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Research Article
Age and Seasonwise Haematological Profile of Little Egret (*Egretta garzetta*) of Chilika Wetland, India

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Abstract

Background and Objective: Wetlands are abode to a number of avian species, both migratory as well as resident. Aves in general represent a sensitive group of faunal population and can act as indicators not only for health of their own population but also for their habitat. The study of the physiological features, like the haematological parameters of a wild avian population can be helpful to comprehend various aspects experienced by it like the effect of environment, pathogenic and parasitic interactions and nutritional deficiencies. The present study aimed at the study of the effect of age and season on the haematological profile of little egret (*Egretta garzetta*), a breeding resident avian species of Chilika lagoon, the largest brackish water wetland of Asia. **Materials and Methods:** Blood samples were collected from 10 juveniles and 10 adults in each season. Haematological parameters like haemoglobin concentration (Hb), Packed Cell Volume (PCV), total RBC count, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), total WBC count, thrombocyte count and differential leucocyte count were calculated using standard procedures and reported as reference values for young and adult little egrets for four different seasons noticed in the tropical wetland, during the year 2015. Statistical analysis like t-test and one way ANOVA were done to test for significant difference at $p<0.05$. **Results:** The study showed that the avian blood profile is influenced by seasonal and age related variations. The values of Hb, PCV, RBC count, WBC count as well as MCHC were higher in adults compared to juveniles in all the seasons except the spring when WBC count was higher in juveniles. The values of MCV and MCH however were higher in juveniles except the rainy season. All the other leucocyte types except heterophils and lymphocytes indicated non-significant influence of age and seasons on their values. The thrombocyte count in adults showed seasonal fluctuations but juveniles, the variation in thrombocyte counts were non-significant. **Conclusion:** It is concluded that birds of two different age groups showed different trends in their blood profiles with respect to the seasonal stress. The data obtained could be useful as references for evaluation of general health and pathological conditions of the species.

Key words: Annual cycle, breeding residents, *Egretta garzetta*, haematological values, wetland ecosystem

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pertinence of haematological study for assessing, monitoring and managing wildlife is a well acclaimed preliminary strategy being an easier, faster, less-invasive, economically sustainable and efficient first hand technique. Wetlands are important sectors of the wildlife of any geographical region for their own geo-climatic specifications giving sustenance to a large number of flora and fauna[1]. The exposure of avian fauna to probable pathogenicity like avian influenza by virtue of the annual conglomeration of migratory birds on one hand and the growing threats on ecosystem on the other hand, whether in form of encroachment or contamination or implications of global warming are major threats to any wetland. The avian fauna being the prominent population of any wetland ecosystem is a crucial link towards the assessment of the health of the region, which needs investigation not only of the ecological factors but also behavioural and physiological aspects of its flora and fauna. Thus, overall wellbeing of a wetland can be monitored by studying various aspects of its avian population like migration of its water birds, their feeding and breeding ecology, annual cycle as well as physiological characteristics including haematology.

The haematological profile is known to be influenced by factors like age, sex, seasons, climatic conditions, species specific annual cycle etc.[2,3]. Influence of season and age on haematological values has been reported for many avian species in captivity[4-19]. A few literature regarding season and age related variations on the blood values in free living or wild species are on record[4,7,8,10,13,14,17,19-25]. The little egret is a common species in most of the wetlands and is a good model for study of haematology throughout the year, being a populous breeding resident or semi migratory species. The literature available on these birds is but very less, again none specifically from the Indian subcontinent. The present study enumerates the seasonal and age related variations, of the haematological parameters of little egret as a representative of the breeding resident species of the Chilika wetland of India, Asia.

The objective of the study was to record the values of the haematological parameters of a normal, apparently healthy little egret population along with variations due to age and season. The baseline data might be useful as a cross reference for studying the health status of the avian population in turn helping towards the conservation and protection of the wildlife of the region.

MATERIALS AND METHODS

The study was conducted in the Chilika lagoon and its nearby areas located between 19°28′ and 19°54′ N and 85°05′ and 85°38′ E in the Odisha state of India which is the second largest brackish water lagoon of the world and is enlisted as a Ramsar site. Characterized by its shallowness, highly productive biomass, relatively stable salinity and high degree of sheltering from the tidal currents, it is one of the important aquatic bodies for wintering in the central Asian Flyway[26]. Its water has been reported to have pH of 6.8-9.6, salinity of 0-35 ppt and temperature ranging between 14-39.9°C[26].

The little egret (*Egretta garzetta*) is a small sized bird found both in swallow muddy water and on land. In a year three different age groups such as first year juveniles, second year juveniles or sub adults and the adults have been taken into account. In their annual cycle, adults and both the groups of juveniles show pre-breeding moult but only the adults exhibit post-breeding moult. The breeding period coincides with the rainy season (Mid-June to September)[27,28].

The study was conducted between January, 2015 and 2016 in four different seasons noticed in the region, winter (October-January), spring (February, March), summer (April-June) and rainy (Mid June-September). However, spring season not being so prominent in the wetland is often merged with early summer. The birds were captured using mist nets and noose traps from their nesting and foraging sites early in the morning by professional bird trappers. Clinically sound adults and juveniles (n = 10) were chosen for the purpose in each season. Blood samples were collected between 6.00 am and 8.00 am in all cases to avoid diurnal variation. Sample collection was undertaken by veterinary personnels, who used the method of venipuncture of the ulnare vein[29] using 2.5 mL disposable syringes. Fresh drops of blood were used to make bloodfilms immediately on the site. Rest of the blood was kept in EDTA vials and then transported in icebox to the laboratory from the collection site, for further study to be completed within 24 h. The blood parameters were studied using standard procedure[30]. Haemoglobin was estimated as oxy-haemoglobin, using Sahli's haemoglobinometer, PCV was estimated by centrifuging the micro haematocrit tube with blood at 2500 rpm for 15 min. The counting of RBC and WBC was performed by manual method using Neubauer's haemocytometer. Erythrocyte indices like MCV, MCH and MCHC were calculated using standard formulae[30]. Blood smears stained with Leishman's stain were used to study the Differential Leucocyte Count (DLC) as well as the total thrombocyte count[2,29].

Statistical analysis: The data presented as Mean±SE (Standard Error) were analysed for significant difference ($p<0.05$) between the blood values of two age groups, using student's t-test (assuming equal variance) in MS Office Excel 2010 and for differences in the values in different seasons one way ANOVA was followed using PAST software (version 2.17, Natural History Museum, University of Oslo). Differences were considered significant at $p<0.05$[17,25,31].

RESULTS

Season wise variation of haematological parameters were analysed for groups of juvenile and adult little egret (Table 1, 2). Marked significant differences (assuming a confidence level of 95%) were found in the blood profiles of the two age groups, both within the groups as well as between the groups in almost all the four seasons.

The study indicated that the values of Hb, PCV, RBC count, WBC count as well as MCHC were higher in adults compared to juveniles in all the seasons except the spring when WBC count was higher in juveniles. The values of MCV and MCH however were higher in juveniles except the rainy season. All the other leucocyte types except heterophils and lymphocytes indicated non-significant influence of age and seasons on their values.

Table 1 shows that Hb and PCV values were the highest in the rainy season and lowest in summer ($p<0.001$) in adults, whereas for the juvenile the highest value were realised in winter season and lowest in rainy season ($p<0.001$). Total RBC value was high in winter and lowest in summer for adults ($p<0.05$). In contrast juveniles had higher number of RBCs in rainy season and lowest number in summer ($p<0.05$). The thrombocyte count in adults showed seasonal fluctuations at $p<0.01$ whereas, for juvenile egrets, the variation in thrombocyte counts with respect to the seasonal changes was non-significant. The WBC count and total thrombocyte count were found to be highest in summer and lowest in rainy season in adults ($p<0.05$), in contradiction the highest values in spring and lowest in winter noted in juveniles ($p<0.001$). The significant differences in H/L ratio were realised at $p<0.05$ with in the age groups, however,between the age groups the difference was highly significant at $p<0.001$ (Table 2).

DISCUSSION

In the present study, the values for Hb concentration, RBC count and the red cell indices like MCH and MCHC are found to be within the ranges proposed earlier for little egret, both for adults and juveniles[31,32]. It is also found to be in accordance with similar studies on other related avian species[4,14,17,19,25,33,34]. However, PCV and MCV values are observed to be lower in comparison to the study by Celdran *et al.*[31] but are close to the PCV value reported by another study[32] on little egret. As has been suggested in other studies[5,21,24,25,35-37], juveniles show a lower value of Hb, PCV and RBC count in comparison to that of adults but without significant differences. The increase in

Table 1: Age and season wise variations in haematological parameters of little egret (*Egretta garzetta*)

Haematological parameters	Age	Seasons: Winter (November-January)	Spring (February-March)	Summer (April- Mid June)	Rain (June-September)	F-value
Haemoglobin (g dL^{-1})	Adult	13.91 ± 0.2^{cB}	13.27 ± 0.24^{cB}	11.23 ± 0.16^{aB}	16.11 ± 0.51^{bB}	40.87***
	Juvenile	11.90 ± 0.33^{bA}	11.58 ± 0.19^{bA}	10.19 ± 0.25^{aA}	9.97 ± 0.1^{aA}	16.95***
PCV (%)	Adult	40.66 ± 0.45^{bB}	37.18 ± 0.83^{dB}	34.00 ± 0.41^{aB}	47.99 ± 0.89^{cB}	76.48***
	Juvenile	38.49 ± 0.27^{cA}	34.71 ± 0.63^{bA}	30.10 ± 1.3^{aA}	29.89 ± 0.24^{aA}	28.52***
RBC (10^6 mm^{-3})	Adult	3.92 ± 0.89^{bB}	3.55 ± 0.11^{bB}	3.086 ± 0.13^{aB}	3.49 ± 0.12^{ab}	8.34*
	Juvenile	2.95 ± 0.11^{cA}	2.66 ± 0.18^{bA}	2.65 ± 0.14^{bA}	3.29 ± 0.06^{a}	5.22*
WBC (10^3 mm^{-3})	Adult	7.41 ± 0.16^{abB}	7.00 ± 0.26^{abA}	7.51 ± 0.22^{b}	6.01 ± 0.65^{a}	3.28*
	Juvenile	6.17 ± 0.18^{aA}	8.96 ± 0.49^{bB}	6.99 ± 0.27^{a}	6.97 ± 0.18^{a}	14.32***
Thrombocytes (10^4 mm^{-3})	Adult	1.92 ± 0.04^{b}	1.78 ± 0.03^{aA}	2.06 ± 0.49^{bB}	1.62 ± 0.08^{a}	10.56**
	Juvenile	1.82 ± 0.04^{NS}	1.93 ± 0.03^{NSB}	1.88 ± 0.04^{NSA}	1.79 ± 0.04^{NS}	2.33^{NS}
MCV (fl)	Adult	104.07 ± 2.23^{aB}	105.17 ± 2.96^{aB}	111.78 ± 4.18^{a}	139.02 ± 5.3^{bB}	17.81***
	Juvenile	131.35 ± 3.79^{bA}	134.64 ± 7.78^{bA}	115.16 ± 5.39^{b}	91.00 ± 1.5^{aA}	14.93**
MCH (pg)	Adult	35.59 ± 0.8^{aB}	37.61 ± 0.25^{aB}	36.84 ± 1.15^{a}	46.63 ± 2.1^{bB}	12.73***
	Juvenile	40.69 ± 1.82^{bA}	44.87 ± 2.6^{bA}	39.21 ± 1.8^{b}	30.33 ± 0.41^{aA}	10.98**
MCHC (g %)	Adult	34.22 ± 0.44^{bB}	35.81 ± 0.91^{aB}	33.02 ± 0.27^{b}	33.51 ± 0.57^{b}	4.1*
	Juvenile	30.90 ± 0.79^{aA}	33.32 ± 0.28^{bA}	34.17 ± 0.83^{b}	33.35 ± 0.12^{b}	5.61*

$^{A,B,\ a\text{-}d}$Different superscripts (A, B) and (a, b, c, d) indicate significant difference (*$p<0.05$, **$p<0.01$, ***$p<0.001$) for each haematological parameter column wise and row wise respectively, NS: Non significant, values given as Mean±SE (n = 10)

Table 2: Age and season wise variations in the leucocyte profile of little egret (*Egretta garzetta*)

Haematological parameters	Age	Seasons: Winter (November-January)	Spring (February-March)	Summer (April- Mid June)	Rain (June-September)	F-value
Heterophil (%)	Adult	51.90±2.15^{aA}	61.20±0.59^{b}	62.40±2.14^{bB}	58.80±1.9^{ab}	6.67**
	Juvenile	55.20±1.45^{abB}	60.70±1.36^{b}	50.00±1.06^{aA}	55.40±2.2^{ab}	7.01***
Lymphocyte (%)	Adult	41.06±2.08^{aB}	34.50±0.49^{b}	32.90±2.01^{bA}	36.80±1.8^{ab}	4.3*
	Juvenile	37.80±1.26^{aA}	35.70±1.06^{a}	44.70±0.95^{bB}	40.70±1.6^{b}	9.53*
Eosinophil (%)	Adult	3.30±0.7^{NS}	2.00±0.49^{NS}	2.50±0.37^{NS}	2.20±0.53^{NS}	1.1^{NS}
	Juvenile	3.60±0.56^{NS}	1.80±0.48^{NS}	3.00±0.06^{NS}	2.10±0.45^{NS}	2.38^{NS}
Basophil (%)	Adult	2.10±0.5^{a}	1.70±0.36^{b}	1.80±0.32^{b}	1.50±0.3^{ab}	3.32*
	Juvenile	2.30±0.32^{NS}	1.10±0.34^{NS}	1.40±0.3^{NS}	1.20±0.29^{NS}	0.98^{NS}
Monocyte (%)	Adult	1.10±0.23^{NS}	0.70±0.36^{NS}	0.20±0.13^{NS}	0.70±0.15^{NS}	0.35^{NS}
	Juvenile	1.00±0.21^{NS}	0.70±0.21^{NS}	0.50±0.22^{NS}	0.60±0.22^{NS}	2.45^{NS}
H/L	Adult	1.29±0.12^{aA}	1.79±0.08^{b}	1.98±0.17^{bB}	1.65±0.12^{ab}	5.00*
	Juvenile	1.48±0.08^{bB}	1.72±0.08^{b}	1.13±0.04^{aA}	1.40±0.11^{a}	7.8*

$^{A,B,a-d}$Different superscripts (A, B) and (a, b, c, d) indicate significant difference (*p<0.05, **p< 0.01, ***p<0.001) for each haematological parameter column wise and row wise respectively, NS: Non significant, values given as Mean±SE (n = 10)

the values of these parameters may be an indication of the increase in oxygen demand, for activities like flying[25] also an increase in bodyweight with a decreasing blood volume[36]. However, when seasonal effect is taken into consideration, the juveniles did not always show a trend of increase in these parameters as they were growing into adults. This may have an answer in the study conducted on bald ibis[19], which suggests that variation due to age are usually restricted to the first few months of life and the differences in values with respect to the age has more to do with differences in diet as well as hydration state of the birds than to age. The Hb and PCV values are the highest in rainy season but showed a gradual decrease from winter towards summer through spring. This corroborates with some previous studies[4,15,16]. Lower ambient temperature during winter results in higher food intake and may be a reason behind the increase in these parameters. In contrast, summer being dry and with a high ambient temperature may be the reason behind dehydration and subsequent respiratory water loss, increased oxygen intake which leads to a decreased erythropoiesis[15]. A decreased Hb and PCV in summer may also be concomitant with food scarcity, poor nutrition and protein deficiency[38]. Rainy season in comparison is a more favourable season in a wetland habitat[38,39] due to higher humidity, lower atmospheric temperature, increased food availability, favouring an increased metabolic rate and erythropoiesis[15,16]. Early rainy season also coincides with the egg laying activities in little egret which suggests that the process of erythropoiesis is simultaneously under hormonal effect in adults[15] during this period of time.

The RBC value shows decrease during spring and early summer which is probably due to pre breeding moulting[8] followed by a rise in rainy season which is the laying period. This has also been reported in pheasants[11]. The juveniles, however, show difference in these processes. They show the highest Hb and PCV along with RBC count in winter which gradually decreases towards spring, probably due to pre-breeding moult, followed by summer, with a negative impact of higher temperature as in case of adults, but in rainy season, unlike the adults they show very low values of these parameters, at times suggesting a state of anaemia[2]. This may be due to the increased competition for food and related deprivation of adequate nutrition as rainy season is the most crowded of all seasons for the little egret. Also it was noticed that juveniles were infested with a large number of ectoparasites during rainy season, which may be a cause behind anaemic condition[9,23,40]. However, as reported by some authors[40,41], the lower Hb and PCV might be compensated by an increase in the RBC count which is realised in this study in the rainy season for the juvenile egrets.

The MCV is the measure of size of red blood cells and shows a negative correlation with metabolic rate[42] as well as RBC count[43,44]. In the present study, it is noted that the MCV in juveniles in all the seasons except rainy season are greater than that of adults, thus, supporting a low RBC count and lower metabolic rate. A high MCV is also indicative of relatively higher proportion of reticulocytes along with mature erythrocytes in juvenile blood where as in adults the MCV is contributed mostly by mature erythrocytes[6]. Low MCV of juveniles in rainy season may be an indicator of iron deficiency and anaemic condition causing an accelerated erythropoiesis to compensate the demand of iron during Hb formation[6]. Earlier studies[5,14,21,35] in other avian species have proposed an increase with age for MCH and MCHC values, the juveniles and nestlings showing lower values. However, it is observed that only MCHC to be agreeing with these studies while MCH is

found to be higher in all three seasons except rainy season and coincides with a higher Hb concentration and is within the normal range reported for birds in general[43]. Increase in MCHC is related to the haemoconcentration of blood in summer than in wet season[4,18,43,45]. Also an increase in MCHC might be a physiological mechanism to increase the oxygen carrying capacity of blood, RBC count remaining constant[43]. As reported in other studies[11,43], a higher MCHC value is found in adults in spring which is the period of growth and absence of breeding activities for little egrets whereas the value decreases in the breeding and moulting periods which falls during summer and rainy season.

The WBC count in the present study matches the range reported for little egret[31] and is also comparable to the values reported for other related avian species[30,33]. The present study, however, differs from other such studies[12,31,34,37,46] in the fact that, juveniles showed higher WBC value than the adults only in spring and rainy season, though the difference was significant only in spring ($p<0.01$). Adults showed a higher count in the summer instead of spring. A simultaneous observation of the heterophil and lymphocyte counts shows that in adults, highest of lymphocytes and lowest of heterophils are found in winter as in other studies[4,10]. The juveniles showed the same pattern, in spring instead. This may be supported by the reports of some studies[12,35] which suggest that the response of the immune system might be expressed differently in juveniles and adults, as the strategies to handle stress differs in the two age groups depending on physiological priorities at a given point of time. Thus, spring which coincides with the growth and maturation of the juvenile birds, has the highest H/L value[4,13,22,31], perhaps because most of the juveniles are having their first exposure to the moulting activities also to the impact of sex hormones[47] and thus, this time of the year may be more stressful for a juvenile than an adult. In contrast, adults have the highest stress and so the highest H/L value in summer, which coincides with pre-breeding preparatory phase in addition to adverse environmental conditions like high ambient temperature, food scarcity etc. The WBC profile as a whole showed significant difference with respect to age as well as season ($p<0.01$, $p<0.001$) only in the early part of the year, i.e., in winter and spring but none towards summer and rain. The basophil as well as eosinophil count are found within range reported for little egret[31], however, the monocytes are less in number in the present study. No significant difference is found for monocyte, basophil and eosinophil for age or season related variation except basophils showing the highest value in winter in adults.

No reports were found on thrombocytes in the few studies undertaken on little egrets. However, thrombocyte count we found in the present study agrees with value reported for other related avian species like white stork[30], scarlet ibis and magauri stork[33], though it is very low in comparison to some related species like white stork and black stork[25]. Significant differences were noticed in the thrombocyte count of juveniles and adults in spring ($p<0.001$) and summer ($p<0.05$) seasons only. It was also observed that the number of these cells are least affected by seasonal variations in juvenile egrets, whereas, in adults, their number fluctuated in accordance with stressful conditions. This fact suggested two concepts, firstly these cells were in their developing stage in juveniles as immune system is not fully developed thus imposing vulnerability to this age group. Secondly, even when fully developed as in case of adults these blood cells are either more stable or less sensitive in nature. Thus their role in stressful environmental conditions needs further investigation.

The leucocyte and thrombocyte profile may be used to assess the level of stress,infection and change in other immune responses while the erythrocyte indices may be useful in indicating other physiological status like dehydration, nutritional deficiency as well as response to drastic change in environmental conditions. These interpretations might be helpful in protection and conservation of habitat and wildlife of the region.

CONCLUSION AND FUTURE RECOMMENDATION

The present investigation re-established the fact of influence of season and age on the haematological values. However, it also put forth the fact that birds of two different age groups showed different trends in their blood profile with respect to seasonal stress. Thus while studying an avian population for implication of probable stress causing factors, age and season during the study needs to be considered.

The present study provide a baseline reference value for the haematological parameters of a common resident species. This data may be useful for future studies relating to the wellbeing of the general avian population in the wetland, which may be affected not only by the outbreak of diseases but also due to change in the ecological factors in the region.

SIGNIFICANCE STATEMENTS

The present study is the first ever haematological study done on any wild free living avian species of the Chilika

wetland of India, Asia, which is a Ramsar site and hence conservation status of its flora as well fauna is of international importance. This study discovers the effect of season and age on the blood profile of a normal and apparently healthy egret population. These values may be used as references in future diagnostic purpose such as in case of outbreak of disease or degradation of the ecosystem, which in turn affects the health of the bird population of the region.

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