Effect of environmental microplastics ingestion on zebrafish (*Danio rerio*) metabolism

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INTRODUCTION

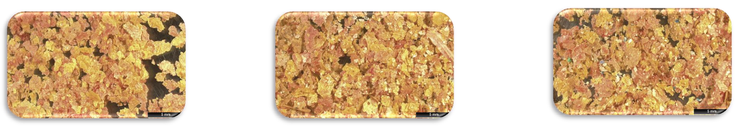
The problematic of microplastic pollution has been studied for years, so that their distribution, composition and the physical hazards they imply for marine organisms have been widely reported. Once all this has been well defined, it would be time to research on polluted MPs' impact on marine organisms' metabolism. Potential damage at cellular levels as well as at different food-web levels should be investigated.

In this study, we studied the effect of beach-stranded microplastics (MPs) on the metabolism and biochemistry of the vertebrate model organism, *Danio rerio* (zebrafish).

METHODOLOGY

DIETS

Microplastics from 2 to Canary Islands beaches, Lambra (La Graciosa), and Porís (Tenerife), and virgin microplastics were grounded to < 500µm and mixed with commercial flakes.



EXPERIMENT

Zebrafish were exposed to treatments for 60 days

- *Treatment A* (Control)
- *Treatment B* (Flakes + virgin MPs)
- *Treatment C* (Flakes + 10% Lambra MPs)
- *Treatment D* (Flakes + 10% Poris MPs)

SAMPLING

- ✓ At the beginning (T0)
- ✓ After 7 days (T7)
- ✓ After 30 days (T30)
- ✓ After 60 days (T60)

ANALYSIS

ELECTRON TRANSPORT SYSTEM (ETS)

Owens and King (1975); Gómez et al. (1996)

- Sample + Substrate solution + INT
- Read absorbance kinetically at 490nm during 8 min

0.48 J/µmol O_2 → Energy consumption (Ec)

PROTEIN CONTENT (PROT)

Smith et al. (1985)

- Sample + Work reagent
- Incubation 37ºC, 30min
- Read absorbance at 540nm

(Standard: BSA in buffer)

24 J/mg

CARBOHYDRATE CONTENT (CARB)

Dubois et al. (1956)

- Sample + Phenol + H_2SO_4
- 10min, vortex
- Incubation 30ºC, 10min
- Read absorbance at 485nm

(Standard: Glucose in buffer)

17.5 J/mg

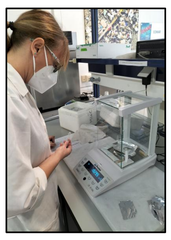
Energy available (Ea) (PROT + LIP + CARB)

39.5 J/mg

LIPID CONTENT (LIP)

- Extraction (1:1:0.9; Bligh and Dyer, 1959; Centrifugation, 2600g, 10min, 4ºC)
- Charring (200ºC, 15min)
- Colorimetric assay (Barnes and Blackstock, 1973):
  - -- Charred sample + Phosphovanillin reagent
  - -- Incubation 37ºC, 15min
  - -- Read absorbance at 525nm

(Standard: Olive oil in chloroform)



ENERGY BUDGET

CEA index = Ea / Ec

RESULTS

- Figures 1 to 7 show that, in all cases, despite an apparent imbalance between treatments from T7 onwards, no significant differences ($p<0.05$) were found between the different treatments, nor during the time-periods of each treatment.
- At T60, all parameters tend to be back on the equal level between treatments.

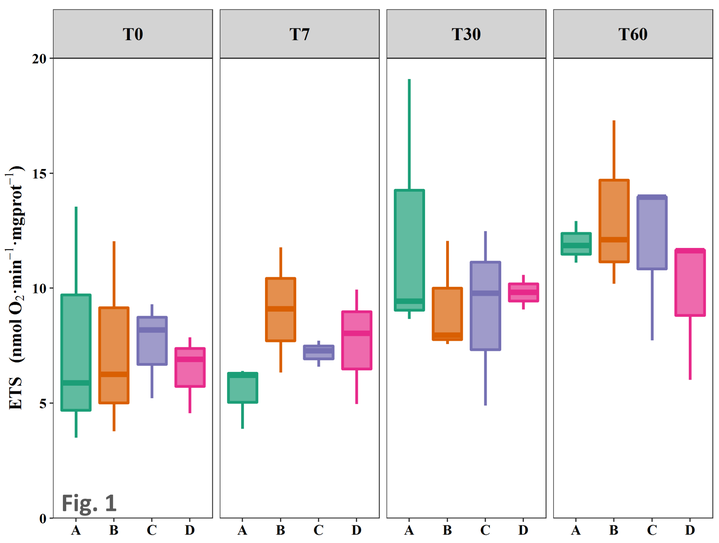


Fig. 1

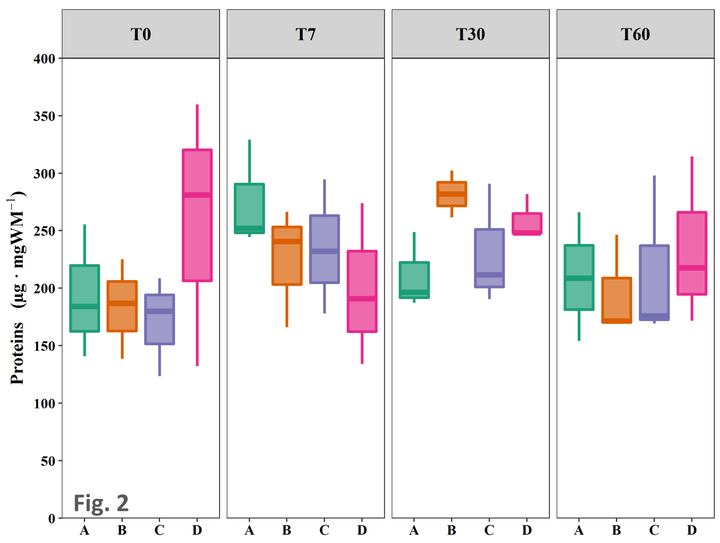


Fig. 2

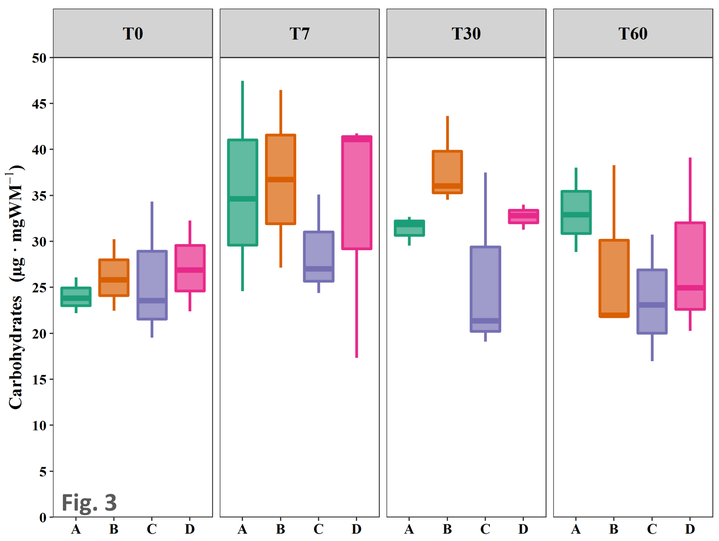


Fig. 3

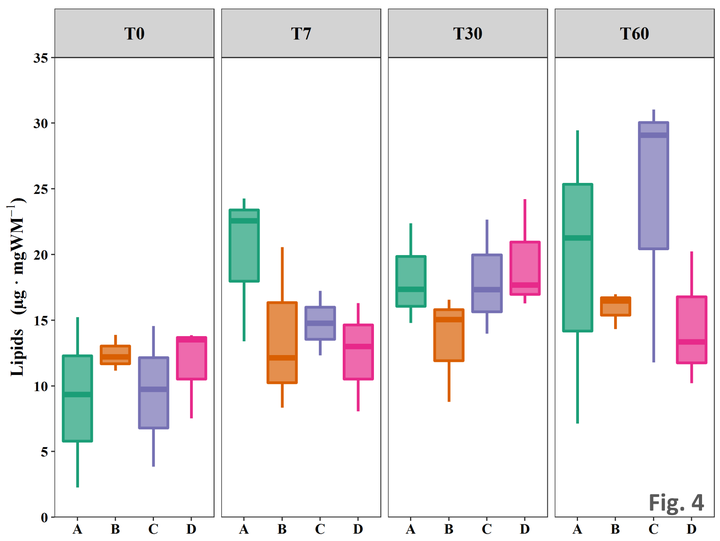


Fig. 4

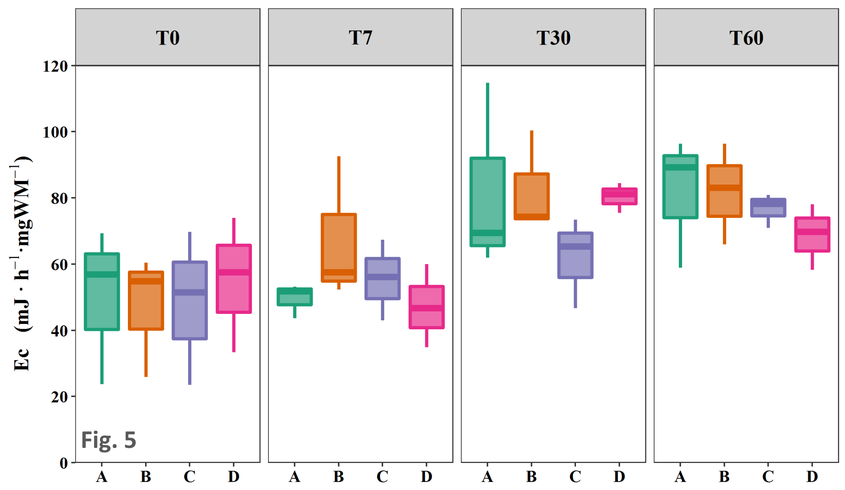


Fig. 5

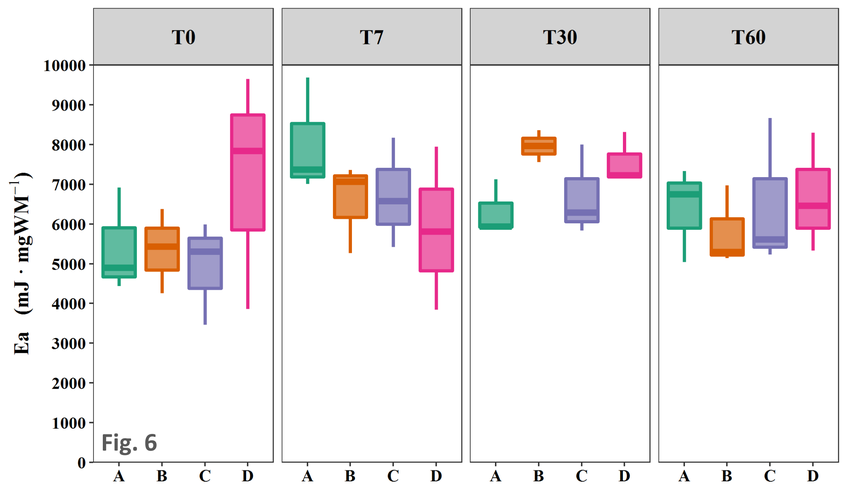


Fig. 6

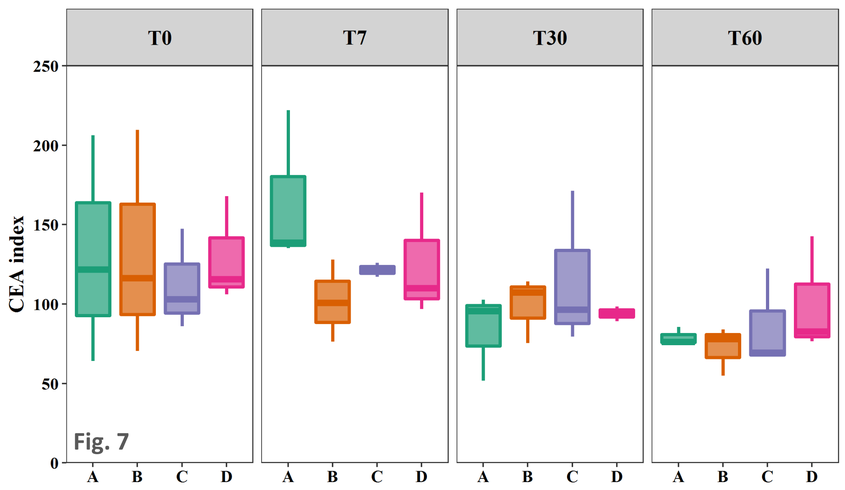
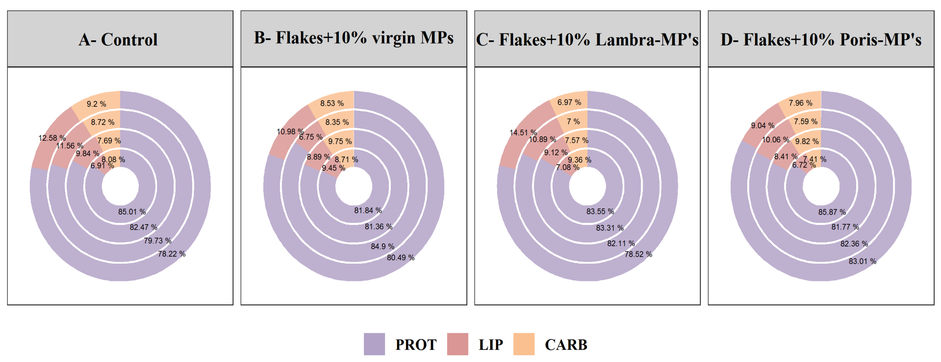


Fig. 7

- Proteins were the most prevalent energy-rich compound (78-86%), followed by lipids (7-14%) and carbohydrates (7-10%). These percentages remained stable over time and treatments. This was evidence that no change in biochemical composition was associated with MP-ingestion (Fig. 8).

Fig. 8
From the center outward: T0 to T60 →



CONCLUSION

Under our conditions, the biochemical composition and metabolism of zebrafish were not significantly affected by the ingestion of weathered MPs after 60 days.

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