Comparison of Salicylic acid and Caffein effect on the digestive glands microtubules of *Mytilus galloprovincialis*

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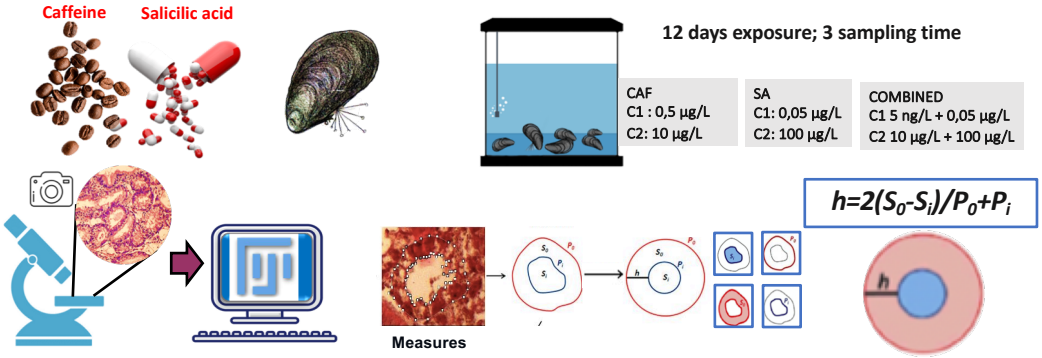
Background

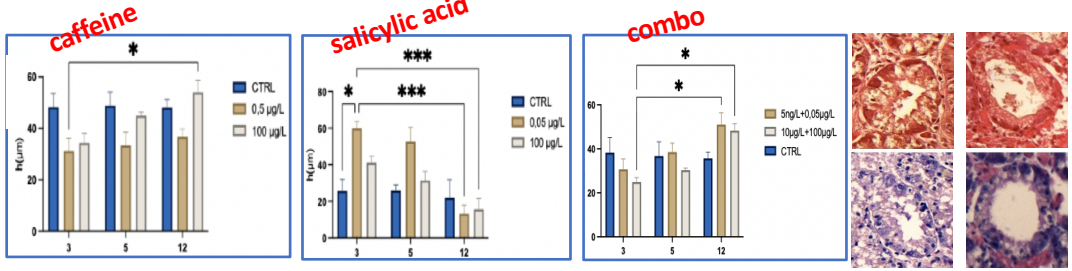
Pharmaceuticals active compounds (**PhACs**) are continuously discharged into aquatic environments, threatening biota. Their impact as single compounds has been widely investigated, whereas few information exists on their effects as mixtures. Among the various detected PhACs, only **caffeine** (**CAF**) and **salicylic acid** (**SA**) were found to be consistently present at the hospital effluent, influent and effluent, and in seawater, at concentrations higher than other pharmaceuticals, likely due to their wide use in daily human life. PhACs have the potential to trigger different types of biological responses in **non-target organisms**.

Aim & Methods

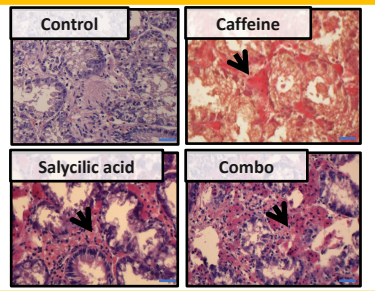


Results

Caffeine at 3, 5 and 12 days causes thinning compared to controls at low concentration. The high concentration induces thinning to 3 days.
Salicylic acid induces thickening at 3 and 5 days at low concentration. Thinning to 12 days caused by both concentrations.
COMBO thinning to 3 days, no effect to 5 days, thickening to 12 days than both concentrations.



Relevant hemocyte infiltration between the microtubules was observed throughout the exposure at the different conditions (CAF, SA, or their combination, different concentration and exposure time). Arrows indicate the hemocyte.



Conclusion

These results confirm that morphological biomarkers based on size cell modification parameters are a useful tool to detect chemical effect on animal system and to employ in systemic biomonitoring program. Here alteration seems may cause **atrophy** and subsequently necrosis. Here emerges that the histological condition of digestive gland microtubules, marker already validated for other contaminants, could be a good biomarkers for investigating the environmental contamination from PhACs.

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A mesocosm study: behavioural and physiological stress responses of *Cherax quadricarinatus* after exposure to acoustic signal

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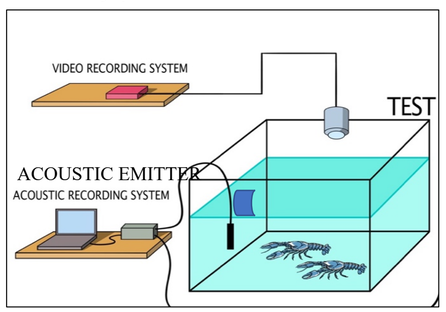
Background

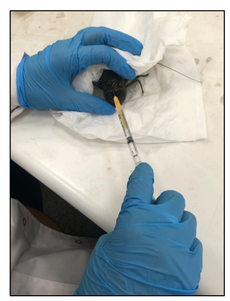
In addition to other stressors, anthropogenic noise is recognised as a significant factor that can have negative consequences for species over time. This recognition led to the identification of anthropogenic noise as a form of pollution in the WFD 2008/56/EC.

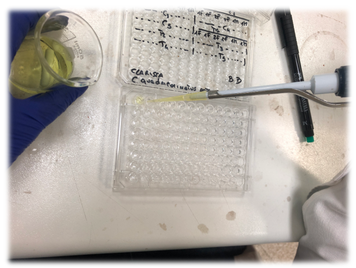
The importance of the acoustic environment has been highlighted by several authors who have assessed the effects of noise pollution at true behavioural, cellular and biochemical levels on various invertebrate species [1,2]. As previously described on other crustacean species [3], *Cherax quadricarinatus* also appears to be sensitive to water vibration frequencies, perceived through sensory hairs. We recently described the characteristics of the haemolymph of this freshwater species: total protein concentration= 2455 ± 824 g/mL; osmolarity value= 409 ± 18.75 mOsm; pH = 7.56 ± 0.105; total haemocyte count (THC)= 1.678x 103 ± 707 x103; cell types. In particular, three types of haemocytes were described: hyaline, semigranular and granular [4].

Aim & Methods

In this study we evaluated if high frequency sound can cause alterations in behaviour and haemolymphatic parameters (pH, osmolarity, total protein concentration and enzymatic activity.)

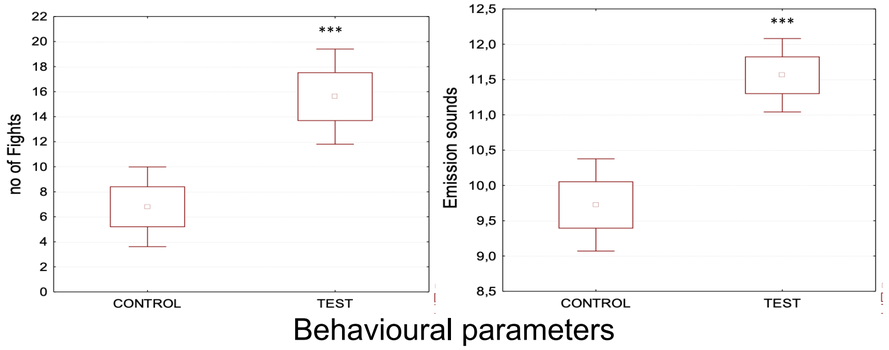




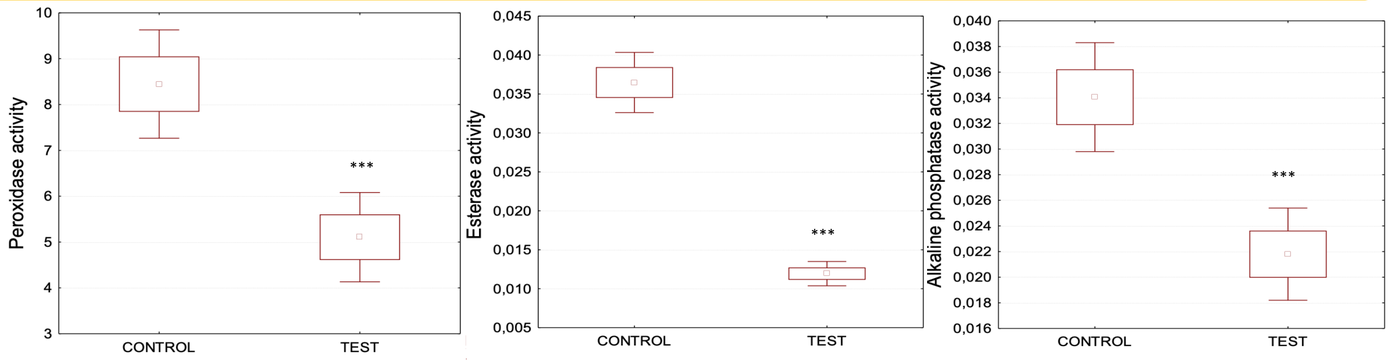


EU directive 2010/63/EU (22 September 2010)

Results



Behavioural parameters



Enzymatic activity (U/µg) Mann-Whitney U Test (***p < .001)

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Conclusion

These results suggest that high-frequency stimuli induce both a behavioural and physiological stress responses, thus suggesting that acoustic noise may have an effect on the species *Cherax quadricarinatus*. This information is essential for ensuring the well-being of the animals and implementing appropriate measures to mitigate.

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Acute exposure of *Artemia salina* to TiO_2 Brookite/CeO_2 Nanoparticles

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Background

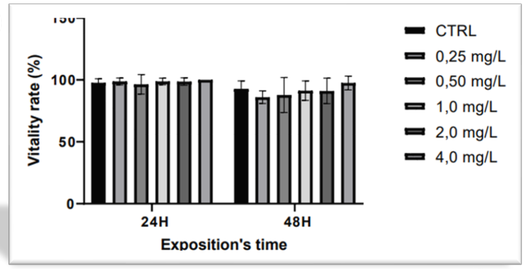
TiO_2 and CeO_2 NPs are widely used in different medical fields, in the administration of drugs[1-2-3], in cancer therapies or in disinfection. Their effects on the marine ecosystem and their impacts on human health are little known but their peculiar properties give them cyto-genotoxic activity and antioxidant activity against oxidative stress. In this study, the effects of the combined nanoparticles, TiO_2 Brookite/CeO_2 on *A. salina* nauplii after acute exposure at 24h and 48h were evaluated.

Aim & Methods

The aim was to examine the effect of the interaction of two combined nanoparticles following acute exposure of *A. salina* in order to understand the type of TiO_2 interaction in the individual phases of Brookite and CeO_2. *A. salina* nauplii were exposed to different concentrations of TiO_2 Brookite/CeO_2 and their effects on viability at 24 hours and 48 hours were evaluated. After 48 hours of exposure, susceptibility to oxidative stress and induction to the apoptotic process were assessed.

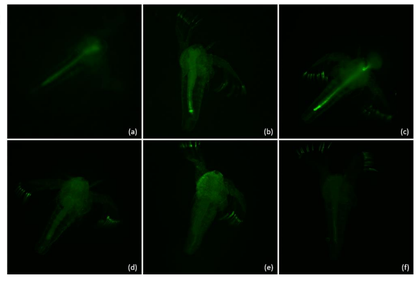
Results

A)



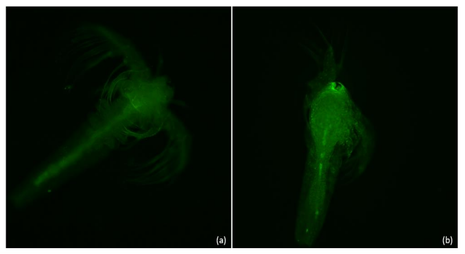
A) Bar diagram shows results for vitality rate (24 and 48h) of *A. salina* nauplii treated with various concentrations of TiO_2 Brookite/CeO_2 NPs.

B)



B) Fluorescence microscope of *A. salina* exposed to different concentrations of TiO_2 Brookite/CeO_2 NPs within 48h of exposure to evaluate ROS generation. (a) CTRL; (b-f) exposed.

C)



C) Fluorescence microscope of *A. salina* exposed to different concentrations of TiO_2 Brookite/CeO_2 NPs within 48h of exposure to evaluate cell damage. (a) CTRL; (b) exposed.

No adverse time-dose-dependent effects were observed on the viability of exposed organisms. However, fluorescence analysis for the detection of ROS revealed that the exposed were more subject to oxidative stress. The positivity detected after apoptosis test was observed only in organisms exposed to the highest concentration.

Conclusion

In order to understand the mechanisms by which NPs act on biological systems, an acute toxicity test was conducted to evaluate the effects that TiO_2 Brookite/CeO_2 nanoparticles may exert on *A. salina* nauplii. Although acute exposure does not result in a statistically significant reduction in viability, the same results, combined with those obtained following qualitative analyses of ROS and apoptosis, could suggest that chronic exposure to NPs TiO_2 Brookite/CeO_2 may have a negative impact on the environment and human health.

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USE OF ARTEMIA SALINA IN TOXICITY STUDIES OF NANOMATERIALS

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Background

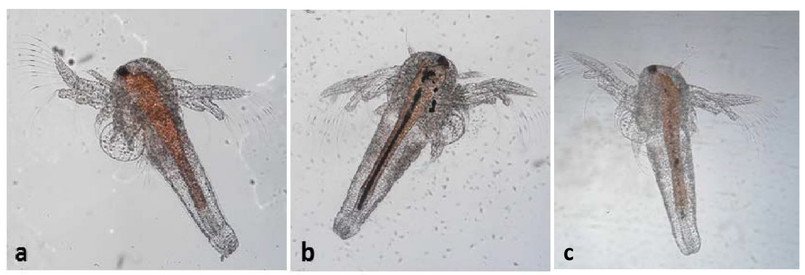
Animal models are employed for in vivo assays of nanomaterials, because they allow to reconstruct the typical routes of exposure of organisms to nanostructured materials (pulmonary, epidermal and oral routes). However, the ethicality of in vivo testing has led to the use of alternative assays conducted in vitro, but even in this case the problem of costs and complexity of the tests remains. Therefore, a good compromise is the use of the marine crustacean *Artemia salina* as a model organism. Toxicity tests on *Artemia salina* are widely used in toxicological research, because they are cheaper, easy and quick compared to *in vivo* and *in vitro* traditional tests[1].

Aim & Methods

Artemia salina were used to evaluate the acute effects (24-48 hours) of MoS2 powders (Sigma Aldrich 90nm). We have selected the nauplii in the first larval stage (instar I) of *Artemia salina* and exposed them to different solution of MoS_2 (0,5 mg/ml; 0,05mg/ml; 0,005mg/ml). We have set up a multi well plates, with ones nauplius per well, for each MoS_2's solution. A control plates have been also included. Until the end of the test, the nauplii were observed through a binocular microscope to record the number of immobile

Results

The results have shown a very low toxicity of all MoS_2's solution, even if the presence of a dark strip inside the gut highlighted the ability of nauplii to ingest MoS_2.



(a) Control larva; **(b)** Larva exposed to concentrations of 0.05 mg/ml of MoS_2 at 24 hours; **(c)** Larva exposed to concentrations of 0.05 mg/ml of MoS2 at 48 hours, in which the reduction of its intestinal content is evident.

% immobile								
	Control		0,5 mg/ml		0,05mg/ml		0,005mg/ml	
	24h	48h	24h	48h	24h	48h	24h	48h
MoS_2	0	0	1%	1%	0	0	2%	4%

Percentage of immobile after 24 and 48 hours of exposure to MoS_2

Conclusion

It was evident that the MoS_2 powders did not have a toxic effect

References

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MORPHOLOGICAL CONDITIONS OF MUSSEL GONADS AFTER EXPOSURE TO POLYSTYRENE MICROPLASTICS ALONE AND CONJUGATED WITH BISPHENOL A

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Background

In the marine environment, plastic contamination is a major contemporary pollution problem. Physical and chemical factors such as temperature waves and photo-oxidation cause the fragmentation process and the formation of microplastics (MP).

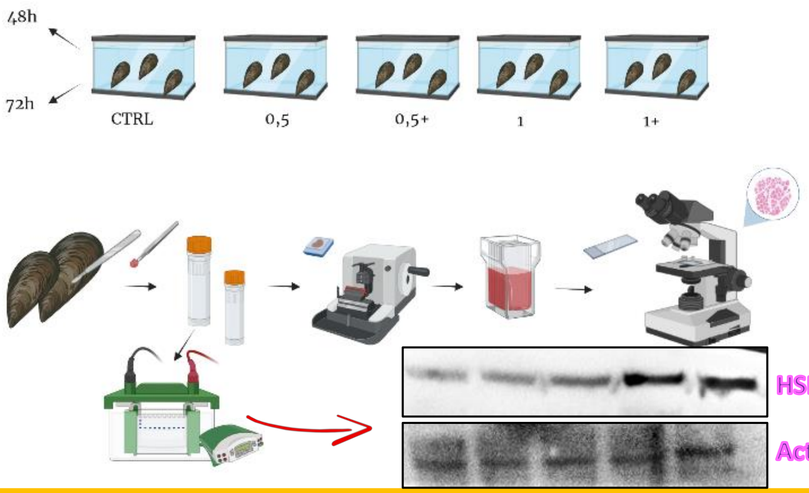
Do microplastics really act as a Trojan horse?

Mytilus galloprovincialis is a bivalve and filter-feeding organism, thus an excellent sentinel organism in marine biomonitoring, and therefore useful to find an answer to this question.

Aim & Methods

The animals were treated with 0.5 and 1 µg/mL of MP (5 µm) alone and conjugated with BPA 25 µM (0,5+ and 1+, respectively) for 48h and 72h.

After opening the shell, the mantle containing the gonad was removed and used for morphological (Hematoxylin-Eosin and PAS staining) and biochemical (Western Blot) investigations.



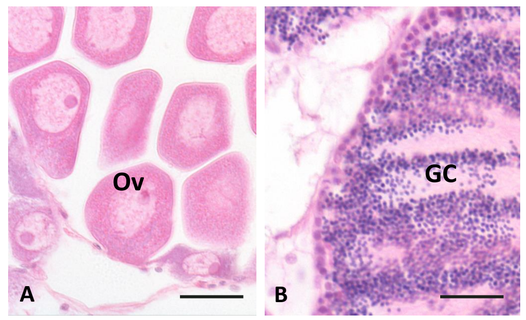
Conclusion

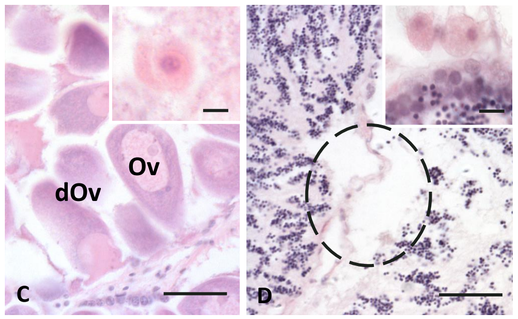
Histological analysis showed that treatment with MP alone and conjugated caused structural changes in ovarian follicles and sperm cysts in a dose-dependent manner. At the highest concentration, the ovary is characterised by mostly degenerated oocytes; the testis shows disorganisation of germ cells within the cysts. Haemolymph cells infiltrates in the connective tissue of all treated samples were recorded.

Western blot analysis showed an increase in HSP70 protein in the treated gonads, in particular with the highest dose of BPA-conjugated MPs. These preliminary data suggest that both MP alone and conjugates induce a stress condition, as evidenced by lipofuscin granules and the strong immunity response that, in turn, may induce toxic effect on mussel reproduction.

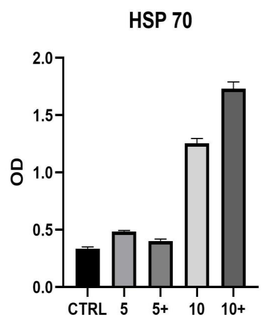
Results

Control gonads. **A**: sections of ovary with normal follicles and cells in various stages of differentiation, including vitellogenic Oocytes (Ov) ready to be expelled. **B**: testis sections with cysts rich in germ cells (GC), including spermatozoa. Scale bars: 20 µm.

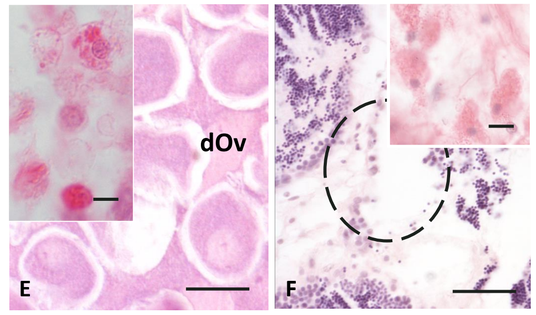


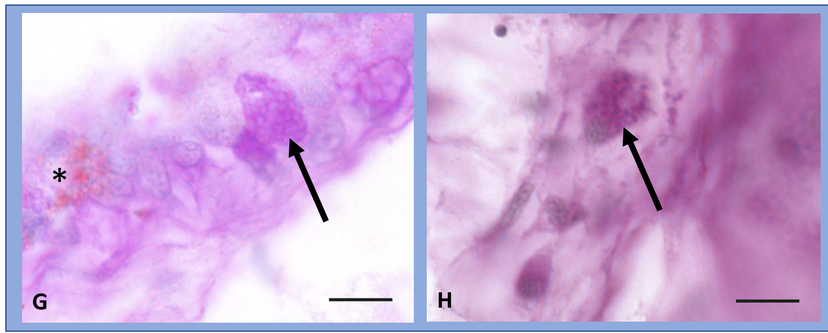


Gonads treated with 0,5 and 0,5+ for 48-72h. Ovary (C): follicles show empty spaces, some degenerated vitellogenic Oocytes (dOv) and infiltrates of haemolymphatic cells are evident in the connective tissue (**insert C**). **Testis** (**D)**: partially emptied spermatic cysts with the presence of uncolored areas indicating disorganization. Also in this case, infiltrates of haemolymph cells are evident in connective tissue (**insert D**).. Scale bars: 20 µm C, D; 5 µm inserts C,D.



Gonads treated with 1 and 1+ for 48-72 h. Ovary (E): disorganised follicles and degenerated vitellogenic Oocytes (dOv). **Testis (F)**: warped and partially emptied sperm cysts. In both ovary and testis, haemolymph cells are evident in the connective tissue. Scale bars: 20 µm E, F; 5 µm insets E, F.





In all animals treated, we recorded in the peripheral part of the mantle the presence of PAS-positive hypertrophic goblet cells (arrows), no matter the dose. Asterisk: lipofuscin granules. Scale bars: 20 µm.

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Comparative composition and distribution of mucins in the mantle edge of bivalves

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Background

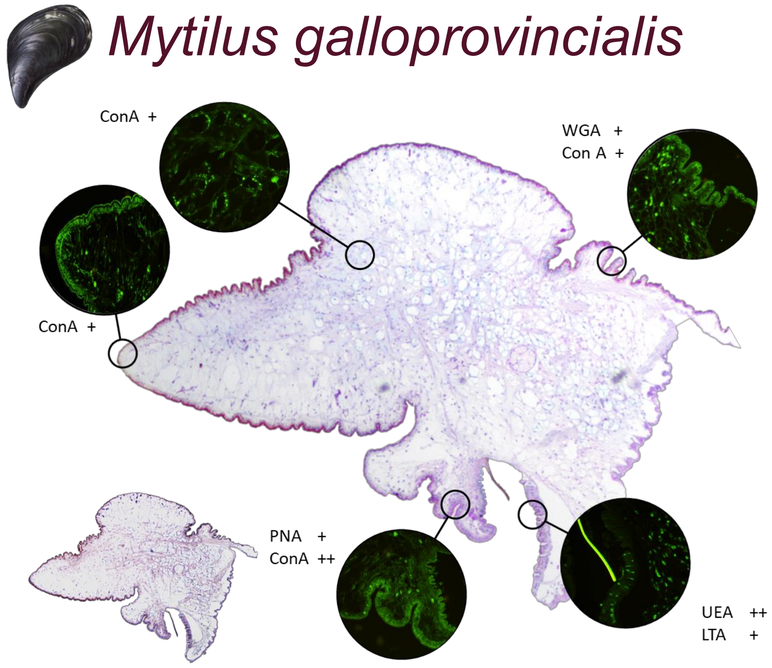
Bivalves are models in environmental[1] and contamination[2] studies, and some contaminants affect the mucus produced by their epithelia[3]. The mantle tissues are exposed to pollutants, with negative impact on many different activities, such as the expression of glycans in the mucous secretion, involved in mucus acidity and viscosity. We compared the mucus composition in three model edible bivalves as a reference for toxicological studies.

Aim & Methods

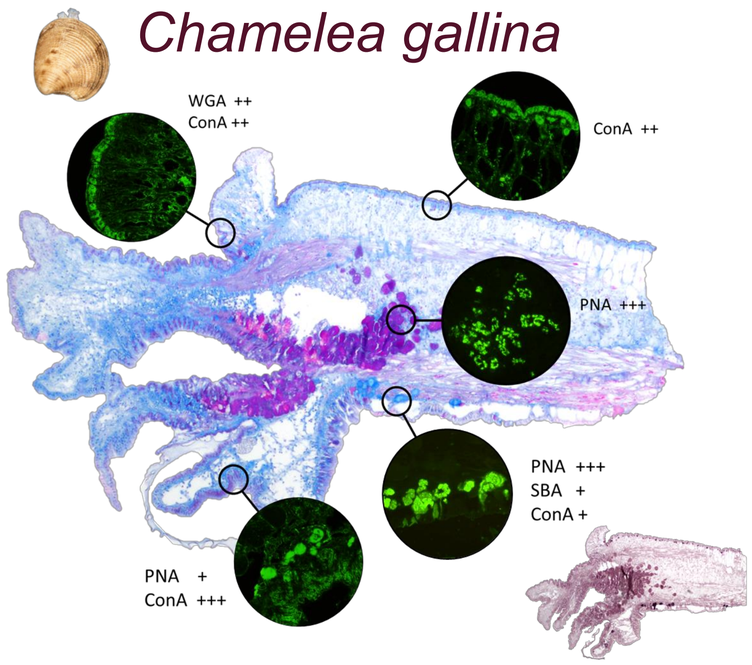
The mantle edge of the mussel *Mytilus galloprovincialis* and the clams *Chamelea gallina* and *Ruditapes philippinarum* was analyzed. Samples were routinely fixed in paraffin and cut into 5 µm thick sections. Mucocytes distribution and secretions were characterized by standard histochemical techniques, (PAS, AB pH 2.5, HID), and FITC lectins histochemistry (PNA, SBA, WGA, ConA, UEA, LTA).

Results

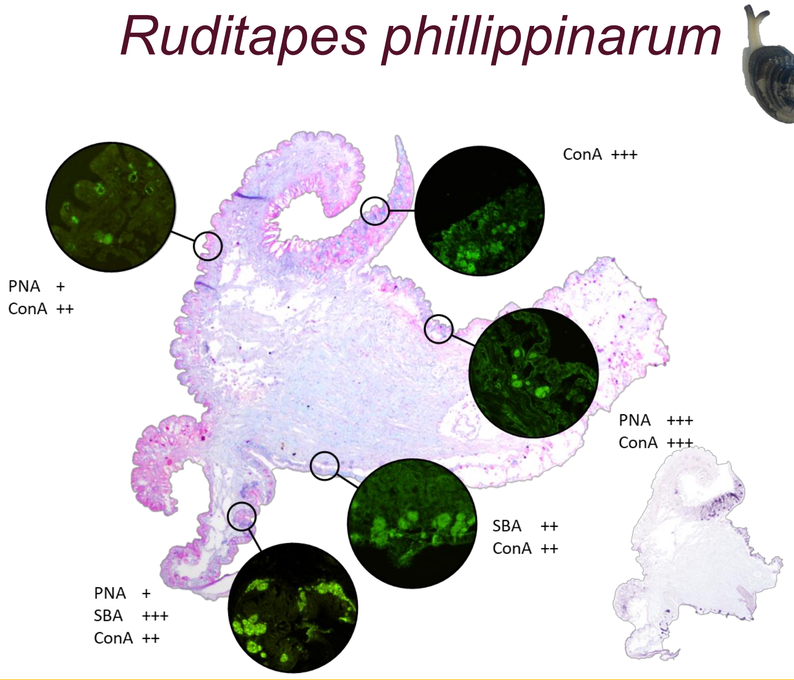
Mytilus galloprovincialis



Chamelea gallina



Ruditapes phillippinarum



Conclusion

The different glycosylation patterns can be related to variable functions among species. Further studies will clear the meaning of the observed variation and response to toxicant exposition, with possible consequences for human health.

References

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TOXICOLOGICAL EVALUATIONS OF GLYPHOSATE IN ZEBRAFISH EARLY-LIFE STAGE

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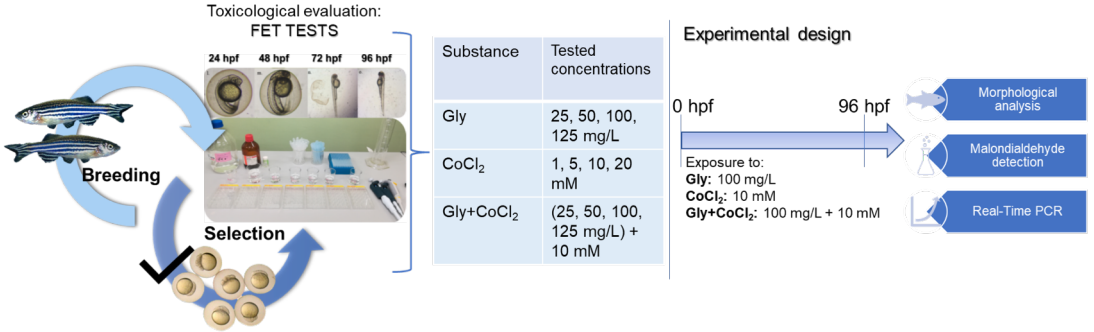
Background

Glyphosate (Gly) is now considered the most widely used herbicide in the world. Traces of this herbicide are increasingly frequent in soil, water, air, and food, and this is becoming a growing concern for human health. A distinctive feature of water environments, particularly those highly polluted, is the low water oxygen concentration. For this reason, the present study aimed to evaluate the potential effects of glyphosate on the zebrafish's early-life stages of development in hypoxic conditions induced by $CoCl_2$.

Aim & Methods



Substance	Tested concentrations
Gly	25, 50, 100, 125 mg/L
$CoCl_2$	1, 5, 10, 20 mM
Gly+$CoCl_2$	(25, 50, 100, 125 mg/L) + 10 mM

Results 1: Toxicological evaluation

A

Critical Doses		0-24 h	0-48 h	0-72 h	0-96 h
Survival					
95%-CL	LD10	88,096	86,990	86,693	85,683
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
95%-CL	LD20	101,452	98,571	97,671	96,974
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
95%-CL	LD50	132,904	125,194	122,696	122,688
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
Survival	LOED	>125,000	>125,000	>125,000	>125,000
	NOED	>=125,000	>=125,000	>=125,000	>=125,000

B

Critical Doses		0-24 h	0-48 h	0-72 h	0-96 h
Survival					
95%-CL	LD10	9,302	9,302	6,399	0,568
	lower	5,374	5,374	3,168	0,039
	upper	13,344	13,344	9,438	1,457
95%-CL	LD20	17,695	17,695	13,962	2,228
	lower	12,417	12,417	9,467	0,567
	upper	36,549	36,549	25,795	4,020
95%-CL	LD50	n.d.	n.d.	n.d.	30,445
	lower	n.d.	n.d.	n.d.	15,432
	upper	n.d.	n.d.	n.d.	175,049
Survival	LOED	>20,000	>20,000	>20,000	>20,000
	NOED	>=20,000	>=20,000	>=20,000	>=20,000

C

Critical Doses		0-24 h	0-48 h	0-72 h	0-96 h
Survival					
95%-CL	LD10	n.d.	n.d.	n.d.	n.d.
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
95%-CL	LD20	n.d.	n.d.	n.d.	n.d.
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
95%-CL	LD50	n.d.	n.d.	n.d.	n.d.
	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
Survival	LOED	>100,000	>100,000	>100,000	>100,000
	NOED	>=100,000	>=100,000	>=100,000	>=100,000

n.d.: not determined due to mathematical reasons or inappropriate data

Fig1. Toxycological end-point of Gly (A), $CoCl_2$ (B) and Gly+$CoCl_2$(C) Data obtained from ToxRat software.

Results 2: Sublethal alterations

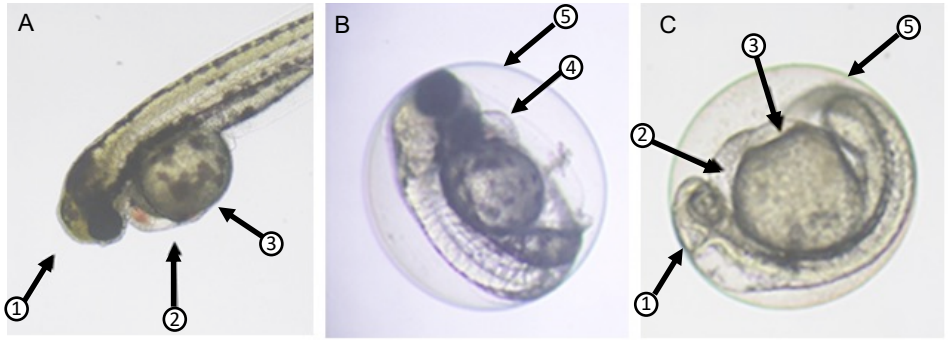


Fig.2 Sub-lethal alterations of Gly (A), $CoCl_2$ (B), and Gly+$CoCl_2$ (C). **1**, smaller head size; **2**, pericardial edema, reduction of blood circulation and blood stasis; **3**, yolk sac edema; **4** pericardial edema; **5**, hatching delay.

Results 3: Oxidative stress

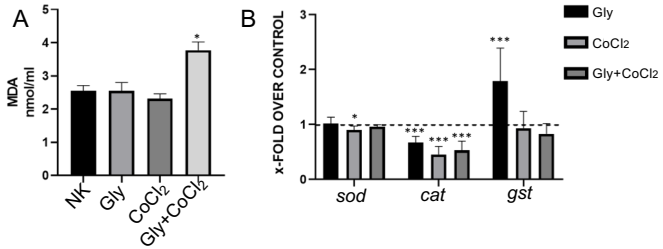


Fig.3 A Malondialdeldehyde detection in zebrafish larvae at 96hpf. B, gene expression analysis of *sod*, *cat* and *gst* in zebrafish larvae at 96hpf

Conclusion

The data obtained confirmed the Gly toxicity in zebrafish. Gly in combination with $CoCl_2$ reduces the death rate compared to single compounds; anyway, Gly+$CoCl_2$ treatment did not recover from the most relevant sublethal alteration and caused an increase in lipid peroxidation accompanied by decreased *gst* expression levels. This data points to a modification of redox status induced by $CoCl_2$, increasing the detrimental effects of Gly. Further analyses are needed to confirm this hypothesis.

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Toxicological effects of microplastics on zebrafish early development stages

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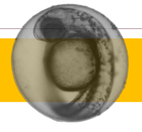
Background

In recent years, scientific research has focused on microplastics (MPs) and the associated effects of their massive diffusion. MPs are found in different environmental matrices, in marine and freshwater ecosystems and are considered toxic materials, as vectors of microorganisms and other potentially toxic chemicals.[1] *Danio rerio*, commonly known as zebrafish, is considered an excellent model organism in ecotoxicological studies for its transparent embryos, which allow to highlight malformation and disorders.[2]

Aim & Methods



In this study, zebrafish embryos were used to evaluate the possible toxic effects of polystyrene MPs of 1µm and 3µm diameter. The concentrations of 0.01, 0.1, 1.0, and 10.0 mgL^{-1} were tested, and the embryos were monitored at 24, 48 and 72 hours. Nile Red staining was used to observe the possible localization of MPs, while Acridine Orange was applied to highlight apoptotic cells.

Results

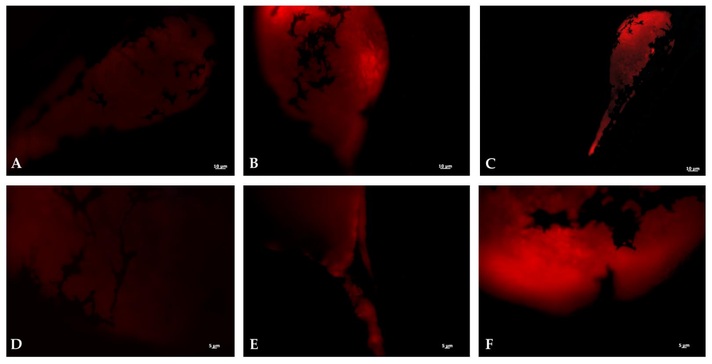


Fig. 1. Nile Red staining at 72 h after treatment where it can be observed MPs localization. (A, D) control larvae; larvae treated with 10.0 mgL-1 of 1 µm (B, E) and 3 µm (C,F) MPs.

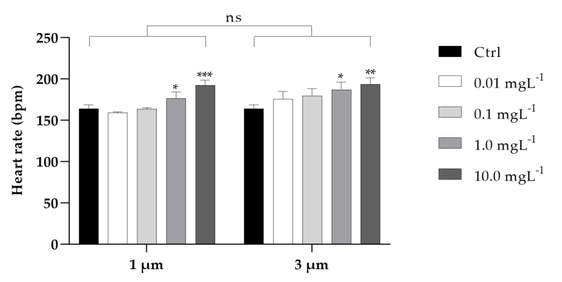


Fig. 3. Heart rate at 72 h after treatment. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

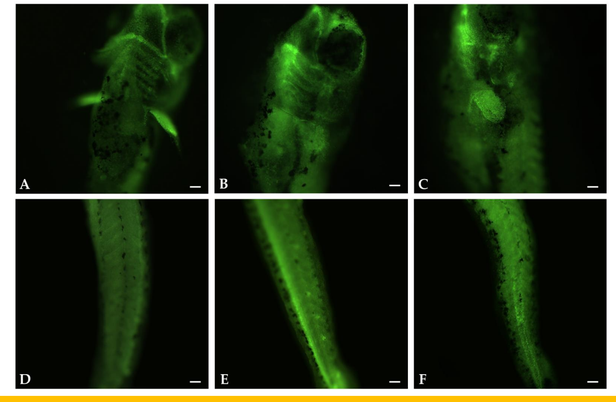


Fig. 4. Acridine orange staining at 72 h after treatment. (A, D) Head and tail of control larvae; head and tail of larvae treated with 10.0 mgL^{-1} of 1 µm (B, E) and 3 µm (C, F) MPs.

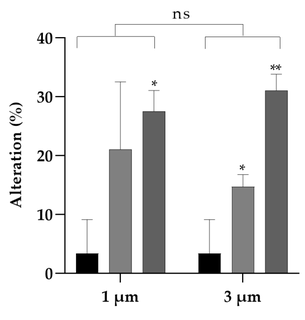


Fig. 2. Phenotypic alterations at 72 h after treatment. (* $p < 0.05$ ** $p < 0.01$)
(A) Control larvae; (B,C) larvae tretaed with 10.0 mgL^{-1} of 1 µm and 3 µm MPs, respectively.

Conclusion

Nile Red staining showed that MPs of both sizes at 10 mgL^{-1} enter and accumulate in the embryos (Fig. 1). Higher concentrations also caused phenotypic alterations (Fig. 2) and accelerated the heart rate frequency (Fig. 3). The Acridine Orange staining highlighted the presence of apoptotic cells in the head and tail, at 72 h at the same concentrations (Fig. 4). In conclusion, no difference was found between the two sizes of microplastics, and both were found to be toxic to developing zebrafish embryos. Future investigations will lead to a better understanding of molecular mechanisms at the base of these results and the effects of long-term MPs exposure.

References

1. Prata, J. C., et al. Science of the total environment 2020, 702: 134455.
2. Capriello, T., et al. Food and Chemical Toxicology 2021, 147: 111877.

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BENZODIAZEPINE DELORAZEPAM INTERFERENCE WITH EARLY PARACENTROTUS LIVIDUS DEVELOPMENT

68° Convegno GEI - SIBSC Messina 2023

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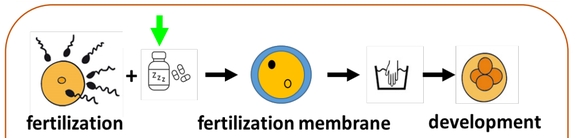
Background and Aims

Benzodiazepines are present in aquatic environments at concentrations ranging from ng to µg/L[1,2]. By binding to the GABA-A and TSPO receptors, they interfere with flora and fauna[3,4,5] with high potency and efficacy. Behavioural and functional alterations are induced in adults[3] and embryonic stages[4].

The growing concern prompted this study on the effect of delorazepam on sea urchin fertilization and early development.

Methods



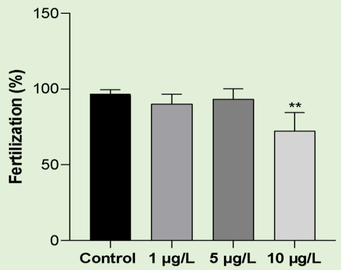
Gametes and **embryos** were obtained from *Paracentrotus lividus*[6].

DLZ (commercial preparation, oral drops) was diluted in seawater at 1, 5, and 10 µg/L[1].

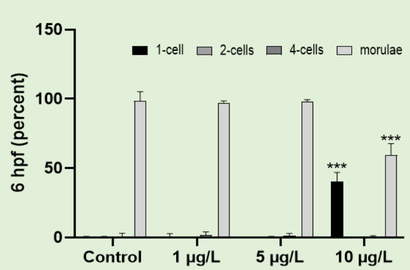
Endpoints: fertilization rate, growth, percent of normal and altered embryos, presence and localization of carbohydrate residues (FITC-lectins staining).

Results

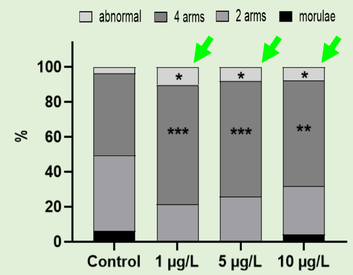
DLZ at fertilization



DLZ (10 µg/L) reduces fertilization rate (**, p<0.01)

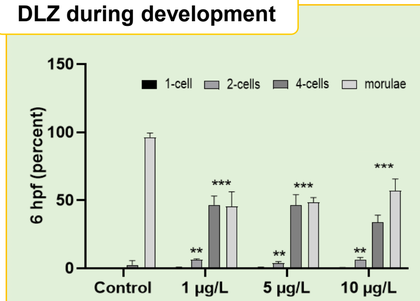


DLZ (10 µg/L) delays early development (***, p<0.001)

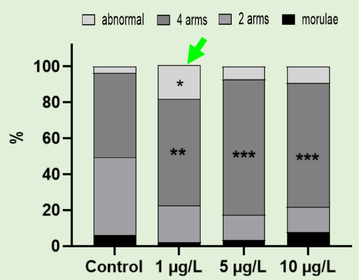


DLZ accelerates late development and increases plutei anomalies (arrows). (*, p<0.05; **, p<0.01; ***, p<0.001)

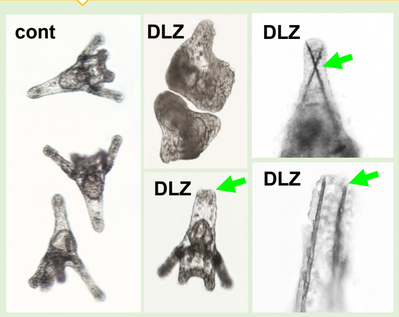
DLZ during development



DLZ, at all concentrations, delays early development (**, p<0.01; ***, p<0.001)

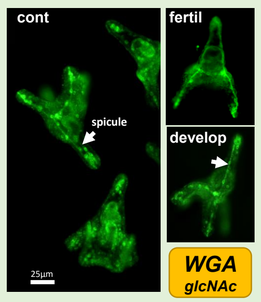


DLZ accelerates late development and increases plutei anomalies (arrow). (*, p<0.05; **, p<0.01; ***, p<0.001)



Most found altered plutei. Anomalies (arrows)

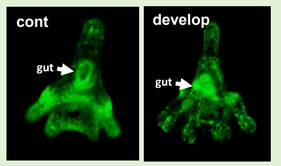
DLZ, at all concentrations tested, does not modify carbohydrate presence and distribution



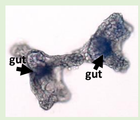
WGA *glcNAc*

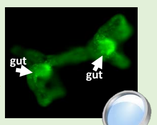
Stains spicules (arrows); no difference between treatments

Stains gut (white arrows); no difference between treatments

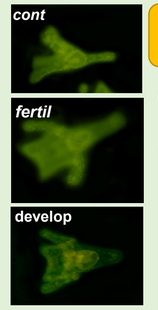


Con A *D-mannose*



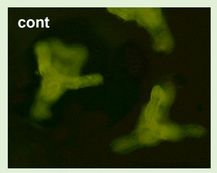


Useful to highlight the possible onset of gut damage



DBA *galNAc*

Plutei remain unstained



Plutei are not autofluorescent

Conclusion

DLZ initially delays and then accelerates development

DLZ at fertilization markedly increases the number of altered plutei

No evidence found, so far, of interference with sugar composition

References

1) Esteban et al. 2012. Gaceta sanitaria 26,457. 2) Fick et al. 2017. Chemosphere 176,324. 3) Fogliano et al. 2023. Env Toxicol Pharm 97,104030. 4) Fogliano et al. 2022. Aquat Toxicol 250, 106244. 5) Overturf et al. 2016. Comp Biochem Physiol C 183,46. 6) Vacquier, 2011. Mol repr dev 78, 553

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MICROPLASTICS AND NANOPLASTICS POLYSTYRENE IMPAIR DIGESTIVE AND BYSSUS GLANDS IN *Mytilus galloprovincialis*

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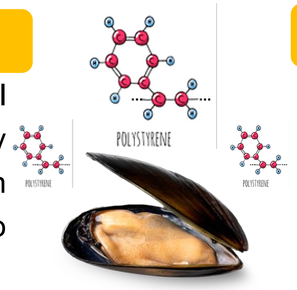
Background

Plastic is the most prevalently used material in our modern society; all socioeconomic activities are supported by plastic production that has exponentially increased in the past 70 years. More than 94% of plastics currently present in oceans are represented by Microplastics (MPs) (<5 mm), further degraded into Nanoplastics (NPs) (< 100nm) (Stapleton et al., 2019).



Aim & Methods

To understand how MPs and NPs interfere with marine organisms' life, *M. galloprovincialis* was used in the present study as a model organism (Cappello et al., 2021). Mussels were exposed to polystyrene (5 or 0.1 µm) for 1, 3 and 11 days at frequent environmental concentrations (MPs 100 or NPs $2.17x10^4$ particles/ml) (Vroom et al., 2017). Samples were processed for light microscopy. Hematoxylin & Eosin, Mallory's trichrome and Picrosirius red stains were performed.

Results

ALTERATIONS IN DIGESTIVE GLAND

Fig.1

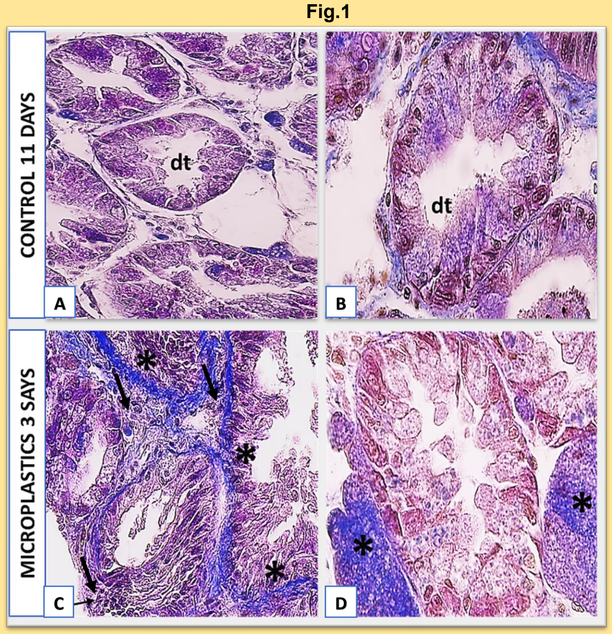
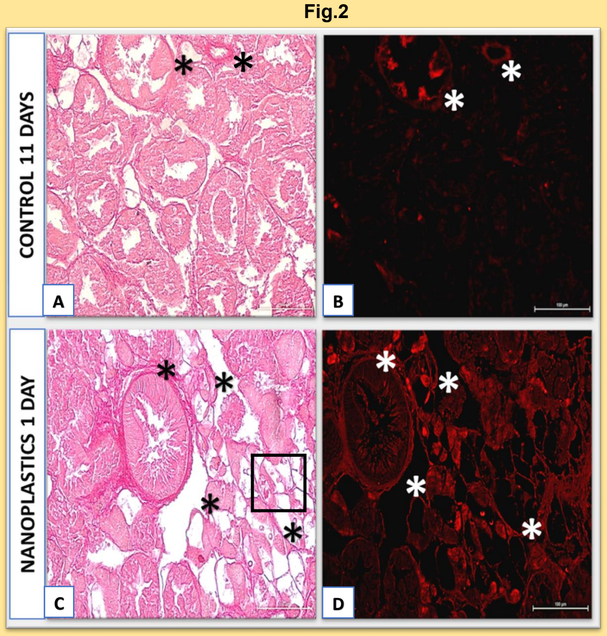


Fig.2



Microplastics and Nanoplastics affect the digestive gland structures in the tubules (dt) and ducts organization, interfering with the hemocytes infiltration (arrows) collagen deposition (asterisks)(Fig.1-2, C-D) within the digestive tubules. Presence of vacuolization (square)(Fig.2,C-D) were also observed.

Fig.1 A-C magnification 600x, B-D magnification 1000x. Fig.2 A-B-C-D magnification 200x.

ALTERATIONS IN BYSSUS APPARATUS

Fig.3

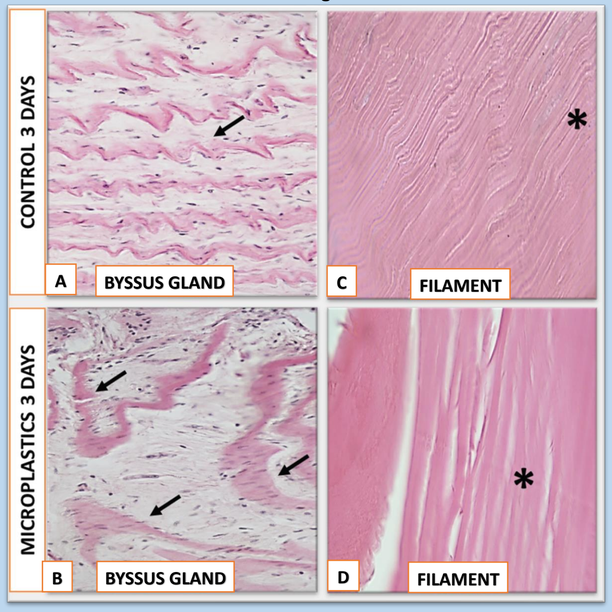
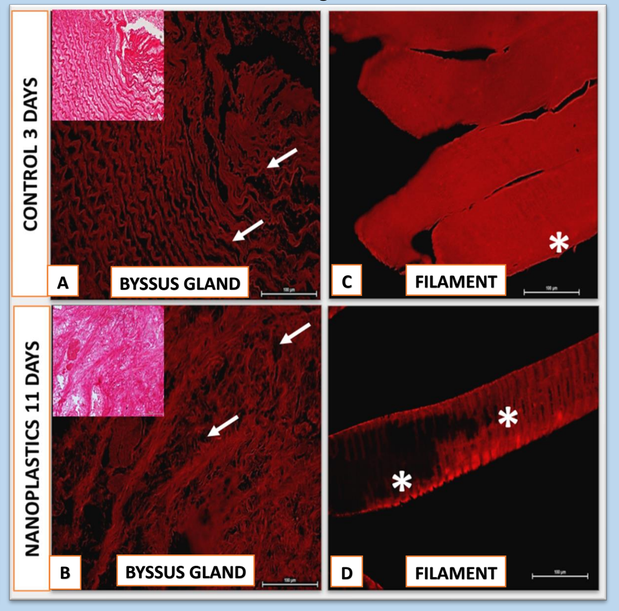


Fig.4



Microplastics and Nanoplastics interfere with byssus gland with strong alterations in lamellae organitazion (Fig.3-4, A-B). Filaments showed discontinuities and loss of collagen deposition (Fig. 3-4, C-D).

Fig.3 A-B magnification 400x, C-D magnification 1000x.Fig.4 A-B-C-D magnification 200x.

Conclusion

MPs and NPs impair the feeding activity and substrate adhesion of *Mytilus galloprovincialis*. Reducing MPs and NPs contamination is a crucial aspect to recover and defend the ecosystems.

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EFFECTS OF AN EXTENSIVELY USED UV-FILTER (OXYBENZONE) ON THE ELIMINATION OF LIPOPHILIC TOXINS IN THE CLAM (*Donax trunculus*)

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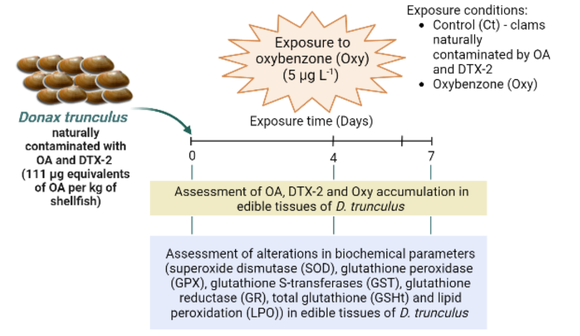
Background

Donax trunculus is a clam with high economic value in the southern Europe, which capture is often interdicted due to the presence of lipophilic toxins [okadaic acid (OA) and dinophysistoxin-2 (DTX-2)]. In southern Portugal, interdiction periods for *D. trunculus* capture have been increasing, mainly in summer. It can be hypothesized that the occurrence of increased levels of contaminants in coastal waters in summer months, such as UV filters, may overload toxin elimination pathways (OA and DTX-2), inhibiting *D. trunculus* ability to metabolize these compounds.

Aim & Methods

To explore the hypothesis that the exposure of *D. trunculus* to oxybenzone (Oxy) could interfere with the bivalve metabolization of lipophilic toxins [okadaic acid (OA) and dinophysistoxin-2 (DTX-2)].



Results and Discussion

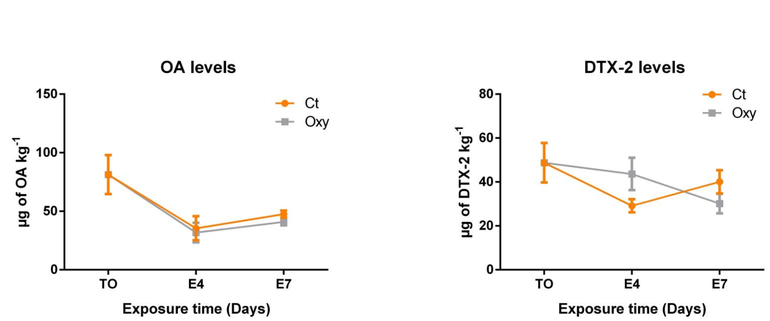


Figure 1. Levels of OA and DTX-2 in the edible tissues of *D.trunculus* at the pre-exposure time (T0, clams naturally contaminated by OA and DTX-2), and after exposure to Oxy for 4 and 7 days (E4 and E7) both in Ct group (clams naturally contaminated by OA and DTX-2) and Oxy group, respectively).

- A reduction of both lipophilic toxins levels was recorded at E4 and maintained at E7. The rate of DTX-2 elimination was slightly higher in the absence of oxybenzone in the water.

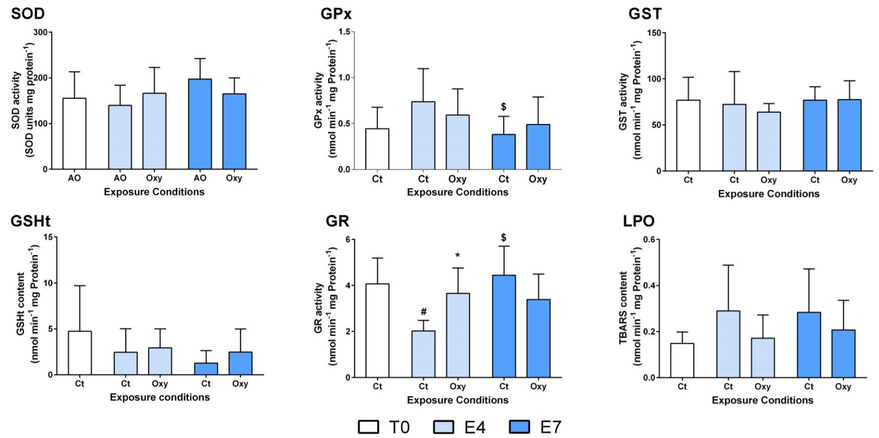


Figure 2. SOD, GPX, GST, and GR activities, levels of GSHt and LPO in the edible tissues of *D. trunculus* at the pre-exposure time (T0, clams naturally contaminated by OA and DTX-2), and after exposure to Oxy for 4 and 7 days (E4 and E7, respectively) both in Ct and Oxy groups. * indicates differences compared to the Ct group at each experimental time, # indicates differences for Ct relative to T0, and $ indicates differences for the same exposure condition relative to E4).

- Only an increase of GR activity was recorded in clams upon oxybenzone exposure (E4), suggesting a very mild effect of this compound, while the interference of oxybenzone with lipophilic toxins metabolism could not be clearly discerned.

Conclusion

The occurrence of oxybenzone in the water may delay the metabolism of DTX-2 by the clam *Donax trunculus*, as pointed out by this toxin toxicokinetics. Pro-oxidant effects of oxybenzone were only mildly discerned.

Acknowledgments

This work was supported by the MAR2020 project SCREEN&TOXIN (MAR2020-P04M03-1475P). P.P. is funded by national funds, through FCT under the Scientific Employment Stimulus (Individual Call) [CEECIND/01144/2017].

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Exploring retinoic acid role in crinoid embryogenesis

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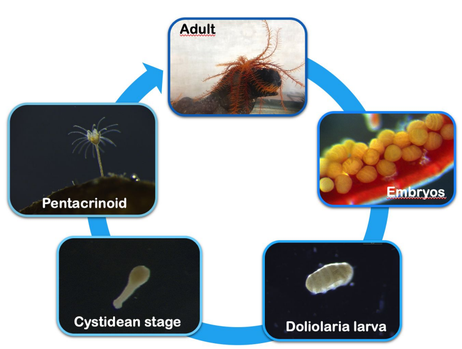
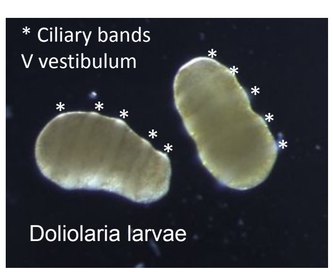
Background

Retinoic Acid (RA) is a Vitamin A-derived molecule which plays fundamental roles in chordate development[1] such as the determination of embryo body axis. It was discovered that RA machinery is present also in invertebrates, even if RA role in this animals is still poorly understood[1-2]. Crinoids are basal echinoderms and occupy a key phylogenetic position to elucidate RA machinery evolution in this peculiar group characterized by secondary pentameral symmetry[3].

Life cycle of *Antedon mediterranea*





Aim & Methods

Here, we exploited the model *Antedon mediterranea* to analyse the effects of RA exposure on development and metamorphosis. We treated embryos and larvae with different concentrations of RA (10^{-7}M, 10^{-6}M). Embryos were treated at cleavage stage for 5 days, while larvae were treated at doliolaria stage and then fixed after four days. SEM (Scanning electrone microscope) and immunolabelling of the nervous system were performed to start characterizing the effects of RA exposure.

Results

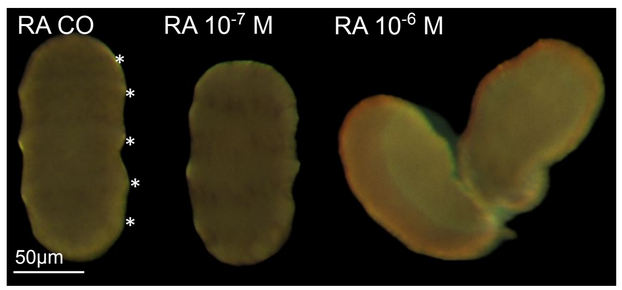


Fig.1 Doliolaria larvae. The treated samples body appeared less elongated compared to controls and this could probably have affected the pattern of the 5 ciliary bands.

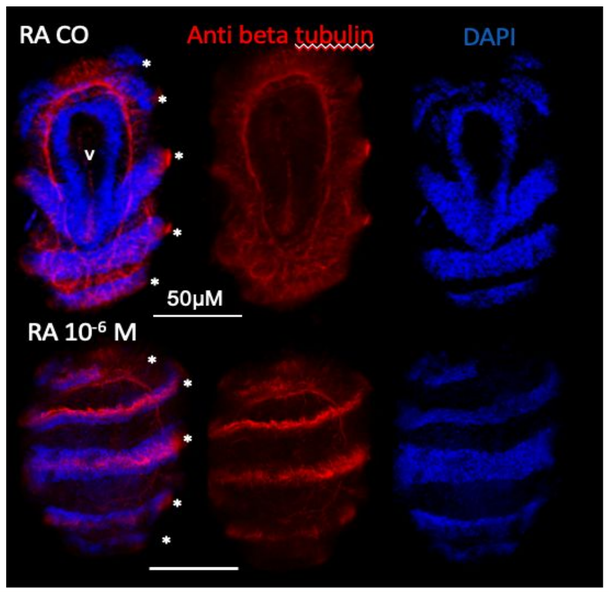


Fig.2 Immunolabeling revealed that the nervous system architectural seemed normal comparing control and treated larvae.

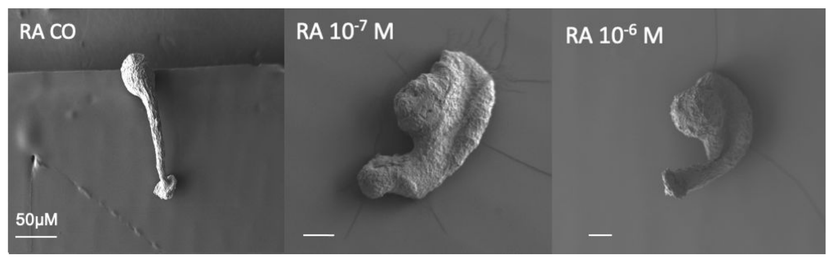


Fig.3 During metamorphosis, RA treatment prevented larval settlement but the process started and proceeded with an altered morphology.

Conclusion

These results provided a first hint on the effects of RA treatment during *A. mediterranea* development. Exposure to RA affected both embryo development and metamorphosis, suggesting that the molecule could play key roles during these processes. Further research will lead to a more comprehensive understanding of the evolution of RA functions in invertebrate deuterostomes.

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DNA DAMAGE BY POLYSTYRENE MICROPLASTICS IN ZEBRAFISH

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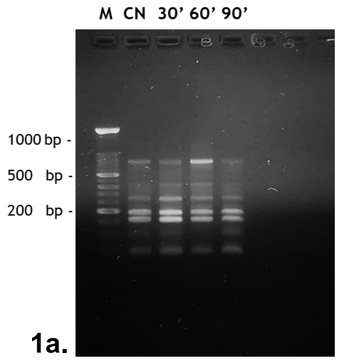
Background

In recent years, plastic pollution has become global environmental concern affecting both terrestrial and aquatic environments. Microplastics (MPs) derived from the degradation of plastic through physical-chemical processes, can be ingested by organisms and reach humans through the food chain. Although the mechanism of action and the health impact on the of exposed organisms are not yet fully understood, the literature data confirm deleterious health consequences following exposure to different types of microplastics.

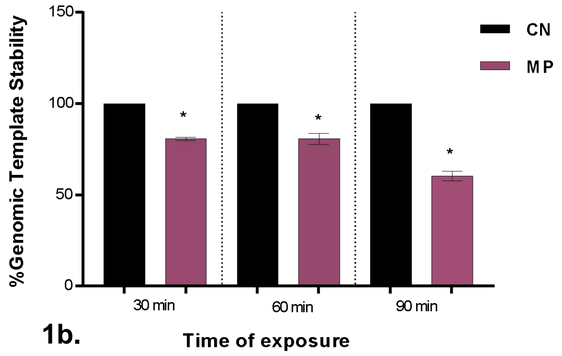
Aim & Methods

The aim of this research was to evaluate the *in vitro* genotoxic effects of polystyrene MPs on *Danio rerio* cells using RAPD-PCR, to quantify the genomic template stability (GTS), TUNEL reaction to evaluate MP-induced DNA fragmentation (DFI) and DCF assay to highlight a possible ROS-dependent mechanism of damage. Zebrafish blood cells were exposed to MPs (105 µg/ml) for 30, 60 and 90 minutes.

Results

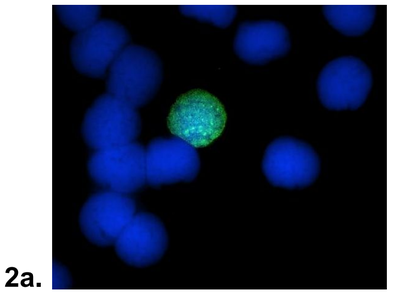


1a.

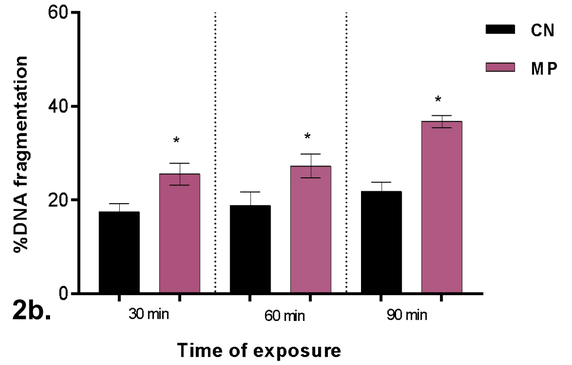


1b.

Figure 1.
a. RAPD-PCR profiles of DNA extracted from zebrafish following treatment with MPs.
b. %GTS after treatment with MPs.

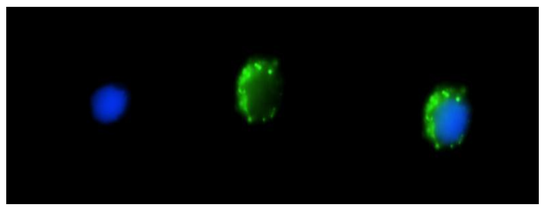


2a.

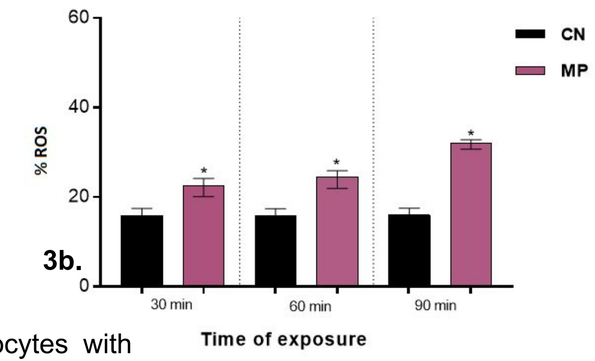


2b.

Figure 2.
a. Representative image of zebrafish erythrocytes with fragmented DNA (green fluorescence) and with intact DNA (blue fluorescence) analyzed by fluorescence microscope.
b. %DFI after MPs treatment.



3a.



3b.

Figure 3.
a. Representative image of zebrafish erythrocytes with intracellular ROS presence (green fluorescence) analyzed with fluorescence microscope.
b. %ROS after MPs treatment.

Conclusion

These results confirm the harmfulness of MPs, in particular their ability to interfere with genetic material causing apoptosis via oxidative imbalances. Further *in vitro* and *in vivo* studies evaluating the bioaccumulation processes will be needed to establish the mechanisms underlying the MPs damage.

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Environmental changes affect transposon expression in zebrafish (*Danio rerio*)

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Background

Human activities alter the environment through the emission of chemical substances that pollute the soil and the waters or contribute to the global warming. Growing scientific evidence suggests that environmental stressors can influence the expression and activity of transposable elements (TEs) [1]. TEs are DNA repetitive sequences able to move from one location to another within the genome. TEs activity can be mutagenic in organisms including humans, but it may also act as driving force at evolutionary level. The zebrafish is a good study model in many research areas, including molecular biology and ecotoxicology. Our research group demonstrated that environmental temperature alteration strongly impacts the brain proteome and behaviour of zebrafish [2-5]. Chen et. al observed that cold temperature induces retrotransposition in zebrafish [6]. Bioinformatics analysis of transcriptomes is a useful tool to identify variations in TEs expression in teleosts [7].

Aim & Methods

The aim of the project is to verify if altered environmental conditions can affect the activity and transcription of TEs. A total of 66 transcriptomes from zebrafish subjected to temperature change or contaminant exposure (pesticides, nanoparticles and drugs) were selected from the NCBI database. We chose the transcriptomes with the highest quality and which had more replicates for each condition. Transcriptomes were processed by RNA-seq data analysis: FASTqc and MULTIqc were used for quality control, Trimmomatic and Cutadapt for quality trimming, Hisat2 for read alignment, TEcount for expression quantification, and DESeq2 for differential expression analysis of both gene sequences and transposons.

Results

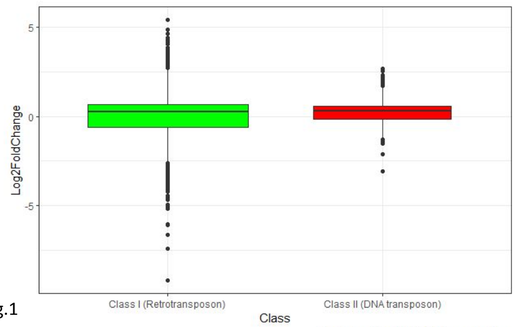

Fig.1

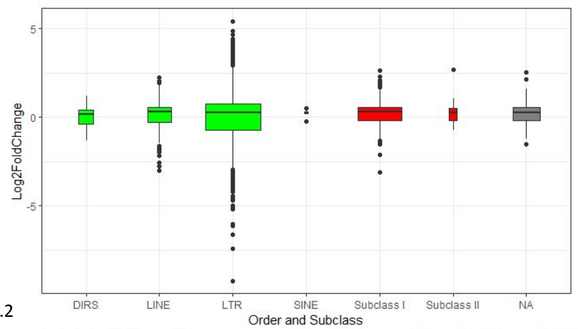

Fig.2

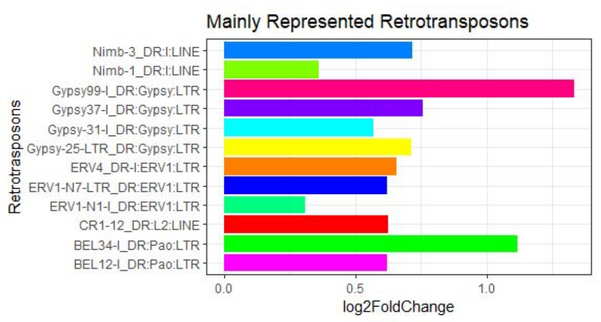

Fig.3

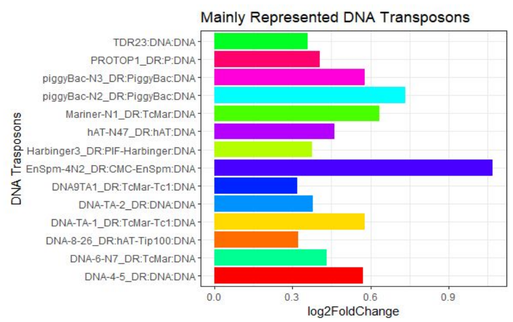

Fig.4

Overall the results show the differential expression of 982 TEs. The treatments analysed alter both the expression of class I (retrotransposons) and class II (DNA transposons) TEs, determining increases or decreases depending on the individual cases (Fig.1). Among class II TEs, the alteration of environmental parameters influences TEs belonging to all the main classes (DIRS, LINEs, LTR, and SINEs). LINEs TEs show a greater tendency to increase expression (Fig.2). The analysis allowed to identify the TEs whose expression increases more often in the analyzed transcriptomes (Figs 3,4). This information is useful for selecting the TEs to be analyzed in real time experiments when it is not possible to provide a complete transcriptomic analysis.

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Conclusion

Results show that the environmental changes affect TEs expression in zebrafish suggesting that such variations can alter gene functionality and determine genomic structural variations that can also be transmitted to progeny if they occur in the germline. So, environmental alterations may have a greater potential impact on individual health than is currently known. The increased activity of TEs following environmental changes could lead to increased phenotypic variability on which the mechanisms of natural selection can act.

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GENOTOXIC EVALUATION IN ZEBRAFISH OF QUINOIN, TYPE 1 RIBOSOME-INACTIVATING PROTEIN FROM QUINOA SEEDS

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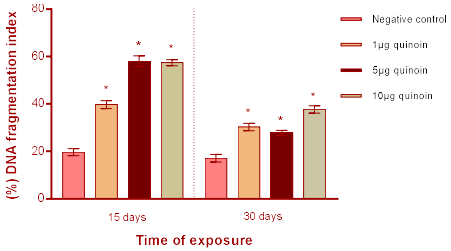
Background

Nowadays, functional foods have greatly increased attention thanks to the numerous benefits for human health. Among them, quinoa contains essential amino acids and minerals, for which is suitable for consumption. However, quinoin (~30-kDa), a toxic enzyme classified as ribosome-inactivating protein, recently found in quinoa seeds (*Chenopodium quinoa* Wild), exhibits *in vitro* cytotoxic action towards both normal fibroblasts and keratinocytes and several tumour cell lines.

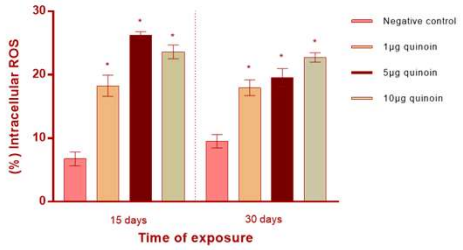
Aim & Methods

Our study aims to evaluate quinoin genotoxicity on zebrafish specimens after intraperitoneal route administration of three different quinoin amounts (1, 5 and 10 µg) for 15 and 30 days of treatment by means: i) TUNEL reaction to evaluate DNA fragmentation index; ii) RAPD-PCR and relative calculation of the genomic template stability (GTS%) to estimate genome stability; and iii) DCF assay to evaluate intracellular ROS occurrence.

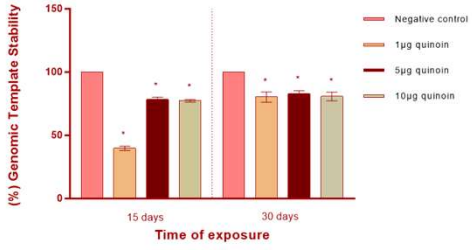
Results



Percentage of DNA fragmentation observed in zebrafish blood cells after 15 and 30 exposure days to different amounts of quinoin. *p<0.05.



Percentage of intracellular ROS observed in zebrafish blood cells after 15 and 30 exposure days to different amounts of quinoin. *p < 0.05.

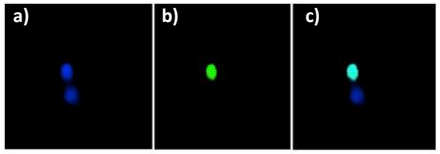


Percentage of Genomic Template Stability in zebrafish specimens after 15 and 30 exposure days to different amounts of quinoin. *p < 0.05.

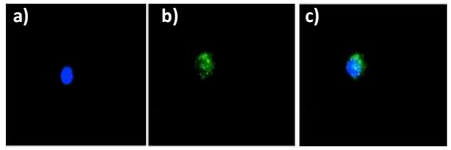
Control bands:
200bp, 220bp, 300bp, 400bp, 520bp.

QUINOIN AMOUNT	DAYS OF EXPOSURE	LOST BANDS	GAINED BANDS
1 µg	15	–	280 bp, 320 bp, 550 bp
	30	520 bp	–
5 µg	15	–	280 bp
	30	520 bp	–
10 µg	15	–	280 bp
		520 bp	–

RAPD-PCR profiles of zebrafish DNA following 15 and 30 days of treatment with different amounts of quinoin.



Quinoin-treated zebrafish erythrocytes with fragmented DNA (green nucleus) and without DNA fragmentation (blue nucleus) following the Tunel technique. a)- DAPI; b)-FITC; c)- merge.



Quinoin-treated zebrafish erythrocyte with intracellular ROS (green fluorescence) following the DCF Assay. a)- DAPI; b)-FITC; c)- merge.

Conclusion

Quinoin can induce genotoxic damage to the zebrafish genome acting through ROS formation. The lower percentage of damage at longer quinoin treatment compared to shorter ones could indicate the activation of detoxifying and/or repair mechanisms and/or a loss of protein activity by enzymatic digestion in the gastrointestinal tract. Our data suggest that the presence of quinoin in quinoa seeds could be very harmful if this pseudocereal is consumed with inappropriate cooking, considering the melting temperature (Tm= 70 °C) of quinoin.

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BENEFICIAL BACTERIA TO COUNTERACT PFOA TOXICITY ON *Danio rerio* DEVELOPMENT

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[4]Department of Biological Sciences, University of Calgary, Calgary, Canada.

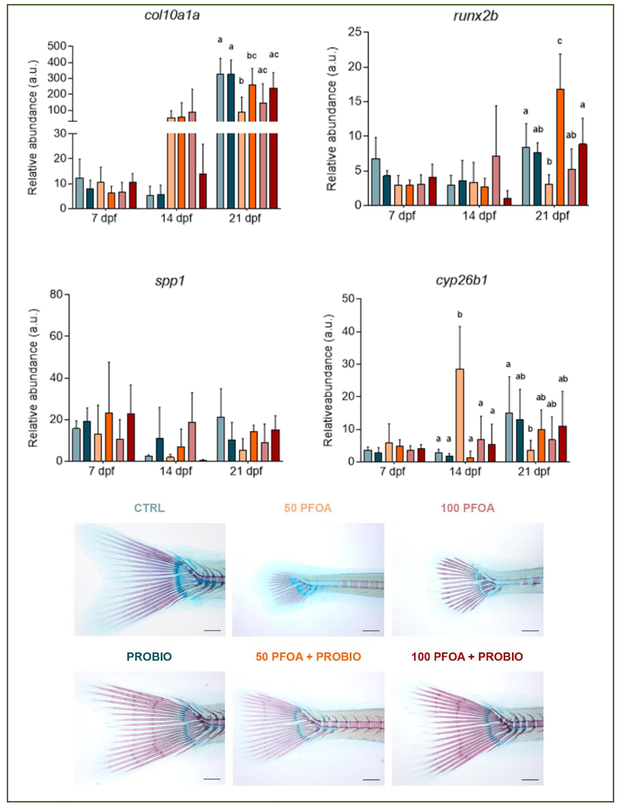
Background

Perfluorooctanoic acid (PFOA) is a surfactant and a flame retardant very resistant towards biological degradation, which is nowadays environmentally ubiquitous. Studies conducted on zebrafish demonstrated its toxic effects on embryo development through reduction of both heartbeat rate and locomotor behaviour[1] and increasing the malformations level in a thyroid hormone dependent way[2]. Probiotics instead were observed to improve *Danio rerio* embryo development, ossification, both *in vivo*[3,4] and *in vitro*[5], and zebrafish fin regeneration[6].

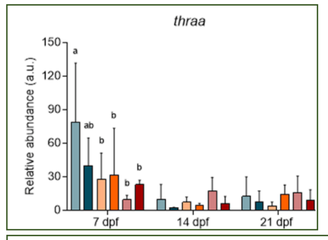
Aim & Methods

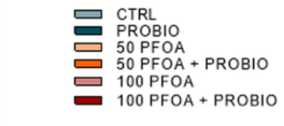
In the present study, the role of *Bacillus subtilis natto* in mitigating PFOA developmental toxicity during zebrafish early development was investigated through morphological (alcian blue and alizarin red (Ab/Ar) staining) and molecular analysis (RT-qPCR transcript analysis). Control and larvae exposed to 50 and 100 mg/L of PFOA were reared, with or without *Bacillus subtilis natto* dietary administration (10^7 CFU/larvae/day), starting from hatching until 21 days post fertilization (dpf). Specimens were sampled at 7, 14 and 21 dpf and further analyzed.

Results

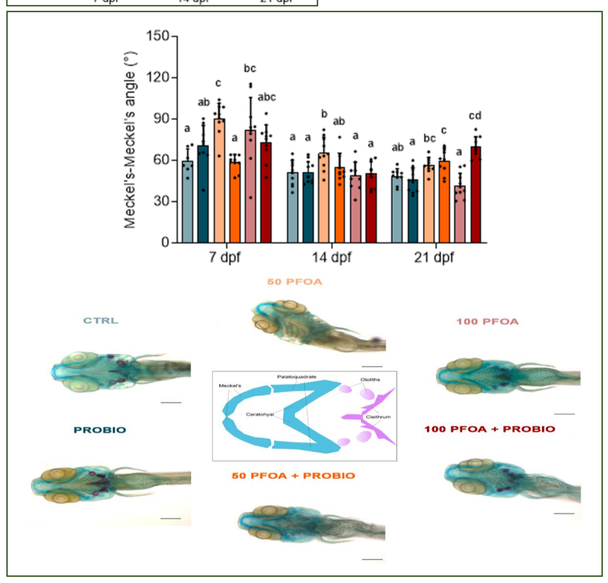


Transcript analysis of master genes involved in ossification and representative Ab/Ar stained larvae

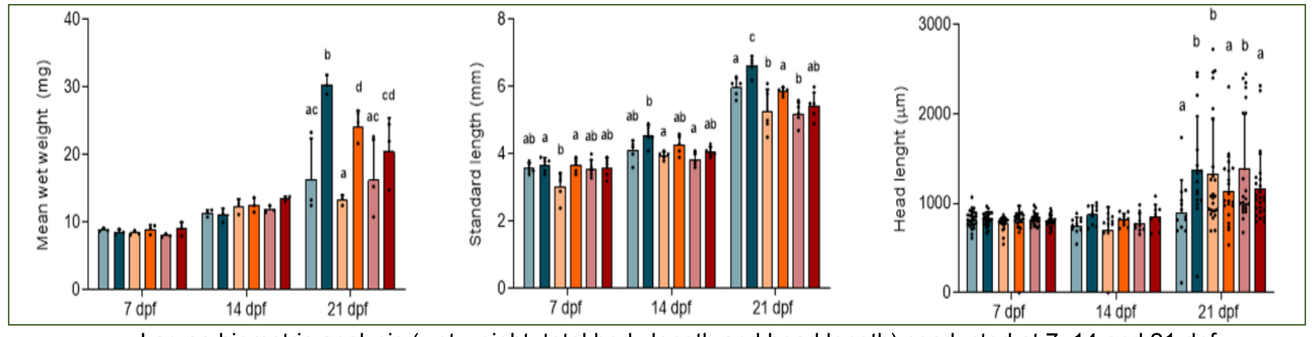




Transcript analysis of thyroid hormone receptor in 7, 14 and 21 dpf larvae



Meckel's-Meckel's angle analysis in Ab/Ar stained larvae and respectively representative microphotographs



Larvae biometric analysis (wet weight, total body length and head length) conducted at 7, 14 and 21 dpf.

Conclusion

Bacillus subtilis natto mitigated PFOA detrimental effects as evidenced by the results of the biometries and ossification genes transcription conducted at 21 dpf, the most impacted endpoint. At 21 dpf, probiotic seemed to exacerbate the contaminant damage on Meckel's-Meckel's angle, while at 7 and 14 dpf the mitigation of PFOA effect is evident. PFOA effect on *thraa* level at 7 dpf could be the cause of the above-mentioned detrimental effects and the trend in increase regarding co-administered groups could be the reason of the recovery

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Tempol-methoxycinnamate, an environmentally-friendly UV filter? Evidence from zebrafish early development

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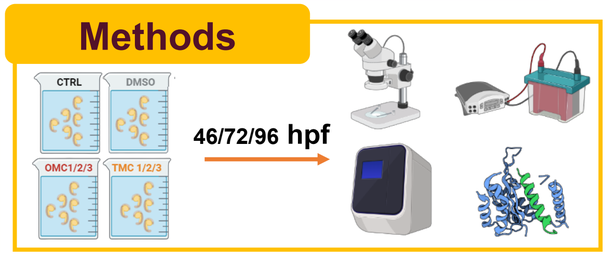
Background

In the last years, the use of ultraviolet (UV) organic filters in personal care products has increased due to the growing need of preventing skin damage caused by UV radiation overexposure. To date, ***octyl-methoxycinnamate*** (**OMC**), has been used worldwide in over 90% of cosmetic products, however its endocrine disrupting effects have been documented in vitro and in vivo studies[1,2].

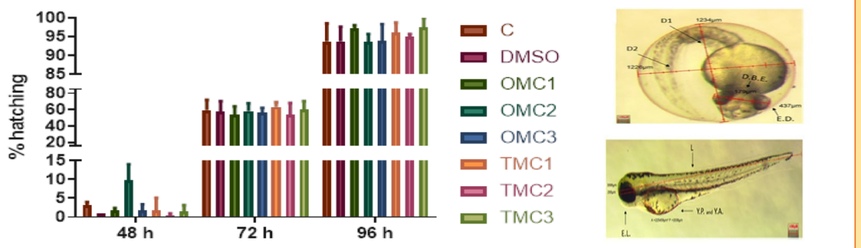
Aim

Verify the suitability of ***Tempol-methoxycinnamate*** (**TMC**), a recently synthesized OMC derivative[3], as alternative, safer, environmental friendly UV filter, on zebrafish, *Danio rerio*, early development.

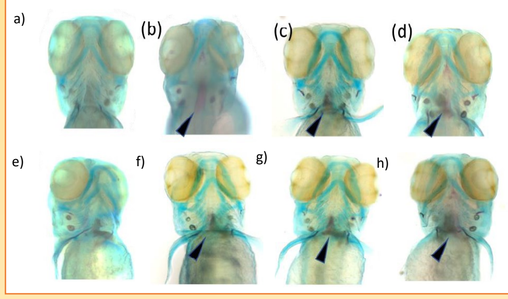
Methods



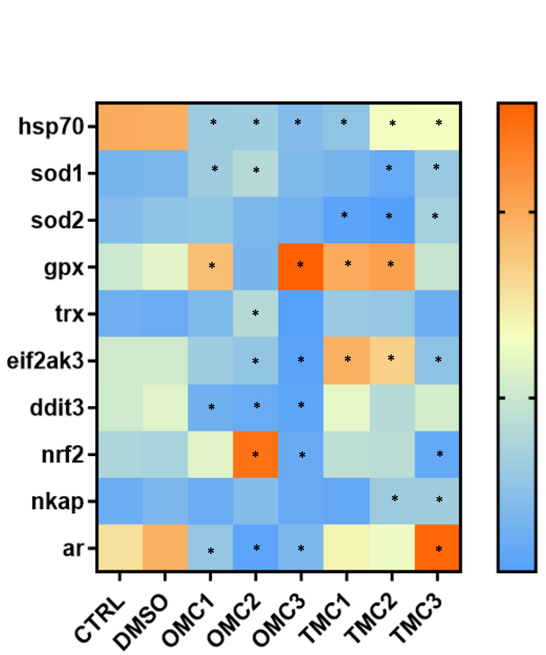
Results

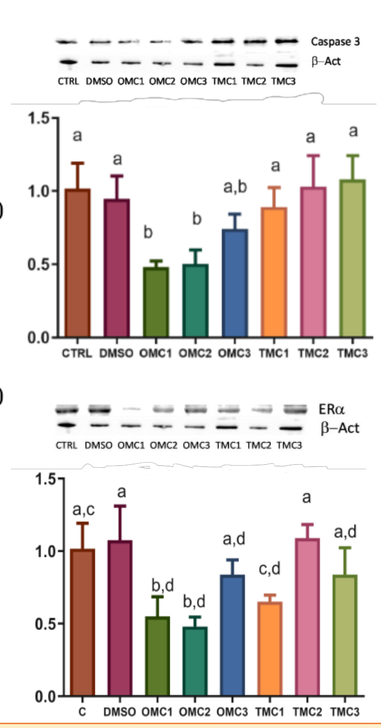


At 48 h in OMC2-exposed embryos, the hatching rate was higher, but not to a significant extent respect to the other experimental groups. At 72 hpf and at 96 hpf, the hatching rate reached 60% and 90% in all groups, but no statistical significances differences were observed among treatment.



At 96 hpf, in larvae exposed to OMC (a–d) and TMC (e–h) evidence of calcified basioccipital articulatory process (BOP) were seen, suggesting a role of both filters in the acceleration development.

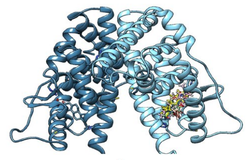




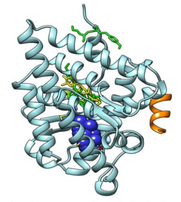
TMC, differently from OMC, which significantly affects both the ar and ERα levels, has scarce hormone-like activity. Only OMC affects apoptosis, which has a key role in embryo shaping at this stage of early development.

Results suggest that TMC, differently from OMC, potentiates the larvae oxidative stress and immune response, suggesting that organisms could be more prone to contrast ROS production.

a)



b)



TMC (a) and OMC were docked to ER and AR (b) in order to evaluate their possible hormone like activity. TMC, differently to OMC presents a lower binding affinity and capacity to both receptor suggesting a possible lower endocrine interference.

Conclusion

In conclusion, TMC does not affect the hatching rate, embryo morphology, ossification process, does not induce ER stress response and apoptosis respect to control fish. In addition, it potentiates the larvae oxidative stress response, suggesting that organisms could be more prone to contrasting ROS production caused by oxidative stimuli, such as UV light exposure.

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AN INSIGHT INTO THE HAZARDOUS EFFECTS OF GLYPHOSATE DIETARY DOSES ON ZEBRAFISH MALE REPRODUCTION

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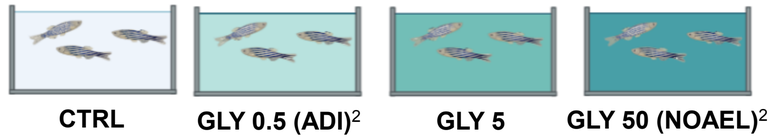
Background

Glyphosate (GLY) is one of the world's leading agrochemicals since it is widely applied for weed control and desiccation. In Italy, although the use of GLY for gardening and pre-harvesting purposes has been banned, this compound is still present in edible products due to its application during harvest and threshing as well as to the import of wheat from countries that are more permissive to GLY use[1].

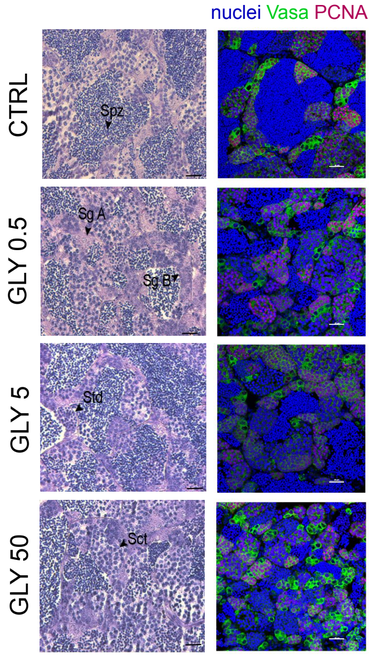
Aim & Methods

The aim of this study is to shed some light on the effects of doses of GLY, which are considered safe by the EFSA, on spermatogenesis and male breeding capacity, using zebrafish as model species.

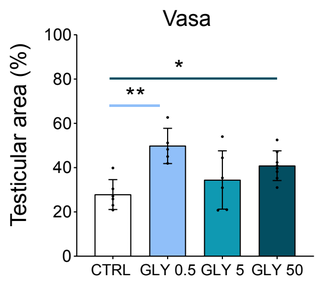


- ✓ Testicular architecture
- ✓ mRNA and protein levels
- ✓ Epigenetic modifications
- ✓ Fertility and F1

Results



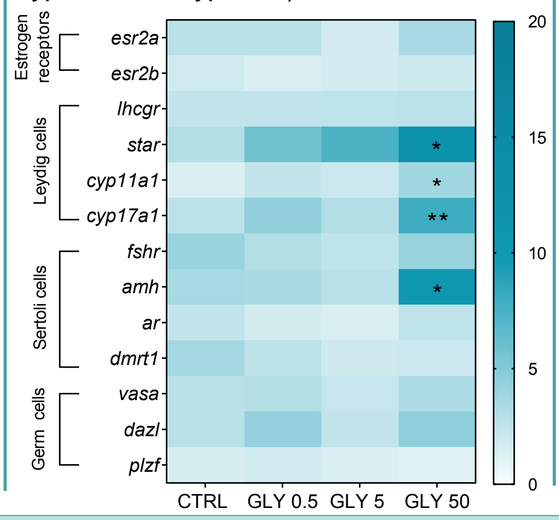
Testicular architecture

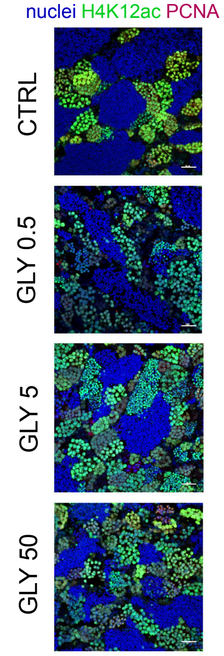


The exposure to GLY 0.5 and 50 leads to an uncontrolled proliferation of spermatogonia (Sg) and decrease the proportion of the spermatozoa (Spz) and spermatocytes (Stc), respectively. Likewise, the testicular area of Vasa-positive cells is significantly increased in both doses.

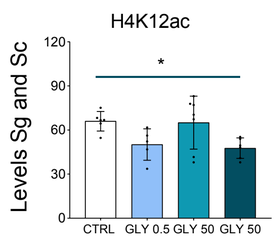
Steroidogenesis

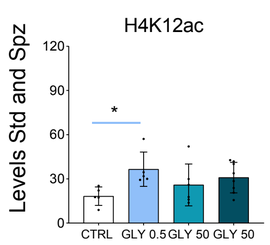
GLY 50 promotes the expression of genes involved in steroid production (*star*, *cyp11a1* and *cyp17a1*) and *amh*

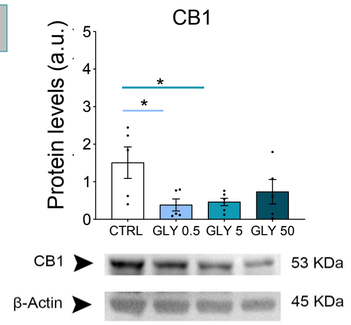




Epigenotoxicity







GLY 50 decreases H4K12ac in Sgs and Scts. GLY 0.5 triggers hyperacetylation of H4K12 in Stds and Spz, this epigenetic change being likely linked to alterations in CB1 levels, as reported in mouse sperm[3].

Fertility

	CTRL	GLY 0.5	GLY 5	GLY 50
Number of fertilized eggs	110 ± 74	115.67 ± 38.56	46.67 ± 50.33	None
Embryo mortality at 3.3 hpf (%)	0	0	6 ± 1.41 *	-
Hatching rate (%)	90.46 ± 14.87	85.43 ± 20.85	82 ± 25.46	-

GLY 50-exposed fishes do not reproduce at all

Conclusion

Altogether, these data support the need of a deeper revaluation of the GLY safety standards by the agencies at National and European level.

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