Temperature variation induces neurotoxicity in *Danio rerio*

Grassi Scalvini F. [1], **Nonnis S.** [1,2], **Maffioli E.** [1], **Tedeschi G.** [1,2], **Fazzina M.** [3], **Frabetti F.** [4], **Cioni C.** [5], **Toni M.** [5]

[1]Dept. of Veterinary Medicine and Animal Science, University of Milano, Milano, Italy, [2]CRC "Innovation for Well-being and Environment" (I-WE), University of Milano, Milano, Italy, [3]Dept. for Life Quality Studies - QUVI, University of Bologna, Rimini, Italy, [4]Dept. of Medical and Surgical Science, University of Bologna, Italy, [5]Dept. of Biology and Biotechnologies "Charles Darwin", Sapienza University, Rome, Italy

Background

Neurotoxicity occurs when exposure to toxic substances or environmental factors alters the normal activity of the nervous system [1]. Our studies demonstrated that environmental temperature variation heavily alters brain proteome and behaviour in adult zebrafish [2-5]. The present results suggest that the molecular and behavioural effects generated by temperature variation can be framed in the context of neurotoxicity.

Aim & Methods

To evaluate whether thermal variation induces neurotoxicity in zebrafish, we extrapolated from our previous works all the data listing the proteins differentially expressed (either increased, decreased or exclusively expressed in one single conditions) in the comparisons: 18°C vs 26°C (kept as a control) and 34°C vs 26°C upon acute [4] and chronic exposure [5], BDNF+/− (HT) vs wild type (WT) and BDNF−/− (KO) vs WT at 26°C and 34°C [3]. A total of eight data set were obtained and a tox analysis was conducted by IPA setting a significance level of $p \leq 0.05$.

Results

	18°C vs 26°C Acute treatment	18°C vs 26°C Chronic treatment	34°C vs 26°C Acute treatment	34°C vs 26°C Chronic treatment	WT at 34°C vs WT at 26°C Chronic treatment	HT at 26°C vs WT at 26°C Chronic treatment	KO at 26°C vs WT 26°C Chronic treatment	HT 34°C vs WT 34°C Chronic treatment	KO at 34°C vs WT at 34°C Chronic treatment
Time immobile	-	NA	-	NA	↓	↓	↓	-	-
Average speed	↓	↓	-	↑	↑	-	↑	-	-
Maximum speed	-	NA	-	NA	-	-	-	-	-
Total distance travelled	↓	↓	-	↑	↑	-	↑	-	-
Meandering	-	NA	-	NA	-	-	↓	-	-
Transition	↓	NA	-	↑	↑	-	↑	-	-
Transition to top area	↓	NA	-	↑	-	-	↑	-	-
Time in top areas	↓	NA	-	↑	-	-	↑	-	-
Distance travelled in top area	↓	NA	-	↑	↑	-	↑	-	-
Latency to enter the top area	↑	NA	-	↓	↓	-	↓	-	-
Time spent in dark area	↑	NA	-	↓	↓	↓	↓	-	-
Transitions	↓	NA	-	-	-	-	-	-	-
Transitions to social area	↓	NA	-	-	-	-	-	-	-
Time spent in social zone		NA		↓	↓				
Distance travelled in social area	-	NA	-	↓	↓	-	-	-	-
Mirror approach zone entries	↓	NA	-	-	-	-	-	-	-
Mirror approach zone time spent	-	NA	-	-	-	-	-	-	-
Mirror bites	-	NA	-	-	↓	↓	↓	-	-
Mirror biting latency	-	NA	-	↓	-	-	↑	-	-

	18°C Acute treatment	18°C Chronic treatment	34°C Acute treatment	34°C Chronic treatment	WT 34°C Chronic treatment	HT 26°C Chronic treatment	KO 26°C Chronic treatment	HT 34°C Chronic treatment	KO 34°C Chronic treatment
Entries in N arm (%)	-	↑	-	-	-	-	-	-	-
Time spent in N arm (%)	-	↑	-	-	-	-	-	-	-

Figure 1

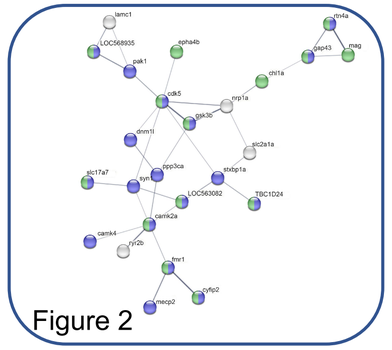


Figure 2

18°C vs 26°C ACUTE	
Generalized seizures	Increased
Coordination	Decreased

18°C vs 26°C CHRONIC	
Generalized seizures	Increased
Coordination	Decreased

34°C vs 26°C ACUTE	
MorphoGenesis of nervous tissue	Decreased
NeuritoGenesis	Decreased
Transport of synaptic vesicles	Increased
Endocytosis of synaptic vesicles	Increased

34°C vs 26°C CHRONIC	
Environmentally induced seizure	Increased
Degeneration of neurons	Increased
Seizures	Increased
Myoclonus	Increased
Tonic-clonic seizure	Increased
Generalized seizures	Increased
Coordination	Decreased

Mutant_BDNF_HT26°C vs WT26°C	
Movement Disorders	Increased

Mutant_BDNF_KO26°C vs WT26°C	
Brain tumor	Decreased
Brain lesion	Decreased
Motor dysfunction or movement disorder	Increased
Convulsion	Increased
Movement Disorders	Increased
Tonic-clonic seizure	Increased
Ataxia	Increased
Seizure disorder	Increased
Seizures	Increased
Generalized seizures	Increased
Coordination	Decreased
Proliferation of neuronal cells	Decreased
Growth of neurites	Decreased
Developmental process of synapse	Decreased

MUTANTE_BDNF_HT34°C vs WT34°C	
Seizure disorder	Increased
Seizures	Increased

MUTANTE_BDNF_KO34°C vs WT34°C	
Abnormality of cerebral cortex	Increased
Tonic-clonic seizure	Increased
Convulsion	Increased
Degeneration of nervous system	Increased
Neurodegeneration	Increased
Motor dysfunction or movement disorder	Increased
Congenital encephalopathy	Increased
Tremor	Increased
Ataxia	Increased
Early-onset encephalopathy	Increased
Early-onset neurological disorder	Increased
Movement Disorders	Increased
Congenital neurological disorder	Increased
Generalized seizures	Increased
Seizures	Increased
Seizure disorder	Increased
Cell viability of nervous tissue cell lines	Decreased
Developmental process of synapse	Decreased
Endocytosis of synaptic vesicles	Decreased
Coordination	Decreased
Formation of brain	Decreased
Proliferation of neuronal cells	Decreased
Growth of neurites	Decreased

Figure3

Thermal variation or reduced expression of BDNF alter adult zebrafish behaviour [1-4] (Fig. 1). The association network analysis of proteins common to all data sets with occurrence ≥ 20% by STRING shows the altered expression of proteins involved in synapsis (blue) and cell projections (green) (Fig. 2). Neurotoxicity related results in terms of pathways, networks and genes among the top 5 most enriched terms found by IPA in each dataset shown in Fig. 3 highlight the neurotoxic effect of acute and chronic thermal treatments and the involvement of BDNF.

Conclusion

The temperature alteration induces neurotoxic effects in adult zebrafish resulting in the alteration of the brain proteome and behaviour. Common targets of the neurotoxic action of all analysed treatments are proteins involved in synapses and cell projections. The increased exposure to the thermal variation increases the neurotoxic effect: the acute exposure reduces morphogenesis and neuritogenesis processes determining the increase of seizure and the reduction of coordination in the chronic treatment.

References

1) Fitzgerald, J.A. et al., Environ Toxicol Chem, 2021; 40(4):989-1006. 2) Angiulli, E., et al., Sci Rep, 2020; 10(1): p. 5385; 3) Maffioli, E., et al. Int J Mol Sci, 2022; 23(10); 4) Nonnis, S., et al. Sci Rep, 2021; 11(1): p. 2521; 5) Toni, M., et al. J Proteomics, 2019; 204: p. 103396; 6) D'Agostino, Y. et al., iScience, 2022; 25(4):104054.

Acknowledgments

We thank Dr. Salvatore D'Aniello and Prof. Cristiano Bertolucci for the help in generating BDNF+/- and BDNF -/- zebrafish [6].

mattia.toni@uniroma1.it

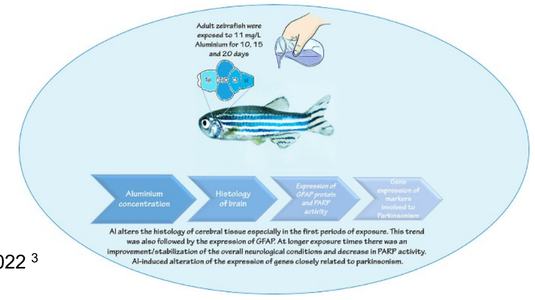
DNA DAMAGE IN THE BRAIN OF ZEBRAFISH EXPOSED TO ALUMINUM

Ferrandino I., Bianchi A. R., La Pietra A., Capriello T., Guerretti V., Lucariello D., De Maio A.

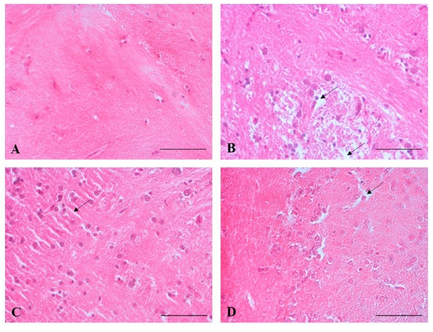
University of Naples Federico II, Department of Biology, Naples, Italy

Background

Aluminum is a widespread metal in the environment. It is harmful for human health and is associated with neurodegenerative diseases. In previous studies we demonstrated that it is toxic in zebrafish embryos[1] and in adult causes changes in the histology of brain, demyelination, neurodegeneration and poly(ADPribosyl)ation hyperactivation.[2,3] Poly(ADP-ribosyl)ation is a covalent and reversible post-translational modification of proteins, catalysed by the poly(ADPR) polymerases (PARPs), of which the activation is correlated to severe water stress resistance, pollution and is also involved in various neurodegenerative diseases.



da Capriello et al., 2022 [3]

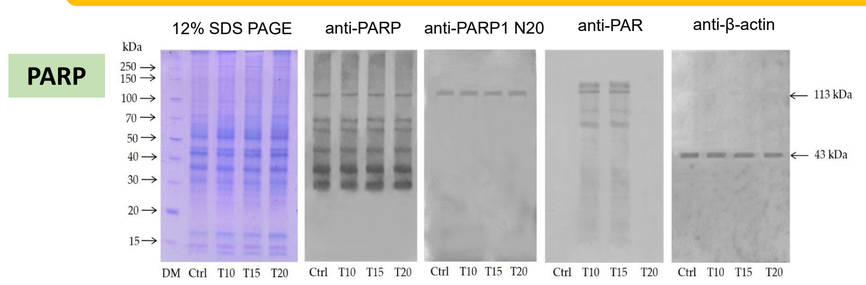


Hematoxylin-Eosin staining. A: section of the control brain. B: at 10 days of treatment, the tissue appeared altered and edematous. C: after 15 days of treatment, the tissue is still disorganized and with diffuse edema. D: section of the fish brain exposed to Al for 20 days. Scale bars: 50 µm.

Aim & Methods

Aim of this study was to investigate the effects of aluminum on the zebrafish brain and specifically on DNA damage by characterization of the polyADPribosylation system. Three groups of fish were exposed respectively for 10 (T10), 15 (T15), and 20 (T20) days to 11mg/L Al and compared with a control group (Ctrl). Three animals were used for each experimental group. SDS-PAGE, Western Blot analysis, PARP and PARG activities were performed on homogenized brains in according to *Capriello et al. 2022 and De Maio et al. 2015.* [3,4]

Results



SDS-PAGE and immunoblotting on control and T10, T15 and T20 samples.

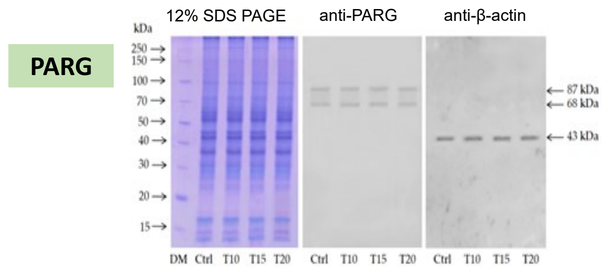


Table 1. Poly(ADPR) degradation percentage.

Zebrafish Brain	PARP activity (mU/mg)	PARG activity (mU/mg)	Poly ADPR degradation (%)
Control group	53.3 ± 4.88^{a}	0.6 ± 0.02^{a}	99
Exposed to Al for 10 days	1086 ± 27.6^{b}	48.3 ± 4.27^{b}	96
Exposed to Al for 15 days	1071 ± 25.4^{b}	24.4 ± 1.56^{c}	98
Exposed to Al for 20 days	56.5 ± 10.69^{a}	1.5 ± 0.36^{a}	97

Activity values are reported as mean ± SD. Data followed by different letters in the same column are significantly different for $p < 0.05$ using ANOVA, followed by Holm-Sidak's multiple comparisons test.

Conclusion

Expression protein analysis revealed the presence of various PARP and PARG isoforms having different molecular weight. Aluminum exposure induces genotoxic damage resulting in increased PARP activity and poly-ADPR degradation at 10 and 15 days of exposure (Table 1). At longer times (20 days), instead, the reduction of PARP activity and PAR synthesis could represent a mechanism of the neuronal cells to avoid intracellular energy consumption and ensure cell survival.

References

1. *Capriello T et al. Aquat Toxicol. 2021, 236:105872*
2. *Capriello T et al. Environ Toxicol Pharmacol. 2021, 85:103636*
3. *Capriello T. et al. Chemosphere 2022, 307:135752*
4. *De Maio et al. Current Proteomics, 2015, 253-263*

ida.ferrandino@unina.it

Exploiting zebrafish tools to evaluate biointeractions and adverse effects of silica- and copper-based nanomaterials

D'Abramo C.[1], Negrini B.[1], Mazzotta R.[1], Floris P.[1], Bonfanti P.[1], Colombo A.[1], Bragato C.[1], Mantecca P.[1]

[1]Dept. of Earth and Environmental Sciences, POLARIS Research Center, University of Milano-Bicocca, Milan, Italy.

Background

The rapid progress of nanotechnology has been facilitating the creation of a plethora of nanomaterials (NMs) with greatly enhanced functionality differing from their bulk counterparts. To guarantee a safe and sustainable development of nanotechnologies, the human and environmental (nano)toxicological outcomes of new NMs should be evaluated since the design phase, with special attention to the relationship between the NM physico-chemical (P-chem) properties and the biological responses.

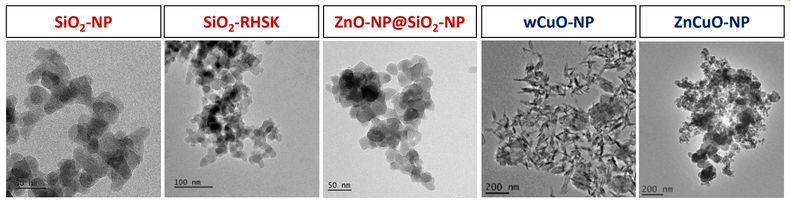
Aim & Methods

D. rerio development is proposed as a valid model to screen and compare the biological effects of SiO2- and CuO-based nanoparticles (NPs), differing for the synthesis process and surface functionalization.

Embryo toxicity: the acute toxicity assessment was made through the Fish Embryo Toxicity (FET) test, exposing fertilized zebrafish embryos at different concentrations for each NP.

Results

NPs TEM morphological investigation



Data from TEM analysis showed that all the NPs differed in size and shape

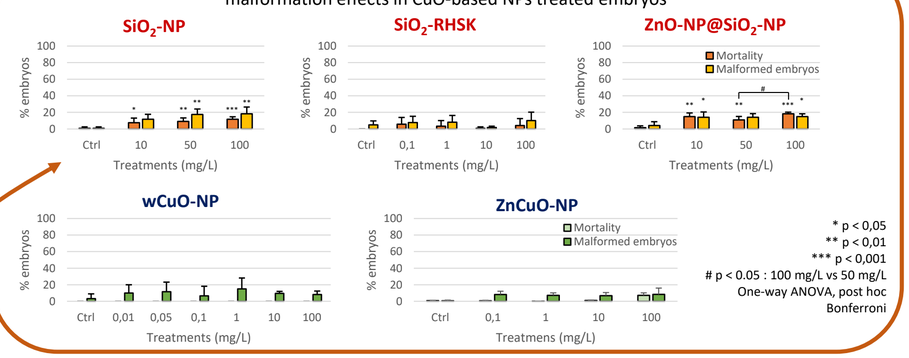
Fish Embryo acute Toxicity (FET) test (*OECD*, n. 236, 2013)

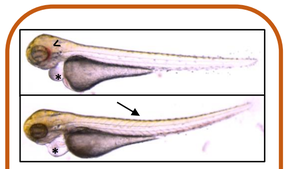
SiO_2-NP, SiO_2-RHSK, ZnO-NP@SiO_2-NP, wCuO-NP and ZnCuO-NP

Newly fertilized zebrafish embryos were exposed to different NPs concentrations until 96 hours post-fertilization (hpf). Every 24 hours, embryos were screened for lethality, in particular checking coagulation of embryos, lack of tail detachment, lack of somite formation and lack of heartbeat. Moreover sub-lethal endpoints were observed from 48 hpf

NPs exposure induced …

… very mild embriotoxic effects in SiO_2-based NPs treated embryos, no lethality and only moderate malformation effects in CuO-based NPs treated embryos





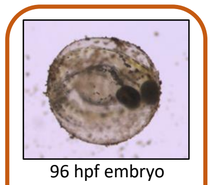
96 hpf embryos treated with ZnO-NP@SiO_2-NP

Legend:
* : edemae;
< : blood congestion;
↘ : axis defects;

… a delay in embryos hatching, especially in the wCuO-NPs and Zn-doped NPs

		Hatching							
	96 hpf EC_{50} (mg/L)	Hatching Time 50							
Treatmens		Ctrl	0,01	0,05	0,1	1	10	50	100
ZnO-NP@SiO_2-NP	73.878	68	-	-	-	-	92	97	97
SiO_2-NP	112.634	68	-	-	-	-	79	80	82
SiO_2-RHSK	n.d.	68	-	-	n.d.	n.d.	n.d.	-	n.d.
wCuO-NP	0,026	78	83	177	n.d.	n.d.	n.d.	-	n.d.
ZnCuO-NP	0,119	78	n.d.	n.d.	104	99	102	-	125

n.d. : not determined



96 hpf embryo treated with 10 mg/L wCuO-NPs

Conclusions

Zebrafish developmental features, like malformation and especially hatching rate are here demonstrated to be predictive tools to assess NMs adverse effects related to P-chem characteristics.

Acknowledgments

EU-H2020 - project BIOMAT (GA n. 953270)

ERA-NET AquaticPollutants Joint Transnational Call – project AMROCE (GA nª869178)

christian.dabramo@unimib.it

Identification of a functional predictive assay to test developmental neurotoxicity in the *Xenopus laevis* tadpole

Di Renzo F. [1], **Battistoni M.**[1], **Bacchetta R.**[1], **Metruccio F.** [2], **Menegola E.**[1]

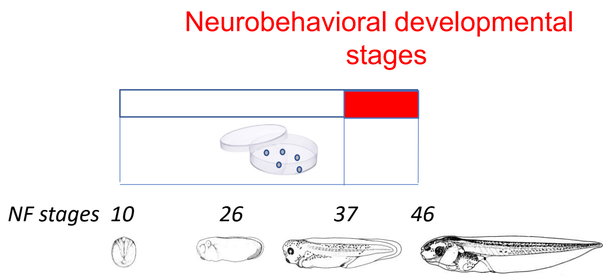
[1] Università degli Studi di Milano, Dept of Environmental Science and Policy, Milan, Italy.

[2]ASST Fatebenefratelli Sacco,ICPS,, Milan, Italy.

Background and Aim

In compliance to animal welfare 3Rs principle there is a great demand for refined tests alternative to classical mammal teratogenicity tests. We propose, using the refined alternative amphibian method (R-FETAX), a neurobehavioral test adapted to evaluate *Xenopus laevis* tadpole swimming profile.

Methods

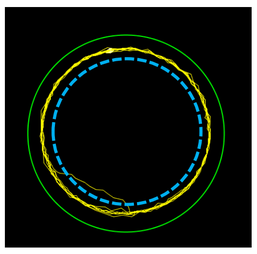


X. laevis embryos were exposed during neurobehavioral development (NF 37-46 corresponding to the spontaneous swimming acquisition period) to molecules involved in developmental neurotoxicity: antiepileptic drug VPA (500-1500 µM), 2 bisphenol-plasticizers (BPA 10-25 µM, BPB 5-10 µM).

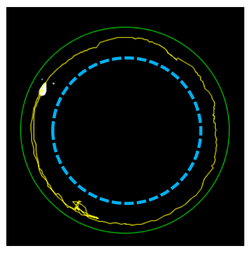
The motor behavior of the tadpoles was evaluated into a 27 mm plastic cylinder, representing the arena. After acclimation tracking was recorded (60s) using a digital camera and videos analyzed using the AnimalTracker plugin and ImageJ.

Results

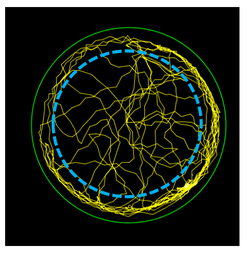
On the basis of free swimming evaluated in controls, we set the region normally not invaded by swim as "inner circles area" (0.75 internal diameter considering 1 the arena diameter). Selected parameters were: total/ outer ring/ inner circle distances, mobility time in the different sectors, time spent in the different sectors, speed.



CONT



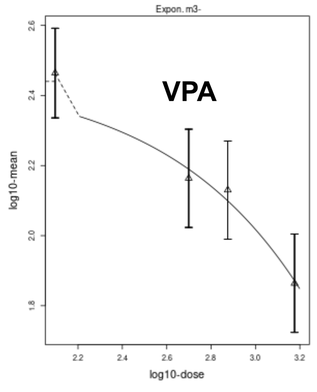
VPA1500 µM



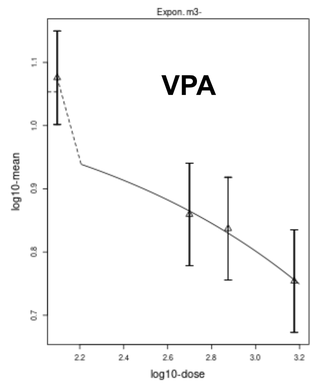
BPB 10µM

The tested molecules induced neurobehavioral deficits in a concentration-related manner:

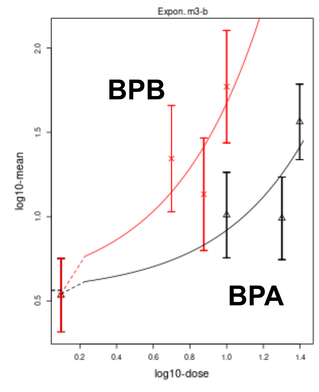
- VPA: total distance and speed reduced
- BPA and BPB: inner circle distance increased, the swimming routes altered.



VPA: reduced total distance



reduced speed



BPA and BPB:
Inner circle distance increased

Conclusion

We suggest the amphibian swimming test as a valid additional test for the evaluation of chemicals suspected to alter the neural development.

francesca.direnzo@unimi.it

CELL PROFILING OF THE EMBRYONIC INTEGUMENT OF THE MODEL FROG *Pelophylax* kl. *esculentus* BY HISTOCHEMICAL AND ULTRASTRUCTURAL METHODS

G. Scillitani[1], D. Semeraro[1], M. V. Guglielmi, D. Mentino[1], M. Mastrodonato[1]

[1]University of Bari Aldo Moro, Dep*artment of Bioscience, Biotechnology and Environment*, Bari, Italy

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

DIPARTIMENTO DI BIOSCIENZE, BIOTECNOLOGIE E AMBIENTE

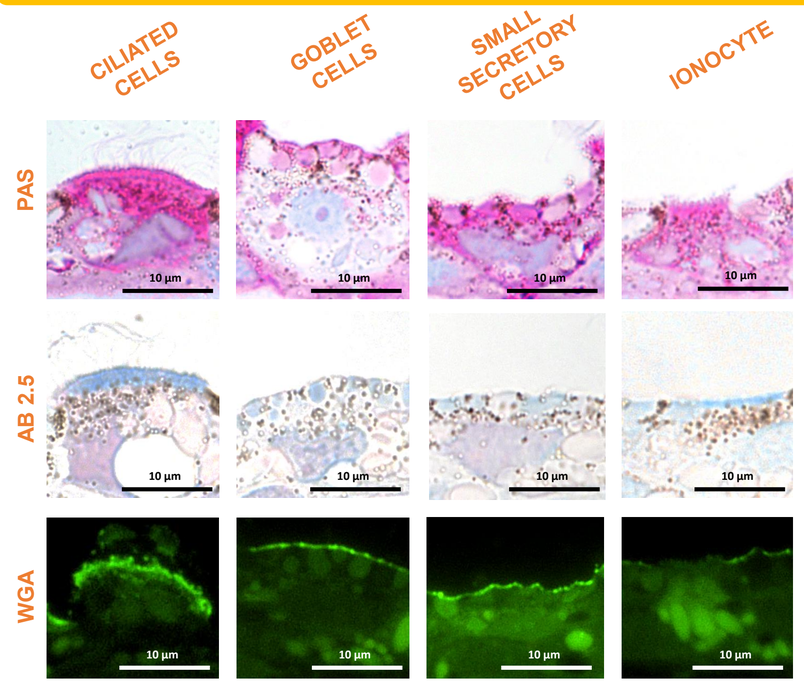
Background

The need to carry out toxicological studies to test emerging xenobiotics focuses attention on what could be the best experimental models to use. Among the amphibians, *Xenopus* is the most used, but it would be appropriate to use autochthonous species as biomarkers and bioindicators. The muco-ciliary epidermis of amphibian embryos is a direct interface system with the environment, that could be useful for conducting ecotoxicological assessments.

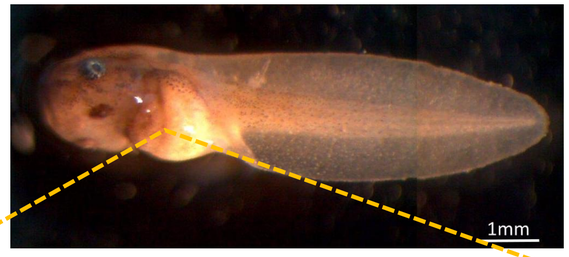
Aim & Methods

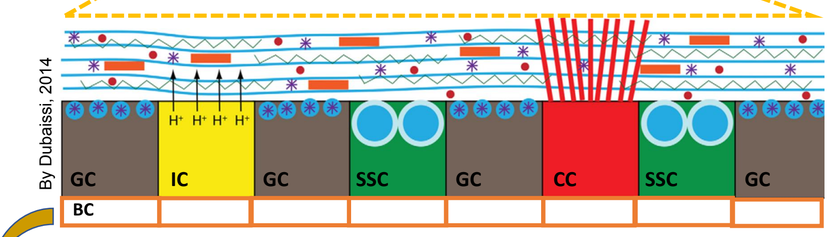
We have evaluated the embryos epidermis of *Pelophylax* kl. *esculentus* (Gosner's stage 21) as a possible model system. Some embryos were fixed in 4% paraformaldehyde and embedded in Technovit 8100 to analyze the cell types of the epithelium through histochemical investigations (PAS; AB pH 2.5, WGA lectin). Other embryos were fixed in 2.5% buffered glutaraldehyde and embedded in Epoxy Resin-Araldite for TEM, and coated with gold for SEM.

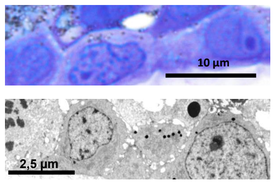
Results



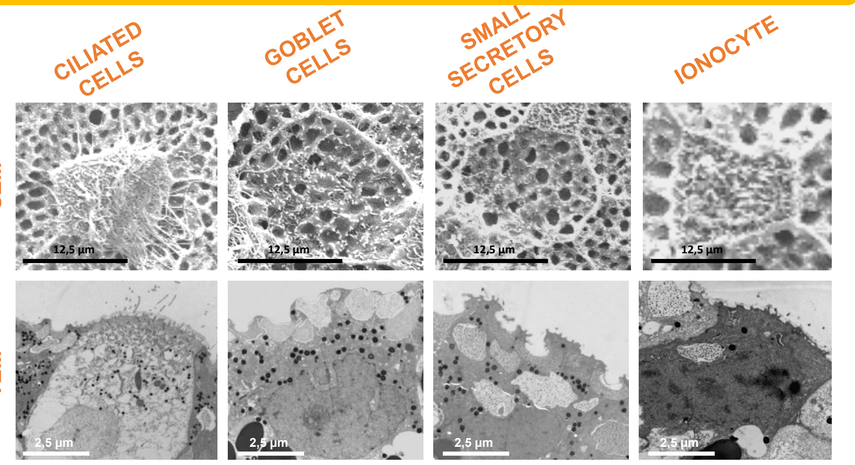
The cells of the embryonic epithelium were PAS and AB pH 2.5 positive on the apical portion. Particularly, goblet cells and small secretory cells produced secretory material with carboxylated acid glycoproteins, characterized by the abundant presence of sialic acid.







Basal cells (BC)
Toluidine Blue; TEM



SEM and TEM images showed ciliated cells (CC) with long cilia and sub-apical vesicles; goblet cells (GC) were characterised by secretory vesicles releasing mucus, small secretory cells (SSC) showed large apical vacuoles and ionocytes (IC) had numerous apical microvilli.

Conclusion

Five cell types were identified in the embryonic epidermis of *P.* kl*. esculentus*, some of which are morphologically representative of muco-ciliary epithelia, also of higher systems, such as those in the human respiratory system. Therefore, they could be useful in the evaluation of responses to toxicants.

daniela.semeraro@uniba.it

EXTRACTS FROM MARINE SPECIES MODULATE GLUCOSE UPTAKE AND CONSUMPTION BY HEPG2 CELLS

G. Abruscato, R. Tarantino, D. Ganci, A. Vizzini, M. Mauro, V. Arizza, M. Vazzana, C. Luparello

Dept. of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy

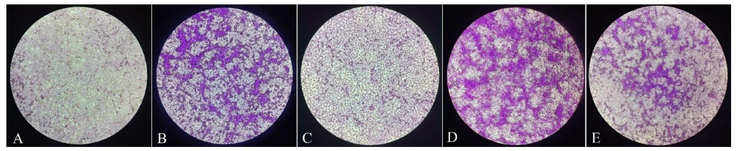
Background

The efficacy of preparations from marine invertebrates, e.g., holothurians, and plants, e.g., *Posidoniacee*, as anti-diabetic remedies is well-know from folk medicine. To get more insight into this beneficial property, HepG2 liver cancer cells which retain many differentiated hepatic functions[1] were exposed for 24 h to sublethal concentrations of aqueous extracts from coelomic fluid of *Holothuria tubulosa*[2] (CFE), or green leaves (GLE) or rhizomes (RE) of *Posidonia oceanica*[3], with or without co-treatment with 10^{-7} M insulin.

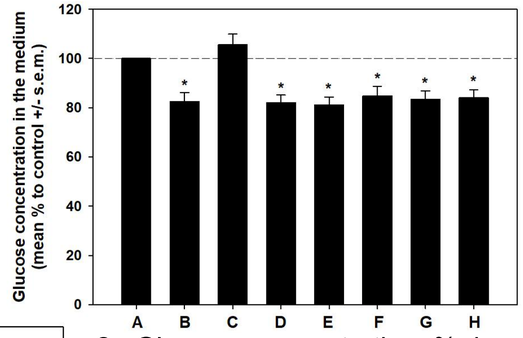
Aim & Methods

To test the potential effect of 24 h-cell exposure to CFE, GLE and RE on glucose consumption and uptake: **1**) the intracellular glycogen accumulation was detected *via* PAS staining; **2**) the consumption of glucose present in the medium was quantitated by enzymatic method; **3**) the 1h-uptake rates of the fluorescent glucose analogue 2-NBDG after exposures were evaluated by flow cytometry; **4**) the expression levels of genes involved in glucose uptake were studied *via* real time-PCR analysis.

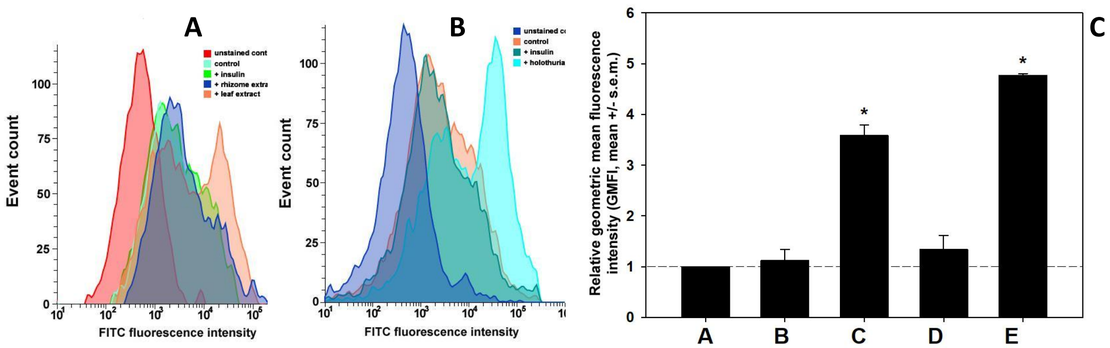
Results



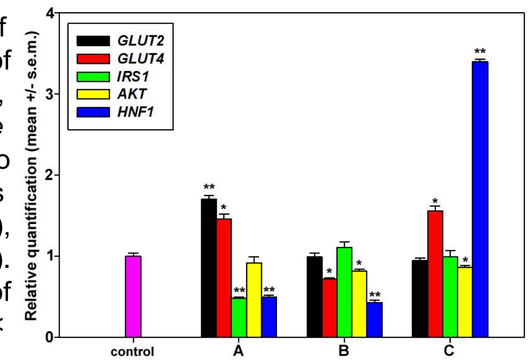
1. Representative micrographs for glycogen staining in control (A), GLE- (B), RE- (C), CFE- (D) and insulin-treated cells (E). Insulin/extract co-treated samples stained as plain insulin (not shown). PAS stain. Original magnification = 20x.



2. Glucose concentration % in the medium of cells exposed to GLE (B), RE (C), CFE (D), insulin (E), GLE + insulin (F), RE + insulin (G) and CFE + insulin (H) compared to control (A). In all conditions, except for (C), glucose consumption was stimulated. Mean ± s.e.m. of triplicate assays. *$p < 0.05$



3. Representative flow cytometric profiles showing the enhanced 2-NBDG uptake after cell treatment with GLE (panel A, pale pink peak) and CFE (panel B, turquoise peak). Panel C reports the relative 2-NBDG GMFI after cell exposure to insulin (B), GLE (C), RE (D) and CFE (E) compared to control condition (A). Mean ± s.e.m. of triplicate assays. *$p < 0.001$



4. qRT-PCR analysis of the modulation of *GLUT2*, *GLUT4*, *IRS1*, *AKT* and *HNF1 gene* expression normalized to that of *ACTB* in cells exposed to insulin (A), GLE (B) and CFE (C). Mean ± s.e.m. of triplicate assays. *$p < 0.05$; **$p < 0.001$

Conclusion

The preliminary data obtained strongly support the anti-diabetic effect of CFE and GLE, but not RE, and suggest the implication of differing molecular signalizations underlying the glucose-lowering properties of the two extracts also in comparison with insulin treatment. Further studies will examine the actual intracellular accumulation of glucose metabolism-related factors and GLUT transporters, and the rate of translocation and exposure of the latter ones on the plasma membrane, in response to CFE and GLE treatment.

References

1) *Donato MT et al. Methods Mol Biol 2015,1250:77-93.*
2) *Luparello C et al. EXCLI J 2022, 21:722-743.*
3) *Abruscato G et al. Biology (Basel) 2023, 12:616.*

claudio.luparello@unipa.it

MACROSCOPIC AND MICROSCOPIC ANALYSES TO EVALUATE THE GONADIC MATURITY OF *Engraulis encrasicolus* IN THE CAMPANIA COAST

M. Karam[1], D. Ciorciaro[1], R. Romano[1], F. Ferrigno[1-2], M. Peruzzi[1], L. Appolloni[1-2], G. F. Russo[1-2], P. Simoniello[1]

[1]Dept. of Science and Technologies, University Parthenope of Naples, Napoli, Italy;

[2] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa) Rome, URL-Centro Direzionale-Isola C4, 80143 Napoli, Italy.

Background

The Campania coast is an important spawning and nursery area for small pelagic fishes in Southern and Central Tyrrhenian Sea (GSA10). The European anchovy (***Engraulis encrasicolus)*** represents an important fishery resource for coastal communities and due to a sharp decline in catches, a specific monitoring program was developed to investigate biological and fisheries aspects of the target stock.

Aim & Methods

To assess the biological aspect of female reproductive cycle in the Gaeta, Naples and Salerno Gulfs (the main fishing areas of purse seiners in Campania), landings samples from April to July were collected and macroscopically analysed to detect the maturity stages. Ovaries were fixed in 4% formalin and processed for histological investigation to evaluate the oocyte developmental stages according to “the six-stage maturity scale”(ICES REPORT, 2008).

Preliminary Results

Fig. 1

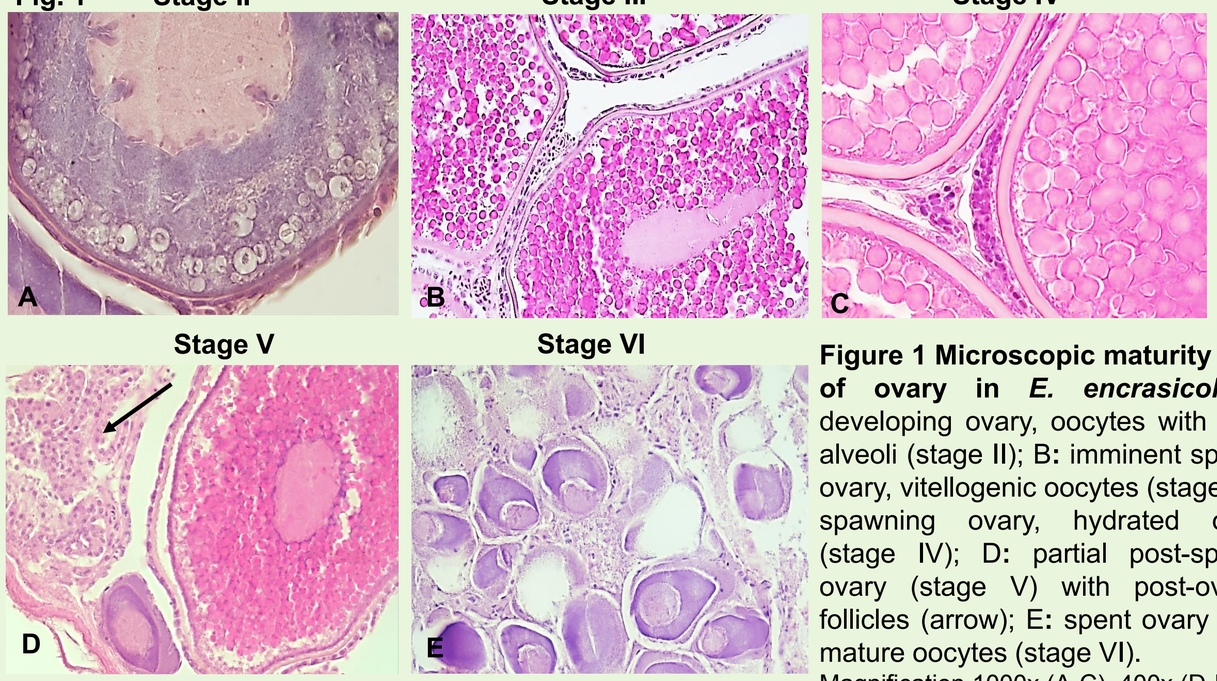


Figure 1 Microscopic maturity stages of ovary in *E. encrasicolus* A: developing ovary, oocytes with cortical alveoli (stage II); B: imminent spawning ovary, vitellogenic oocytes (stage III); C: spawning ovary, hydrated oocytes (stage IV); D: partial post-spawning ovary (stage V) with post-ovulatory follicles (arrow); E: spent ovary without mature oocytes (stage VI).
Magnification 1000x (A-C), 400x (D-E).

Fig. 2a

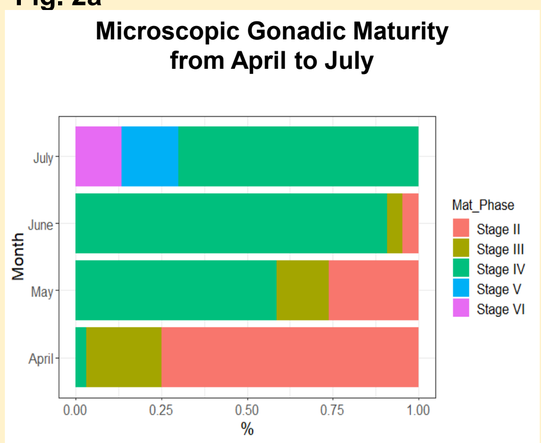


Fig. 2b

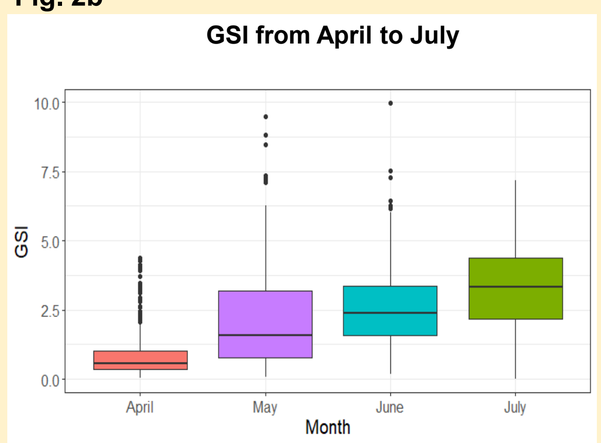


Figure 2a Percentage of Anchovy specimens for each Maturity Stage evaluated by microscopic analysis from April to July**.**

Figure 2b Box-Plot of *E. encrasicolus* Gonado Somatic Index (GSI) caugth in the period from April to July;
GSI = gonad weight/(total weight - gonad weight)*100.

Conclusion

The preliminary analysis on microscopic gonadic maturity showed that in June whole of the specimens were in active spawning (stage IV) while in July the level of active spawners were reduced of 20%. Further investigations are ongoing to establish the batch fecundity, the length at first maturity (L50) and the age at length key. The characterization of these aspects is essential to improve new approaches for the management of fisheries resources also based on variability in spatial distribution of target stock.

myriam.karam001@studenti.uniparthenope.it

HISTOLOGICAL ANALYSIS TO INVESTIGATE THE EFFECTS ON HEALTHY MUSCLE TISSUE AFTER IRRADIATION WITH FLASH THERAPY IN A MURINE MODEL

P. Simoniello [1], S. Belardo [1], U. Weber[2], A. Puspitasari[2], A. Abdollahi[3], C. Schuy[2], M. Durante [2], W. Tinganelli [2]

[1] Dept. of Science and Technology of University Parthenope, Napoli, Italy; [2] GSI Helmholtzzentrum für Schwerionenforschung, Biophysics Department, Darmstadt, Germany; [3]Clinical Cooperation Unit Radiation Oncology, Heidelberg Institute of Radiation Oncology, National Center for Tumor Diseases, Heidelberg University and German Cancer Research Center (DKFZ), Heidelberg, Germany.

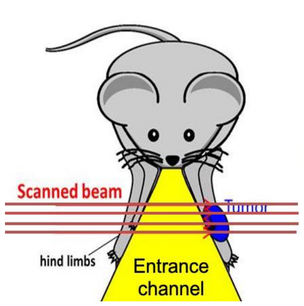
Background

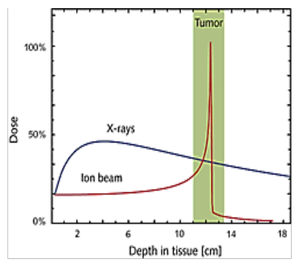
Radiotherapy is the main treatment for cancer diseases. However, the efficacy of radiation treatment has a limitation due to the toxicity in the surrounding normal healthy tissue. Recent studies are considering as a new strategy to treat cancer sparing normal tissue, FLASH therapy that use ultra-high dose rates radiation delivery[1-2].

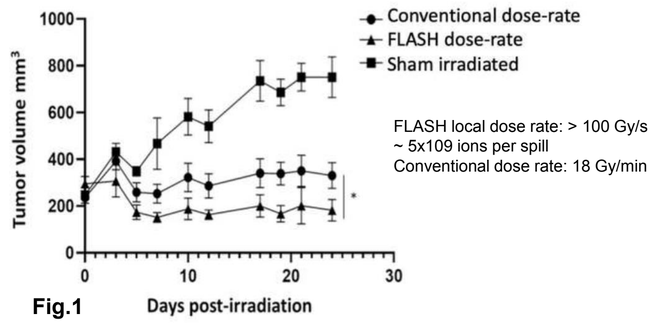
Aim & Methods

In this work we studied the high-energy ^{12}C-ions delivered at an ultra-high dose rate in mouse as model system (Fig.1). Mouse osteosarcoma LM8 cells were injected in the posterior limb of mice, then divided into three groups: FLASH dose-rate, conventional dose-rate, and sham irradiated. Healthy muscle tissues were processed for light microscopy.

Results







FLASH local dose rate: > 100 Gy/s
~ 5x109 ions per spill
Conventional dose rate: 18 Gy/min

Fig.1

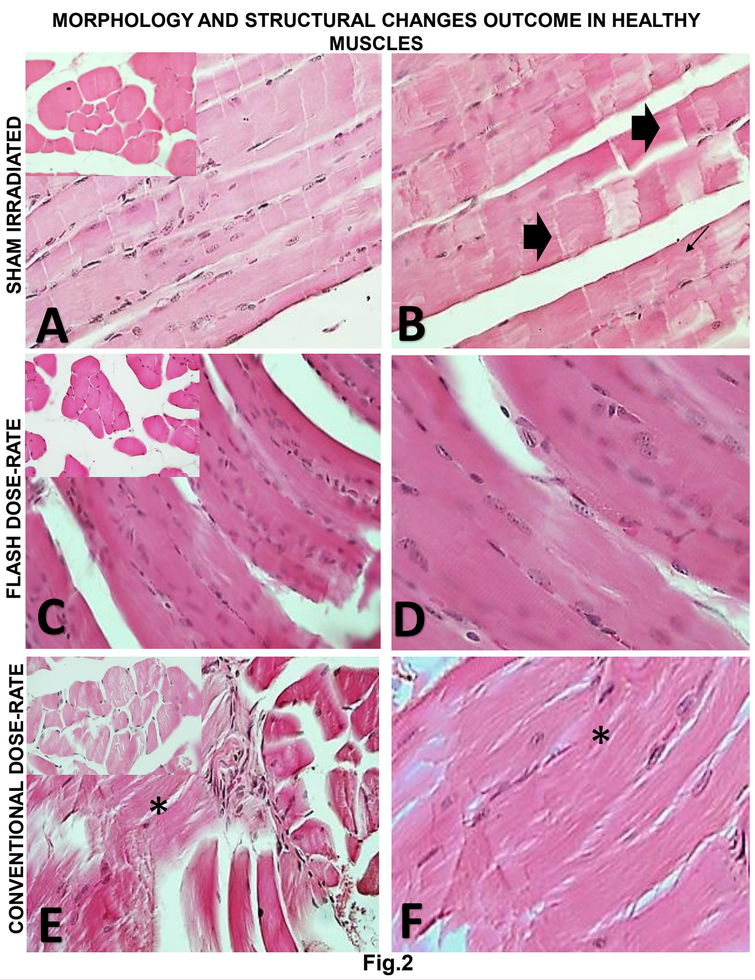


Fig.2

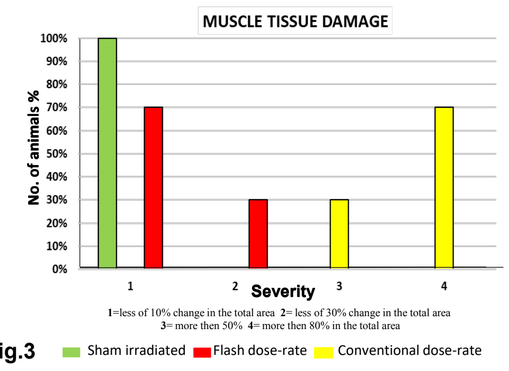


Fig.3

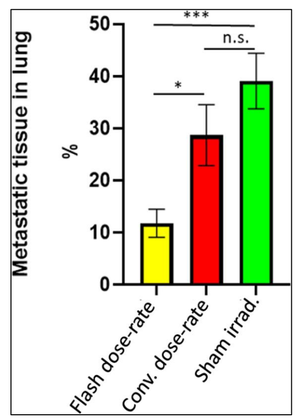
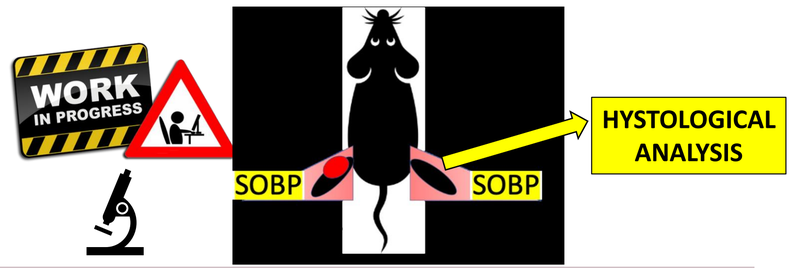


Fig.4

- Irradiation with carbon ions was able to control the tumour, both at conventional and ultra-high dose rate (Fig.1).
- FLASH decreases normal tissue toxicity, demonstrated by significant reduction of lung metastasis (Fig.4) and reduced tissue damage in healty muscle compared to the conventional dose-rate irradiation (Fig.3).
- Histological analysis (Fig. 2) shows normal structure of myofibril (A-B, arrowhead) and a stronger alteration (*) in the muscle tissue after conventional dose-rate irradiation (E-F) versus FLASH (C-D).



Conclusion

FLASH therapy could revolutionize the future of cancer treatment and widen the therapeutic window of radiotherapy.

References

1. Weber et al., Med Phys, 2022, 49(3):1974-1992
2. Vozenin et al., Nat Rev Clin Oncol, 2022, 19, 791–803

salvatore.belardo002@studenti.uniparthenope.it

IN SITU PROTEOMICS: IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS DURING MOUSE FOLLICULOGENESIS

Fiorentino G.[1], Smith A.[2], Nicora G.[3], Bellazzi R.[3], Magni F.[2], Garagna S.[1], Zuccotti M.[1]

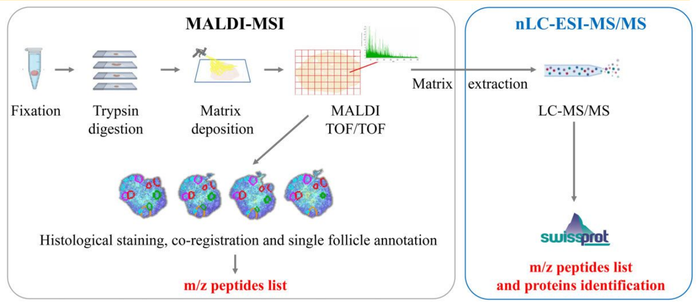
[1]University of Pavia, Department of Biology and Biotechnology, Pavia, Italy; [2]University of Milano-Bicocca, Department of Medicine and Surgery, Vedano al Lambro, Italy; [3]University of Pavia, Department of Electrical, Computer and Biomedical Engineering, Pavia, Italy

Background

Mouse folliculogenesis proceeds from primordial type 1-2 (T1-T2) to the preovulatory T8 follicle. A deeper knowledge of proteins involved in the cross-talk between oocytes and surrounding somatic cells will help our understanding of folliculogenesis.

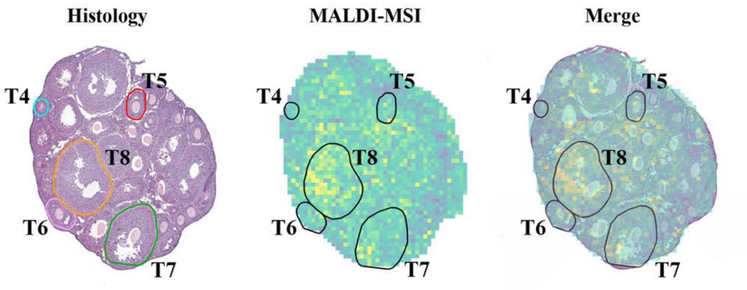
Aim & Methods

For the first time, we used *in situ* MALDI mass spectrometry imaging (MALDI-MSI) to build the proteome landscape of folliculogenesis.[1]

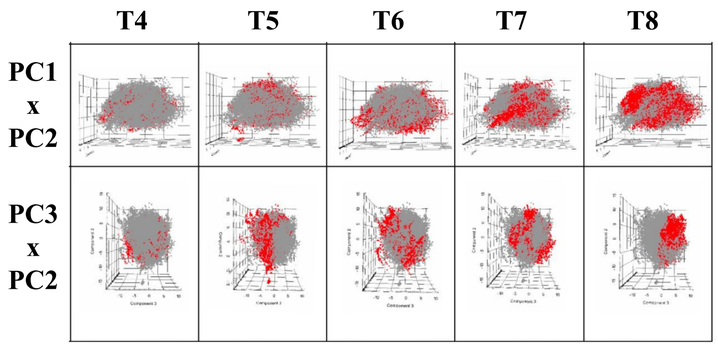


Results

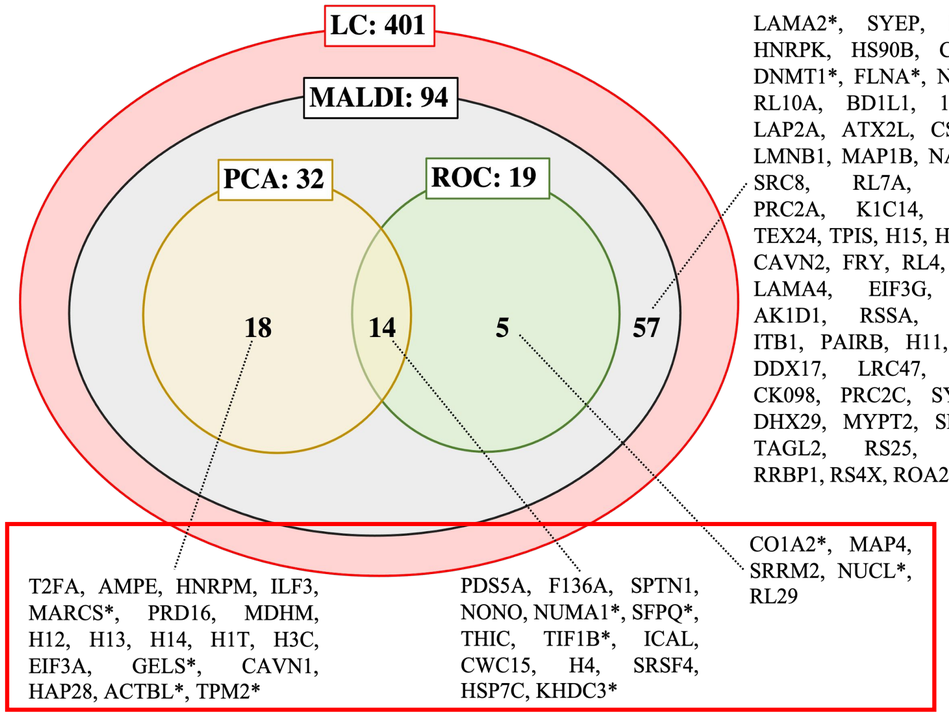
MALDI-MSI of serial ovarian sections



3D PCA on the mass spectra indicates a gradual shift in the protein profiles from T5 to T8 follicles



37 candidate proteins displaying a quantitative change during folliculogenesis



Conclusions

MALDI-MSI spatial information indicated changes during folliculogenesis of proteins with a known function and of some whose ovarian role may be subject of future studies.

References

1. Fiorentino G et al. *Mol Hum Reprod* 2023, 29:gaad006.

maurizio.zuccottii@unipv.it

Mitochondrial DNA copy number as biomarker of human and environmental health

Calogero G. S.[1], Giuga M.[1,2], D'Urso V.[1], Ferrito V. [1], Pappalardo A. M.[1]

[1]University of Catania, Department of Biological, Geological and Environmental Sciences – Section of Animal Biology "M. La Greca", Catania, Italy.

[2]Institute for the Study of Anthropic Impact and Sustainability in the Marine Environment, IAS-CNR, Trapani, Italy.

Dipartimento di Scienze Biologiche, Geologiche e Ambientali

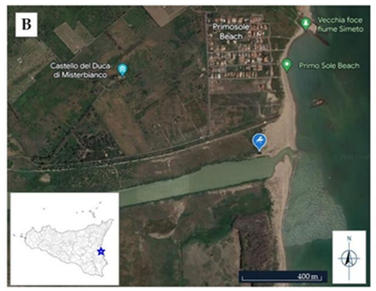
Background

Mitochondrial DNA (mtDNA) is particularly vulnerable to various types of damage due to the lack of protective histones but especially because it is in the proximity of ROS generation-sites located within OXPHOS complex I and III[1]. It has been hypothesized that an increase or decrease of the mtDNA copy number (cn) could precede the onset of mitochondrial dysfunction. The mtDNAcn has proved to be easy to use as a biomarker of mitochondrial damage and has confirmed to be also an excellent biomarker in various human pathologies [2-4] preceding the eventual collapse of mitochondrial function[5]. *Opsius heydeni is a* sap-feeder insect on *Tamarix* species, a plant classified as metal accumulator[6-8], and is a good bioindicator to study the mtDNAcn variation related to the negative anthropic impact.

Aim & Methods

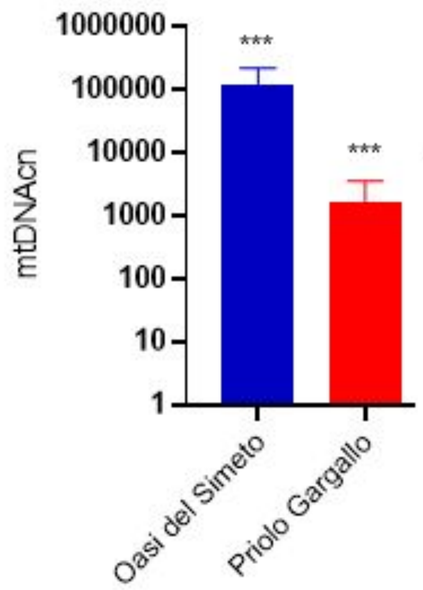
The aim of this study is to extend the use of mtDNAcn variation to determine the effects of environmental pollutants on populations of *O. heydeni.* A total of 20 samples were collected from two sites in eastern Sicily: Priolo Gargallo (polluted site) and the Simeto river Oasis (control site). The relative mtDNAcn was determined by qPCR through the comparative Ct method[9], using the mt *COI* mini barcode as target gene and the nuclear *18S* gene as reference gene.

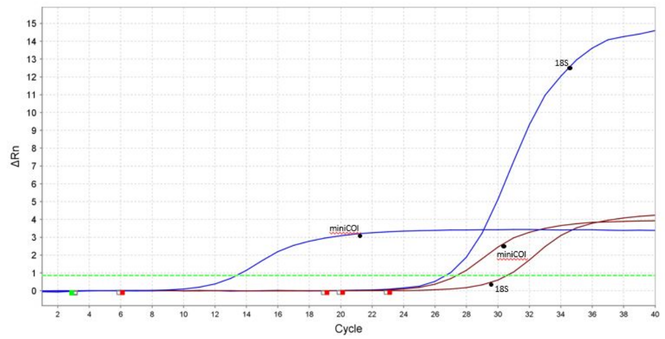
Results



A Priolo Gargallo listed as a Site of National Interest (SNI) by the Italian Ministry of the Environment in 2003.

B Oasi del Simeto control site.





Conclusion

The present study highlights the statistically significant decreasing of the mtDNA copy number in insects living in the area of Priolo Gargallo with respect to those living in the control site. These findings indicate that the mtDNAcn could be used as a biomarker of exposure to identify the genomic damage caused by pollutants in animals. As a future perspective, the early assessment of mtDNA alterations caused by pollutants should be the subject of more intensive research in the fields of health and environmental toxicology than has been done so far.

References

1, Gredilla, R. *et al*. Stevnsner, T. *Exp. Gerontol.* 2010, 45, 478–488
2. Ashar, F.N. *et a*l. *JAMA Cardiol.* 2017, 2, 1247–1255
3. Taylor, R.W. & Turnbull, D.M. *Nat. Rev. Genet*. 2005, 6, 389–402
4. Fazzini, F. *et al*. *J. Intern. Med*. 2021, 290, 190–202
5. Malik, A.N. & Czajka, A. *Mitochondrion* 2013, 13, 481–492
6. Fawzy, E.M. *et al*. *Toxicol. Environ. Chem*. 2006, 88, 603–618
7. Manousaki, E. *et al*. *Environ. Res*. 2008, 106, 326–332
8. Jeddi, K. *et al. Environ. Sci. Pollut. Res*. 2021, 28, 28598–28605
9. Hartmann, N. *et al*. *Aging Cell* 2011, 10, 824–831

giada.calogero@phd.unict.it

Exploring the epithelial-to-mesenchymal transition in breast cancer by proteomic and *in silico* investigations

Peri E.[1], **Buttacavoli M.**[1], **D'Amico C.**[1], **Marino S.**[1], **Vaglica F.**[1], **Pucci-Minafra I.**[2], **Roz E.**[3], **Feo S.**[1,2], **Cancemi P.**[1,2]

[1]University of Palermo, STEBICEF Department, Palermo, Italy [2]Experimental Center of Oncobiology (COBS), Palermo, Italy
[3]Oncological Hospital La Maddalena, Palermo, Italy

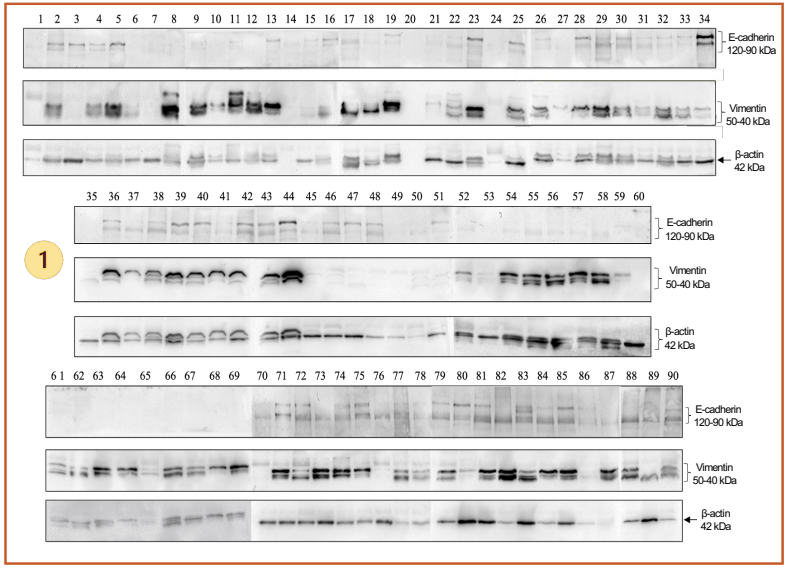
Background

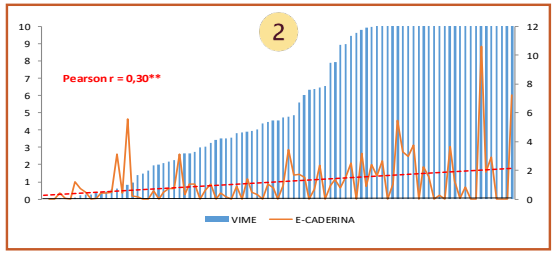
The epithelial-to-mesenchymal transition (EMT) is a biological process in which the epithelial cells lose their polarized phenotype to gain mesenchymal portraits. Essential in embryogenesis and wound healing process, the EMT also plays key roles in breast cancer (BC) progression, enabling cancer cells to acquire invasive and metastatic behaviour and enhancing cell survival[1,2].

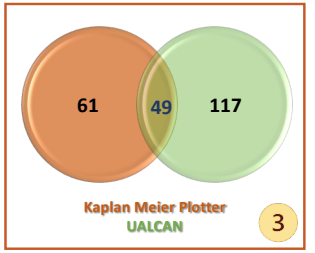
Aim & Methods

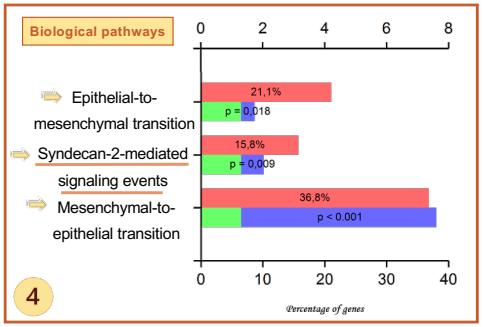
In this study, a combination of proteomic and *in silico* investigations was carried out to investigate and better understand the complexity of EMT in BC. Firstly, profiling of Vimentin and E-cadherin expression, two master genes of the mesenchymal and epithelial phenotypes, was performed in 90 BC tissues by western blotting. Then, the biological connectivity of the EMT-related gene signature was evaluated by a bioinformatic approach.

Results









(1) Western blot analysis performed on 90 BC tissues;

(2) Histogram of Vimentin and E-cadherin expression levels among patients (Pearson r = 0.30);

(3) Venn diagram showing the 49 EMT-genes differentially expressed between normal and BC tissues and significantly associated with prognosis;

(4) Biological pathway enrichment of the 49 EMT-genes obtained using FunRich platform.

Conclusion

In conclusion, the obtained results suggest that the EMT in BC could be more complex than previously assumed and influence the cross talk between cancer cells and extracellular matrix. Additional studies are required to disclose the interconnection between EMT and proteoglycans, focusing on the role of Syndecan-2.

References

1. Brabletz S The EMBO journal 2021 40(18), e108647
2. Liu F Oncology letters 2016 12(6), 4869-4876

emanuela.peri@unipa.it

TERATOGENIC EFFECTS OF DIMETHOATE ON EMBRYONATED EGGS *GALLUS GALLUS*

Salvaggio A.[1] , Scalisi E.M.[2], Pecoraro R.[2], Indelicato S.[2], Giuffrida G.[2], Coppa F.[2], Coco G.[2], Brundo M.V.[2]

[1]Experimental Zooprophylactic Institute of Sicily "A. Mirri", Palermo, Italy

[2]Department of Biological, Geological and Environmental Science, University of Catania, Catania, Italy

Background

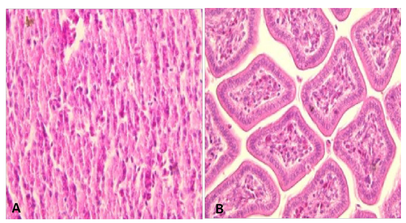
Pesticides are useful to control any type of organism that attack and damage crops, as consequence their intensive use has raised considerable questions in the scientific field. Several studies have shown that the exposure to pesticides is a risk to the health of farmers, children and the environment[1]. Among pesticides, the organophosphates are anticholinesterase insecticides widely used in agriculture.

Aim & Methods

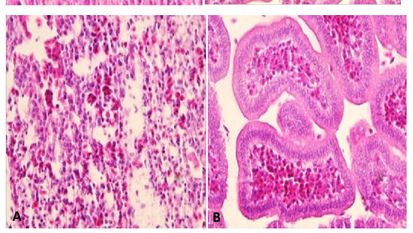
Embryonated eggs of *Gallus gallus domesticus* were used to evaluate the toxicity of the Dimethoate, acetylcholinesterase inhibitor. A stock solution of dimethoate 0,04g/10 ml, was used to obtained the working solutions: 0,004 g/10 ml, 0,0004 g/10 ml and 0,00004 g/10 ml. The solutions were inoculated into the fertilized chick eggs by insulin syringe and placed in an incubator, we have included also control samples. On 5th, 10th and 19th day after incubation, window was made in the shell to taken the embryos, thus they were fixed for histological analyses

Results

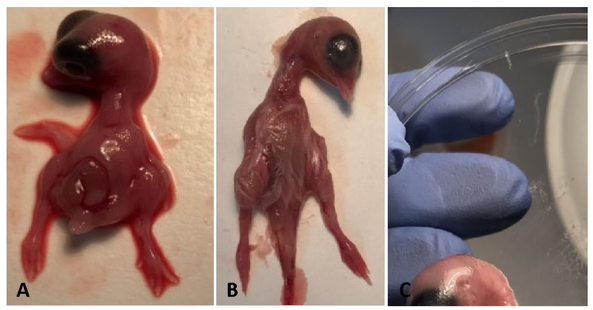
We have observed that the treated samples were viable, but showed evident anomalies such as: visceral ectopia, caudal hernia and lack of resorption of the yolk sac. The histological analyzes have highlighted alterations in the liver, intestine and lungs tissue.



Sample on 15th day ($4x10^{-3}$): A) Liver with evident perivascular extravasation. **B)** Intestinal villi with hemorrhagic areas



Sample on 15th day ($4x10^{-4}$): A) Lung with evident hemorrhage. **B)** Intestinal villi with hemorrhagic areas



Living samples: **A)** Evident visceral ectopia on 10° day ($4x10^{-4}$), **B)** On 15° day ($4x10^{-4}$), **C)** Evident caudal hernia on 15th day ($4x10^{-3}$)

Conclusion

It is evident that exposure to dimethoate leads to an increased risk of teratogenic effects during embryonic development.

References

1 Fenske RA et al. Environ Health Perspect 2022, 108,515-520

antonio.salvaggio@izssicilia.it

Discovering new lncRNAs in Zebrafish: characterization of *LOC100535512* in the developing and adult central nervous system

Fazzina M.[1], Cogliandro L.[2], Bergonzoni M.[3], Casadei R.[1] and Frabetti F.[2]

[1]Department for Life Quality Studies - QUVI, University of Bologna, Rimini, Italy

[2]Department of Medical and Surgical Sciences - DIMEC, University of Bologna, Bologna, Italy

[3]Department of Biomedical and Neuromotor Sciences - DIBINEM, University of Bologna, Bologna, Italy

Supported by FONDAZIONE CASSA DI RISPARMIO IN BOLOGNA

Background

Long non-coding RNA (lncRNAs) are transcripits longer than 200 nucleotides that are not translated into protein with regulatory functions at the transcriptional, post-transcriptional and epigenetic levels[1]. Despite lncRNAs sequence is not evolutionary conserved across different species[2], putative orhtologs can be traced in syntenic loci[3]. The analysis of genomic traits neighboring a human lncRNA, which we found probably associated with Parkinson's disease (PD)[4], led to the identification of a syntenic region on Zebrafish chromosome 17, harbouring the lncRNA *LOC100535512* as a potential orthologue.

Aim & Methods

We aim to characterize the physiological role of LOC100535512 in Zebrafish embryology and to investigate its involvement in the developing and adult central nervous system (CNS) through the following methods:

1) Sanger sequencing of LOC100535512;
2) RT-qPCR analysis of the lncRNA during Zebrafish development and in adult tissues;
3) Set-up of an in vivo model to study LOC100535512 modulation following Rotenone treatments.

Results

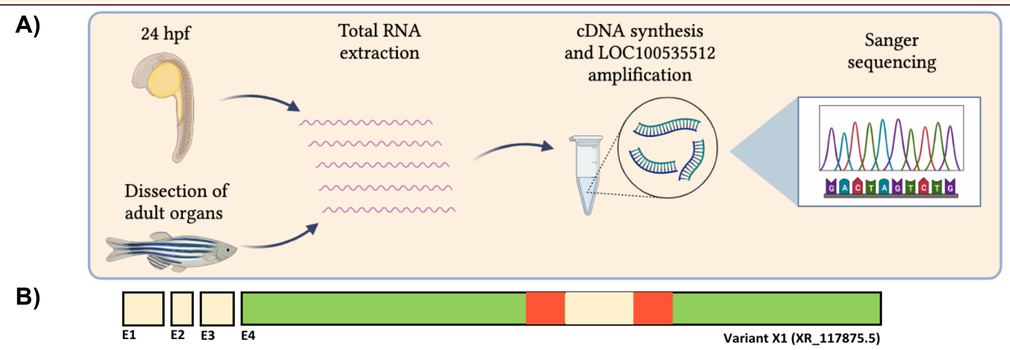


Fig 1 A) Experimental workflow of Sanger sequencing for LOC100535512. To obtain the sequence, specific primer pairs were designed based on the predicted transcript variant X1 (accession number XR_117875.5). **B)** The transcript structure with each of the 4 exons is depicted and yellow, red, and green boxes, respectively, indicate not sequenced, partially and fully sequenced portions.

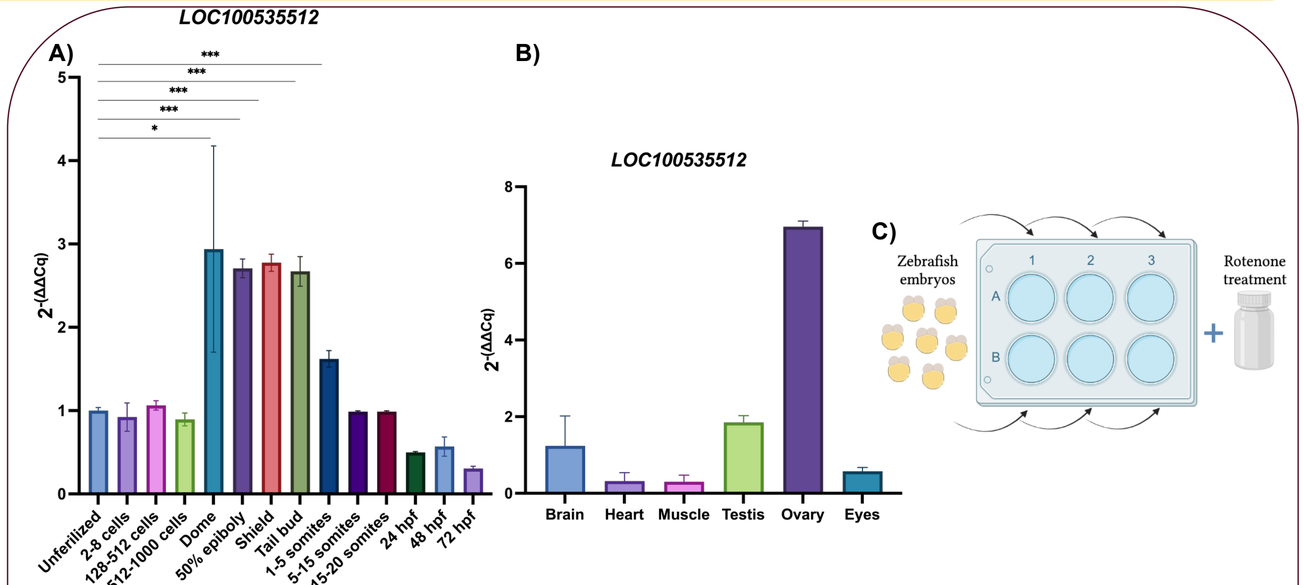


Fig. 2 A) Time course of differentially expressed LOC100535512 in Zebrafish embryos, starting from unferitlized eggs to 72 hpf (hours post-fertilization). **B)** RT-qPCR analysis in Zebrafish adult organs. **C)** Schematic representation of Rotenone treatments for subsequent gene expression studies during early development. Data referred to up three biological replicates and normalized on two reference genes (*βactin2*, *slc25a5*). Student's t-test (unpaired, two-tailed), *p<0,05; ***p<0,001 vs unfertilized eggs.

Conclusion

Our findings indicate a crucial role for *LOC100535512* during Zebrafish development, particularly in the phases when the neural plate is formed. In the adult Zebrafish brain, the lncRNA remains still highly expressed, suggesting a function for it in the neuronal regulatory circuits. It will be useful to understand the physiological role of an uncharacterized lncRNA in Zebrafish embryology and in the adult CNS and to confirm its putative orthology with a human lncRNA.

References

1. Statello L., et al. Nat Rev Mol Cell Biol 2021 Feb;22, 96–118; ***2.*** Ulitsky I. et al., Cell. 2011 Dec;23, 147(7):1537-50; ***3.*** Szcześniak MW. et al., Essays Biochem. 2021 Oct 27;65(4):741-749; ***4.*** Mariani E. et al., PLoS One. 2016, Sep 9;11(9); ***5.*** Robea MA. et al., Oxid Med Cell Longev. 2020 Aug 18;2020:1370837.

Acknowledgments

This research was supported by a grant from Fondazione Cassa di Risparmio in Bologna, Italy (cod. SIME: 2020.0388).

martina.fazzina2@unibo.it

EVALUATION OF THE REPRODUCTIVE BIOLOGY OF THE EUROPEAN SARDINE IN THE ADRIATIC SEA

Chemello G.[1], Cerrone G.[1], Tavolazzi V.[1], Donato F.[2], Tiralongo F.[3], Carnevali O.[1] and Gioacchini G.[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona (Italy). [2]Institute for Biological Resources and Marine Biotechnologies (IRBIM), National Research Council (CNR), 60125, Ancona (Italy). [3]Dipartimenti - scienze biologiche, geologiche e ambientali, Università di Catania, 95131, Catania (Italy).

Background

The European sardine (*Sardina pilchardus*) is one of the most economically and ecologically relevant small pelagic species in the Mediterranean Sea. In the Adriatic area, fluctuations in total catches have been registered over the years. The decline of stock biomass is usually the consequence of high exploitation rates combined with the variation of environmental parameters that could affect food quantity and quality and consequently influence the reproduction and health status of small pelagic stocks.

Aim & Methods

This study aimed to evaluate the current status of sardines' reproductive cycle with a particular focus on female gonads' development and maturation. Samples were collected once a month along the coast of the Marche region from April 2021 to March 2022 in collaboration with fishermen of the Ancona fleet. Ovary reproductive stage was identified through histological analysis and the gonadosomatic index (GSI) was calculated. Otolith analysis identified fish age successively related to the ovary reproductive stage.

Results

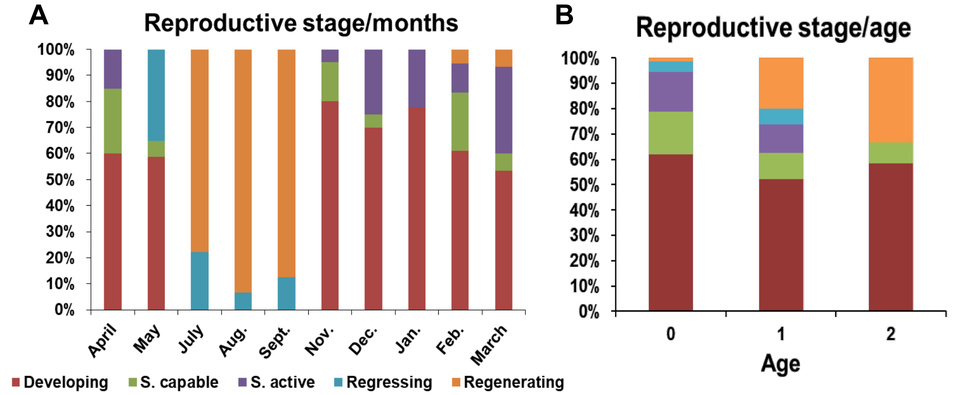


Figure 1. Ovarian reproductive stages' frequency per each sampling month (**A**) and per age (**B**). **0**, age assigned to fish that have not completed the first year; **1**, age assigned to fish between the first year and the second year; **2**, age assigned to fish between the second year and the third year.

Month	*GSI*
April	$2.72^{a,e} \pm 0.84$
May	$1.61^{b,e} \pm 0.77$
July	$\mathbf{0.50^{b} \pm 0.11}$
August	$\mathbf{0.31^{b} \pm 0.13}$
September	$\mathbf{0.62^{b} \pm 0.24}$
November	$3.40^{a,c} \pm 1.16$
December	$4.75^{c,d} \pm 1.57$
January	$5.10^{d} \pm 2.32$
February	$3.59^{c,d} \pm 2.02$
March	$4.52^{c,d} \pm 2.11$

Table 1. GSI mean values of females per each sampling month. $p \leq 0.05$. Different letters indicate a significant difference.

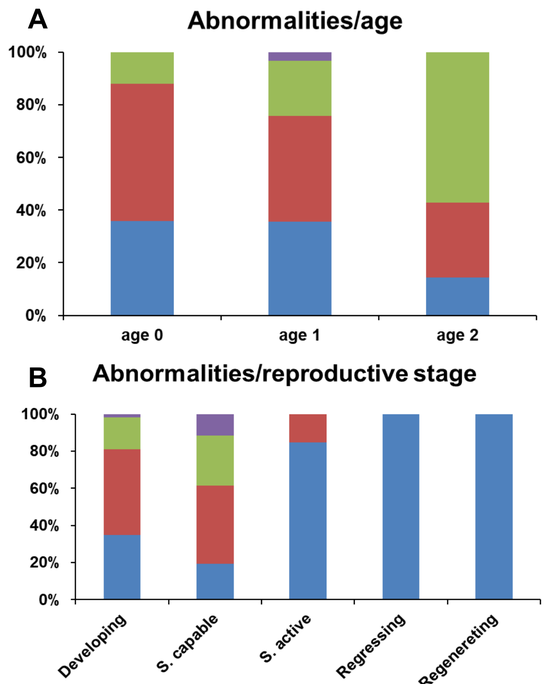


Figure 2. Grading of abnormalities per age (**A**) and per ovarian reproductive stages (**B**). (**blue**) no abnormalities; (**red**) one abnormality; (**green**) two abnormalities and (**violet**) three abnormalities identified.

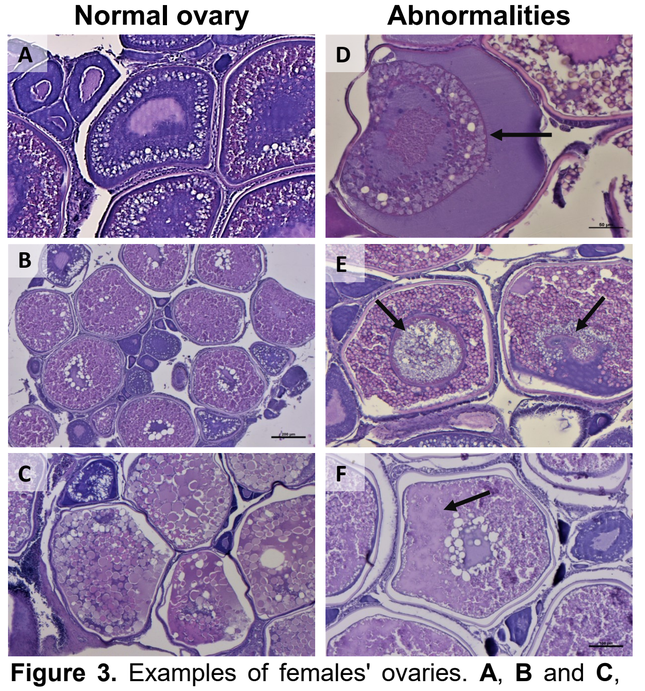


Figure 3. Examples of females' ovaries. **A**, **B** and **C**, normal ovaries at previtellogenic, vitellogenic and late vitellogenic stage respectively. **D**, previtellogenic atresia; **E**, vitellogenic atresia; **F**, vitellogenic atresia.

Conclusion

Sardines' reproduction resulted altered. Focusing on females, they appeared to reach maturity at the end of the first year and a longer reproductive period was observed compared to records of previous years. The presence of abnormalities waas related to females' age and ovarian reproductive stage, older females showed more abnormalities than the younger ones.

g.chemello@staff.univpm.it

Identification of a new developmental scoring system applicable to FETAX (Frog Embryo Teratogenicity Assay: *Xenopus*).

Battistoni M.[1], **Di Renzo F.**[1], **Metruccio F.**[2], **Menegola E.**[1]

[1]Dept of Environmental Science and Policy Università degli Studi di Milano, Italy

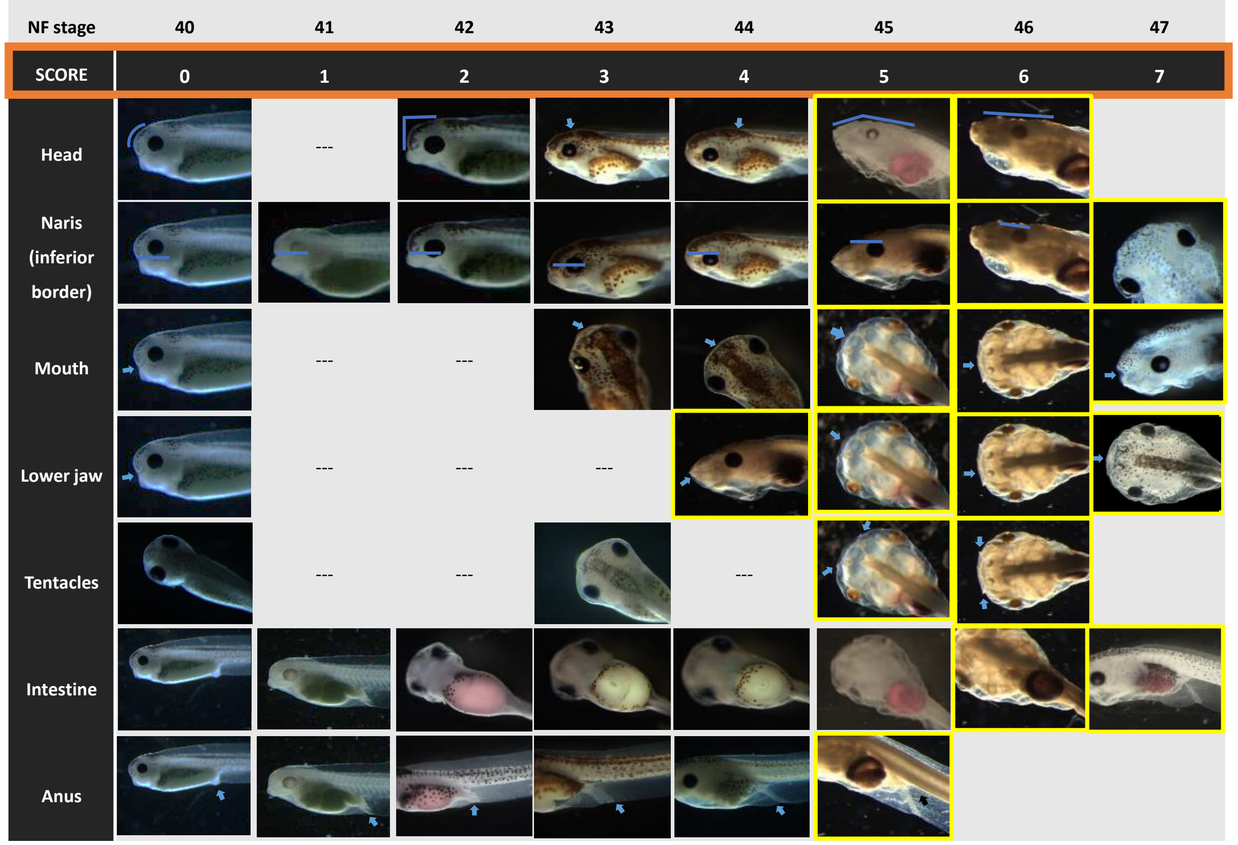
[2]ICPS, ASST Fatebenefratelli Sacco, Milan, Italy

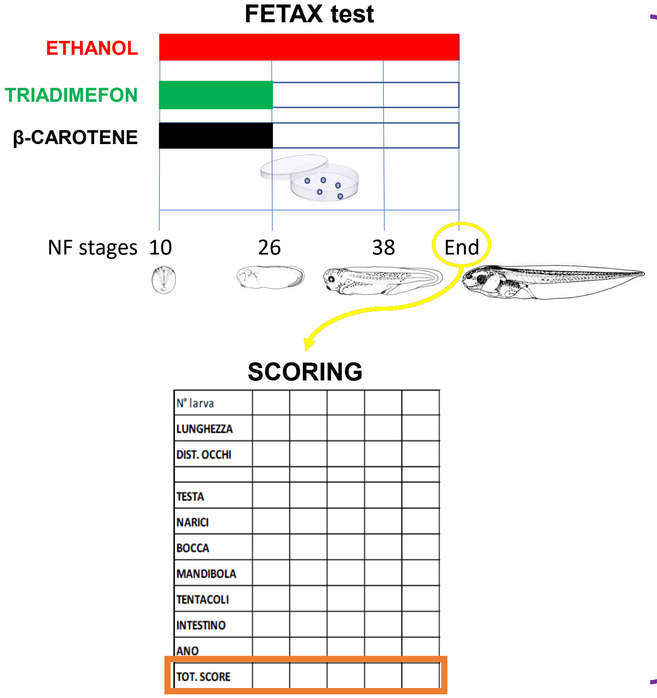
ICPS
Centro Internazionale per gli Antiparassitari e la Prevenzione Sanitaria
International Centre for Pesticides and Health Risk Prevention

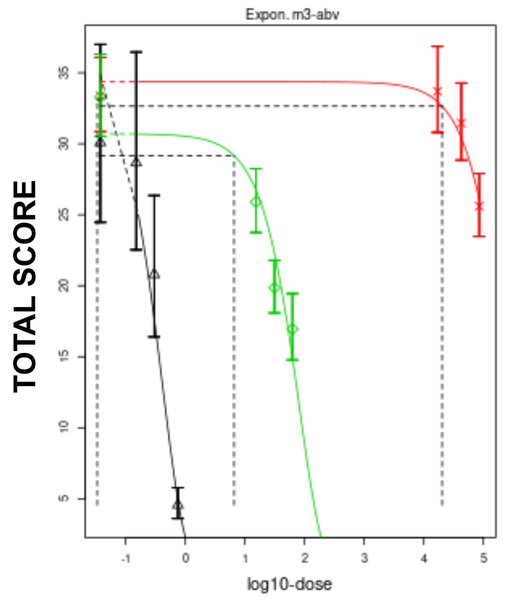
Background and Aim

The quantitative estimation of embryonic growth and development is a major concern in developmental toxicity evaluation caused by exposure to xenobiotics in pregnancy. A precise estimation of overall embryonic development and the evaluation of treatment-related deviation from the normal developmental is possible by application of quantitative morphological methods (scoring systems). The most accurate score for embryotoxicity evaluation is the Brown and Fabro scoring system designed for rat embryos cultured *in vitro*: it provides significant indication on substance- and dose-related developmental impairments. **The aim of the present work is to describe a quantitative assessment of the development of *Xenopus laevis* embryos applicable to FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*) methodology.** To test the applicability of this new scoring system, we evaluated samples exposed to different teratogens: **ETHANOL (17 - 85 mM)**, **TRIADIMEFON (15.625 - 62.5 µM)**, **NANOENCAPSULATED β-CAROTENE (0.15 - 0.75 µM)**.

Methods and Results







Conclusion

Results support the use of the present scoring system to quantitatively assess *X. laevis* development variations in embryotoxicity studies.

maria.battistoni@unimi.it

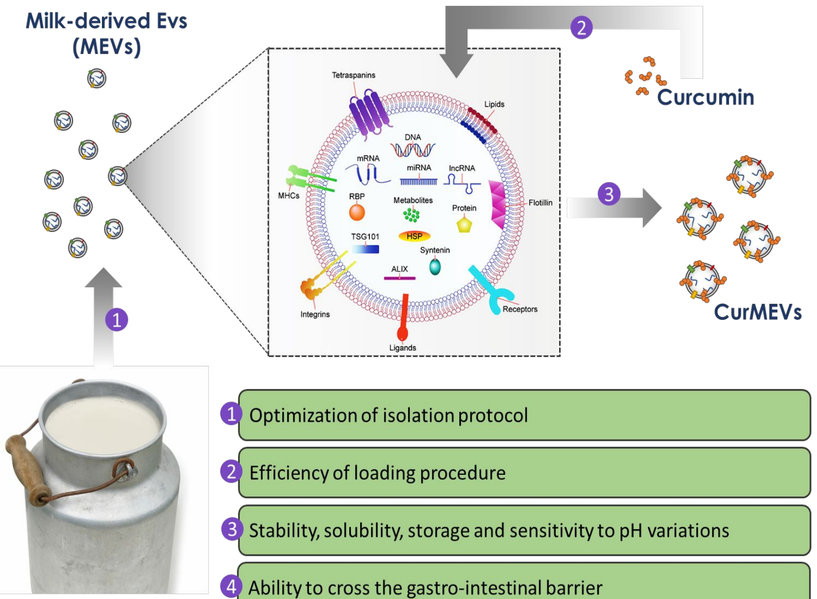
68° CONVEGNO GEI - SIBSC 2023

BOVINE MILK EXTRACELLULAR VESICLES: AN ORAL DRUG DELIVERY SYSTEM FOR BIOACTIVE COMPOUNDS

S. Tacconi[1], F. Vari[2], L. Buccini[3], S. Sennato[2], S. Dinarelli[4], M. Zuccotti[5], L. Dini[6,7]

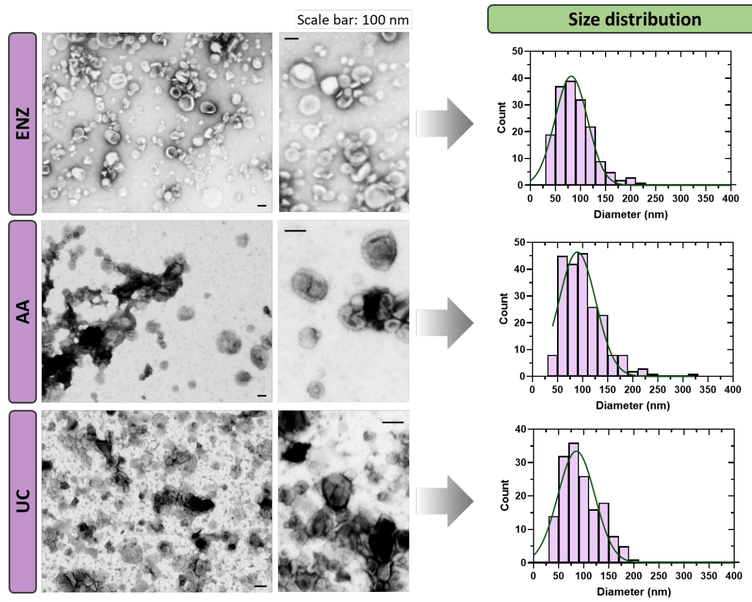
[1]INRAE CarMeN laboratory, Lyon, France; [2]DFFVE Sapienza University of Rome, Rome, Italy; [3] SBAI, Sapienza University of Rome, Rome, Italy ; [4] ISC, CNR Rome, Italy; [5]DBBLS, University of Pavia, Pavia, Italy; [6]DBBCD, Sapienza University of Rome, Rome, Italy; [7]CNIS, Sapienza University of Rome, Rome, Italy

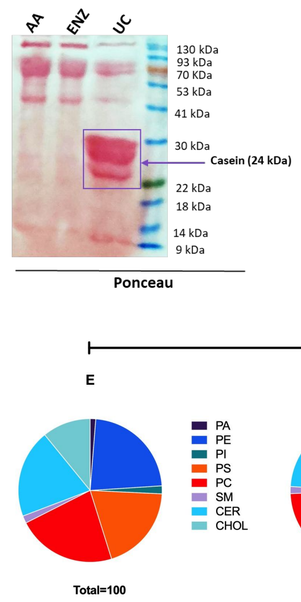
Aim

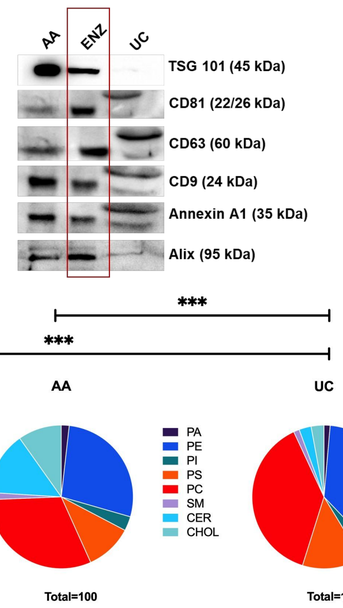


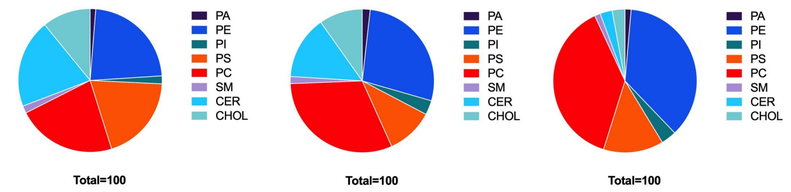
1. Optimization of isolation protocol
2. Efficiency of loading procedure
3. Stability, solubility, storage and sensitivity to pH variations
4. Ability to cross the gastro-intestinal barrier

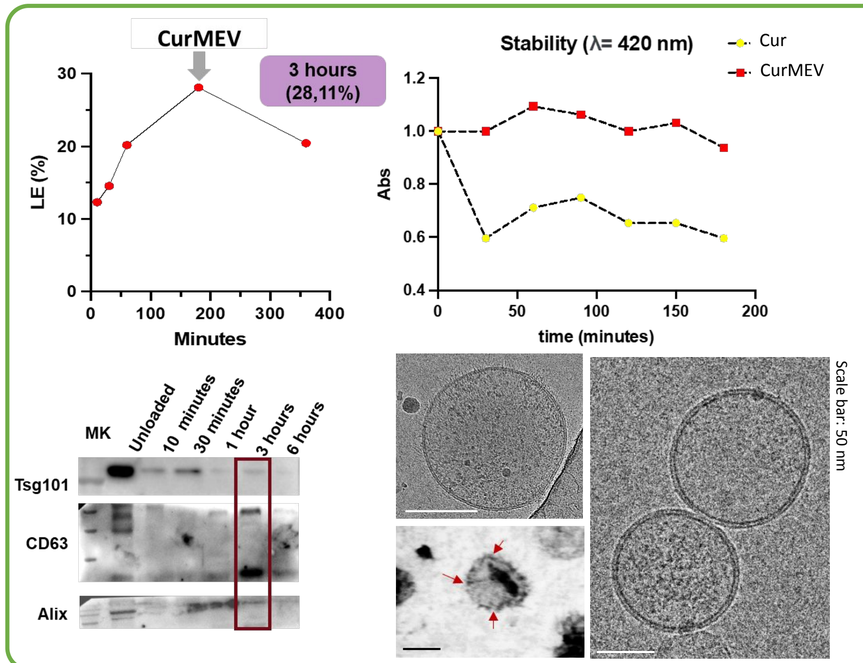
Results

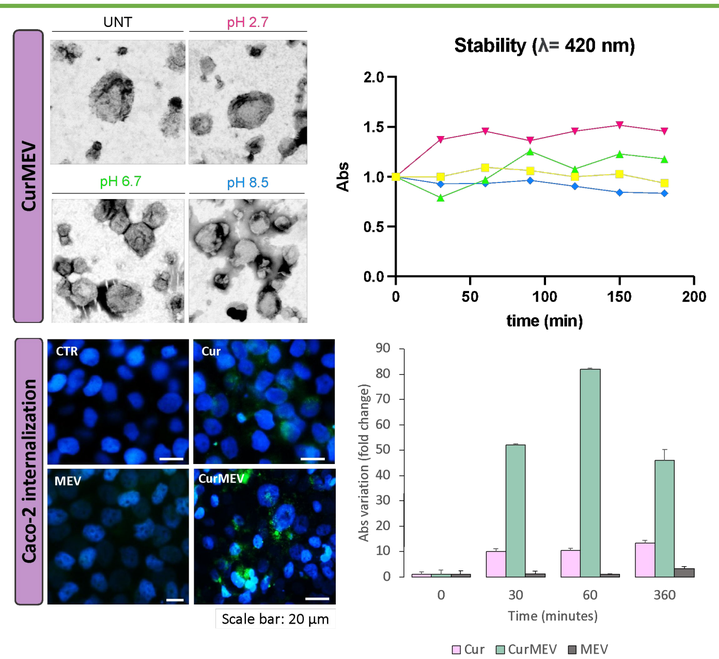


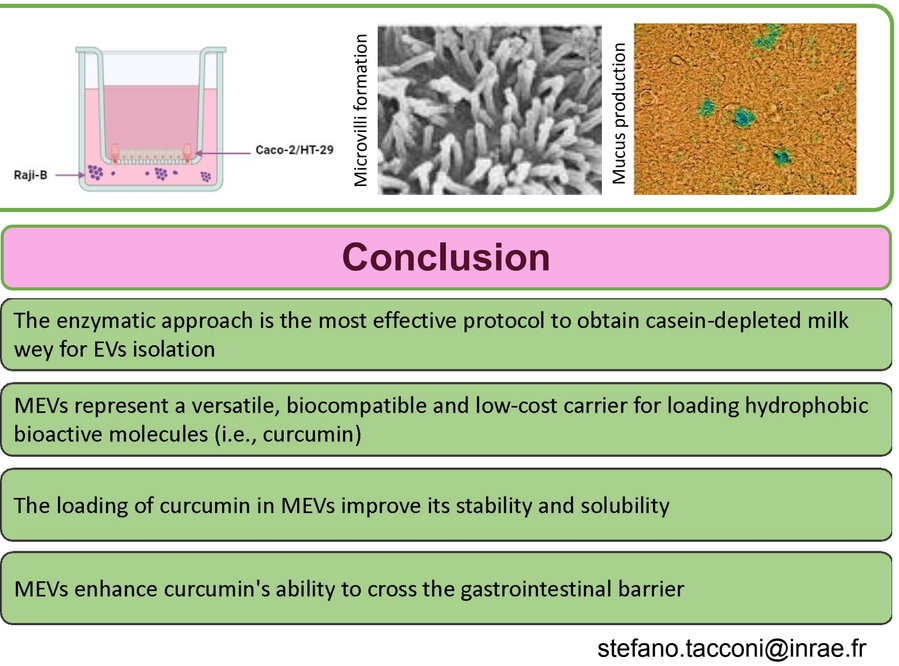












Conclusion

- The enzymatic approach is the most effective protocol to obtain casein-depleted milk wey for EVs isolation
- MEVs represent a versatile, biocompatible and low-cost carrier for loading hydrophobic bioactive molecules (i.e., curcumin)
- The loading of curcumin in MEVs improve its stability and solubility
- MEVs enhance curcumin's ability to cross the gastrointestinal barrier

stefano.tacconi@inrae.fr

DOES MICROPLASTICS INDUCE SUFFERENCE IN *XENOPUS LAEVIS* EMBRYOS?

F. Melfi[1], V. Impagliazzo[1], C. Artiaco[1], M.G. Orsi[1], G. Rusciano[2], B. Avallone[1], C. Fogliano[1], R. Carotenuto[1]

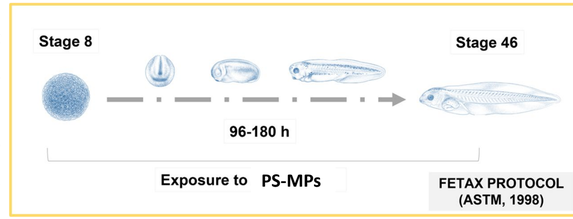
Department of Biology[1] - Department of Physics[2] - University of Naples Federico II - Italy

Background

Microplastics (MPs), from industrial and household products or from the degradation of larger plastics, are now pollutants of global concern. These particles are found in aquatic environments in high concentrations[1,2]. The ability of MPs to adsorb pollutants and subsequently release them into marine and freshwater systems is an additional cause for concern[3]. Because of their small size, they are easily ingested by aquatic organisms and could cause problems not only to the gastrointestinal level but also up the food chain.

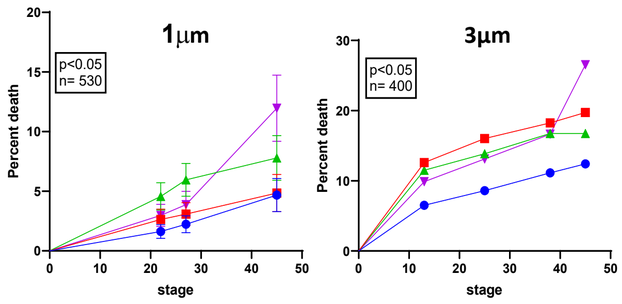
Aim & Methods

The aim of this study was to test the effects of Polystyrene microplastics (PS-MPs) of 1 and 3 µm diameter on *X. laevis* development. We used 0.1, 1 and 10 mg/L concentrations considering the environmental accumulation. The conventional FETAX test was performed[4]. Heart rate, oxidative stress and gene expression were also evaluated. Raman spectroscopy was performed on embryo extract to reveal the presence of internalized PS-MPs.

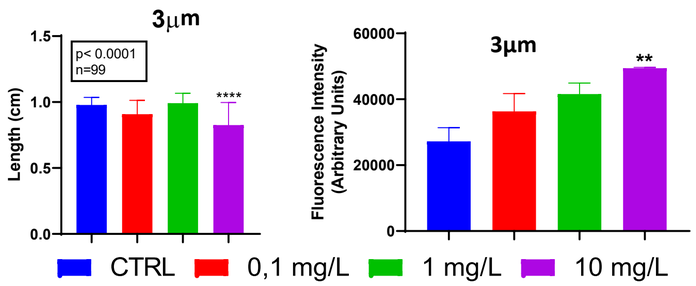


Results

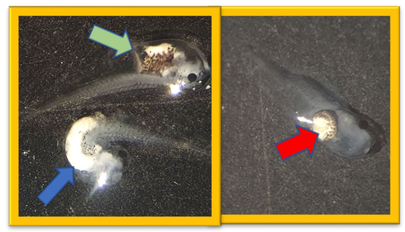
PS-MPs produce a low mortality rate



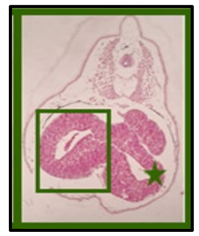
PS-MPs induce growth retardation



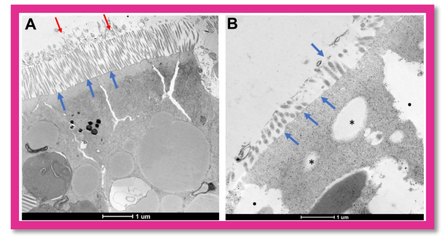
PS-MPs stimulate the ROS production



***In toto* images**
trunk and tail folds
abnormal intestinal maturation
extensive oedema

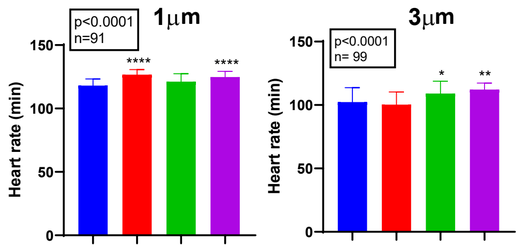


Hematoxylin-eosin staining
PS-MPs are present in some sections of the intestines.

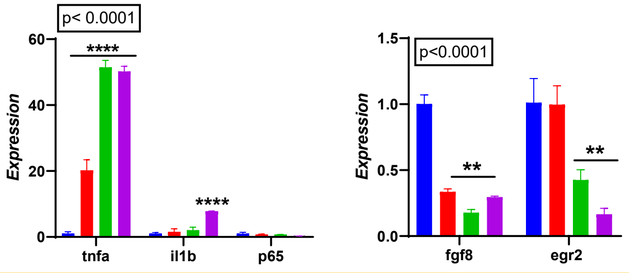


Electron Microscopy
PS-MPs cause suffering in the intestinal brush border

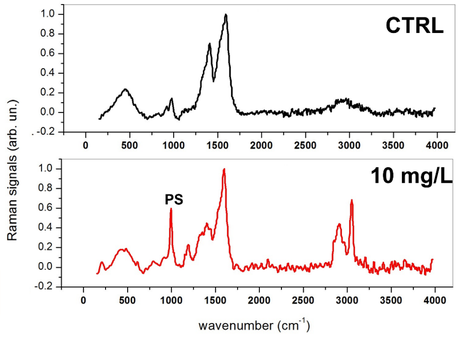
PS-MPs induce tachycardia



PS-MPs modify the expression of pro-inflammatory cytokines and some early developmental genes



PS-MPs penetrate the embryos



Conclusion

PS-MPs are not very lethal and have a low toxic/teratogenic effect. Low percentage of malformation (oedemas, tail and intestinal abnormalities) were observed. However intestinal brush border shows suffering and a dose-dependent increase in ROS production and upregulation of pro-inflammatory cytokines were detected. More evident results were seen at 10mg/L concentrations. MPs adverse effect certainly depend on their size, but concentration is the most important aspect to consider.

References

1) Shen Xu et al., 2020 Sci Total Environ 703 (2020) 134699; 2) Salthammer,2022 Angew. Chem. Int. Ed. 61,e202205713
3) Acarer, 2023 Water Science & Technology Vol 87 No 3, 685; 4) Carotenuto et al., 2022 Chemosphere 289 (2022)133233.

fmelfi19@studenti.unina.it

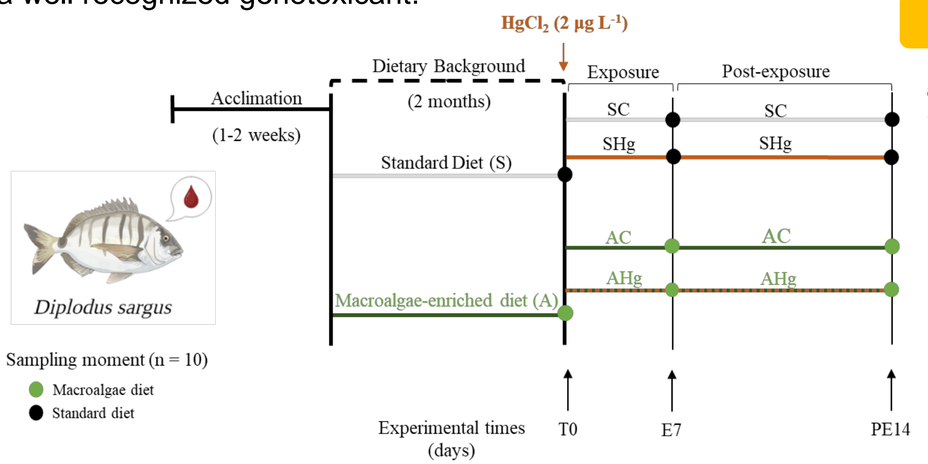
Marine macroalgae dietary supplementation provides genoprotection in fish (*Diplodus sargus*) against inorganic Mercury

Neto A.[1], Brandão F.[1] , Cesário R.[2], Marques A.[1], Pereira V.[1], Pacheco M.[1], Pereira P.[1]

[1]CESAM and Dept. of Biology, University of Aveiro, Campus Universitário de Santiago, Aveiro 3810-193, Portugal [2]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa 1049-001, Portugal.

Background

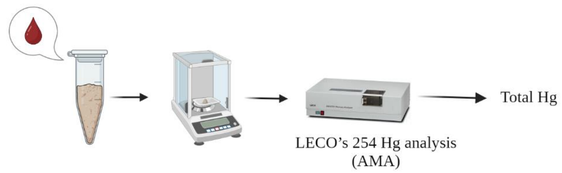
The benefits of marine macroalgae on human health are well recognized, while more studies are needed focusing on their advantages to improve fish condition, namely under water contaminants exposure. Genoprotection of macroalgae consumption in fish has been demonstrated, but this effect remains unexplored under realistic exposure scenarios to mercury (Hg), a well recognized genotoxicant.



Aim & Methods

AIM: To evaluate if a macroalgae-enriched diet can provide genoprotection to fish (*Diplodus sargus*) exposed to waterborne inorganic mercury (iHg) by the determination of **total Hg levels** in blood and by the assessment of **the erythrocytic nuclear abnormalities**.

Total Hg levels



Erythrocytic nuclear abnormalities

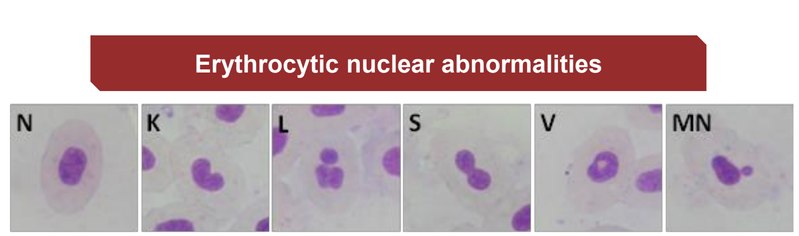


Figure 1- Mature fish erythrocytes: normal (N) and with abnormalities: Reniform/Kidney (K), Lobulated/Lobed (L), Segmented/Segmented (S), Vacuolated/Vacuolated (V) and Micronucleus/Micronuclei (MN).(Adapted from: Maceda-Veiga et al., 2015).

Results & Discussion

Hg toxicokinetics in the blood

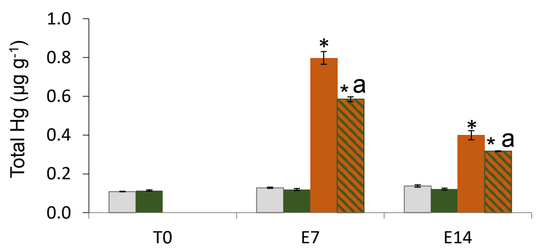


Figure 2 – Total Hg levels in the blood of *D.sargus*.

Fish with a macroalgae-enriched diet background accumulated lower levels of Hg in the blood that those under a standard diet, both at E7 and PE14

Erythrocytic nuclear abnormalities

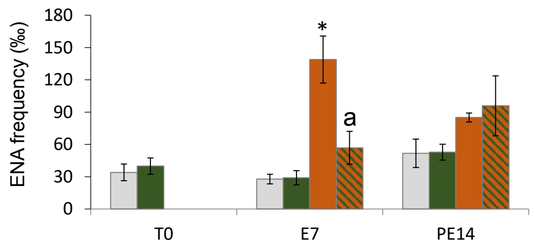


Figure 3 –Total frequency (‰) of erythrocytic nuclear abnormalities in *D.sargus*.

iHg triggered the enhancement of ENAs
A decrease of iHg genotoxicity at the group feed with the macroalgae-enriched diet were observed

Control
Standard diet
Macroalgae diet

Exposed to iHg
Standard diet
Macroalgae diet

* differences between control and exposed to iHg within each diet
a differences between diets

Conclusions

Results are promising by revealing the genoprotection of a macroalgae dietary supplementation against the genotoxicity of iHg in fish erythrocytes, as well as by diminishing the levels of Hg in the blood.

References

Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N., Pacheco, M., 2015. Inside the Redbox: Applications of haematology in wildlife monitoring and ecosystem health assessment. Sci. Total Environ. 514, 322–332. https://doi.org/10.1016/j.scitotenv.2015.02.004

Acknowledgments

CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds. P.P. is funded by national funds through FCT, under the Scientific Employment Stimulus (Individual Call) [CEECIND/01144/2017].

acneto@ua.pt

Peroxisomal alterations in a mouse model of amyotrophic lateral sclerosis (ALS)

Terricola M.[1,2], Price R.[1,2], Scaricamazza S.[3], Salvatori I.[3], Cervelli M.[1,2], Berardinelli F.[1,2], Ferri A.[3], Valle C.[3], Moreno S.[1,2]

[1]Dept. of Science-LIME, University Roma Tre;
[2]Laboratory of Neurodevelopment, Neurogenetics and Molecular Neurobiology, [3]Laboratory of Neurochemistry, IRCCS Fondazione S. Lucia, Rome, Italy.

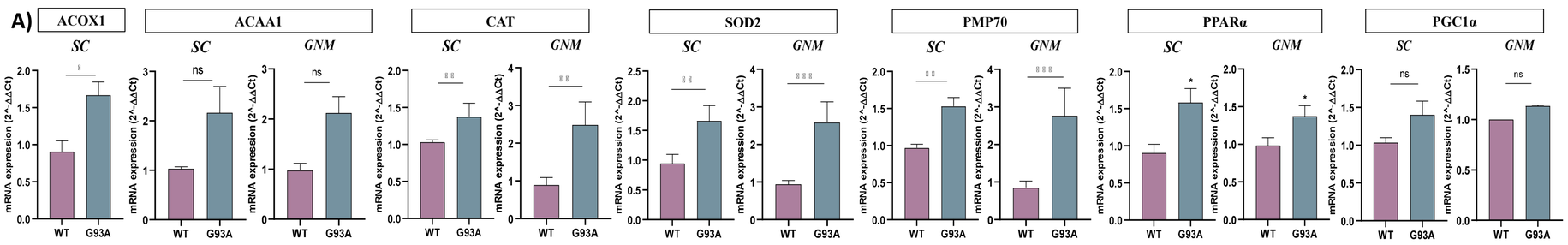
Background

Peroxisomes are dynamic organelles, involved in numerous anabolic and catabolic pathways, such as lipid (*e.g.*, fatty acids β-oxidation) and reactive oxygen species (ROS) metabolism, which are shared with mitochondria. Impaired ROS scavenging and altered lipid catabolism hallmark ALS, a neurodegenerative disorder affecting motor neurons and skeletal muscle. Of all cases, ~2% are linked to superoxide dismutase 1 (SOD1) mutation. The role of mitochondria impairment in the pathogenesis of ALS is thoroughly being investigated, while peroxisomal (dys)function is yet to be assessed.

Aim & Methods

In this study we addressed peroxisomal involvement in ALS pathogenesis, performing Western Blot and Real-Time PCR analyses, of peroxisomal markers in the spinal cord and muscle of *SOD1^{G93A}* transgenic mouse model at the symptomatic stage. Specifically, we have analyzed markers of lipid transport and metabolism (peroxisomal membrane protein of 70 kDa, PMP70, acyl-Coenzyme A oxidase 1, ACOX1, and acetyl CoA acyltransferase 1, ACAA1), antioxidant response (catalase, CAT, and superoxide dismutase 2, SOD2) and mitochondria-peroxisome biogenesis (PGC1-α and PPARα).

Results



(A) Real-Time PCR analysis of selected markers on GNM and SC of SOD1^{G93A} and WT mice. Data presented as mean ± SEM, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤0.0001, Mann-Whitney test.

(B) Western Blot analysis of GNM, normalized to GAPDH protein expression. Data presented as mean ± SEM, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, Mann-Whitney test.

(C) Western Blot analysis of SC, normalized to GAPDH. Data presented as mean ± SEM, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, Mann-Whitney test.

Conclusion

Our data suggest peroxisomal induction in ALS, in both muscle and nervous tissue. Both fatty acyl β-oxidation and ROS metabolism are upregulated, possibly by by PPARα, emphasizing mitochondria-peroxisome interplay. Overall, the pathological role played by peroxisomes in ALS progression deserves further attention.

References

Islinger et al. 2018 Histochem Cell Biol 150:443. Ravits et al. 2014 Exp Neurol 262:121.

Scaricamazza et al. 2021 Cells 10:525. *Wahli & Michalik 2012 TEM 23:351.*

Acknowledgments

REGIONE LAZIO

martina.terricola@uniroma3.it

YOLK INTERNALIZATION PATTERNS IN EMBRYOS OF LOGGERHEAD SEA TURTLE (*Caretta caretta*): HOW ARE NUTRIENTS TRANSFERRED TO EMBRYOS?

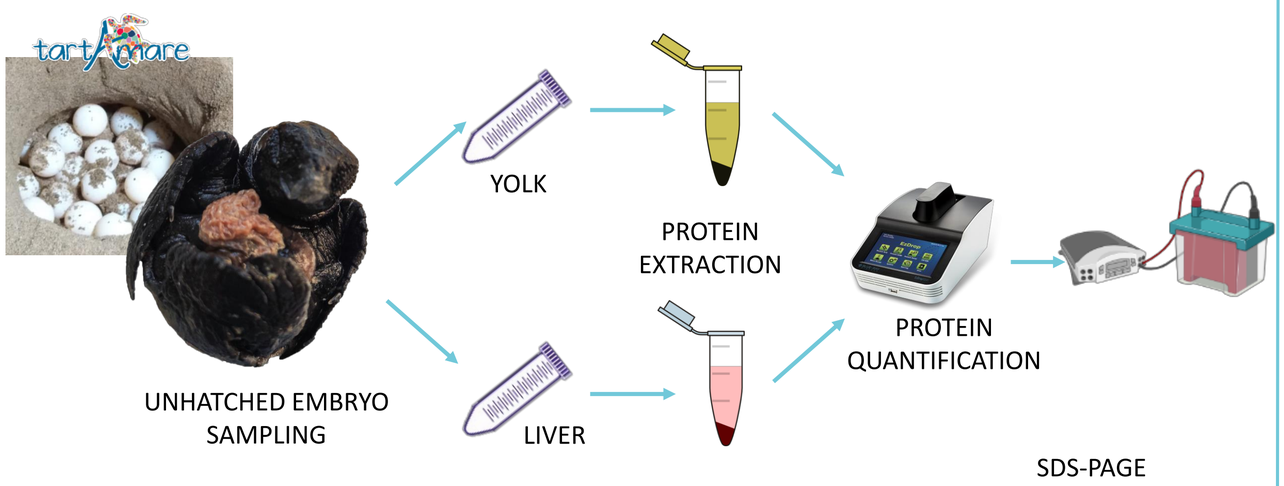
E. Trotta[1], G. Chemello[1], L. Papetti[2], L. Di Renzo[3], M. Matiddi[4], C. Silvestri[4], O. Carnevali[1], G. Gioacchini[1]

[1]Deptartment of Life and Environmental Science, Polytechnical University of Marche, Ancona, Italy; [2] CRTM "tartAmare", Marina di Grosseto, Italy;
[3]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy;
[4]ISPRA, National Institute for Environmental Protection and Research, Rome, Italy

Background & Aim

The embryogenesis of Loggerhead sea turtles (*C. caretta*) is strictly related to energy sources stored in yolk necessary to sustain all the developmental processes until hatching. The yolk is mainly composed by lipids, proteins, carbohydrates and some inorganic ions but its internalization pathway is still unclear.
This study aimed to investigate potential internalization patterns of protein yolk components in embryo's livers highlighting variation between all the developmental process. To this, death embryos from 4 different nests laid in Tuscany (Italy) during all the nesting season in 2021 were analysed.

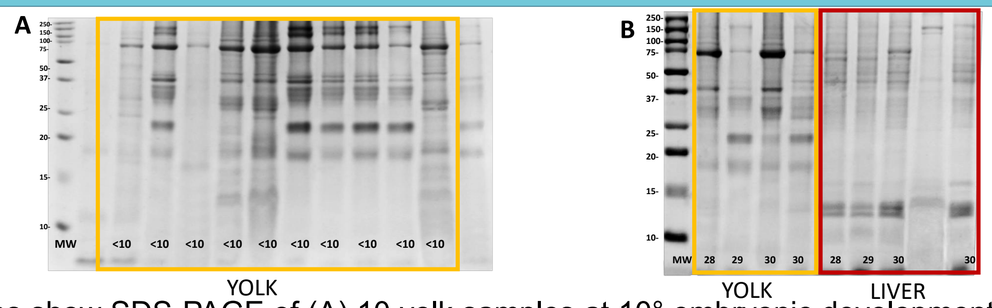
Methods



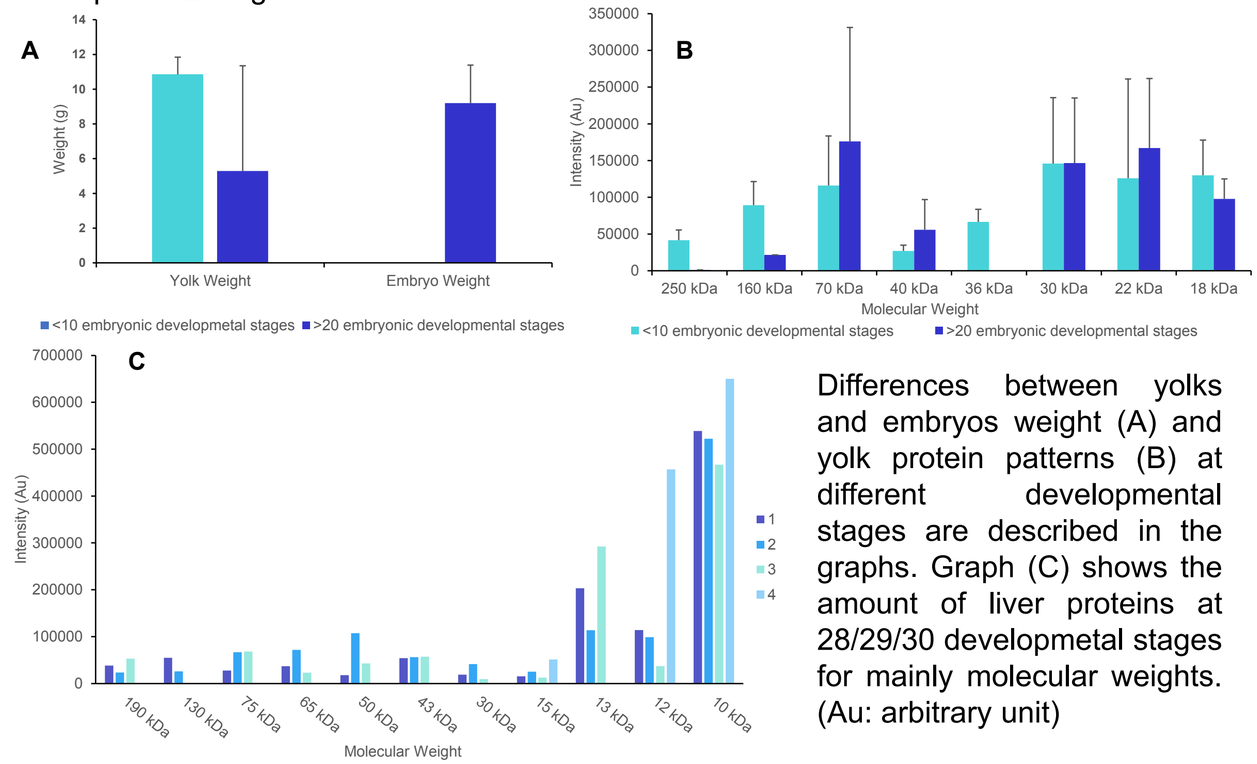
Conclusion

These preliminary results show for the first time the protein pattern of yolk and liver of embryos of loggerhead sea turtles highlighting differences during the developmental process. Results from liver protein patterns suggest an highest presence of lower molecular weight protein which could imply a metabolic role of liver in order to nourish organism.

Results



Images show SDS-PAGE of (A) 10 yolk samples at 10° embryonic developmental stage, (B) 4 yolk samples (yellow) and 4 liver samples (red) at more than 20° embryonic developmental stage.



Differences between yolks and embryos weight (A) and yolk protein patterns (B) at different developmental stages are described in the graphs. Graph (C) shows the amount of liver proteins at 28/29/30 developmetal stages for mainly molecular weights. (Au: arbitrary unit)

e-mail: trotta.erica@gmail.com