Monitoring *Eleonora's falcon* conservation status both at its breeding and non-breeding grounds, using biological (stress indices) and environmental data

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1. Introduction

Eleonora's falcon (*Falco eleonorae* Géné, 1839; Fig. 1) is a colonial breeding raptor of the Mediterranean that overwinters in SE Africa, mainly in Madagascar. Greece hosts over 85% of its world population (estimated at 14700-15400 pairs) during the breeding season (Dimalexis et al., 2007). *F. eleonorae* is classified as "Least Concern" by the IUCN, however it is included in Annex I of the Directive 2009/147/EC on the conservation of wild birds, in Annex II of the Bern Convention and belongs to the category "SPEC 2" of BirdLife International. In the framework of the LIFE ElClimA project, an extensive eco-toxicological study was carried out in order to assess the quality of its breeding and reproductive habitants, as well as to identify significant biomarkers and tools for the assessment of its conservation status. The study was carried out on Antikythira island, which is one of *F. eleonoarae's* largest colonies worldwide (Fig. 2;) [1].

Fig 1. Eleonora's falcon (*Falco eleonorae*).

2. Materials & methods

Determination of stress indices on avian blood

2.1 Samples

- ✓ Blood samples were collected from wild birds caught at a known pond used by the falcons for bathing and drinking on Antikythira, during May (N=13) and September (N=19) 2017.
- ✓ Samples collected in May are considered indicative of the habitat quality at the species' wintering and/or staging areas, while the one collected in September are considered indicative of the habitat quality at the species' breeding grounds.

2.2.1 Determination of cellular abnormalities –MN assay

- ✓ A total number of 1000 erythrocyte per bird per smear, stained with 5% Giemsa (N=2 per slide) was examined **[2].**
- ✓ .Nuclear and cytoplasmic abnormalities: Binucleated cell-BN, Lobed nucleusl-LB, Notched nucleus-NT, Nuclear bud-Nbu, Nucleoplasmic bridge-Nbr, Vacuolated nucleus-VN, Nuclear tails-NTls, Micronucleus-MN. Echinocytes-EC, Acanthocytes-AC, Notched cell-NC, Vacuolated cytoplasm-VC, Microcyte-MC.

2.2.2 Measurements of enzyme activity (CHEs, AChEs, BChEs)

- ✓ Enzyme activity was determined in triplicate, according to the Ellman's method [3] adapted to 96-well spectrophotometric plate, [4].

Study area

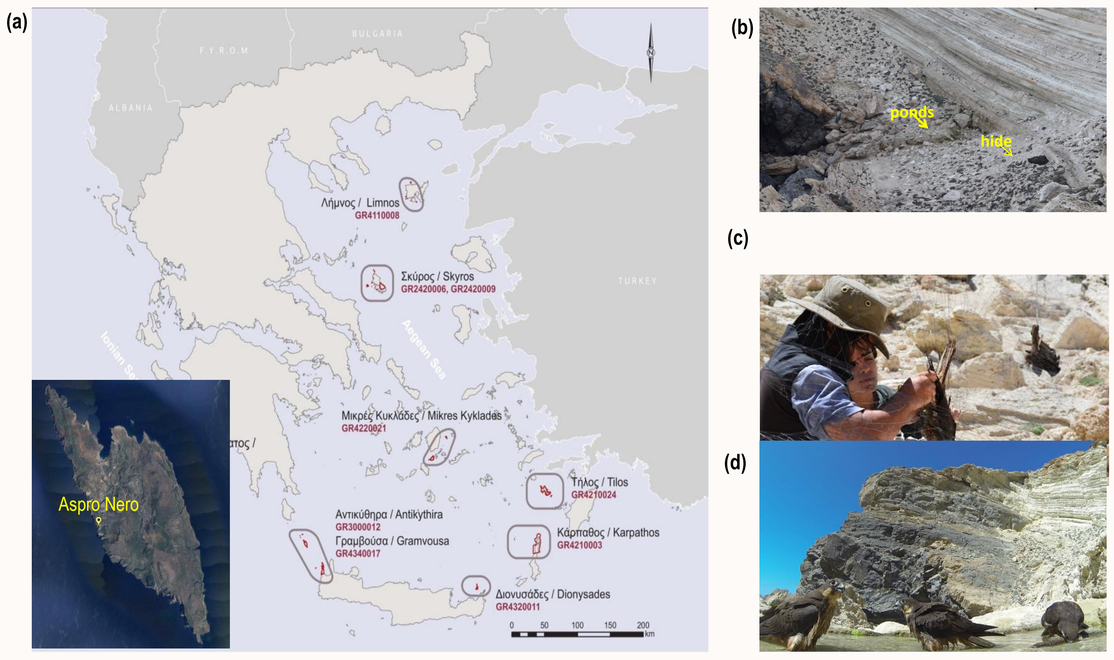


Figure 2. (a) Antikythira Island. One of the seven study areas of the LIFE ElClima project. (b)Capturing site from the above (© C.Kassara/UOP) (c) trapping *F. eleonorae* (© P.Rosta/ABO HOS), (d) Eleonora's falcons drinking and bathing in the ponds (© P.Rosta/ABO HOS)

Determination of heavy metals

2.3.1 Investigation of drinking water's quality in the breeding area:

- ✓ Samples from the pond were collected in September 2017 (Fig 2b-c).
- ✓ Cu, Cd, Cr and Pb levels were determined with the use of a Perkin Elmer Analyst 300 Atomic Absorption spectrometer (AAS), equipped with a graphite furnace and air-acetylene flame after digestion with HNO_3 .

2.3.2 Estimation of heavy metals in liver of *F. eleonorae*

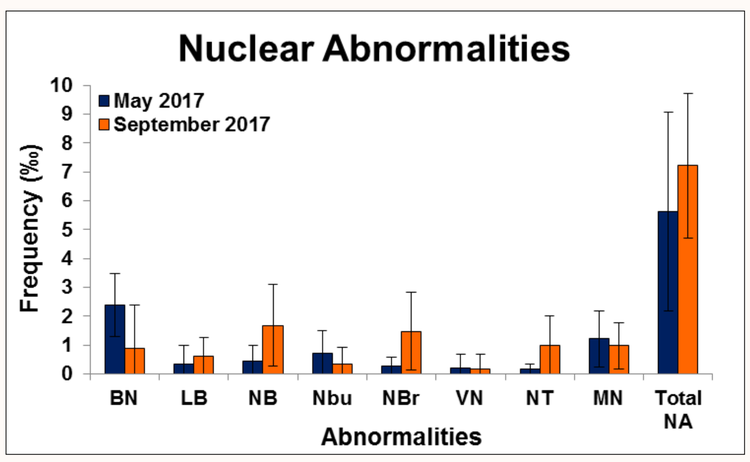
- ✓ Metal analysis was carried out as described above on the liver of a falcon encountered dead near the pond.

Statistical analysis

2.4 Statistical analysis

- ✓ Non Parametric Analysis ($p<0.05$), SPSS IBM.

3. Results and Discussion



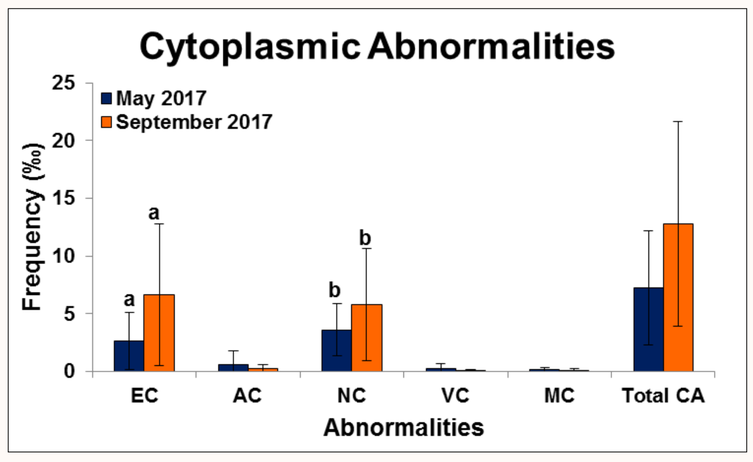


Fig. 3a-b. Mean nuclear (a) and cytoplasmic abnormalities during May) and --September 2017.

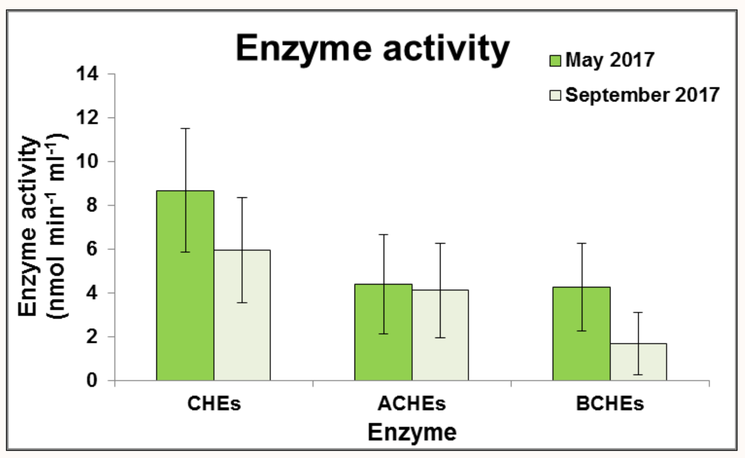


Fig. 4. Total enzyme activities during May and --September 2017.

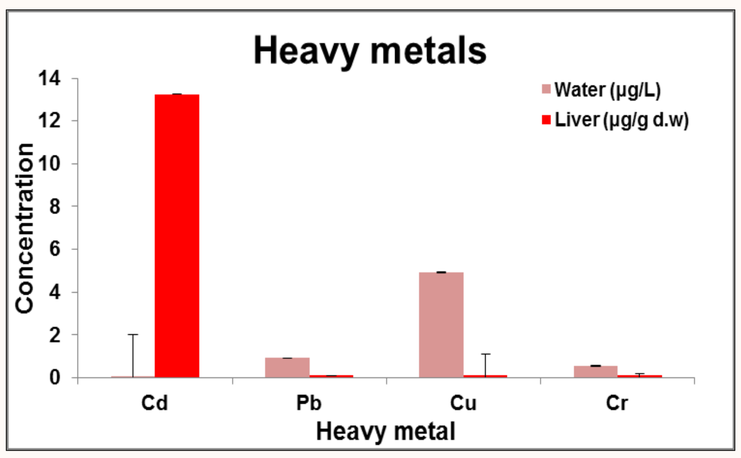


Fig. 5. Heavy metals levels during May and -September 2017.

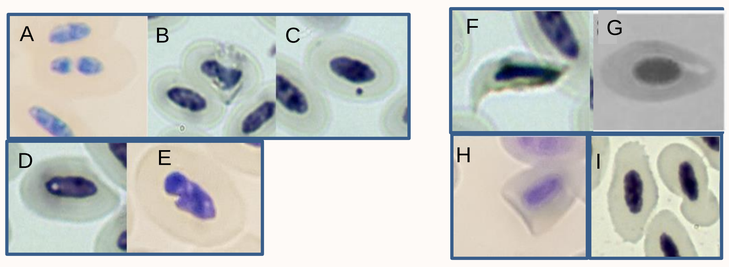


Fig. 4. Main nuclear A) BN (B) LB (C) MN (D) VN (E) NT and cytoplasmic abnormalities (F) AC (G) VC (H) NC (I) EC.

3.1 Investigation of cellular abnormalities

- ✓ More cytoplasmic abnormalities were observed in September than in May (Fig. 3B), with echinocytes frequency exceeding the normal levels of 1‰.
- ✓ Total NA appeared to be similar in both periods, however higher BN cell frequency was detected in May and higher NB and NBr cell frequency was observed in September.
- ✓ Mean MN values in all samples were within the limits of normal levels. However individual cases of elevated MN have been observed.

3. 2 Estimation of enzyme activity (CHEs, AChEs, BChEs)

- ✓ Total plasma ChE activity lower than enzyme activity estimated in other avian species, probably due to interspecies variations.
- ✓ , AChE levels are usually lower than BChE in avian species [5] .Maul and Farris, 2004), however increased AChE activity compared to BChE has been observed in species of the family of Falconidae [6].
- ✓ AChE activity constant between the two sampling periods.
- ✓ BChE activity in May was significantly higher than September.

3. 3 Heavy metals in water and liver samples

- ✓ Cr and Cu concentration in water samples were found to be within natural limits, while Cd and Pb concentrations were lower than the MAC-EQS [7].
- ✓ All heavy metals levels measured in liver samples were found to be within acceptable limits [8]. Although Cd concentration seemed to be elevated compared to other metals, it did not exceed the threshold value of adverse effect in avian species [9].

Conclusions

❖Overall, the preliminary results of the present study indicate a rather good habitat quality at both the species' breeding and wintering grounds.

❖Since no difference have been observed among individuals of different sex or age class (class 5 & 6), variations between the two sampling periods could be attributed to either seasonal variations or to other factors, such as the reproductive phase and the nutrition status.

❖Alterations due to external factors, such as the exposure to chemical compounds, could not be excluded.

❖Stress indices used in this study have proved a valuable tool for assessing Eleonora's falcon health status and consequently the impact of different factors, such as the land use on it.

❖However, given the low sample size analyzed herein and the lack of reference values from previous studies, the present data are considered useful baseline information, while more field surveys will be conducted in the future to elucidate the impact of any internal and/or external factors.

Acknowledgements
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