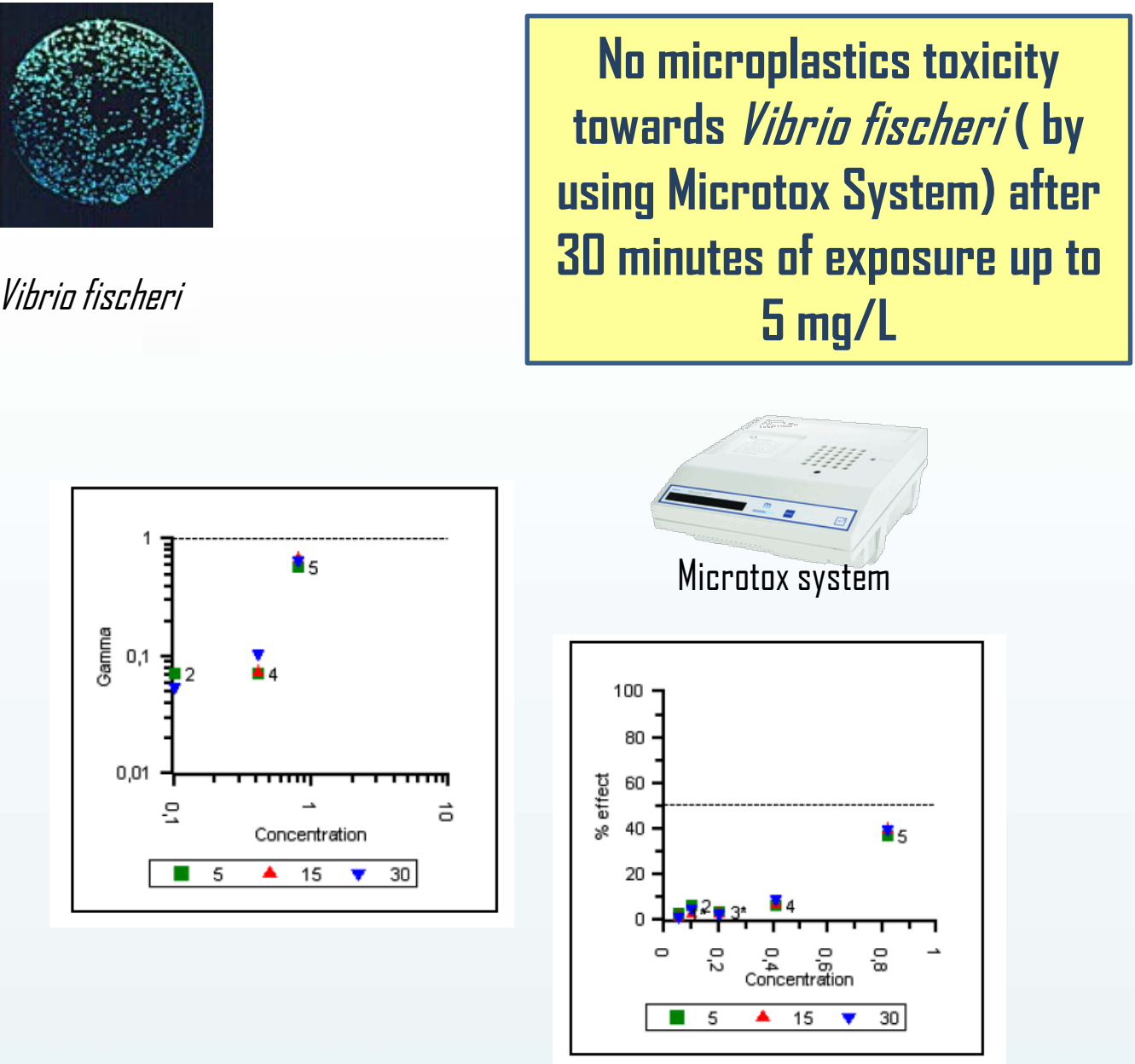


CIIMAR: A. Pacheco, L. Ghlhermino; **IED:** D. Rial, S. Garrido, M. Linares, J. Bellas, M. Albentosa; **ISMAR:** C. Gambardella, V. Piazza, F. Garaventa, E. Costa; **UALg:** S. O'Donovan, N. Mestre, M.J. Bebianno; **UBor:** N. de Lima, C. Clérandeau, B. Morin, J. Cachot; **UHei:** A. Batel, P. Heinrich, T. Braunbeck; **UMU:** C. Espinosa, M.A. Esteban A. Cuesta; **UiO:** A. Bour, K. Hylland; **UVi:** S. González, R. Beiras

TOXICITY ASSESSMENT
WP3 Organism Level WP4 Cellular and Molecular levels

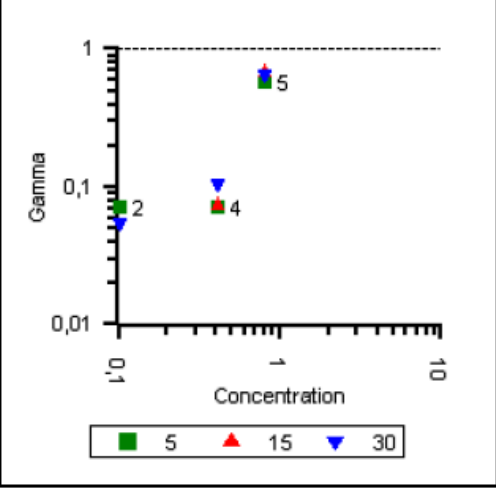
NO FEEDERS

MICROTOX

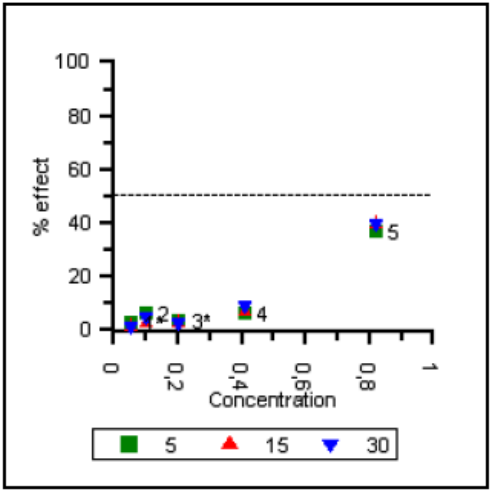


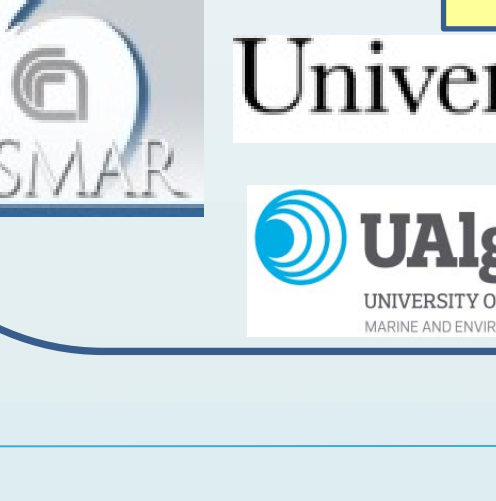
No microplastics toxicity towards *Vibrio fischeri* (by using Microtox System) after 30 minutes of exposure up to 5 mg/L

Only very high concentrations (g/L) of virgin and oxidized MPs cause toxic effects on bacteria




Microtox system






Universidade de Vigo



CELL LINES




Exposure of DLB-1 cells (seabass brain cells) to virgin (4-6 µm) or oxidised (6-8.5 µm) PE produced no effects at 30 or 100 mg/mL. However exposure of these cells to PE DMSO extracts equivalent to 1 or 10 mg/mL induced DNA damage. The same is observed when DLB-1 cells are exposed to extract MPs spiked with benzo[a]pyrene (BaP) or PFOS (eq. 1-10 mg/mL) as well as to extract of MP-BP3 eq. 10 mg/mL. MPs extracts at 10 mg/mL spiked with BaP lead to a significant ROS production

Exposure of primary culture of European sea bass head-kidney leucocytes (HKLs) with different sizes of PE (virgin or oxidized) MPs produced a decrease in cell viability.


After exposure of RTL-W1 cells to MPs spiked with benzo[k]fluoranthene the release of this molecule to the culture medium in absence of supplemental surfactants was shown via EROD induction. Several fluorescent reporter cell lines to further quantify DNA damage as well a novel *in vitro* assay for neurotoxic potential were developed.

MICROALGAE

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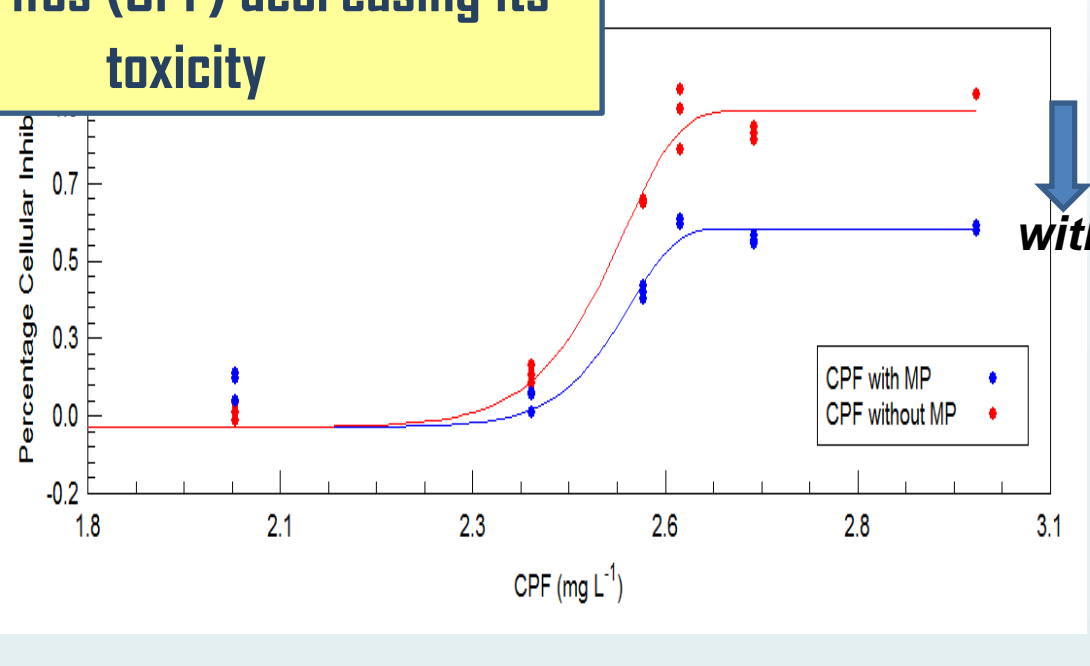


Phaeodactylum tricornutum
Isochrysis galbana




No effects of microplastics in any case on algal growth at the highest concentrations tested

The presence of MPs interacts with chlorpyrifos (CPF) decreasing its toxicity

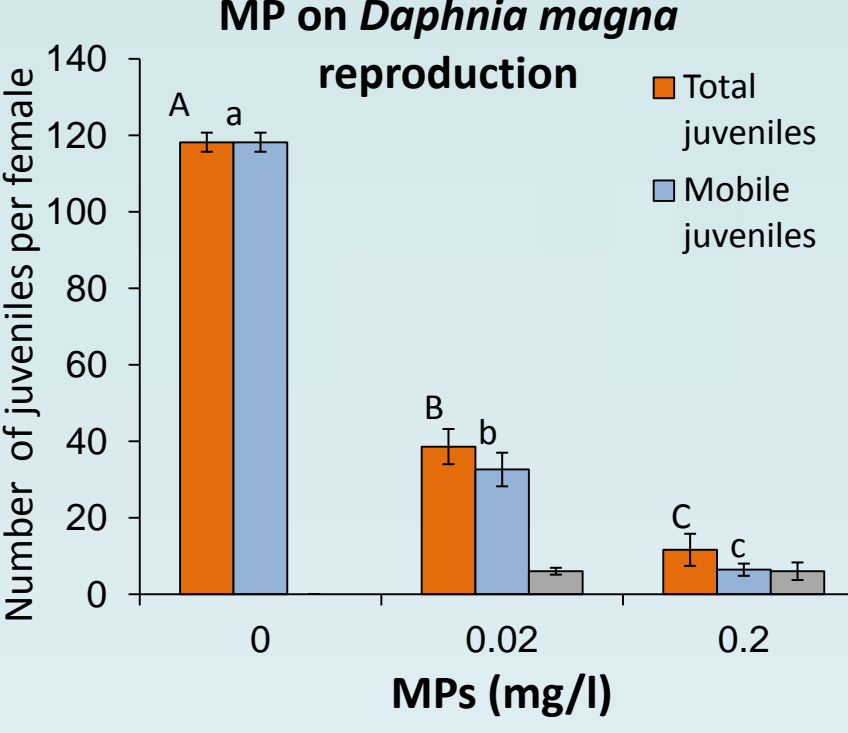


PLANKTONIC and BENTHIC ORGANISMS

DAPHNIA

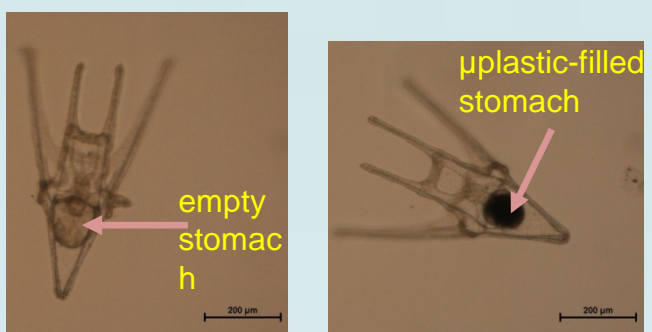


MP on *Daphnia magna* reproduction



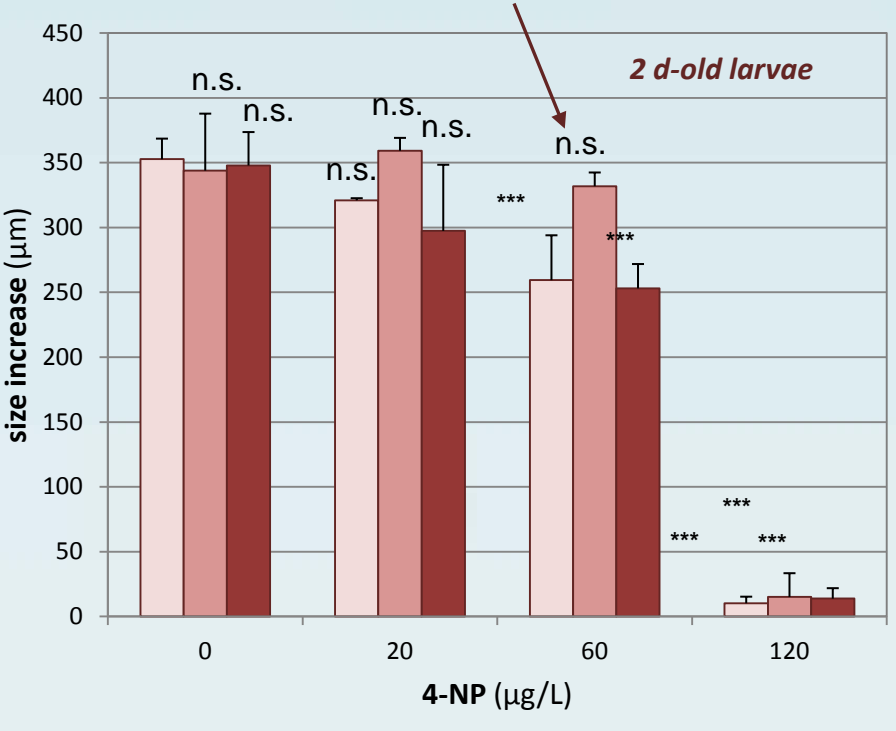
MP significantly decreased the reproductive output by reducing the number of viable juveniles and inducing the production of immobile juveniles

SEA-URCHIN LARVAE



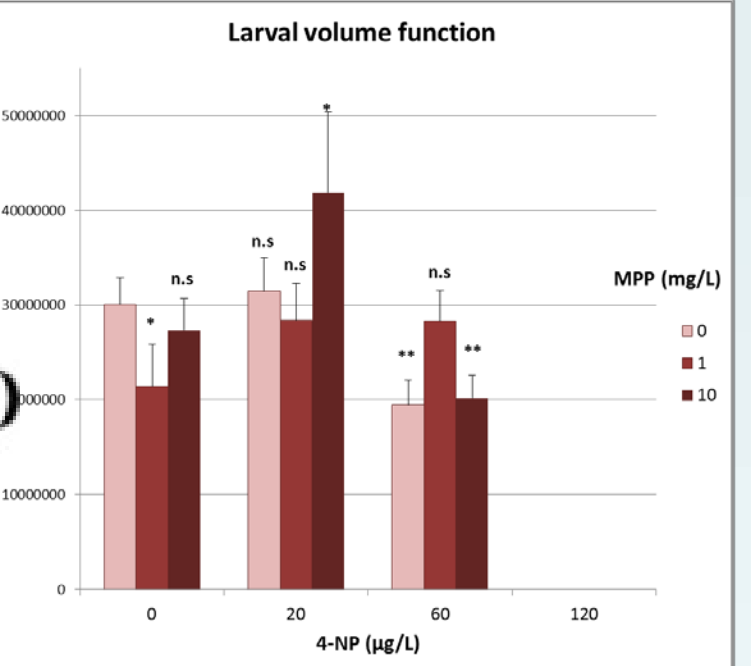
Sea-urchin larvae do ingest microplastics but this does not increase nonylphenol toxicity

In fact 1 mg/L MP significantly decreased toxicity (enhanced larval growth)




Two way ANOVA

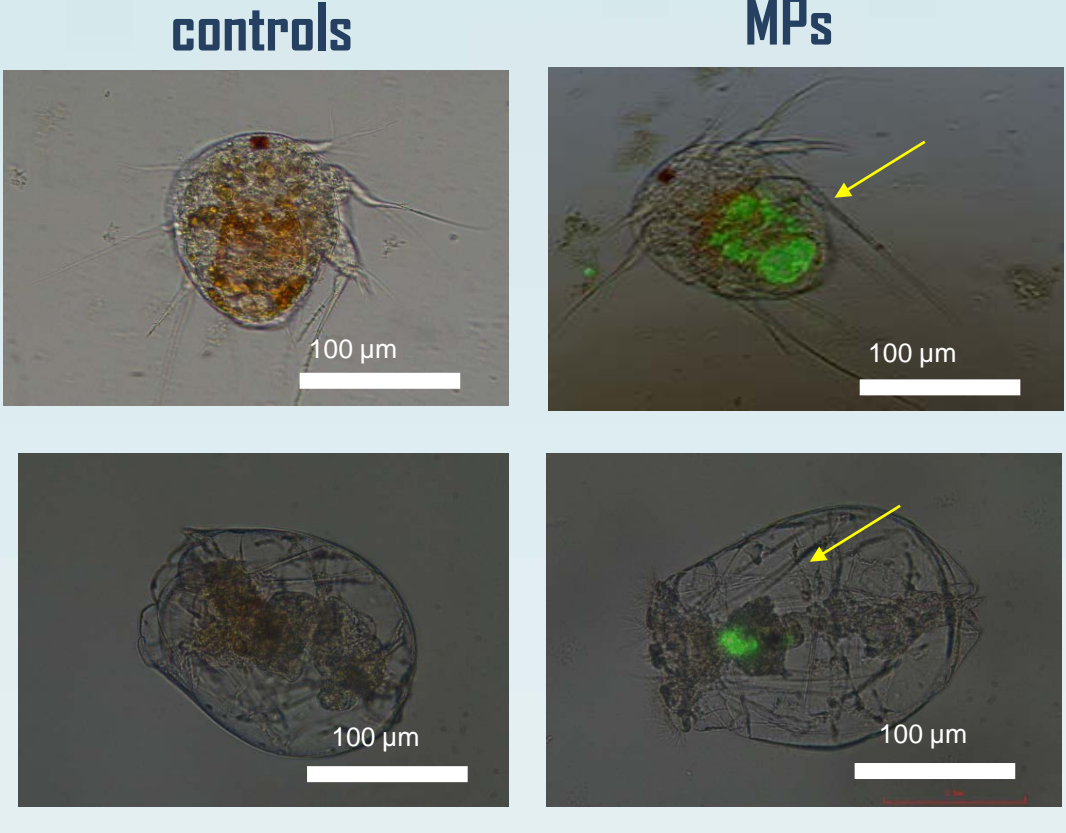
	P	Sig.
MPP	.258	n.s.
4-NP	.000	***
MPP * 4-NP	.000	***



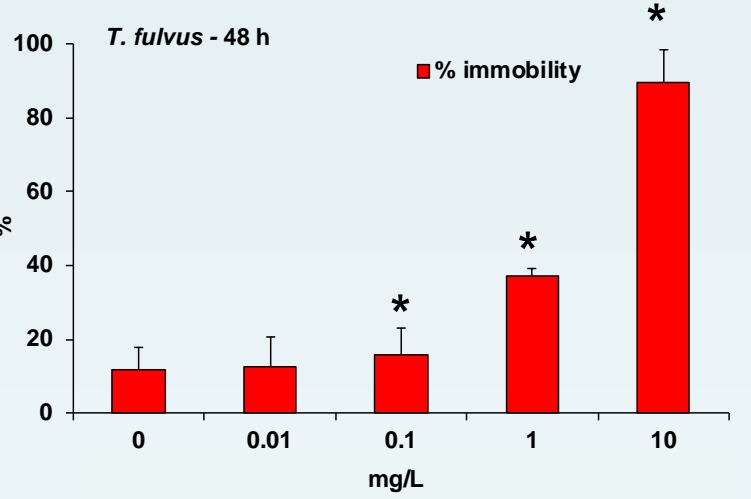
COPEPODS and ROTIFERS



Copepod nauplii and rotifer neonates ingest microplastics (from 0.01 mg/L up to 10 mg/L) after 24 h of exposure



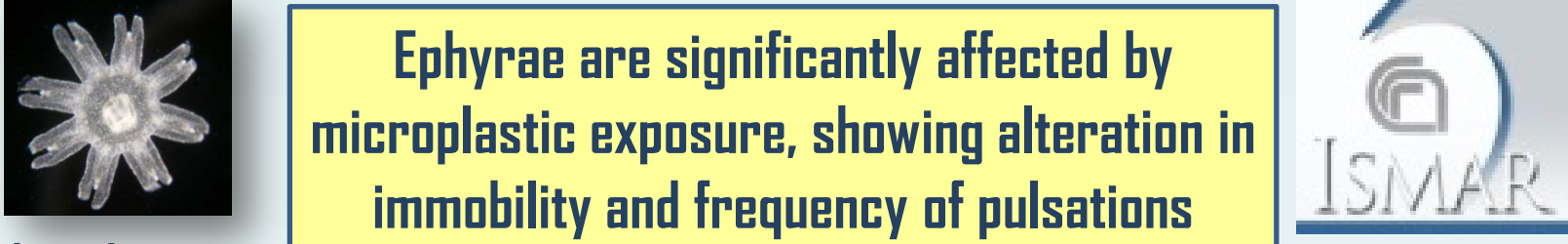
Tigriopus fulvus
Brachionus plicatilis



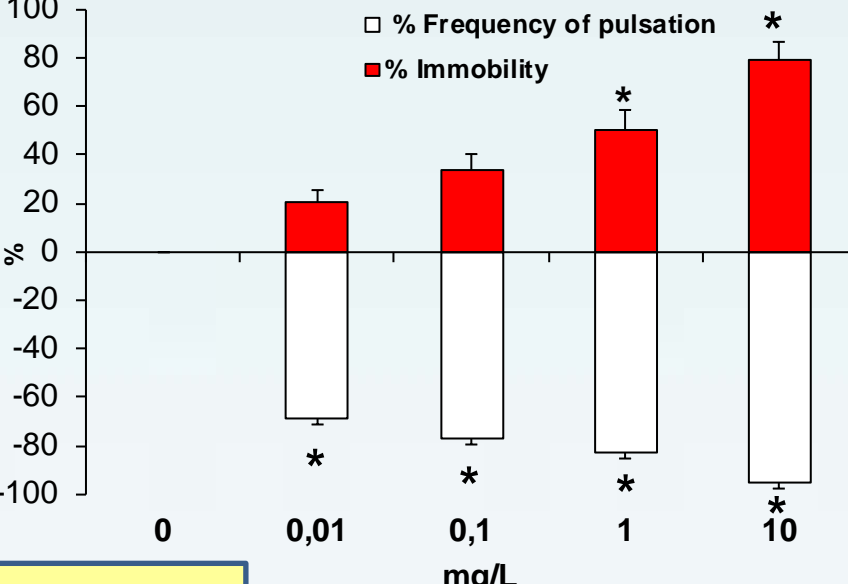
This ingestion, observed in the gut of both species, only affects copepod's survival, causing a toxic effect

EC50 = 1.82 (1.34-2.48) mg/L

JELLYFISH




Ephyrae are significantly affected by microplastic exposure, showing alteration in immobility and frequency of pulsations



Microplastic exposure allows to highlight a toxic effect on both acute and behavioural response

EC50 (Imm) = 2.10 (1.64-2.80) mg/L
EC50 (Fp) = < 0.01 mg/L

DEPOSIT-FEEDERS

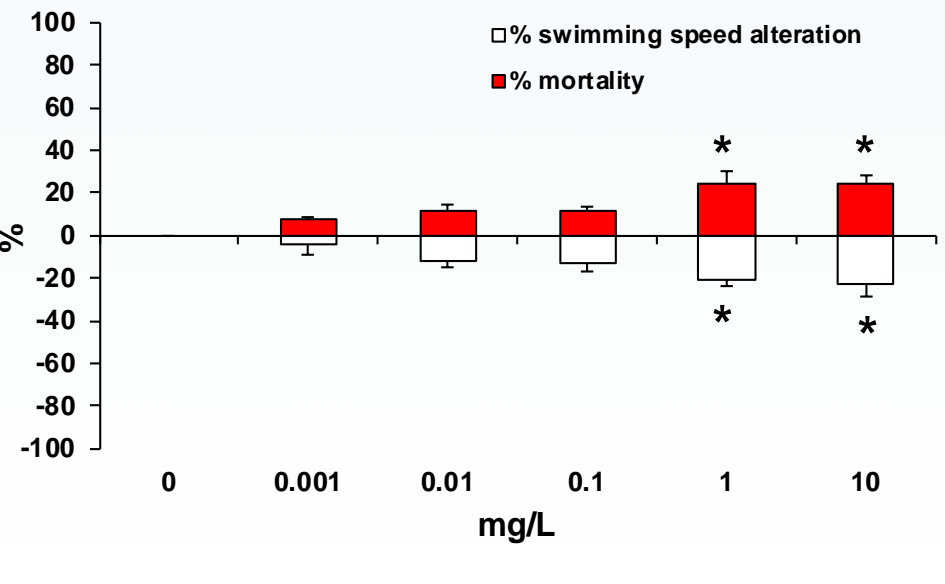


Major effects with Large MPs, at every concentration:


	<i>Ennucula tenuis</i>	<i>Abra nitida</i>
Lipid contents	-	-
Protein contents	-	-
Carbohydrate contents	-	-
Total Energy reserves	-	- (trend only)
Burrowing	-	-
Condition Index	-	-

MPs affect energy reserves in a size-dependent manner (p<0.05). No effect of concentration was observed.

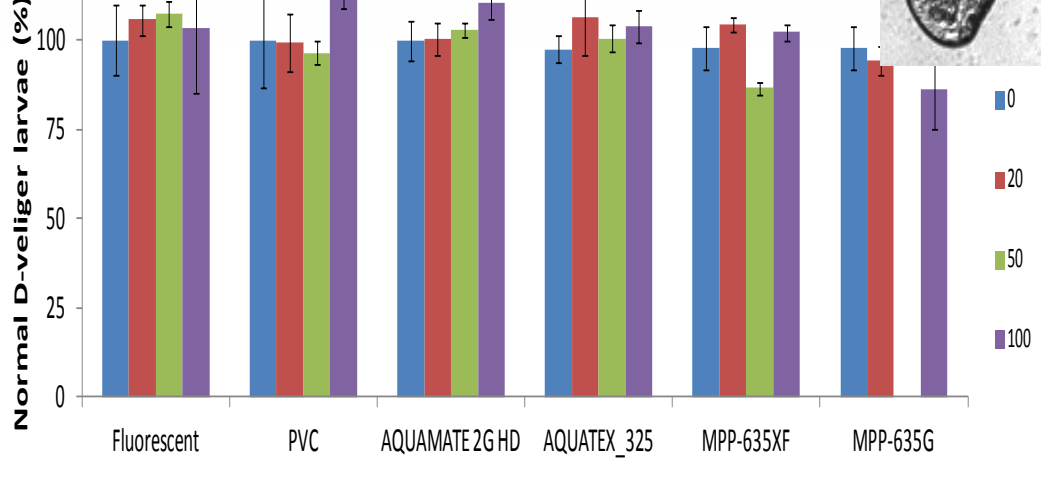
However, a significant effect (p<0.05) due to MPs can be also observed in rotifer acute and behavioural responses (swimming alteration) from 1 mg/L onwards



MUSSEL LARVAE



No significant effects of the tested MPs was observed on the embryonic development of the Mediterranean mussel.





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