

## PROJECT TITLE: EVOTELOX

### *Objectives of the project and methodology*

The project 'EVOTELOX' had two clear and well defined objectives. First, I aimed to assess the extent to which antioxidant and micronutrient availability in the diet affects telomere dynamics and oxidative status during early life (first phase of the project) and secondly, whether nutritional conditions and telomere dynamics during early life affect the speed of sexual maturation and reproductive investment during adulthood (second phase). To that end, firstly, I performed an experiment where I modified the availability of dietary micronutrients (e.g. antioxidants and essential minerals) during early development and sexual maturation of zebra finches (*Taeniopygia guttata*), and I measured and blood sampled these birds periodically. I used blood samples to determine the effects of the experimental treatment on oxidative status (i.e. non-enzymatic and enzymatic antioxidant levels and oxidative damage on lipids) and telomere dynamics (telomere length and loss) with age, and I also examined these in relation to growth patterns of these birds. At the same time, I also assessed the development of secondary sexual characters (i.e. adult plumage, bill colour and intensity) of these birds until they were fully adults. I combined these data together with the results of a second experiment to understand how early life antioxidant and micronutrient during development influenced the speed of sexual maturation and the investment in reproduction. During the second experiment (second phase of the project), I allowed the birds that were reared on different micronutrient regimens to breed with a standard partner. This second experiment allowed me to assess different reproductive traits of the experimental birds (i.e. clutch size, brood size, latency to start reproduction, etc.) as well as key life history traits (i.e. growth) in the offspring.

### *Main results of the project*

In brief, the main findings of this project can be summarised as follow (a detailed description of the main results is provided in the Supplemental Material):

-1- *The adult antioxidant defence system is influenced by the availability of dietary micronutrients received during early and later development and such effects seem to fit with an 'Environmental-matching' model; during the period of late development only those birds that continued with the same dietary treatment as during early development showed a significant increase in their level of antioxidant defences.*

-2- *The availability of diet micronutrients during sexual maturation affected the rate of telomere loss and final telomere length but such effects differed between sexes; females reared on a high micronutrient diet had longer telomeres and experienced lower telomere loss than males. Irrespective of the sex, birds in the low micronutrient diet experienced higher rate of telomere loss and had shorter telomeres. Moreover, the family of origin had a strong effect on both telomere length and loss suggesting a genetic influence (heritability) on both traits.*

-3- *The speed of development and intensity of antioxidant-demanding secondary sexual characters is influenced by the availability of diet micronutrients; in agreement with the*

findings for antioxidant defences, those birds that maintained the same diet during both the growth period and sexual maturation had a faster development of adult bill colour. Also, during the period of sexual maturation, those birds fed with a low micronutrient diet had a redder bill colour (bill hue) suggesting a greater investment in sexual maturation. Importantly, our preliminary analyses revealed a marginally significant positive trend between the rate of telomere loss and the investment in sexual maturation (bill hue) during this period.

*-4- Early and late dietary conditions experienced by the mother affect the investment in reproduction and offspring growth trajectories;* although these data are still being analysed, my preliminary analyses show that mothers reared on a high micronutrient diet during both early development and the period of sexual maturation seem to invest less in reproduction (e.g. fewer breeding attempts) than any other experimental group. However, when reproducing, these females performed better (higher fertility rates, hatching success and clutch size) and had offspring with higher growth rates.

In terms of publication, I am just finishing writing the first two manuscripts about the effect of nutrition on oxidative status and telomere loss (results 1 and 2) which I plan to submit for publication in the next few months to the Journal of Experimental Biology and to Biology Letters. Additionally, during these two years I have published four different manuscripts in top journals of my field of research - three of them as co-author (Journal of Evolutionary Biology 2013, 26, 1341-1352; Animal Behaviour 2013, 85, 1359-1366; Proceeding of the Royal Society of London 2014, 281, 20133151) and one as the main author (Hormones & Behaviour 2013, 64, 19-25) (see supplementary information for a summary of main findings). Moreover, over the last year and complementary to the planned tasks, I assessed different aspects of personality of my birds as well as their reproductive behaviour. These two complementary experiments have allowed me to test the effect that nutrition can exert in the development of adult personality traits and reproductive strategies. As a result of these studies, I have already submitted a new manuscript (to the journal Animal Behaviour; under review) and I am currently preparing a second manuscript on this topic which I plan to submit for publication in the next months. I have also successfully achieved the other planned tasks related to my own development: acquiring new skills, attendance at conferences and dissemination of results. Thus, for instance, most of the results obtained during this two years have also been presented in national and international congresses (i.e. The International Congress of Animal Behaviour, Newcastle, 2013; the Mini-Symposium on Oxidative Stress founded by the Rank Prize Funds, Grasmere 2014; the Scottish Ecological Ageing Research Meeting, Durham 2012; the Scottish Conference on Animal Behaviour, Glasgow 2013, Edinburgh 2014; The international conference of the Society for Experimental Biology, Manchester 2014).

Finally, as I mentioned before, some of the data sets from the second phase of the project are still being analysed which means that the results and potential publications from this project will increase in the next few months. A part of the publication record and dissemination of the results, an important personal achievement during the duration of the project has been the acquirement of a new 2-year post-doctoral fellowship funded by AXA and to be held at the University of Glasgow. This new research project has been designed on the basis of the Marie Curie project and therefore, it will allow me to

continue my work on the effects of nutrition on telomere dynamics, senescence and life-history strategies. In summary, I think this project has been very successful and the results are very promising. Given the increasing general interest in understanding the role of nutritional components on human health and diseases around the world and particularly within the EU, I really think that the outcome of this project will be of great interest not only for other researchers working on ageing but also for the general public and policy makers.

## SUPPLEMENTARY INFORMATION

### ABSTRACT OF MAIN RESULTS AND PUBLICATIONS ACHIEVED DURING THE DURATION OF PROJECT

1. **Title of the study:** Forecasting the future: diet micronutrients during growth period prime the adult antioxidant defence system

**Status:** Manuscript *in prep.* (2014)

**Authors:** Jose C. Noguera, Pat Monaghan & Neil B. Metcalfe

#### *Summary*

Accumulating evidence suggests that the individual's antioxidant system and oxidative status is influenced by the availability of dietary micronutrients. Importantly, it is thought that the programming effect that early life nutritional conditions may have on physiological traits could be particularly negative, if a mis-match between the programmed developmental trajectory and the future environmental conditions occurs ('Environmental-matching hypothesis'). In this study, we investigate this possibility by using zebra finches. We specifically manipulated the micronutrient intake during either early or late development and assessed how the micronutrient environment affected the level of antioxidant defences and oxidative damage. We found that those birds that were reared under low micronutrient diet had lower total antioxidant capacity (TAC) than those reared under a high micronutrient diet. However, birds in the low micronutrient group showed a compensatory response, and at the end of the early development period both experimental groups had the same level of total antioxidant capacity. Interestingly, we found an interactive effect of early and late micronutrient treatment on birds' antioxidant system; during the period of late development only those birds that continued with the same dietary treatment (low or high) than during early development showed a significant increase in their level of total antioxidant capacity. A similar effect was also found on the level of enzymatic antioxidant defences (GPx). The dietary treatment during late development did not affect the level of oxidative damages (MDA). These findings demonstrate the importance of early and late developmental condition in shaping multiple aspects of the antioxidant system. Furthermore, they suggest that the programming effects that early nutritional environment may have on the antioxidant system might be under the 'environmental-matching' effects on fitness.

2. **Title of the study:** are you what you eat? micronutritional deficiencies during development influence adult personality-related traits

**Status:** Manuscript *submitted* to Animal Behaviour (2014)

**Authors:** Jose C. Noguera, Neil B. Metcalfe, Peter F. Surai & Pat Monaghan

#### *Summary*

Stressful environmental conditions such as periods of poor nutrition have been shown to affect a variety of life history traits. Although nutrition-induced effects on the phenotype can appear through the entire life of an individual, it is becoming evident that there are sensitive periods during development when phenotypic traits have heightened sensitivity to nutritional conditions. Very few studies have investigated how nutrition can affect an important aspect of an organism's phenotype: the development of its 'personality'. In this study we manipulated the availability of the main micronutrients (i.e. vitamins and essential minerals) present in the diet of zebra finches during their postnatal development and/or during their sexual maturation. Later, once the birds were fully adult, we assessed key personality traits. We found that low availability of dietary micronutrients during the postnatal period resulted in reduced boldness in males once they reached adulthood, but had no effect on adult stress responses or neophobic behaviour. No such effects were found in females. In contrast, a low micronutrient diet during sexual maturation led in both sexes to reduced stress responses and neophobic behaviours in adulthood. Interestingly, we also found that females became more aggressive as adults if they had received a low micronutrient diet during development, irrespective of the time period during which the availability of micronutrients was modified. Overall, our results emphasise the importance of the availability of essential nutrients in the development of male and female personalities, which could have important consequences for fitness.

3. **Title of the study:** Stress exposure in early post-natal life reduces telomere length: an experimental demonstration in a long-lived seabird

**Status:** Manuscript published; Proceeding of the Royal Society of London Series B (2014), 281, 20133151.

**Authors:** Katherine A. Herborn, Britt J. Heidinger, Winnie Boner, Jose C. Noguera, Aileen Adam, Francis Daunt and Pat Monaghan

#### *Summary*

Exposure to stressors early in life is associated with faster ageing and reduced longevity. One important mechanism that could underlie these late life effects is increased telomere loss. Telomere length in early post-natal life is an important predictor of subsequent lifespan, but the factors underpinning its variability are poorly understood. Recent human studies have linked stress exposure to increased telomere loss. These studies have of necessity been non-experimental and are consequently subjected to several confounding factors; also, being based on leucocyte populations, where cell composition is variable and some telomere restoration can occur, the extent to which these effects extend beyond the immune system has been questioned. In this study, we experimentally manipulated stress exposure early in post-natal life in nestling European shags (*Phalacrocorax aristotelis*) in the wild and examined the effect on telomere length in erythrocytes. Our results show that greater stress exposure during early post-natal life increases telomere loss at this life-history stage, and that such an effect is not confined to immune cells. The delayed effects of increased telomere attrition in early life could therefore give rise to a 'time bomb' that reduces longevity in the absence of any obvious phenotypic consequences early in life.

4. **Title of the study:** Maternal testosterone influences a begging component that makes fathers work harder in chick provisioning

**Status:** Manuscript published; *Hormones and Behaviour* (2013) 64, 19-25

**Authors:** Jose C. Noguera, Sin-Yeon Kim & Alberto Velando

*Summary*

In species with biparental care, parents disagree evolutionarily over the amount of care that each of them is willing to provide to offspring. It has recently been hypothesised that females may try to manipulate their mates by modifying offspring begging behaviour through yolk hormone deposition, shifting the division of labour in their own favour. To test this hypothesis we first investigated how yellow-legged gull (*Larus michaellis*) parents feed offspring in relation to each component of complex begging behaviour and if feeding behaviour vary between sexes. Then we investigated the effect of yolk testosterone on chicks' begging by experimentally increasing yolk testosterone levels. Our results revealed that yolk testosterone has a component-specific effect on chicks' begging, specifically increasing the number of chatter calls. Parental feeding effort was influenced by the number of chatter calls emitted by chicks, but most importantly, the influence was stronger in male than in female parents. Moreover, chick body mass increased with the number of paternal feeds. In conclusion, these results show that female gulls may use yolk testosterone deposition to exploit their partners as predicted by the 'Manipulating Androgen Hypothesis (MAH)'.

5. **Title of the study:** Vitamins, stress and growth: the availability of antioxidants in early life influences the expression of cryptic genetic variation

**Status:** Manuscript published. *Journal of Evolutionary Biology* (2013), 26, 1341-1352

**Authors:** Sin-Yeon Kim, Jose C. Noguera & Alberto Velando

*Summary*

Environmental inputs during early development can shape the expression of phenotypes, which has long-lasting consequences in physiology and life history of an organism. Here, we study whether experimentally manipulated availability of dietary antioxidants, vitamins C and E, influences the expression of genetic variance for antioxidant defence, endocrine signal and body mass in yellow-legged gull chicks using quantitative genetic models based on full siblings. Our experimental study in a natural population reveals that the expression of genetic variance in total antioxidant capacity in plasma increased in chicks supplemented with vitamins C and E despite the negligible effects on the average phenotype. This suggests that individuals differ in their ability to capture and transport dietary antioxidants or to respond to these extra resources, and importantly, this ability has a genetic basis. Corticosterone level in plasma and body mass were negatively correlated at the phenotypic level. Significant genetic variance of corticosterone level appeared only in control chicks nonsupplemented with vitamins, suggesting that the genetic variation of endocrine system, which transmits environmental cues to adaptively control chick development, appeared in stressful conditions (i.e. poor antioxidant availability). Therefore, environmental inputs may shape evolutionary trajectories of antioxidant capacity and endocrine system by affecting the expression of cryptic genetic variation.

6. **Title of the study:** Begging response of gull chicks to the red spot on the parental bill

**Status:** Manuscript published. *Animal Behaviour* (2013), 85, 1359-1366.

**Authors:** Alberto Velando, Sin-Yeon Kim & Jose C. Noguera

*Summary*

In some animals, offspring begging is elicited by parents through behavioural or morphological signals. The red spot on the lower mandible in adult gulls is one of the best-known examples of a signal triggering chick begging. We examined whether the begging response of chicks (pecking for food and the chatter call for drawing parental attention) was affected by the spot size within the natural range of variation on a dummy head. Using a cross-fostering experiment, we examined whether these responses covary with the size of the genetic or social parent's spot. We found that the natural variation in size of this parental signal strongly influenced intensity of chick begging. Pecking increased when chicks were stimulated by a larger red spot. Additionally, pecking intensity increased in chicks reared by mothers with a large red spot, suggesting that this begging component is influenced by previous experience. In contrast, chick hatching order affected the number of chatter calls produced in relation to the size of the red spot on the dummy, suggesting the presence of different begging strategies according to brood hierarchy. The differential call response to a small/large red spot on the dummy was positively correlated with the original mothers' red spot size and negatively with that of the original fathers. These results suggest a genetic correlation between biased chick response for a large spot and parental signal in contrasting patterns for mothers and fathers. Our results suggest that the parental red spot and offspring begging are traits subject to coevolution.

7. **Title of the study:** When nutrition matters: Zebra finch males prefer females that were reared on a similar diet to them

**Status:** Manuscript *in prep*.

**Authors:** Jose C. Noguera., Neil B. Metcalfe & Pat Monaghan

### *Summary*

Traditional mate choice models assume that individuals should prefer mating with the highest quality partner available. However, condition-dependent models of mate choice have challenged this view showing that for low-quality individuals it may be advantageous to change the direction of their preference toward a partner of similar quality (i.e. low-quality). It is becoming clear that an important factor determining an individuals' quality is the availability of dietary micronutrients during development. Here, we investigate whether early life micronutrient availability may affect the direction of males' mating preferences. We predict that males reared under a low micronutrient diet will prefer to mate with females reared under the same regimen. Zebra finch males were reared under high or low micronutrient diets from hatching to the end of sexual maturity (90 days). Two months after the dietary treatment finished (150 days), we assessed the males' mating preferences in a standardized two-choice arena. For a period of 90 min each male was allowed to choose between two different females that only differed in the dietary treatment they had received during their development (High vs Low micronutrient diet). Two months after the preference test, half of each group of experimental males was paired with a female of the same dietary treatment than themselves (matching quality) and the other half with a female of the opposite treatment (non-matching quality). During this reproductive event, the latency to lay the first egg, clutch size and the mean egg mass was recorded for each breeding pair. We found that males reared on a high quality nutrition did not show any preference for those females that received the same dietary treatment

that them. However, when males were reared on a low quality nutrition they systematically preferred females that were reared on a similar diet to them; low-males not only spent more time affiliated to low-females but also spent more time courting and singing. Importantly, our results also revealed that such assortative mating in the low group may have important fitness benefits since low-females laid larger clutches only when they were paired with a low-male. These results support condition-dependent models of mate choice and emphasize the importance that early life nutrition may have on adult reproductive strategies.

### **Contributions to national and international conferences**

1. **Title:** The influence of early and late environmental conditions on the antioxidant system of birds.  
**Place:** The international conference of the Society for Experimental Biology. Manchester, 2014. UK.  
**Type of communication:** *Oral communication.*
2. **Title:** The influence of early and late environmental conditions on the antioxidant system of birds.  
**Place:** Mini-Symposium on Oxidative Stress. The Rank Prize Funds. Grasmere, 2014, UK.  
**Type of communication:** *Oral communication.*
3. **Title:** When nutrition matters: Zebra finch males prefer females that were reared on a similar diet to them.  
**Place:** The Scottish Conference on Animal Behaviour. Edinburgh, 2014, UK.  
**Type of communication:** *Poster communication.*
4. **Title:** Begging on the spot: preferences for parental red-spot of gull chicks.  
**Place:** The Scottish Conference on Animal Behaviour. Glasgow, 2013, UK.  
**Type of communication:** *Poster communication.*
5. **Title:** The nutritional modulation of adult personality.  
**Place:** The international Conference of Animal Behaviour. Newcastle, 2013. UK.  
**Type of communication:** *Oral communication.*
6. **Title:** The effect of age on antioxidant allocation strategies: sacrificing sperm quality for sexual attractiveness.  
**Place:** The nutritional the Scottish Ecological Ageing Research Meeting. Durham, 2012. UK.  
**Type of communication:** *Oral communication.*

### **Invited talks carried out in other institutions**

7. **Title:** Manipulating your partner: Can mothers make fathers work harder?  
**Place:** Department of Ecology. University of Elx. Elx 2013, Spain.  
**Type of communication:** *Invited talk*
8. **Title:** Days of future past: how early life nutrition affect our personality and matting strategies  
**Place:** Department of Ecology. University of Elx. Elx 2014, Spain.  
**Type of communication:** *Invited talk*
9. **Title:** Environmental conditions in early life and phenotypic development in birds

**Place:** Department of Biology. University of Bern, Bern 2013, Switzerland.

**Type of communication:** *Invited talk*

### **Teaching, lecturing and supervising tasks**

#### *Teaching*

-Guest Lecturer 'Environmental conditions in early life and phenotypic development: nutrition, physiology and behaviour'. February 2014. February 2014. G. E. course in Behavioural Ecology coordinated by Prof. Pat Monaghan. University of Glasgow. University of Glasgow.

-Guest Lecturer 'Physiological mechanisms mediating life history trade-offs: the role of oxidative stress'. February 2014. G. E. course in Behavioural Ecology coordinated by Prof. Pat Monaghan. University of Glasgow.

-Co-supervisor of Ms Laurie Glen (2014). Project title: 'The effect of nutrition in song development and quality in zebra finches'. Nuffield Science summer project. University of Glasgow.

-Co-supervisor of Mr Stewart Cowan (2013-2014). Honours project title: 'Do Male Zebra Finches Display an Assortative Mating Strategy? University of Glasgow.

-Co-supervisor of Ms Alex Ritchie (2013). Project title: 'The effect of nutrition during early development in birds'. Nuffield Science summer project. University of Glasgow.