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ORIGINAL ARTICLE

Leukocyte Differential Variation Of *Anguilla bicolor* McClelland, Exposed To Varied Salinities

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ABSTRACT
Eel (Anguilla bicolor bicolor) is a carnivorous fish that have high economic value due to the high nutrient content, and the demand for fish availability in the global market was increasing drastically nowadays. The anguillida eel is a catadromous eel equipped for possessing freshwater development living space and seawater generating territory for the duration of their life cycle. At the adolescent to develop stage, they possess freshwater at that point move to marine water to generate. Changes in saltiness, which is one of the unpleasant natural components for the eel, influence their physiological condition by expanding the leukocytes number. This increment is a transformation technique to improve their insusceptible framework as a reaction to saltiness change. This examination expected to assess the leukocyte differential of anguillia eel (Anguilla bicolor McClelland) presented to different salinities. This exploration applied a Completely Randomized Design. The cycle of testicle development is described by the event of silvering, which is the adjustment in the shade of the ventral to silver and furthermore followed by the increment in eye size. The treatment was three degrees of saline media including 4 ppt, 15 ppt, and 30 ppt with five duplicates. The autonomous variable was the diverse saltiness, and the reliant variable was the leukocyte differential. The boundaries estimated comprised of the distinctive level of neutrophils, lymphocytes, monocytes, and eosinophils in which the estimations. The increment in saltiness diminished the neutrophils and monocytes, yet expanded the lymphocytes, and showed no impact on eosinophils..
Keywords: *Anguilla bicolour, salinity, lymphocytes, monocytes, eosinophils.*

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INTRODUCTION

The freshwater eel of the class Anguilla, being catadromous, move between fresh water development living spaces and seaward producing zones. The freshwater eels are broadly disseminated all through the world. Nineteen species/subspecies of Anguilla have been accounted for around the world, 13 of which happen in tropical districts Anguilla bicolor species is part into two subpopulations. Anguilla bicolor is far and wide in the Indian and western Pacific seas. Two subspecies are for the most part perceived, the Indonesian shortfin eel, A. bicolor McClelland, 1844, and the Indian shortfin eel, A. bicolor pacifica Schmidt, 1928, in spite of the fact that assessment is partitioned on the legitimacy of the last as an animal groups or subspecies [1]. The body of the Anguilla bicolor is round and lengthened, ussualy will look like Monopterus eels that are normally found in rice fields. One of the body part characters of the Anguilla bicolor which recognizes it from basic eels is the presence of a little pectoral blade that situated behind the head which resembles an ear cartilage. In view of that one of a kind character, Anguilla bicolor additionally called eared eel [2]. The Elongated body shape that resembles a snake makes it simple for Anguilla bicolor to swim between restricted holes and openings [3]. Anguilla bicolor's body length differs relying upon their stage, which is between 1-125 cm. The dorsal blade, butt-centric balance and caudal balance are melded to be one design. The contrasts between Anguilla bicolor species and the other Anguilla species can be seen from the correlation between preanal length (before the butt-centric blade) and predorsal (before the dorsal balance), tooth structure, head shape and the quantity of the spines [4].

In light of examination directed already, it was realized that during relocation to the bringing forth zone, develop, European eel avoid food and free significant measure of bone. Minerals in bones are reused as energy materials while relocating. The cell bone fills in as a wellspring of mineral delivery following osteoclastic resorption, while the mineralized notochord sheath, which is unavailable for resorption measures because of an unmineralized cover layer, guarantees adequate mechanical dependability as a piece of the notochord, sheath. Unmistakably, an eel's skeleton is basically upgraded to address the metabolic difficulty of fasting and synchronous sexual advancement during a debilitating excursion to producing zones, while the capacity of the vertebral segment is kept up to accomplish [5]. The interaction of relocation and outrageous swimming from anguilla will trigger the turn of events and development of the balls [6]. Histologically, the oocyte inside the balls is likewise developed and developed alongside movements that arrived at a distance of 2000 km. hormonally, testosterone and luteinizing chemical likewise increment as long as the fish moves. *Anguilla* bicolor, is a catadromous fish with an extremely convoluted life history and during their transient developments they need to make due in marine, estuarine and freshwater environments [7]. At this movement course they are presented to assaults of microbes, for example, microorganisms, infections and parasites and henceforth, they should have all around created safeguard mechanisms. The platelet assumes a significant part in giving resistance taking all things together vertebrates including fishes and consequently the interaction of haematopoiesis is critical for the ordinary improvement of all the platelet heredities [8]. In the current investigation an endeavour has been made in identifying the platelet genealogies in the head kidney of freshwater eel, A. bicolour bicolor as a piece of better comprehension of their insusceptible framework. The formed eating routine was defined from creature and plant based protein sources, for example, fish dinner, cornmeal, soybean supper, fine grain, ebi-shrimp feast, brilliant snail supper, blood meal, and custard flour. Nutrients and min-erals were added into the eating regimen. Furthermore, probiotics and dad torment compound and its mix were additionally included into the test diets to help the development execution, endurance and feed use of the fish. Like every living specie, fish also need nutritious food. There are various assortments of fish food accessible in the stores today. The taking care of fish and their nourishment is perhaps the main components in keeping them solid.

MATERIAL METHODS

Tools used in this research were tarpaulin cloth tanks, fiber tanks, aerator, light microscope, object glass, scissors, scale, and hand refractometer. Materials consisted of silver stage anguillid eel *Anguilla bicolor* McClelland, seawater, freshwater, labels, tissue, distilled water, earthworms, Giemsa 7% and methanol PA. Indian fresh water eel *Anguilla bicolour* were collected from January 2010 to December 2011 at Lower Anaicut, in the river Cauvery. The water samples were taken in plastic containers and brought to the laboratory. The river Cauvery is a major river in South India. The Coleroon River is the northern distributary of the Cauvery as it flows through the delta of Cuddalore, Thiruvarur and Nagapattinam. The fishery potential is more in Lower Anaicut. The fishing activities provide employment to the local fishermen and a permanent fish landing centre is in practice. This research applied a Complete Randomized Design with three salinity treatments and five replicates (15 units). Salinity levels of the growth media were based on [9]. as follows:

A = 4 ppt (control)
B = 15 ppt
C = 30 ppt

The perception of leukocyte profile was led following two months openness. The autonomous factors were the media of different salinities, though the reliant variable was the leukocyte differential. The boundaries estimated were the level of leukocyte differential of the anguillid eel. The fish were set in three units of covering material tanks with the size of 130 x 100 x 100 cm3 furnished with aerators. Each tank was cleaned before use to forestall the development of molds and growths. The readiness of media saltiness (4 ppt, 15 ppt, and 30 ppt) followed [10].

$$Sn = \frac{S1.\ V1 + S2.}{V2\ V1 + V2}$$

where,
Sn = Salinity level planned
S1 = Salinity of stock water
S2 = Salinity of blended freshwater
V1 = Volume of stock water
V2 = Volume of blended freshwater

The fish silver arena (15 people) with a normal body length of 60 cm and a normal load of 480 g were chosen. The fish were from The stream Cauvery is a significant waterway in South India. The Coleroon River is the northern distributary of the Cauvery as it courses through the delta of Cuddalore, Thiruvarur and Nagapattinam and were solid, had no handicaps, and dynamic. The fish were accustomed for at any rate multi week in a fiber tank estimating 70 cm in stature and 125 cm in distance across. The fish presented to high salinities needed to adapt steadily from 4 ppt, 10 ppt, 15 ppt, and 30 ppt for multi week in each degree of saltiness. As numerous as eight fish were set in one cylinder containing media. The fish were taken care of with worms once every day on not indispensable at 16.00 pm. The openness took into consideration two months. The air circulation and saltiness in every tub were routinely controlled to guarantee the proficient oxygen flow and keep up the correct degree of saltiness. The fish weight was estimated with a scale, though the length was estimated utilizing an estimating tape. The blood was taken by cutting the caudal blade and set in on an item glass to spread. The blood smear technique followed [11]. The article glass utilized was inundated in methanol to eliminate any overabundance fat. The blood was dropped on the article glass and spread utilizing another item glass with a point of 45° and dried. The dried smear was then fixed with methanol for 3–5 minutes at that point colored with Giemsa for 20–30 minutes, washed and dried. We notice the smear under a magnifying instrument at 400x amplification. Each kind of leukocyte was determined up to the 100th cells [12].

% neutrophil = number of neutrophil checked (100) x 100%
% lymphocyte = number of lymphocyte checked (100) x 100%
% monocyte = number of monocyte checked (100) x 100%
% eosinophil = number of eosinophil checked (100) x 100%

STATISTICAL ANALYSIS

The obtained data were analyzed using the SPSS program, version 24. Data were figured as mean ± SE (for data of the biological study n=10, where for data of the in vitro study 3 replicates were used). ANOVA test was used to compare results among groups and $P < 0.05$ was significant analysis was continued to the LSD [13].

RESULTS AND DISCUSSION

The cells saw in the head kidney, incorporates the haemocytoblast-the foundational microorganism and the formative phases of various platelet types: supportive of erythroblasts, favorable to erythrocyte and erythrocytes in the erythropoietic arrangement, lymphoblasts and the lymphocytes (both enormous and little) in the lymphopoietic arrangement, monoblasts, and monocyte in the monopoietic arrangement and granuloblast, favorable to neutrophils and neutrophils in the granulopoietic arrangement. Just thrombocytes are seen in the thrombopoietic arrangement. Macrophages and deteriorating erythrocytes were additionally noticed. The morphogenesis of creating platelets and their heredities are like different teleosts [14]. The glucose in cells will be catabolized soon to satisfy the requirements of fish body physiology and energy. The neutrophil diminished when the fish were in 30 ppt saltiness. Notwithstanding, this neutrophil sums fell between the typical scope of marine fish. At the point when the fish adjusted to the change, cortisol levels inside the blood diminished, brought about the neutrophil decline [15]. Expressed that saltiness was one of numerous abiotic factors influencing the leukocyte differential in fish. Saltiness stress may cause a high expansion in leukocyte and result in the increment of lymphocyte. The lymphocyte increment invigorated neutrophil decline. This neutrophil reduction may happen because of the adjusting component of different extents of leukocytes, for example, the lymphocyte [16]. The anguillid eel lymphocyte presented to salinities of 4, 15, and 30 ppt were 49.2 ± 5.97%, 58.8 ± 3.47% and 78.2 ± 2.77% separately. The lymphocyte expanded as the saltiness expanded. They estimated the lymphocyte level of the freshwater fish that ran somewhere in the range of 42 and 51%. Further, bitter fish showed the lymphocyte somewhere in the range of 60 and 70%, and marine fish somewhere in the range of 60 and 80%. Distinction in the lymphocyte rate between *anguillia eel* presented to various salinities. This saltiness probably impacted the lymphocyte sum [16]. The expansion in lymphocyte proposed transformation towards the saltiness change in the fish climate [17]. Lymphocyte of the *anguillia eel* looked like the lymphocyte measure of different fishes in the given waters detailed that the safe framework reacted to outer obstructions, for example, modification in saltiness, with the increment of lymphocyte sum [18]. The measure of lymphocyte could increment during stress. Stress meddled vague safe reactions, for example, lymphocyte multiplication (increment in cell sum and changes into T cells and B cells).

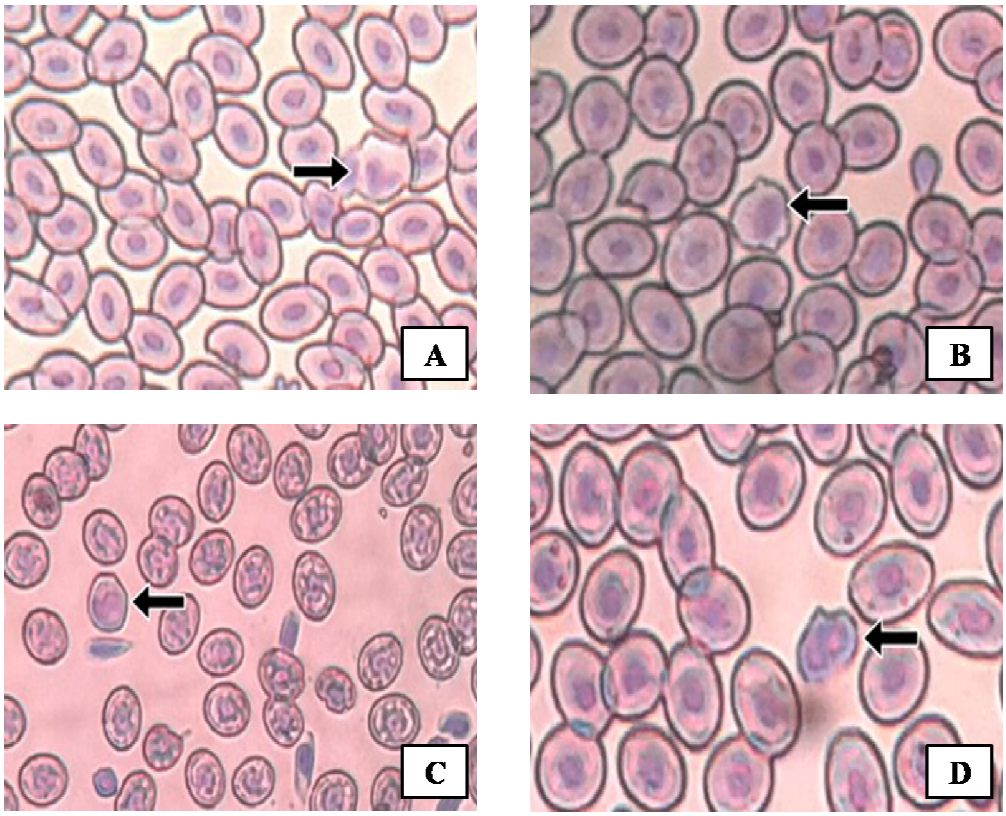

Fig: 1A. Neutrophil (N) (arrow) of anguillid eel *Anguilla bicolor* McClelland (400x)
Fig: 1B. Lymphocyte (arrow) of anguillid eel *Anguilla bicolor* McClelland (400x)
Fig: 1C. Monocyte (arrow) of anguillid eel *Anguilla bicolor* McClelland (400x)
Fig: 1D. Eosinophil (arrow) of anguillid eel *Anguilla bicolor* McClelland (400x)

This Each saltiness possibly affected lymphocyte sum. That to defeat pressure, fish needed to adjust. One kind of which was to build their lymphocyte level as a reaction to climate stressors, for example, changes in temperature, saltiness, and thickness. Leukocyte increment was identified with the lessening in cortisol levels in the body [19]. At the point when cortisol level diminished, DNA combination of the lymphocyte cells happened and prompted the high measure of lymphocyte. A few amphibian creatures, increment in the proportion of lymphocyte and neutrophil could be utilized as a pointer of long haul feelings of anxiety of the creature (persistently) [20]. Referenced that lymphocytes in the fish body were not phagocytic but rather hold a significant part in the arrangement of antibodies [21]. The lessening in lymphocyte sum could prompt a decrease in antibodies and inclined to sickness. The normal monocyte in 4, 15, and 30 ppt salinities were 23.8 ± 2.59%, 15 ± 4.37% and 10.2 ± 2.29% individually. Monocyte diminished with the increment of saltiness. guaranteed that monocyte level of Nile tilapia fish went somewhere in the range of 17 and 25% when introduced in freshwater. Monocyte of marine fish, for example, groupers was 9–15%. A lessening in monocyte could be brought about by the expansion in lymphocyte sum that created antibodies, thusly prompted an obstacle of the monocyte creation. The creation of antibodies was urgent for the insusceptible framework. Anguillid eel presented to various salinities [5,19]. The monocyte diminished when the saltiness expanded. A decrease in monocyte conceivably was on the grounds that monocytes were brief (14–36 hours in the blood framework) [18-21]. They moved to the tissues and separated into macrophages. expressed that phagocytic movement of macrophages was the underlying advance of the following stage in insusceptibility reaction, the immunizer creation. LSD showed no critical distinction ($P < 0.05$) in monocyte level of 15 and 30 ppt salinities. Notwithstanding, salinities 15 and 30 ppt from the 4 ppt saltiness. Monocyte sum in salinities 15 and 30 ppt was less than that of 4 ppt. A reduction in monocyte may happen on account of the blood harmony reaction to the increment of lymphocyte. The monocyte level showed comparable qualities for fish in the given climate. This outcome demonstrated that anguillid eel adjusted to a wide scope of saltiness. The eosinophil in salinities of 4, 15, and 30 ppt were 2 ± 0.75%, 4.4 ± 1.68% and 1.8 ± 1.10% individually. Eosinophil in the saltiness of 4 ppt was lower than that of 15 ppt, and the most minimal was in 30 ppt saltiness. All things considered, the eosinophil of 4 ppt, 15 ppt, and 30 ppt was inside the typical reach. An eosinophil of freshwater fish, for

example, goldfish and catfish were 2–8%. In euryhaline fishes, for example, Nile tilapia, the eosinophil was somewhere in the range of 1 and 3% when creatures experienced pressure, cortisol levels inside the body expanded. The expanded cortisol levels could cause eosinopenia, which prompted a diminishing in eosinophil rate.

SUMMARY AND CONCLUSION

This exploration showed that leukocyte differential of *anguillia eel* Anguilla bicolour McClelland) changed after presented to various salinities. The expansion in saltiness prompted the abatement of neutrophil and monocyte, however the increment of lymphocyte. Notwithstanding, eosinophil level was inside the typical reach. The level of leukocyte differential of anguillid eel in every saltiness was generally like that of other fish occupying waters with that given saltiness. It contains all the platelet genealogies aside from eosinophils, basophils and thrombocytes. The construction and the ancestries of creating platelets ref lect the physiological state of the fish. A blend of light and transmission electron minuscule portrayal makes it conceivable to perceive distinctive platelet heredities present in the head kidney with a serious level of assurance [17]. Modification in the level of leukocyte differential demonstrated of variation exertion because of the adjustment in saltiness of the fish climate. It was reasoned that saltiness affected the leukocyte differential of the anguillid eel Anguilla bicolor McClelland. Expanded saltiness prompted decline the level of neutrophil and monocyte, increment the level of lymphocyte, however no impact on eosinophil rate that fell inside the ordinary reach.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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