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A RESPONSE STUDY ON BRAIN, GILLS, AND (SUPRABRANCHIAL CAVITY) ACCESSORY RESPIRATORY ORGANS OF FRESHWATER AIRBREATHING SNAKEHEAD FISH CHANNA PUNCTATUS FOLLOWING EXPOSURE TO CLOVE OIL

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ABSTRACT

Channa argus, also known as the northern snakehead, is an air-breathing fish species with a large distribution in East Asia. The fish has a distinctive respiratory system known as the suprabranchial chamber, which enables it to respire air off the water's surface by a cough-like process. Main aim of the study is A Response Study on the Brain, Gills, and (Suprabranchial Cavity) Accessory Respiratory Organs Of Freshwater Airbreathing Snakehead Fish Channa Punctatus Following Exposure To Clove Oil. The fish underwent acclimation inside a controlled rearing system located at the Fisheries Science Institute of the University. The water temperature was maintained at a mean value of 25°C with a standard deviation of ±1°C.In conclusion, when northern snakeheads are exposed to air, their survival is positively associated with several factors.

1. INTRODUCTION

1.1 OVERVIEW

Channa argus, also known as the northern snakehead, is an air-breathing fish species with a large distribution in East Asia. The fish has a distinctive respiratory system known as the suprabranchial chamber, which enables it to respire air off the water's surface by a cough-like process. This unique adaptation allows the fish to temporarily survive outside of water. The Channa species has a suprabranchial chamber that is characterized by a highly vascularized wall, facilitating efficient gas exchange. The organism establishes a connection with the buccopharyngeal cavity by means of a ventral opening that is protected by a plate-like bone protrusion originating from the first branchial arch. This bone structure can seal up the inhalant aperture of the suprabranchial chamber when the organism exhales. While it is true that several air-breathing fish exhibit comparable rates of oxygen consumption in both air and water, some species of air-breathing fish actually decrease their oxygen consumption rate (VO2) while in an aerial environment. The findings of our current research indicate that the northern snakehead species can lower its metabolic rate when exposed to air. This suggests that the snakehead may use other methods, apart from its suprabranchial chamber, to fulfill its metabolic requirements and adapt to terrestrial environments.

In conjunction with the process of ventilation, fish may need additional physiological adaptations to facilitate aerial respiration. Fish can augment their blood oxygen-carrying capacity in order to compensate for reduced oxygen absorption and/or improve anaerobic metabolism, as seen by increased lactic acid concentration, when exposed to air. The extent to which the northern snakehead exhibits regulatory mechanisms during aerial exposure to facilitate air respiration of the suprabranchial organ is currently not well understood.

1.2 ACCESSORY RESPIRATORY ORGANS OF FRESHWATER AIRBREATHING SNAKEHEAD FISH CHANNA PUNCTATUS FOLLOWING EXPOSURE TO CLOVE OIL

In the context of freshwater air-breathing snakehead fish (Channa punctatus), the Suprabranchial Cavity is an unusual anatomical feature that plays a critical role in the adaptation of these fish to oxygen-depleted aquatic settings. We need to go into some background information about these exceptional fish and the usage of clove oil in scientific studies in order to appreciate the importance of this cavity and its link with exposure to clove oil. This is necessary so that we can comprehend the connection between the two.

Freshwater Air-breathing Snakehead Fish (Channa punctatus): The Channa punctatus is a species of freshwater fish that may be found in a number of different places in South Asia. It is also known as the spotted snakehead or the northern snakehead. These fish, which belong to the family Channidae, are distinguished by their capacity to maintain normal respiration in both water and air. This characteristic allows them to survive in a variety of habitats. One of the unique adaptations that helps make this dual respiratory capacity feasible is called the suprabranchial cavity.

Suprabranchial Cavity: The suprabranchial cavity is a distinguishing anatomical characteristic that is present in a wide variety of fish that breathe air, including the Channa punctatus. This chamber serves as an auxiliary respiratory organ, and it is located slightly above the gills in the body of the animal. In addition to the normal process of aquatic respiration that takes place via the use of gills, it enables these fish to directly absorb oxygen from the surrounding air. This adaptability is necessary for their survival in settings with varying oxygen levels, such as ponds that do not circulate water and shallow waters, both of which may have low oxygen concentrations.

Clove Oil Exposure: Clove oil is a naturally occurring chemical that is extracted from the clove plant (Syzygiumaromaticum) and has been used extensively in a variety of scientific studies involving fish. One of its most prominent uses is in the medical fields of anesthesia and sedation. This is because it has the ability to create a state of serenity and immobility in fish, which makes them easier to work with during a variety of treatments.

2. LITERATURE REVIEW

Deepak Varma, Hira Yadav, Sangam Kumar, Vijeta Chaturvedi, Virendra Kumar Tripathi, (2023) Copper sulphate is employed in farming as well as fisheries; but, when present in quantities that are too high, it is harmful to the health of fish and other aquatic life and constitutes a threat to such industries. Through the use of this experiment, we will investigate whether or not copper is detrimental to fish. While the first tank acted as a control for the experiment, the second tank was used for the experiment itself. The fish were thoroughly anaesthetized by anesthetic during the surgery. After surgery, patients got a reduced dosage of an aesthesia (60 mg L 1 MS-222, pH neutralized) to recover for two to three hours, which was retained throughout the studies to guarantee consistency. Each test utilized dechlorinated and oxygenated water at 6.5–9.0 degrees Celsius. In the first series, fish were anaesthetized and placed on an operating table before having a neutralized MS-222 solution irrigated over their gills through opercular pores. The results of the investigation provide credence to this interpretation of the data. This conclusion is supported by the fact that it is easy to arrive to the conclusion that copper plays a significant part in neurodegeneration. This conclusion is supported by the fact that this conclusion is easy to get to. The fact that it is not difficult to reach this conclusion lends credence to the assertion that it is correct.

Duan, Ting & Shi, Chenchen(2018) The primary objective of this work was to empirically examine the theory positing that the aerial survival of the northern snakehead species is influenced not just by suprabranchial chamber respiration, but also by physiological regulatory mechanisms. The study aimed to assess the airborne survival duration and oxygen consumption rate (VO2) in snakeheads with intact suprabranchial organs compared to those with damaged suprabranchial organs. During aerial exposure, many hematological and biochemical markers were evaluated. The findings of the study indicate that there was a drop in resting VO2 while transitioning from a water environment to an air environment in both the control group and the group of fish with suprabranchial organ injuries. The decrease in resting VO2 was measured to be 22.4% and 23.5% for the control group and the damaged group, respectively. There was no significant difference seen in the resting VO2 levels in air between the control group and the group of fish with suprabranchial organ injury. There were no significant alterations seen in the red blood cell (RBC) count and hemoglobin content, however an increase in RBC size was observed with exposure to air. The concentration of lactate in the liver did not show any significant change, but the concentration of lactate in the white muscle reduced when transitioning from an aquatic environment to an aerial one. There was a tendency for the blood ammonia content to rise during aerial breathing. The findings indicate that the ability of the snakehead fish to survive in aerial environments is influenced by several factors. These factors include the functioning of suprabranchial organs and other accessory organs involved in respiration, reduced metabolic demands, enhanced oxygen transport, and the absence of anaerobic metabolism. Additionally, the accumulation of blood ammonia appears to have a negative impact on the aerial survival of the snakehead fish.

Zaccone, Giacomo & Lauriano, Eugenia (2018) The use of aerial gas exchange in fish has independently evolved many times, resulting in the development of diverse air-breathing organs. Air-breathing has been seen in a minimum of 49 documented families of fish. Several aquatic vertebrates exhibit trimodal respiration throughout certain stages of their development, using different combinations of respiratory surfaces to extract oxygen from both water (such as skin and/or gills) and air (such as skin and/or lungs). This current study investigates the evolutionary ramifications of air-breathing organs in fish, as well as the morphology of peripheral receptors and the neurotransmitter content of the cells responsible for regulating air-breathing. The regulation of respiration, whether by gill ventilation or air-breathing, is impacted by feedback received from receptors in the peripheral and/or central nervous system. These receptors are sensitive to changes in the levels of PO2, PCO2, and/or pH. The precise identification of the chemoreceptors responsible for mediating respiratory reflexes remains uncertain. However, research conducted on teleosts that breathe underwater suggests that the neuroepithelial cells (NECs) present in gill tissues may serve as the oxygen-sensitive chemoreceptors responsible for initiating cardiorespiratory reflexes in aquatic vertebrates. Certain species of air-breathing fishes, including Protopterus, Polypterus, and Amia, have been shown to possess neuroepithelial cells (NECs) inside their gills and/or lungs. However, the specific function of these receptors and their neural connections in regulating respiration remains unknown. Necrotizing epithelial carcinomas (NECs) have also been documented in the specialized respiratory epithelia of auxiliary respiratory organs (AROs) in some species of catfish, as well as in the gill and skin of the mudskipper Periophthal modonschlosseri. In contrast to teleosts, which exhibit a breathing system that is mostly influenced by ambient oxygen levels, lungfish possess both central and peripheral H+/CO2 sensors that regulate the acid-base equilibrium of the blood.

Nakkrasae, La-iad & Wisetdee, Khanitha(2015) The dissolution of rock salt, which is high in NaCl, in a natural water source results in fluctuations in salinity that have a significant impact on freshwater ecosystems and the aquatic life within them. The snakehead species, scientifically known as Channa striata, has the capability to survive in saline water; however, the precise osmoregulatory processes responsible for this adaptation have yet to be fully elucidated. In this study, it was shown that the exposure to salinities equal to or more than 10 ‰ NaCl resulted in a significant increase in plasma cortisol and glucose levels, as well as muscle dryness. In a research investigation examining the time-dependent reaction subsequent to the transition from fresh water (0 ‰ NaCl, FW) to brackish water with dissolved salt (10 ‰ NaCl, SW), it was shown that cortisol levels saw a fast rise, concomitant with increases in plasma glucose and lactate concentrations. It is noteworthy that plasma cortisol levels reverted to their initial levels after a longer period of exposure, subsequently followed by a further surge that likely increased the activity of the branchial Na(+)/K(+)-ATPase. In the SW-FW condition, there was no significant alteration seen in the Na(+)/K(+)-ATPase activity as compared to fish accustomed to SW. In summary, changes in salinity, particularly the transition from freshwater to saltwater, have been shown to elicit a stress response in C. striata, leading to the production of cortisol. This release of cortisol may subsequently elevate levels of plasma glucose and lactate, which serve to provide energy for the functioning of the branchial Na(+)/K(+)-ATPase.

John, Maina &Maloiy, Geoffrey (2011) The examination of the gas exchange organs of the African air-breathing catfish (Clarias mossambicus) (Peters) has been conducted using several microscopic techniques, including gross examination, light microscopy, electron microscopy, and scanning microscopy. The respiratory structures found in Clarias consist of a gill system, as well as other organs like as the labyrinthine organ and the suprabranchial chamber membrane. The observed resemblance in the architecture of the marginal channels and the transverse capillaries, which serve as the terminal respiratory components, across the three respiratory organs, indicates a significant developmental association between the gills and the auxiliary respiratory organs. The morphometric examination conducted on the respiratory organs indicated that the average weight-specific surface area of the gills was found to be 17.30 mm2/g, which was higher than that of both the labyrinthine organs (4.65 mm2/g) and the suprabranchial chamber membrane (7.79 mm2/g). Nevertheless, as a result of the comparatively dense water-blood barrier, the average harmonic mean thickness of the gills is 1.97 µm, whereas the accessory respiratory organs have an average thickness of 0.30 µm. Consequently, the gills only account for 15% of the overall morphometric diffusing capacity of the respiratory organs. In contrast, the labyrinthine organs contribute 50% to this capacity, while the suprabranchial chamber membrane contributes 35%. The auxiliary respiratory organs make about 85% of the total diffusing capacity. This observation may explain the physiological adaptation of C. mossambicus as an obligate air-breather, since the gills may potentially be insufficient in oxygen uptake even under conditions of adequate water aeration.

Hughes, G. & Munshi, J. (2009) Electron microscopy was used to explore the structural characteristics of the air-breathing organs of Indian fish species, including Channa punctatus, Channa Striatus, Amphipnouscuchia, Clarias batrachus, and Saccobranchusfossilis. In every species, the division between the air and the blood is comprised of three primary layers, namely the epithelium, basal lamina, and endothelium. The overall thickness varies from 0.78 µm in C. punctatus to 1.6 µm in S. fossilis. The existence of pillar cells, which are characteristic of gill secondary lamellae, provides evidence supporting the hypothesis that the formation of these organs occurred via the alteration of a typical gill structure in Clarias and Saccobranchus. In the case of Amphipnous and two species of Channa, the available information indicates that the auxiliary organs are likely to be gills that have undergone modifications. The visual observation of pillar cells under the light microscope was attributed to the existence of valve-like structures located between the afferent and efferent blood spaces of the vascular papillae. The correlation between the anatomy of these organs and physiological research is indicative of the animal's life value and the extent of gill growth.

3. METHODOLOGY

The snakeheads were acquired. The fish underwent acclimation inside a controlled rearing system located at the Fisheries Science Institute of the University. The water temperature was maintained at a mean value of 25°C with a standard deviation of ±1°C. The photoperiod consisted of alternating periods of 12 hours of light and 12 hours of darkness. The concentration of oxygen exceeded 6 mg l^{-1}, whereas the quantity of ammonia was below 0.015 mg l^{-1}. The School of Life Sciences at Southwest University (LS-SWU-1612) granted approval for the animal handling and studies, which were carried out in compliance with the guidelines outlined in the Environment and Housing Facilities for Laboratory Animals (GB/T14925-2001).

In order to examine the impact of suprabranchial organ damage on VO2, the snakehead fish were partitioned into two distinct cohorts: a control group consisting of 14 individuals, and an injured group also including 14 individuals. The fish were subjected to a 24-hour fasting period, after which their body mass was measured, with each fish weighing around 3 grams. Subsequently, the fish were individually relocated into respiratory chambers and allowed to acclimate overnight prior to the assessment of VO2Water and VO2Air. Li et al. (2017) provided a comprehensive description of the respiratory chamber's construction and the techniques used for determining VO2. In a concise manner, the chamber with a volume of 30 ml has the capability to transition between a flow-through water phase and a closed air phase via the control of the intake and output valves. To assess water respiration, the concentration of dissolved oxygen (mg O2 L−1) was quantified at the outflow using a fiber optic sensor system (Mircrox TX3, PresSens Precision Sensing GmbH, Regensburg, Germany). Additionally, the water flow rate (L h−1) was determined by collecting water for a specified duration. The VO2Water (mg O2 h−1) was determined by multiplying the rate of water flow by the disparity in dissolved oxygen content between the fish chamber and the control chamber. To prevent the occurrence of hypoxic stress, efforts were made to ensure that the concentration of dissolved oxygen in the discharge water remained above 70% of its saturation level. To determine VO2Air, the water inside the chamber was expelled, and a state of near-saturated humidity was maintained within the chamber. The measurement of VO2Air was conducted using an intermittent flow pattern. The initial partial pressure of oxygen in the air was quantified, after which the chamber was hermetically sealed for a duration of 90 minutes. Subsequently, the final oxygen concentration was determined. The measurement of the change in air volume resulting from air respiration was obtained by observing the reading on a 1-ml syringe that was linked to the intake. The measuring process included the recording of atmospheric pressure. Subsequently, the chamber's air was subjected to a 30-minute period of refreshment, following which the subsequent determination loop was initiated.

The determination of the oxygen concentration in the air (expressed in mg O2 l−1) was achieved by using the oxygen partial pressure, temperature, and atmospheric pressure data. The calculation of VO2Air (mg O2 h−1) included the use of the formula VO2Air=(O2i×Vi−O2f×Vf)/t, where O2i and O2f represent the initial and final oxygen concentrations of the air (mg O2 l−1), respectively. Similarly, Vi and Vf denote the initial and final air volume, respectively. Lastly, t represents the breathing duration in hours. The estimation of the fish body volume was conducted on the assumption of a body density of 1 kg l−1. A control chamber, devoid of fish, was designated to assess the effects of water or air respiration. The mass-specific value of VO2 was determined by dividing it by the individual's body mass in kilograms. The resting VO2Water was determined by measuring VO2Water at 1-hour intervals for a duration of 8 hours in both groups, and calculating the mean value. In the case of the wounded cohort, the ventral and posterior walls of the suprabranchial chamber were excised using surgical scissors. Following a 30-minute period of adaptation, the VO2Air of both groups was assessed at 2-hour intervals throughout a duration of 24 hours. The resting VO2Air was determined by calculating the mean values.

4. RESULTS

The oxygen consumption rate (VO2) of both the control group and the group of fish with suprabranchial organ injuries exhibited a reduction upon transitioning from respiration in water to respiration in air, as seen in Figure 1. The resting oxygen consumption rate in air (VO2Air) was found to be considerably lower than the resting oxygen consumption rate in water (VO2Water), exhibiting reductions of 22.4% in the control group and 23.5% in the damaged fish (refer to Table 4.1). There was no significant difference seen in resting VO2Air between the control group and the group of fish that had sustained injuries. The duration of airborne survival for the damaged fish was recorded at 19.0 hours, which was almost 4 hours less than the control group's survival length of 22.9 hours.

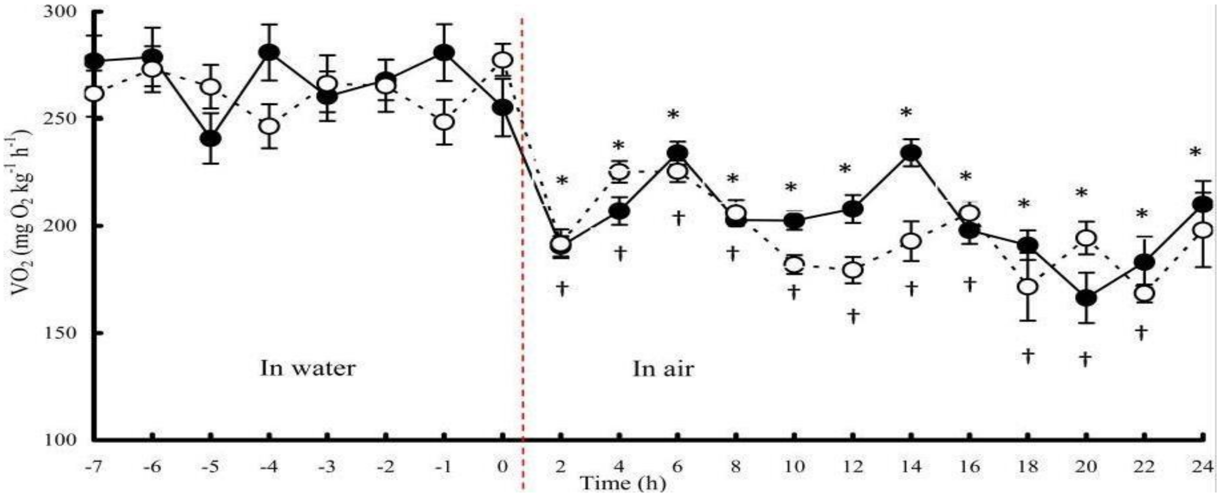

Fig 4.1 Northern snakeheads' oxygen consumption rate (VO2) changed when they transitioned from aquatic to aerial respiration. Each set has n=14. Data are shown as mean±s.e.m. A paired samples t-test revealed that the values with symbols (*, control group;,suprabranchial organ group) were different from the resting VO2 in water (P <0.05). Circles with solid fills represent the control group, whereas circles with dashed fills represent the suprabranchial organ group.

Table 4.1Water and aerial respiration parameters of northern snakeheads

Treatment	Control	Suprabranchial organ injured	*t*	*P*
Number	14	14		
Body mass (g)	3.20±0.04	3.19±0.04	0.290	0.774
Resting VO_{2Water} (mg O_2 kg^{-1} h^{-1})	266.4±10.3	263.1±9.0	0.240	0.812
Resting VO_{2Air} (mg O_2 kg^{-1} h^{-1})	202.2±4.9	198.6±4.4	0.551	0.587
Change in resting VO_2 from water to air (%)	22.4±4.1	23.5±2.9	−0.232	0.819
Survival time (h)	22.9±0.5[a]	19.0±0.8[b]	4.281	0.000224

The measures associated with the ability of oxygen transportation, such as the count of red blood cells (RBCC), concentration of hemoglobin (Hb), and mean cellular hemoglobin content (MCH), exhibited little changes in the fish while transitioning from respiration in water to respiration in air (Table 4.2). The length (RBCL), width (RBCW), and area (RBCA) of the fish's red blood cells (RBCs) were found to be substantially greater after many hours of aerial respiration compared to water respiration. The liver exhibited a variation in lactate level, ranging from 2.6 to 3.1 mg g−1, whereas the white muscle of the fish had a range of 1.4 to 1.9 mg g−1 (Fig. 4.2). There was no significant alteration seen in the lactate content of the liver, but the lactate content of the white muscle exhibited no change at 3 or 6 hours and a substantial drop at 1.5 and 12 hours after the transition from water respiration to aerial respiration.

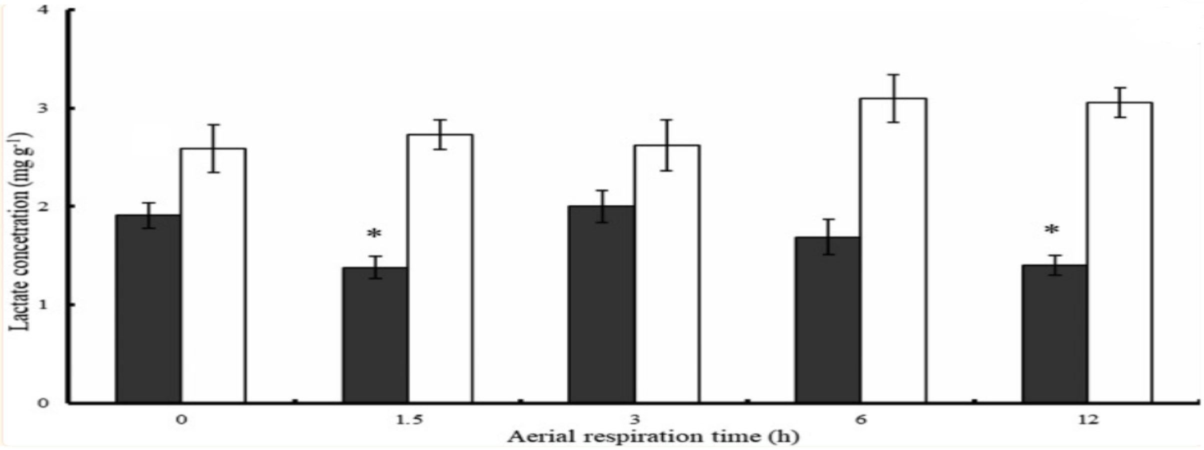

Fig. 4.2 Northern snakeheads' white muscle and liver lactate concentrations changed from water to aerial respiration. For the fish post-aerial respiration for 0 (in water), 1.5, 3, 6 and 12 h, respectively, n=10, 9, 10, 10 and 9. Data are shown as mean ±s.e.m. An independent samples t-test revealed that the values of each organ indicated by an asterisk were different from the values of the fish in water (0 h) (P <0.05). open column, liver; filled column, white muscle.

Table 4.2Northern snakeheads' hematological indicators shifted from water respiration to aerial respiration.

Time (h)	0	1.5	3	6	12	*F*	*P*
Number	10	9	10	10	9		
Body mass (g)	8.39±0.1	8.02±0.1	8.31±0.1	8.45±0.1	8.37±0.1	1.761	0.154
RBCL (μm)	8.84±0.08^{a}	8.67±0.12^{a}	9.33±0.18^{b}	9.22±0.15^{b}	9.36±0.12^{b}	5.326	0.001
RBCW (μm)	6.01±0.10^{a}	6.52±0.16^{bc}	6.80±0.17^{b}	6.36±0.14^{a,c}	6.37±0.10^{ac}	4.534	0.004
RBCA (μm^2)	41.76±0.68^{a}	44.45±1.46^{a,d}	49.93±1.86^{b}	46.09±1.28^{c,d}	46.87±1.16^{b,c,d}	5.146	0.002
RBCC (10^9 ml^{-1})	2.31±0.12	2.41±0.31	2.45±0.31	2.56±0.17	2.74±0.17	0.486	0.746
Hb (mg ml^{-1})	60.44±2.00	56.56±3.66	57.28±4.25	53.65±3.37	59.58±2.63	0.678	0.611
Mean cellular Hb (pg)	26.73±1.51	24.89±1.98	24.82±1.93	21.98±2.24	22.33±1.42	1.151	0.346

Fish in water had blood ammonia concentrations of 0.29 mmol l1 that tended to rise during aerial respiration (Fig. 4.3). Following 12 hours of aerial respiration, the blood ammonia level was 0.50 mmol l-1.

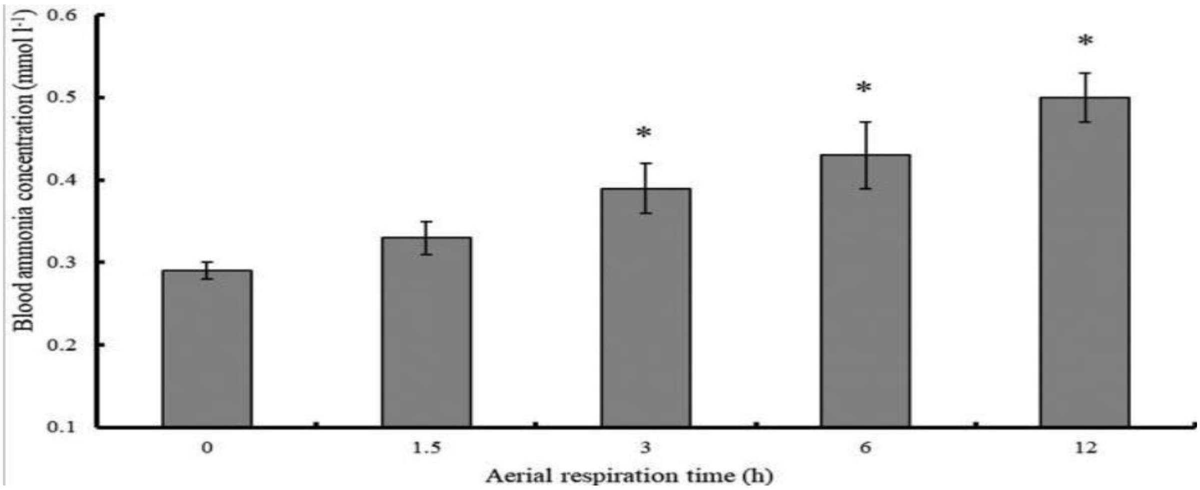

Fig 4.3 Northern snakeheads' blood ammonia levels changed when they transitioned from aquatic to aerial respiration. For the fish post-aerial respiration for 0 (in water), 1.5, 3, 6 and 12 h, respectively, n=10, 9, 10, 10, and 9. Data are shown as mean ±s.e.m. An independent samples t-test revealed that the results with asterisks were different from those of the fish in water (0 h) (P <0.05).

The suprabranchial chamber of the snakehead fish serves as a vital auxiliary respiratory organ, facilitating the exchange of gases in an aerial environment. Nevertheless, our research findings indicate that the northern snakehead's VO2Air remained unaffected by damage to the suprabranchial chamber. This implies that there are other organs involved in the process of aerial respiration, apart from the suprabranchial chamber. Historical histological investigations have consistently shown that the roof of the buccopharynx and the surface of the tongue in snakehead species exhibit vascularization.

In accordance with our prior investigation, the snakeheads exhibited a lower VO2Air in comparison to VO2Water, as seen in Figure 4.1. The Atlantic silverside, Menidiamenidia, has also been shown to have a similarly decreased VO2 in air. The reduced oxygen consumption (VO2) seen in fish may be ascribed to either a decreased ability for gas exchange or a downregulated metabolic demand. A proposition has been put out suggesting that the ventilation mechanism of the snakehead fish is not effective when exposed to air, hence restricting its ability to survive on land. However, it is possible that the decreased VO2 of the snakehead fish in air might be attributed to other processes. This is supported by the observation that the group with lesions to the suprabranchial organ had a VO2 level comparable to that of the fish with undamaged organs. These findings imply that the snakehead fish has an adequate capability for oxygen exchange. Hence, the reduced oxygen consumption (VO2) seen by the snakehead fish in an aerial environment may be attributed to a decrease in metabolic requirements. A decreased metabolic rate has significance for fish inhabiting severe settings as it enables them to save energy, minimize the buildup of waste products, and enhance their survival capabilities over extended durations.

5. CONCLUSION

In conclusion, when northern snakeheads are exposed to air, their survival is positively associated with several factors. These factors include the respiration of suprabranchial chambers and other accessory respiratory organs, a decrease in metabolic demands, and an increase in oxygen transport. On the other hand, survival is negatively associated with the accumulation of blood ammonia. There is just a marginal relationship between the control of anaerobic metabolism and the aerial survival of northern snakeheads.

REFERENCES

1. Deepak Varma, Hira Yadav, Sangam Kumar, Vijeta Chaturvedi,Virendra Kumar Tripathi,(2023) A Response Study On Brain, Gills And (Suprabranchialcavity) Madhya Bharti -Humanities and Social Sciences, Vol-83 No.8 (II) January – June, ISSN: 0974-0066.
2. Duan, Ting & Shi, Chenchen & Zhou, Jing &Lv, Xiao & Li, Yongli & Luo, Yiping. (2018). How doesthe snakehead Channa argus survive in air? The combined roles of the suprabranchial chamber and

physiological regulations during aerial respiration. Biology Open. 7. bio.029223. 10.1242/bio.029223.

3. Zaccone, Giacomo & Lauriano, Eugenia & Capillo, Gioele &Kuciel, Michał. (2018). Air- breathing in Fish: Air-breathing Organs and Control of Respiration. Nerves and Neurotransmitters in the air-breathing organs and the skin..
4. Nakkrasae, La-iad & Wisetdee, Khanitha & Charoenphandhu, Narattaphol. (2015). Osmoregulatory adaptations of freshwater air-breathing snakehead fish (Channa striata) after exposure to brackish water. Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology. 185. 10.1007/s00360-015-0902-z.
5. John, Maina &Maloiy, Geoffrey. (2011). The morphology of the respiratory organs of the African air-breathing catfish (Clarias mossambicus): A light, electron and scanning microscopic study, with morphometric observations. Journal of Zoology. 209. 421 - 445. 10.1111/j.1469-7998.1986.tb03602.x.
6. Hughes, G. & Munshi, J.. (2009). Nature of the air-breathing organs of the Indian fishes Channa, Amphipnous, Clarias and Saccobranchus as shown by electron microscopy. Journal of Zoology. 170. 245 - 270. 10.1111/j.1469-7998.1973.tb01377.x.
7. Cruz A. L., Silva H. R., Lundstedt L. M., Schwantes A. R., Moraes G., Klein W. and Fernandes M. N. (2013). Air-breathing behavior and physiological responses to hypoxia and air exposure in the air-breathing loricariid fish, *Pterygoplichthysanisitsi*. *Fish Physiol. Biochem.* 39, 243-256. 10.1007/s10695-012-9695-0
8. Gao Z. X., Wang W. M., Abbas K., Zhou X. Y., Yang Y., Diana J. S., Wang H. P., Wang H. L., Li Y. and Sun Y. H. (2007). Haematological characterization of loach *Misgurnusanguillicaudatus*: comparison among diploid, triploid and tetraploid specimens. *Comp. Biochem. Physiol. A.* 147, 1001-1008. 10.1016/j.cbpa.2007.03.006
9. Ginneken V. and van den Thillart G. (2009). Metabolic depression in fish measured by direct calorimetry: a review. *Thermochim. Acta.* 483, 1-7. 10.1016/j.tca.2008.09.027
10. Lefevre S., Wang T. and Jensen A., Cong N. V., Huong D. T. T., Phuong N. T. and Bayley M. (2014). Air-breathing fishes in aquaculture. What can we learn from physiology? *J. Fish Biol.* 84, 705-731. 10.1111/jfb.12302
11. Li Y. L., Lv X., Zhou J., Shi C. C., Duan T. and Luo Y. P. (2017). Does air-breathing meet metabolic demands of the juvenile snakehead, *Channa argus*, in multiple conditions. *Biol. Open.* 6, 642-664. 10.1242/bio.024448