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QUANTIFICATION OF HETEROPHIL: LYMPHOCYTE RATIO IN THE BLOOD SAMPLES OF NOISY MINER

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ABSTRACT

Many biologists have used heterophil-to-lymphocyte ratio (HLR) cells from peripheral blood as an indicator of stress in many animals, including both wild and captive birds. However, there has been little research on Australian native birds to date, so the purpose of this study was to see if HLR could be used as a stress indicator in the Noisy Miner (Manorinamelanocephala), and to ensure that HLR could be equated to other likely stress indicators. For that, we investigated how leukocytes responded to some different indicators of stress that might affect these birds, such as age, sex, and season. Blood samples of 48 Noisy Miner birds were collected from the regions of Sydney and Armidale (New South Wales, Australia), and the correlation of body condition and HLR in this bird species was tested. The result indicated that there was no significant relationship between body condition and HLR (R2 = 0.001, F1, 46 = 0.031, P = 0.86), but there was a significant relationship between location and HLR. The result showed that bird colonies in Sydney had lower HLR and thus were less stressed than those in the Armidale colonies. This difference may be a result of factors such as night temperature, climate, and food availability differing for the Armidale samples. The result of Spearman's correlation analysis between HLR age, sex, moult, location, season and the presence of blood parasites indicated that there was no significant relationship. However, blood parasites were detected in blood samples (1.04%; n = 48), including Haemoproteus (n = 10), only one Plasmodium was found, and none of the microfilaria worms or Leukocytozoan were found. The study concluded that the HLR method can not be used to detect stress indicators in this bird species. This is maybe because HLR is a variable measure and a finer-scale measure of likely stress indicators was likely needed for this particular study.

Keywords :HLR, Leukocytes, Stress

الملخص:

تهدف هذه الدراسة للتحقق من امكانية استخدام تقنية قياس نسب خلايا الدم البيضاء المختلفة الى الليمفاوية كدليل لدراسة الاجهاد في الطيور، تم استخدام هذه التقنية بنجاح على أنواع من الطيور البرية والمحجوزة في الأقفاص. إضافة الى ذلك، تهدف هذه الدراسة الى البحث عن استجابة خلايا الدم البيضاء في الدم لمختلف العوامل والمؤشرات التي تعمل على تحفيز الاجهاد والتي قد تؤثر على الطيور والتي من ضمنها

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العمر، الجنس،الموسم الفصلي والإصابة بالطفيليات الدموية،تم دراسة نوع من الطيور تعرفب الطيور ذات الصوت المزعج. تم تجميع48 عينة من الدم من مناطق مختلفة من منطقتي سيدني وارميدال (جنوب استراليا)، تم القيام باختبار لمعرفة اذا كانت هناك علاقة تبادل بين ⍰الة الجسم و معدل خلايا الدم المختلفة الى الليمفاوية، ولكن ثبت من خلال النتائج انه لا توجد علاقة ذات دلالة معنوية،أيضا وجدت علاقة ذات دلالة معنوية بين الموقع ومعدل خلايا الدم المختلفة الى الليمفاوية، ففيطيور منطقة ارميدال كان المعدل اكثر ارتفاعا من طيور منطقة سيدني المتأثرة ببعض العوامل مثل درجة الحرارة اثناء الليل وكذلك وفرة الغذاء. في ختام هذه الدراسة، نوصي بعدم استخدام تقنيةقياس نسب خلايا الدم البيضاء المختلفة الى الليمفاوية في دراسة هذا النوع من الطيور في الوقت الحاضر، وذلك لعدم وجود مؤشرات ذات رابط متقارب. ولكن مع ذلك فإن هذه الطريقة استخدمت بشكل ناجح في عينات الدم لدى بعض الطيور الداجنة، علاوة على ذلك، هناك طرق أخرى قد تعطي معلومات اكثر دقة مثل استعمال عدد كبير من عينات الدم وتجميع العينات من الطيور من مناطق مختلفة.

الكلمات المفتاحية: نسب خلايا الدم المختلفة: خلايا الدم الليمفاوية، خلايا الدم البيضاء، الإجهاد.

1. INTRODUCTION

Quantification of stress in animalssuch as birds has become crucial in understandingtheirwelfare, life history and production. Hans Selye first introduced stress in the 1930s. In fact, many researchersare stillunable to agree on a single definition of "stress.”. Stress can bedefined in manydifferentways. It is a reaction by an organism to pressure, bothexternal and internal pressures that are self-imposed, thatresults in physiological, psychological, and behavioural changes in the animal (Virginina, 2000; Mary, 2009). Stress has also been defined as an orchestrated set of bodilyresponses to differentforms of noxious stimuli, such as changes in body condition or emotionalstressors, such as the sight of predators (Cockrem, 2007; Mary, 2009). The stress responseis the sum of physiological changes thatoccur in response to stressorssuch as handling, immigration, and crowding of birds (Cirule et al., 2012). Moreover, assessing stress is important in understandingbehavioural changes as well as physiological changes in birds. Suchstudies can beused to predict the adaptability of birds to environmental changes, production, and susceptibility to diseases (Davis et al., 2008).

2.LITERATURE REVIEW:

Manystudies have proposedseveral techniques for assessingphysiological stress with the measurement of levels of body chemicalssuch as adrenalglucocorticoid hormones and plasma corticosterone in birds, providing a reliable means of quantifying stress in animals (Davis et al., 2008; Muller et al., 2010). Corticosterone can bemeasuredeither by analysingbirdfeathers, whichreflectcorticosteronelevelsduring moult when the feathers are growing, or by takingbloodsamplesfrombirds to measurecorticosteronelevels (Muller et al., 2010;Lattin et al., 2011).
Hematologicalassessments of stress rely on this close relationshipbetweencorticosterone and eitherheterophil to lymphocyte ratios in birds or neutrophil to lymphocyte ratios in othervertebrates. This use of leukocytecountsfrombloodsmears has recentlyemerged as an

alternative technique for measuringpsychological stress in vertebrates, as ithelpsresearchersovercome the challenges presented by previousmethods (Davis et al., 2008).However, other cheap and simple techniques have thus far been proposed, including white bloodcellcounts and the quantification of HLR, which are commonlyused to assess the welfare of birdsunderdifferentlivestock and rearing conditions (Altan et al., 2000; Davis et al., 2008).Application of the HLR in the assessment of stress has been demonstrated as applicable by researchers in almost all vertebrates, includingbirds, fish, and reptiles (Davis et al., 2008).The HLR isrecognized as a simple and preciseway of assessing stress in avianspeciesowing to the evidence-based support and theoretical basis of itsmechanism. The increase in the number of heterophils in the circulatingbloodisexplained by the influx of heterophilsfrom the bonemarrow, a phenomenonattributed to the stress-induced release of glucocorticoids (Manhiani et al., 2011).The exodus of lymphocytes from the circulatingbloodthrough the sequestration process contributessignificantly to the reduction of lymphocyte cells as determined by the HLR technique (Dhabhar, 2002). This responseaids the immune system responses as itensures the cells are deposited in areas wherethey are more effective in responding to body changes triggered by the effects of stressfulexperiences (Manhiani et al., 2011).

Stress occurspotentiallywith infestations of diseases or parasites. One of the mostcommon parasites found in birds are the blood parasites, particularly the genera of *Haemoproteus*, *Leukocytozoan*, *Plasmodium*, and microfilariaworms. Infestation withthesemayinduce a stress response. These parasites have diverse effects on the avian hosts, includingcausingdiseases, amongother life-threateningeffects (Brown M. and Brown C., 2009; Petra et al., 2011). Further, the parasite load has been identified as a reliable technique for determining the quality of the immune system in birds as well as an evaluation of the susceptibility of differentbirdspecies to disease (Marzal et al., 2004;Ishak et al., 2008). Somestudies have shownthatdespitesome parasites' limitedability to cause acute disease at lowlevels, high blood parasite loads have shownsignificantnegative impacts on the wellbeing of the host (Marzal et al., 2004; Lay et al., 2011). Theseincludereducedproductivity, delays in breeding and reducedhatchingsuccess (Marzal et al., 2004;Ishak et al., 2008; Lay et al., 2011).

The birdspecies of focus for thisstudyis the Noisy Miner (*Manorinamelanocephala*)**,** whichbelongs to the Meliphagidaefamily but inhabitsdrier, wooded country in eastern and southernAustralia (Higgins et al., 2001; Kennedy et al., 2009). Thesebirds are recognized for their large colonies, social organization, and aggressive and territorial behavior (Higgins et al., 2001; Sarah, 2011). Noisy miners are mainlynectivorous but alsofeed on smallinsects as part of theirdiet (Higgins et al., 2001). In the colonies formed by Noisy Miners, helpers fromwithin the colony assist in raising the offspring. Adultmembers of the colonyplay a criticalrole in keepingpredators as well as foodcompetitionsawayfrom the colony'sterritory (Kennedy et al., 2009; Sarah, 2011).

The breedingseason of Noisy Minersoccursfrom July to December, sothesespeciesbreed in small to large colonies (Higgins et al., 2001; Ewen et al., 2003). Femalesincubate the eggs and build the nest. Both sexes perform displays during dominance disputes. Eye patch exposureis important in intimidation displays (Dow, 1975). There are more males thanfemales in the colony of Noisy Miners, and both sexes care for youngbirds (Barati et al. 2018). Manyresearchers have usedheterophil-to-lymphocyte ratio (HLR) cellsfromperipheralblood as an indicator of stress in manyanimals. However, to ourknowledge, there has been littleresearch on Australian native birds. Therefore, the aim of thisstudyis to investigate if HLR couldbeused as a stress indicator in Noisy Miner species (*Manorinamelanocephala*). In addition, wewill examine if we can correlate relative HLR withknown stress factorssuch as age, sex, season and blood parasite load. This will enable us to determine if HLR is an effective tool for monitoring stress in thesebirdspecies.

3.METHODOLOGY:

Blood sampleswerecollected in Sydney near Cumberland SF. ($33^0$44′ 43 S, $151^0$2′ 50' E) and in Armidale at twolocations:Newholme (30^0 25′ 23″S, 151^0 38′ 33″ E) and Hillgrove (30^0 31′ 49″S, 151^0 53′ 00″E). Whenbloodwasobtained, birdswerealsoweighed to the nearest gram, and head-to-tarsusmeasurementsweretaken to the nearest 0.1mm. Avianblood can becollected by manydifferentmethods. In thisstudy, a simple blood collection methodwasused, and the bloodwastakenfrom the venipuncture of the ulnar (wingvein). The bloodsampleswerecollected by capillary tubes following the method (Campbell and Ellis, 2007). Thinbloodsmearswerepreparedafterblood collection and air driedbeforebeingfixed in absolutemethanol (Medway et al., 1969). The fixedsmearswerethenstainedwith Quick Dip solution I (Fronine, Sydney) five times each for one second, followed by Quick Dip Solution II (Fronine, Sydney) five times each for one second. The benefit of using Quick Dip Solution isthatitprovides consistent and high qualityblood film staining, enablingresearchers to differentiatebetween the different types of white bloodcells and theirdetails, such as nuclear and cytoplasmic structure (Campbell and Ellis, 2007). The bloodsmearswerethenrinsedthoroughlywithdistilled water untilclear and air dried.

The preparedsmearswereexaminedunder light with a bifocal microscope (Axioskop 50, Zeiss, Germany) at a power of 1000 X magnification withoil immersion. Twenty-five fields of viewwerethenviewed, and the different types of leukocytespresentwereidentified. Cellscountedincluded granulocytes (heterophils, eosinophils, basophils) and non-granulocytes (monocytes and lymphocytes) and thrombocytes, using a manualcounter (No. 51369, Laboratory Counter, Clay-Adams, Inc., New York). HLR wasthencalculated by dividing the number of heterophils by the number of lymphocytes. Blood parasites wereassessedusing a manualcountingmethod, as wereerythrocytespresent in the sametwenty-five fields of viewused to quantifyleukocytes. By countingerythrocytes, weaimed to view a minimum of 2000 cells in the twenty-five fields of view for eachbloodsmear. The blood parasites foundwereidentified to the genuslevel and photos weretaken of different parasites using a digital camera (Coolpix 5400, Nikon, Korea). To confirmthat consistent scoring of differentforms of leukocyte, 30 photos, comprised of tworandomlychosenfields of viewfrom 15 differentbirds, werescored. Five of thefive species of thesebirdswerescored. The number of lymphocytes and heterophils in eachpicturewasthencountedfrom the screen of the computer for threedifferentdays, and the data wasassessed for consistency. The result of thisanalysisshowedthat the consistency of counting the leukocyteswas correct and reliable, with 100% repeatabilityacross all the samples.

4.STATISTICAL ANALYSES:

The relationshipsbetween HLR and specificmeasuressuch as moult, age, season, sex, and infection of parasites for thesebirdspecieswereanalyzedusingGeneralizedLinear Mixed Models ($GLMM_s$). Biologically relevant two-way interactions werefitted for all data sets, but theywereonlypresented if theyweresignificant, and termswerethenremovedusingbackwardsystematicelimination. Prior to data analysis, the HLR data was square root transformed to reachnormality. The HLR meanwas ($\overline{x}$ = 0.50 ± 0.32 SD; n = 48). Further, a linearregressionwasconducted to assess the relationshipbetween body condition and HLR in birds, with the expectation of a negativerelationshipbetween HLR and condition. All statisticalanalysis has been calculatedusing SPSS (v. 19, IBM Statistics, Chicago). Meanings are presentedwith one standard errorthroughout.

5.RESULTS:

There was no significantrelationshipbetween body condition and HLR (R^2 = 0.001, $F_{1, 46}$ = 0.031, P = 0.86). In addition, before HLR wasassessed relative to specificfactorswhichmightbeindicators of stress, such as age, sex, moult, location, season, and the presence of blood parasites, theywere first assessed for correlationusing Spearman’s correlation coefficient (Table 2). No significantrelationshipswerefound. A GLMMwasconducted to examine if HLR differedsignificantlyaccording to theselikelyindicators of stress (Table 1);termswereremoved via a backwardstepprocedure. There wasonly one significantrelationshipbetween location and HLR:bird colonies in Sydney hadlower HLR and thusappeared to belessstressedthanthosebirdssampled in Armidale colonies thathadhigher HLR (Fig.1).

Table 1: Individualcharacteristicsthatmaycorrelatewith stress and HLR in Noisy Miners are presented as termsthatwereremovedusing a backwardstepprocedure. Mixed model F-ratios (d.f.) – degree of freedom and P-values for all Noisy Miner birds are shown for mixed model results in all Noisy Miner birds.

Factor	F	Df	P
Location	4.602	2,45	0.015
Moult	0.565	1,44	0.456
Infested	0.93	1,43	0.762
Age	0.089	2,41	0.915

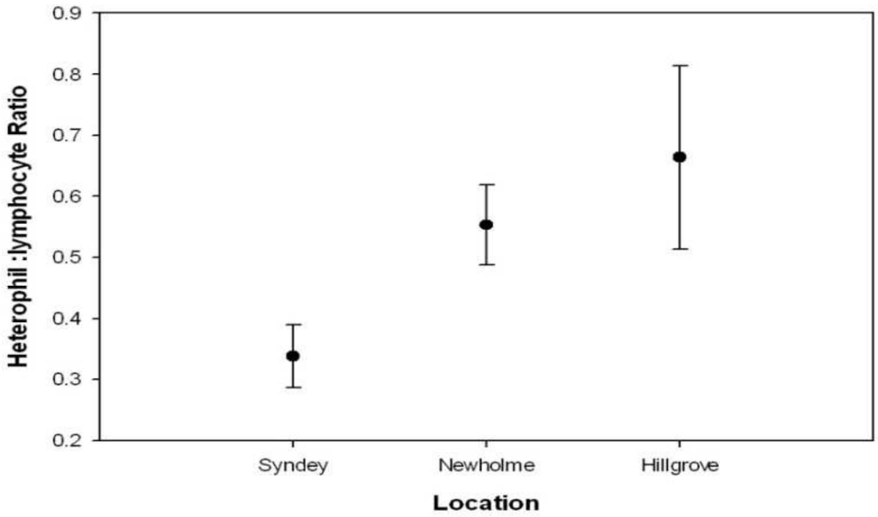

Fig.1: The relationshipbetween sampling location and the HLR of noisyminers.

Blood parasites thatweredetected in Noisy Miner samples (1.04%; n = 48) included*Haemoproteus* (n = 10), only one *Plasmodium*wasfound, and none of the microfilariaworms or*Leukocytozoan*werefound in Noisy Miner bloodsmears. HLR levelsweresignificantlyhigher in Armidale colonies (Newholme and Hillgrove), while HLR levelswerelower in Sydney colonies.

Table 2: Spearman's coefficient of correlationbetweenlikelymeasuredindicators of stress in the Noisy Miner.

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			season	Age	Infested	Moult	Location
Spearman's	Season	Correlation Coefficient	1.000	0.157	0.237	-0.239	0.553**
		Sig. (2-tailed)	-	0.286	0.105	0.103	0.000
		N	48	48	48	48	48
	Age	Correlation Coefficient	0.157	1.000	-0.041	0.084	0.234
		Sig. (2-tailed)	0.286	-	0.781	0.572	0.109
		N	48	48	48	48	48
	Infected, yes/no	Correlation Coefficient	0.237	-0.041	1.000	-0.038	0.215
		Sig. (2-tailed)	0.105	0.781	-	0.799	0.142
		N	48	48	48	48	48
	Moult	Correlation	-0.239	0.084	-0.038	1.000	0.059

		Coefficient					
		Sig. (2-tailed)	0.103	0.572	0.799	-	0.692
		N	48	48	48	48	48
	Location	Correlation Coefficient	0.553**	0.234	0.215	0.059	1.000
		Sig. (2-tailed)	0.000	0.109	0.142	0.692	-
		N	48	48	48	48	48

**. Correlationissignificant at the 0.01 level (2-tailed).

6.DISCUSSION:

This studyaimed to determine if HLR_s can beused as an indication of stress in Noisy Miner birds and to look for a relationshipbetween body condition and HLR.Moreover, thisstudyaimed to examine if HLR correlateswithlikelyindicators of stress such as age, sex, season, moult, and the presence of blood parasites in thisbirdspecies. As the resultindicated, therewasonly one significantrelationshipbetween location and HLR**.** The result of thisstudy of thisbirdspeciesshowedthat none of the noisyminershad a significantrelationshipbetween body condition and HLR. This mayexplainwhy body mass changes rapidly relative to stress response, resulting in the two not beingcloselylinked.Similareffects of asynchronyweirediscovered in a studyconducted by Taylor (1994), whodiscovered a rapidincrease in body mass changes in males and females of Little Auks (Alle alle), due to fat depositionassociatedwith high levels of lipid in the diet. However, instead of measuring body mass, measuring fat levels as long-termstoragemightbe a better variable to measure. Anotherpossibilityisthat all the birdsmeasuredwere in relatively good condition, althoughthisseemsunlikely. In addition, conditions are an indirect measure of stress, and thus, anyrelationshipmightbeclouded by manyfactors, such as environmental conditions, age, and sex. In this case, the data collectedis not sufficient to indicate HLR as a reliable indicator of stress associatedwithlow body condition, sothisstudyrecommendsusing the direct method of corticosterone relative to HLR to confirm or regulate stress status. Further, the significantrelationshipwaslimited to location. Birdssampledfrom Sydney colonies hadlowerlevels of HLR and likelylowerlevels of stress thanthose in Armidale. This differenceislikely due to environmentalfactors and the climate. Sydney has a far milderclimatethan Armidale, whichistemperatewithovernighttemperaturesregularlysub-zero over winter (Andrew et al., 2011). The climatemightalso influence foodavailability. For example, harshwinter conditions in wintering areas can reducefoodresources, thuselevating HLR during the breedingseason of somebirds.

7.CONCLUSIONS:

Overall, the results of the presentstudycannotrecommendusing the method of HLR in Noisy Miner birdspecies, as littleevidencewasfound to relate current HLR to likely stress levelsdirectly. However, the HLR measurement has been successful in otherbirdspecies, such as poultry and a range of othervertebrates. Therefore, itislikelythat a physiologicallinkbetween HLR and stress for thisbirdspeciesdoesexist and couldbeelucidatedwithcarefulassessment. The use of HLR and direct stress assessments of corticosteronetogether can provide a more comprehensivepicture of the relationshipsbetween stress and HLR in thisbirdspecies. To conclude, whatisneededis to have a finer-scalemeasure of stress thatcorrelateswith HLR or, betteryet, directly relates HLR to corticosterone. Using large samplesfromdifferent parts of the study areas and usingdifferentseasonal variation to exactlydetermine if thereis a correlationbetween the differentmeasured variables withinindividualswouldalsoaid in determining if HLR reflects stress in thesespecies.

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REFERENCES:

Altan, O., Altan, A., Cabuk, M. and Bayraktar, H. (2000). Effects of heat stress on somebloodparameters in broilers. *Turkish Journal of Veterinary Animal Science*, **24**, 145-148.

Andrew, N.R., Hart, R. A. and Terblanche J. S. (2011). Limited plasticity of lowtemperaturetolerance in an AustralianCantharidbeetle*Chauliognathuslugubris*. PhysioloigcalEntomolgy, **36** (4), 385-391.

Brown, M. B. and Brown, C. R. (2009). Blood sampling reduces annual survival in cliff swallows (*Petrochelidonpyrrhonota*). *Auk*, **126**: 853-861.

Barati A, Andrew RL, Gorrell JC, McDonald PG. (2018). Extra-pair paternity is not driven by inbreeding avoidance and does not affect provisioning rates in a cooperatively breeding bird, the noisy miner (*Manorinamelanocephala*). *Behavioral Ecology* 29: 244-52.

Campbell, T. W. and Ellis, C. K. (2007). *Avian & Exotic Animal Haematotlogy& Cytology*. (3rd Edition): Blackwell publishing.

Cirule, D., Krama T., Vrublevska, J., Rantala, M. and Krams, I. (2012). A rapideffect of handling on counts of white bloodcells in a wintering passerine bird: A more practicalmeasure of stress? *Journal of Ornithology*, **153**, 161-166.

Cockrem, J.F. (2007). Stress, corticosterone responses and avian personalities. *Journal ofOrnithology*, **148** (suppl. 12): S169-S 178.

Davis, A. K., and Maerz, J. (2008). Sex-relateddifferences in haematological stress indices of breeding, paedomorphic mole salamanders, *Journal of Herpetology*, **42**,197-201.

Davis, A. K., Maney, D. and Maerz, J. (2008). The use of leukocyte profile to measure stress in vertebrates:*A review for ecologists. FunctionalEcology*, **22**, 760-772.

Dhabhar, F.S. (2002). A hassle a daymaykeep the doctoraway: stress and the augmentation of immune function. *Integrative and Comparative Biology*, **42**,556-264.

Dow, D.D. (1975). Displays of the honeyeater*Manorinamelanocephala*. *Z Tierpsychol*, **38**(1):70-96.

Ewen, J. G., Crozier, R. H., Cassey, P., Ward-Smith, T., Painter, J. N., Robertson, R. J., Clarke, M. F. (2003). Facultative control of offsring sex in the cooperatively breeding Bell Miner, *Manorinamelanophrys*. *Behavioura Ecology*, **14** (2): 157-164.

Higgins, P.J., Peter, J.M. and Steele, W.K. (2001). *Handbook of Australian, New Zealand and AntarcticBirds, Volume 5 (Tyrant-flycatchers to Chats).* Oxford UniversityPress, Melbourne.

Ishak, H.D., Dumbacher, JP, Anderson, NL, Keane, JJ, Valkiu¯ nas, G, et al. (2008). Blood Parasites in Owls with Conservation Implications for the Spotted Owl (*Strix occidentalis*). *PLoS ONE***3**(5): E2304.

Kennedy, R. A. W., Evans, C. S. and McDonald, P. G. (2009), Individual distinctiveness in the mobbing call of a cooperative bird, the noisy miner *Manorinamelanocephala*. *Journal of Avian Biology*, **40**: 481–490.

Lay, Jr. D., Fulton, R. M., Hester, P. Y., Karcher, D. M., Kjaer J. B., Mench J., Mullens B., Newberry R., Nicol C., O’sullivan N. and Porter R. E. (2011). Emerging Issues: Social sustainability of egg production symposium: Hen welfare in different housing systems. *Poultry Sciences*, **90 (1)**,278-294.

Lattin, C. R., Reed, J. M., DesRochers, D. W., and Romero, L. M. (2011). Elevated corticosterone in feathers correlate with corticosterone-induced decreased feather quality: a validation study. *Journal of Avian Biology*, **42**: 247-252.

Manhiani, P., Northcutt, J., Han, I., Bridges, W., Scott, T and Dawson, P. (2011). Effects of stress on carnosinelevels in brain, breast, and thigh of broilers. *Poultry Science*, **90**, 2348-2354.

Mary, P. K. (2009). Stress & Adaptation. In Carol MattsonPorth, Glenn Matfin (Eds), Pathophysiology: Concepts of AlteredHealth States (pp.198-213). Philadelphia, PA: Wolters Kluwer Health / Lippincott Williams & Wilkins.

Marzal, A., Lope, F., Navarro, C &Moller, A. (2004). Malarial parasites decrease reproduction success: An experimentalstudy in a passerine bird. *Oecologia*, **142**, 541-545.

Matuna, N. (2001). The pacificdecadaloseillation, In:McCracken, M. C., Perry J. S. (Eds.).

Encyclopedia of Global Environmental Change. The Earthsystem: Physical and Chemical Dimensions of Global Environmental change, vol.1 Wiley, New York, pp, 592-594.

McDonald, P. G., (2012). Cooperativebirddifferentiatesbetween the calls of differentindividuals, evenwhen vocalisations werefromunfamiliarindividuals. *BiologicalLetters*, **8**, 365-368.

McDonald, P. G. and Griffith S. C. (2011). To pluck or not to pluck: The hiddenethical and scientificcosts of relying on feathers as aprimary source of DNA. *Journal of AvianBiology*, **42**, 197-203.

Medway, W., Prier J., Wilkinson J., (1969). A Text Book of Veterinary Clinical Pathology. The Williams &Wilkinis Company, USA.

Muller, C., Jenni-Eiermann, S. and Jenni, L. (2010). Heterophils/ lymphocytes- ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings. *Functional Ecology*, volume 25, Issue 3, pages 566-576.

Petra, Q., Elena, A., Javier, M., Jaun, F. and Santiago, M. (2011). Prevalence of blood parasites in seabirds- a review. *Frontiers in Zoology*, 8: 26.

Sarah, C. (2011). The noisy Native: a miner menace? Noisy Miner habitat preferences and influence on woodland bird species richness.

Taylor, J. (1994). Changes in body mass and body reserves of breeding Little Auks (*Alle alle* L.). *Polish Polar Research*, **15**: 147-168.

Virginia, H. (2000). Handbook of Stress, coping, and Health; Implications for Nursing Research, Theory and Practice.