped catfish had recovered, but there were still traces of damage after being infected with *A. hydrophila* bacteria. This is due to the arginine content in moringa leaves, which has a role in the proliferation phase. According to Arnold & Barbul (2006), arginine is a precursor to proline in collagen synthesis. Methionine and cysteine are two amino acids that also play a role in wound healing by stimulating fibroblast proliferation and collagen synthesis. Stechmiller (2010) states that vitamin A also plays a role in stimulating epithelialization and increasing collagen deposits in the proliferation phase.

There is also a brownish lipofuscin pigment in the structure of fish skin tissue. Lipofuscin is an intralysosomal substance consisting mainly of protein residues and is formed due to oxidative processes catalyzed by iron (Terman & Brunk, 2004). This lipofuscin pigment occurs due to damage to the fish skin after infection with *A. hydrophila*. After the skin condition improves, this pigment will remain in the skin tissue structure because this pigment is one of the signs of damage to the skin. Jung et al. (2007) mentioned that lipofuscin accumulation can occur in damaged skin cells and exposure to sunlight or other environmental factors.

3.2. Kidney Tissue Structure of Striped Catfish

The kidney is a vital organ for fish. It regulates water levels in the fish body (osmoregulation). The kidney generally comprises a bowman capsule containing glomerular cells that filter water and body fluids. If a toxicant enters the body, the fish will filter it, but the result is often damaged or abnormal kidney tissue (Wahyuni et al., 2017). This study showed that the structure of the kidney tissue of striped catfish showed several disorders, such as bleeding and necrosis. This occurs allegedly due to infection from *A. hydrophila* bacteria, which causes bleeding in kidney cells. The kidney tissue of jambul fish can be seen in Figure 2.

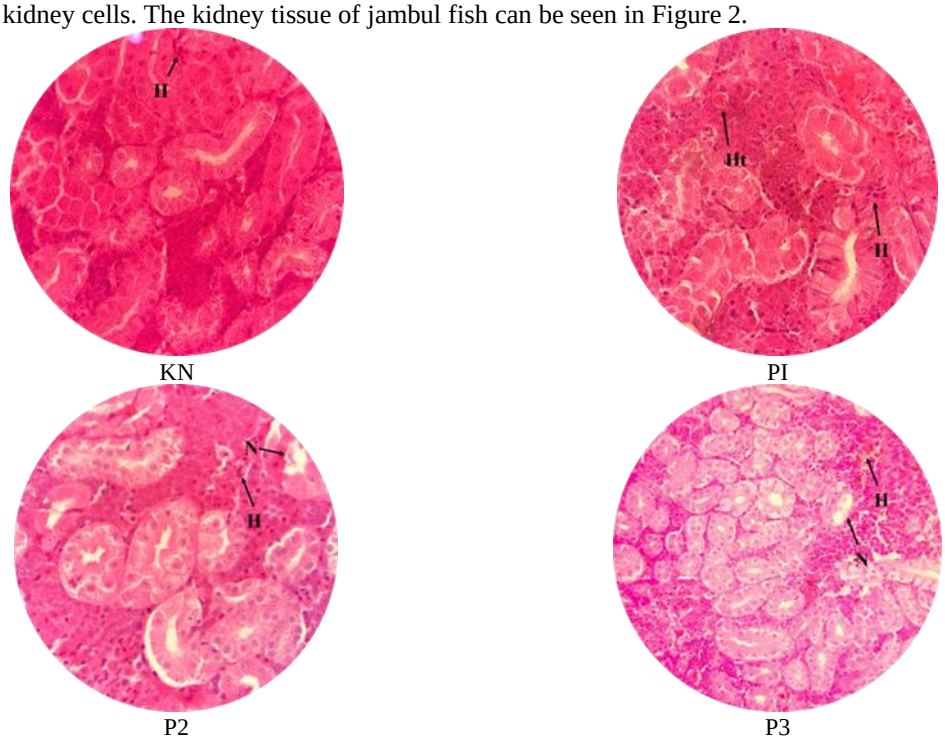

Figure 2. Kidney tissue structure of striped catfish *1000x
Description: H: Hemorrhage, Ht: Hypertrophy, N: Necrosis

The structure of the kidney tissue of striped catfish after being challenged with *A. hydrophila* bacteria in the KN treatment shows that the fish kidney structure has hemorrhage. The P1 treatment showed bleeding (hemorrhage) and cell swelling (hypertrophy). This indicates that in P1, the fish kidney is still in average condition. While in the P2 and P3 treatments, there is hemorrhage and cell death (necrosis). Wahyuni et al. (2017) stated that the attack of *A. hydrophila* bacteria begins with the attachment of bacteria to the skin surface by utilizing pili, flagellum, and hooks to move and attach to the body's outer layer. Then, *A. hydrophila* produces the enzyme chitinase to degrade the chitin layer so bacteria can enter the fish's body. *A. hydrophila* bacterial infection enters the blood, quickly reaching essential organs such as the kidneys, and causes cell death.

In KP, all fish died on day five, so no observations were made. Feeding enriched with moringa flour increases the immune system of striped catfish to minimize damage to the kidneys of fish. According to Ridwan et al.

(2020), vitamin C can strengthen the fish's immune system, accelerate the maturation of erythrocytes, play an essential role in maintaining the structure and formation of collagen, and trigger repairing fish body tissues.

3.3. Survival Rate

The survival rate of striped catfish reared for 60 days with the addition of moringa leaves to the feed ranged from 94.45 to 98.89%. After the challenge test, the fish survival rate ranged from 93.33 to 97.67%, and the positive control (Kp) mortality reached 100%. For more details, see Figure 3.

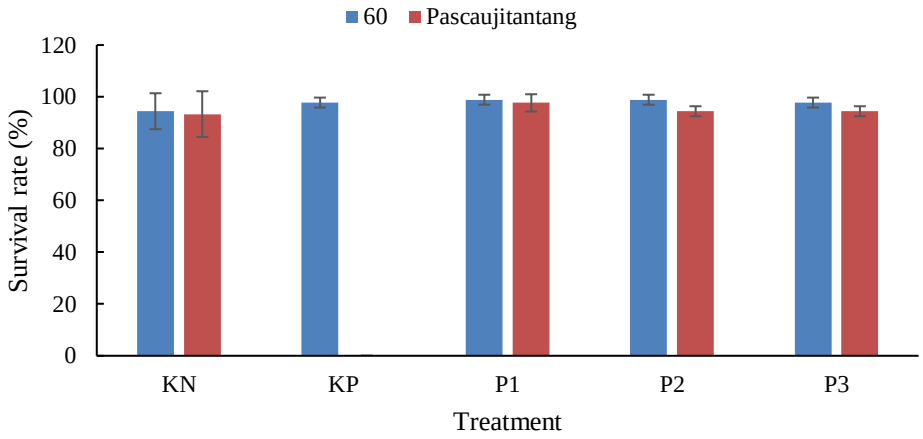

Figure 3. Survival Rate of striped catfish

Feeding with the addition of moringa leaves can increase fish immunity. This is because the addition of moringa leaves can increase the immunity of the body of striped catfish, making it healthier. This can be seen from the high value of feed efficiency every day. The high survival value is likely because the fish adapt well to the environment. In addition, good water quality, by the adaptability of striped catfish, also greatly supports their survival. Water as a living medium for fish must have properties suitable for fish life because water quality can influence the growth of living things in water (Djatmika in Shafitri, 2019).

The survival rate of each treatment is not significantly different. It is suspected that the test feed given contains fermented Moringa leaf flour or does not contain fermented moringa leaf flour, which affects the growth of striped catfish but does not significantly affect the survival rate. Based on the results of the analysis of variance (ANOVA) test, the addition of moringa leaves in the feed does not significantly affect the survival of striped catfish seeds with a probability value (P>0.05).

4. Conclusions

Based on the study's results, it can be concluded that adding moringa flour to feed and in the challenge test with A. hydrophila bacteria damages kidney and skin tissue. The best dose of moringa flour addition is 10 g/kg feed (P1), as seen from the structure of the kidneys and skin. The skin structure in the P2 treatment has lipofuscin pigment in the skin layer, and the kidney tissue has damage such as hypertrophy and hemorrhage and 97.67% survival.

5. References

Arnold, M., & Barbul, A. (2006). Nutrition and Wound Healing. *Plas Recon Surg*: 117: 42-58

Bbole, I., Mumba, C., Mupenda, N., & Kefi, A.S. (2016). Analysis of Growth Performance and Haematological Parameters of *Oreochromis niloticus* Fed on a Varying Diet of *Moringa oleifera* Lam. Leaf Meal as an Additive Protein Source. *International Journal of Fisheries and Aquaculture, 8*(11): 105-111.

Campos, A.C.L., Groth, A.K., Branco, A.B. (2008) Assessment and Nutritional Aspects of Wound healing, *CurrOpinClinNutrMetab Care*,11:281-8.

Jung, T., Bader, N., & Grune, T. (2007). Lipofuscin: Formation, Distribution, and Metabolic Consequences. *Annals of the New York Academy of Sciences*, 1119(1): 97-111.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015) Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of *Moringa oleifera* Leaves: An Overview. *International Journal of Molecular Sciences*, 16(6):12791–12835.

Li, P., Ray, B., Gatlinn III, D.M., Sink, T., Chen, R., & Lochmann, R. (2007). Effect of Handling and Transport on Cortisol Response and Nutrient Mobilization of Golden Shiner, *Notemigonus crysoleucas. Journal of the World Aquaculture Society*, 40(6): 803-809.

Magwa, R.J., Windarti, W., & Siregar, M.R. (2020). Pengaruh Manipulasi Fotoperiod dan Pakan yang diperkaya Kunyit terhadap Pertumbuhan Ikan Jambal Siam (*Pangasius hypophthalmus*). *Jurnal Ruaya*, 8(2).

Ridwan, M., Lukistyowati, I., & Syawal, H. (2020). Hematologi Eritrosit Ikan Patin Siam (*Pangasius hypophthalmus*) yang Diberi Pakan dengan Penambahan Larutan Biji Mangga Harumanis (*Mangifera indica* L.). *Jurnal Ruaya*, 8(2): 114 –121.

Shafitri, D.K. (2019). *Pengaruh Pemanfaatan Tepung Daun Sente (Alocasia macrrhiza) yang Difermentasi Menggunakan Aspergilus niger dalam Pakan terhadap Pertumbuhan Benih Ikan Patin Siam (Pangasius hypopthalmus).* Fakultas Perikanan dan Kelautan. Universitas Riau. Pekanbaru. p70.

Sjamsuhidajat, R. (2010). *Buku Ajar Ilmu Bedah Sjamsudihajat-De Jong (3rd)*. EGC, Jakarta

Stechmiller, J. K. (2010) Understanding the Role of Nutrition and Wound Healing. *Nutr Clin Pract:* 25:61-8

Terman, A., & Brunk, U.T. (2004). Lipofuscin: Mechanisms of Age-Related Accumulation and Influence on Cell Function. *Free Radical Biology and Medicine*, 33(5): 611-619.

Wahyuni, S., Windarti, W., & Putra, R.M. (2017). Comparative Study on Histological Structure of Gill and Kidney of Snakehead Fish (*Channa striata*, BLOCH 1793) from The Kulim and Sibam Rivers, Riau Province. *Jurnal Online Mahasiswa*, 4(2): 1-14.

Windarti, W., & Simarmata, A.H. (2015). *Buku Ajar Struktur Jaringan*. Unri Press. Pekanbaru.

Windarti, W., Siregar, S.H., Simarmata, A.H., & Samiaji, J. (2023). Survival and Growth of *Pangasianodon hypophthalmus* Fed with *Moringa oleifera* Enriched Pellets and Reared in a Tank with an Aquaponic System and Dark Condition. *Jurnal Natur Indonesia*, 21(1), 1-6.

Windarti, W., Amin, B., & Simarmata, A.H. (2021). Growth and Health Status of *Pangasionodon hypophthlamus* Reared under manipulated Photoperiod Conditions. *F1000research*, 10:154p.