



MORPGHOLOGICAL AND BEHAVIOURAL ANALYSIS OF BMAA IN ZEBRAFISH LARVAE

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BMAA (β -N-methylamino-L-alanine) is a toxin produced mainly by cyanobacteria and the subject of several hypotheses suggesting a link with amyotrophic lateral sclerosis (ALS). It has historically been found in cycad roots, consumed in various ways by the Chamorros on the island of Guam, where an incidence of ALS much higher than the global average was detected. Other clusters have also been identified by the scientific community (Spencer *et al.*, 2019) and several studies have highlighted multiple mechanisms of toxicity of this molecule (Courtier *et al.*, 2022).

In France, in the Occitanie region, BMAA has been detected in the Thau lagoon in bivalves (Réveillon *et al.*, 2015) while its isomers have been found in water and/or air samples (Courtier thesis, 2022). Other global studies show the possible presence of this toxin in the environment at nanogram concentrations (Abbes *et al.*, 2022). As highlighted by the 2017 ANSES report, most of the toxicological studies have been developed at relatively high concentrations and investigations on the effects of environmental concentrations remain to be completed.

Keywords: BMAA; Zebrafish larvae; *Artemia salina*

1 BMAA:

- Non-proteinogenic amino acid
- Demonstrated pathways of action:

Concentrations already studied are representative of bioaccumulation but not of an environmental exposure (ng.l⁻¹ to μ g.l⁻¹)

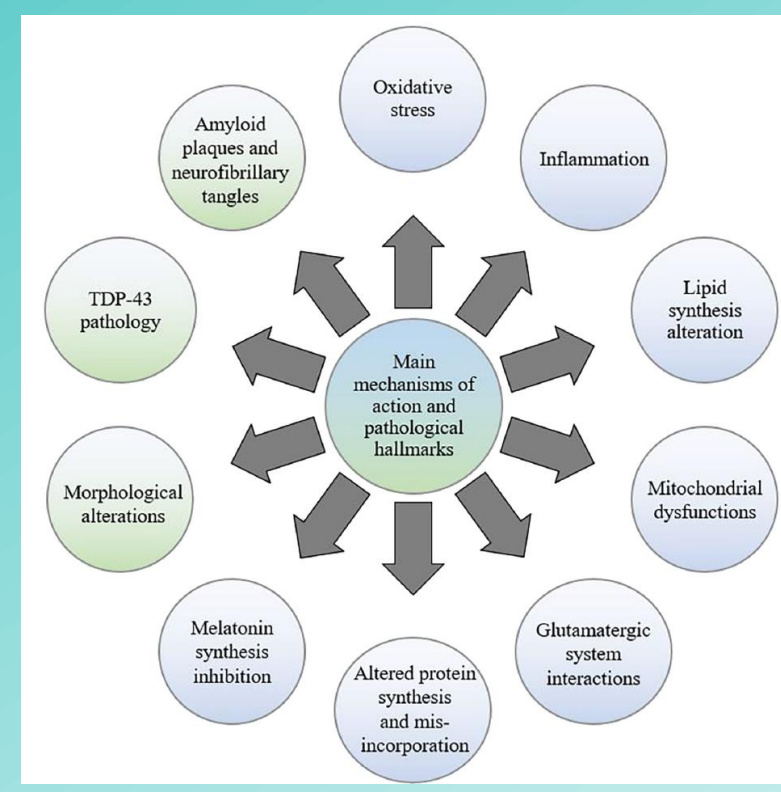
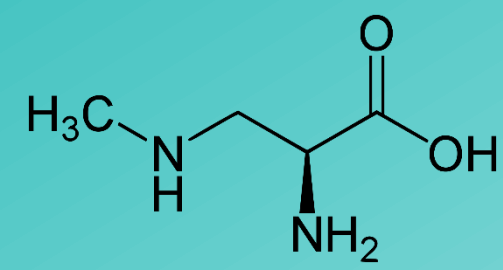
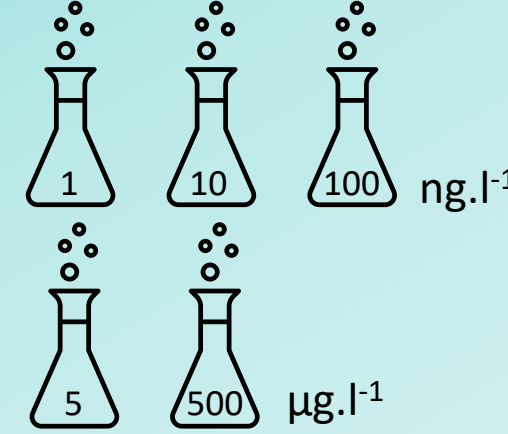


Fig 1. From Courtier *et al.*, 2022

Concentrations tested

in our study:



5 *Artemia salina*

Objective: Comparaison of results with another model organism

Method

- Artemia survival test (nauplii);
- Exposition for 24 and 48 hours;
- 10 nauplii per concentration per experiment. Every experiment was repeated 6 times.



Results

- A non-linear response is evidenced, confirming the same trend observed in zebrafish larvae:
- Non-linear response;
- Heterogeneity of results.
- No statistical significance.

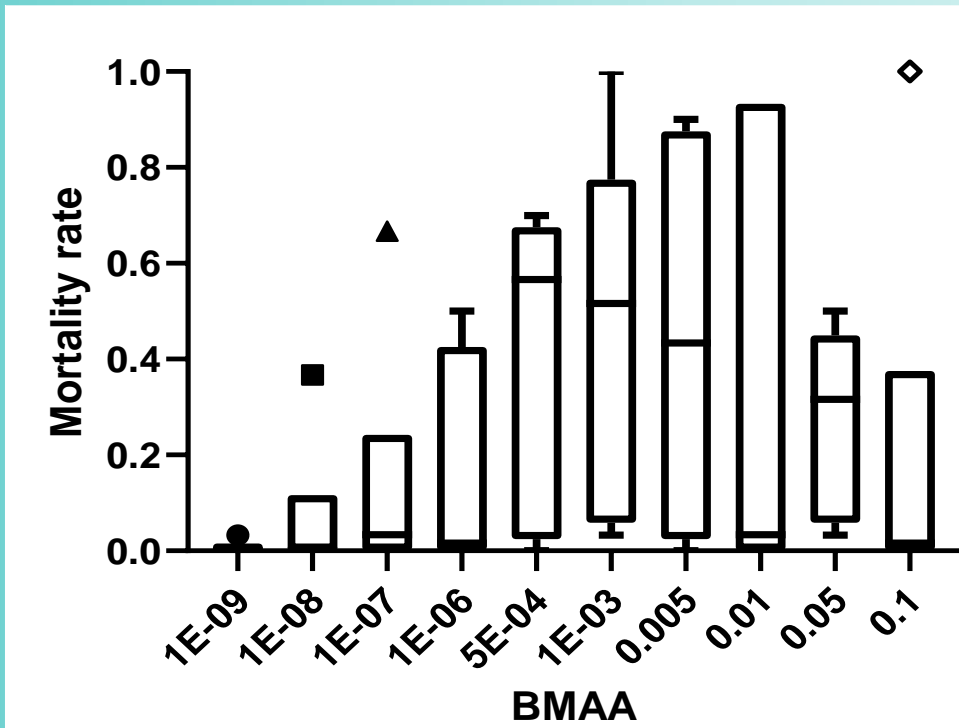


Fig 7. Mortality rate of *Artemia* nauplii after 24h exposure to different concentrations of BMAA. (n=60). Tukey representation. No significant differences. Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks.

4 Additional observations

In accordance with the 3R rule, these observations might help in the refinement of protocols and analysis

- The age of the spawners can influence the post fertilization mortality rate.

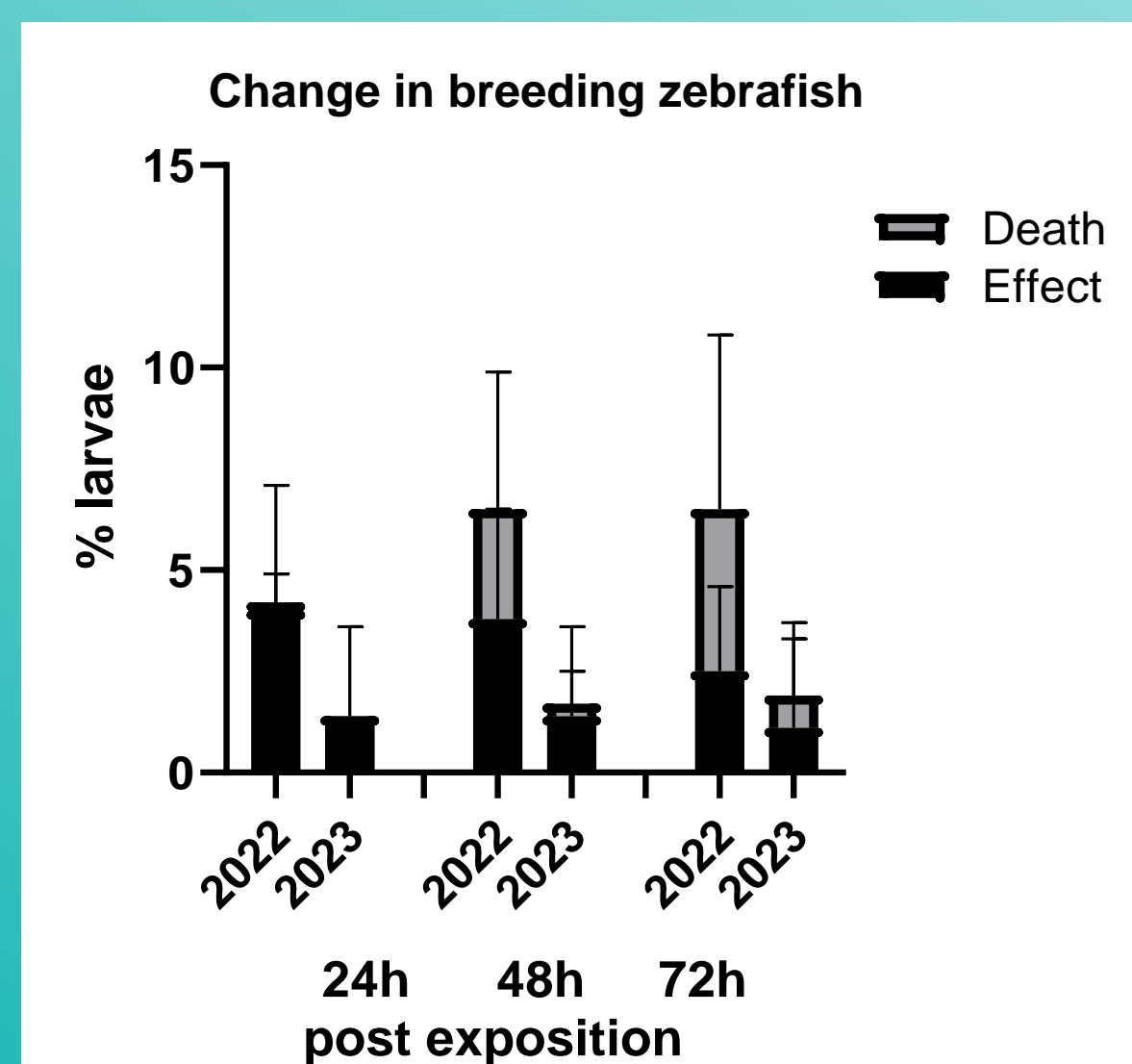


Fig 5. Effects observed on *Danio rerio* larvae after 24h, 48h and 72h exposure to control medium E3 (2022 n=723, 2023 n=482). Mean \pm SD for 21 replicates. No significant differences. Mann-Whitney test.

- A possible reabsorption of edema was noticed in affected larvae depending on the time of observation.

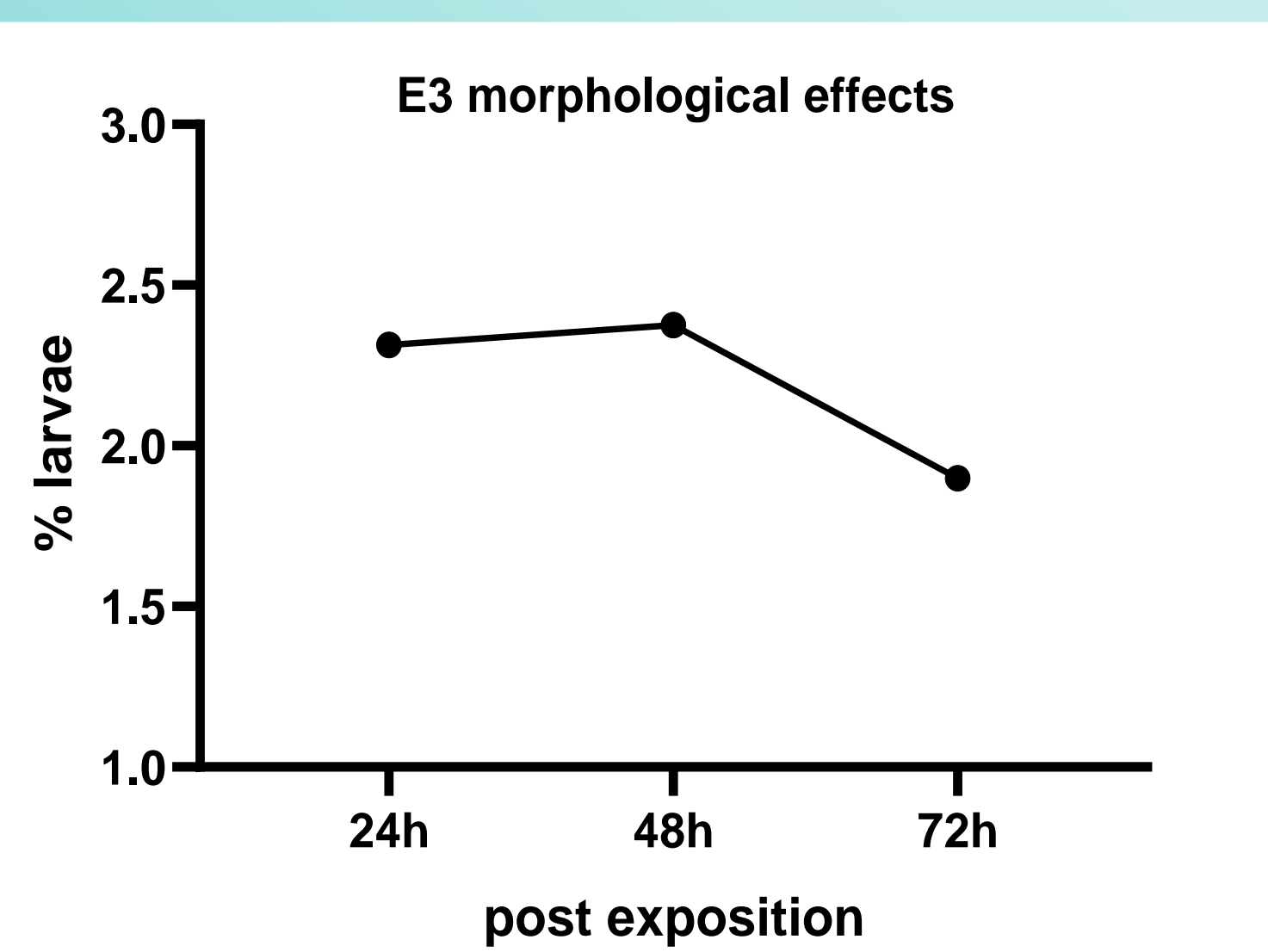


Fig 6. Effects observed on *Danio rerio* larvae after 24h, 48h and 72h exposure to control medium E3 (n=1141). No significant differences. Wilcoxon test.

2 Morphological effects

Method

- OCDE 236; 20 larvae per condition; all experiments were repeated 4 times;
- Dechorionated larvae at 28 hpf (hours post-fertilization);
- Exposition to BMAA from 30 hpf until 72 hpf;
- Semi-static conditions.

Observed effects

- Yolk sac edema / opacity;
- Pericardial edema;
- Tail deformation.



Results

BMAA showed a certain degree of toxicity even at the lowest concentration studied, although the observed effects were not statistically significant :

- Non-linear response;
- Heterogeneity of results;
- NB: In each experiment there was at least one larvae that was affected at the lowest concentration studied.

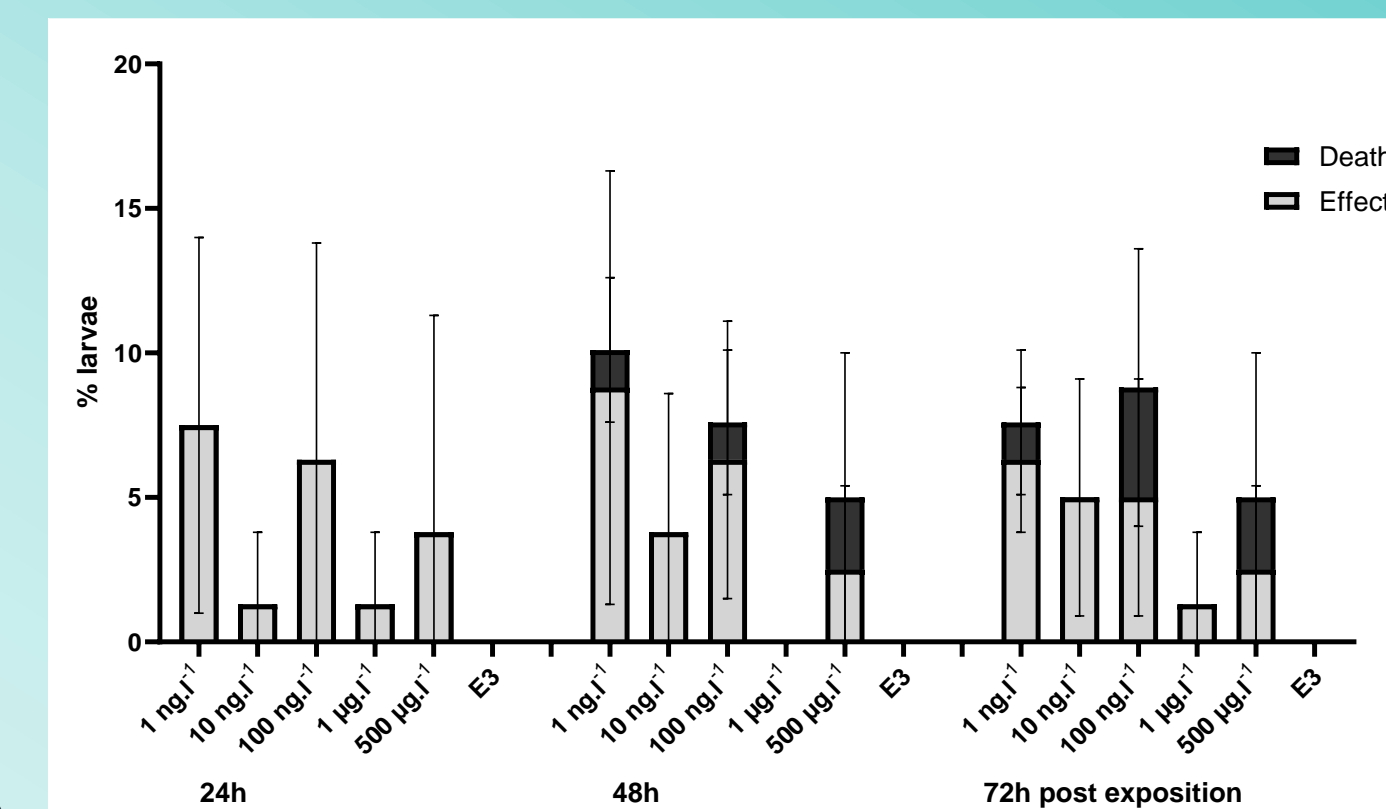


Fig 2. Effects on *Danio rerio* larvae after 24h, 48h and 72h exposure to different concentrations of BMAA (n=80). Mean \pm SD for four replicates of 20 larvae. No significant differences Kruskal-Wallis multiple comparison test.

3 Behavioural analysis



Investigations

- Total distance moved (5 minutes of observation);
- Startle response (distance covered 1 second before and 1 second after a tap stimuli (level 8, maximal force by Daniovision®)).

Method



- Dechorionated larvae at 28 hpf, 72h of exposure in semi-static conditions;
- DCA (3,4-dichloroaniline 4 mg.l⁻¹), is used as positive control.

Results

Larvae exposed at the lowest concentration studied (1 ng.l⁻¹) covered a significantly smaller distance if compared to the negative control (E3). Results show the same trend when analysing both the total distance (Fig 3) and the startle response (Fig 4).

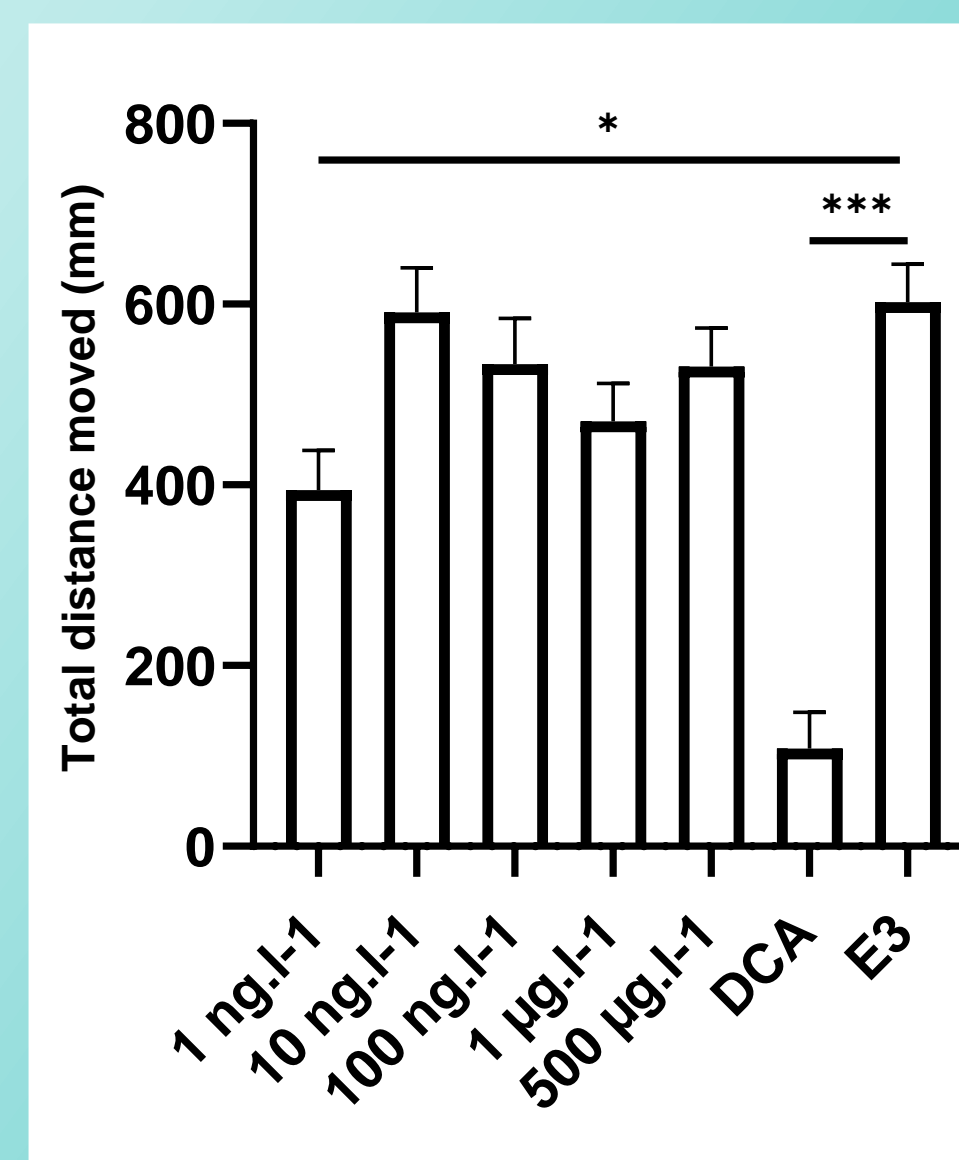


Fig 3. Effect of different concentrations of BMAA on *Danio rerio* behaviour during a total test of 5min performed after 72h of exposure and 10 min of adaptation (n=80). Mean \pm SEM (P<0.05, Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks)

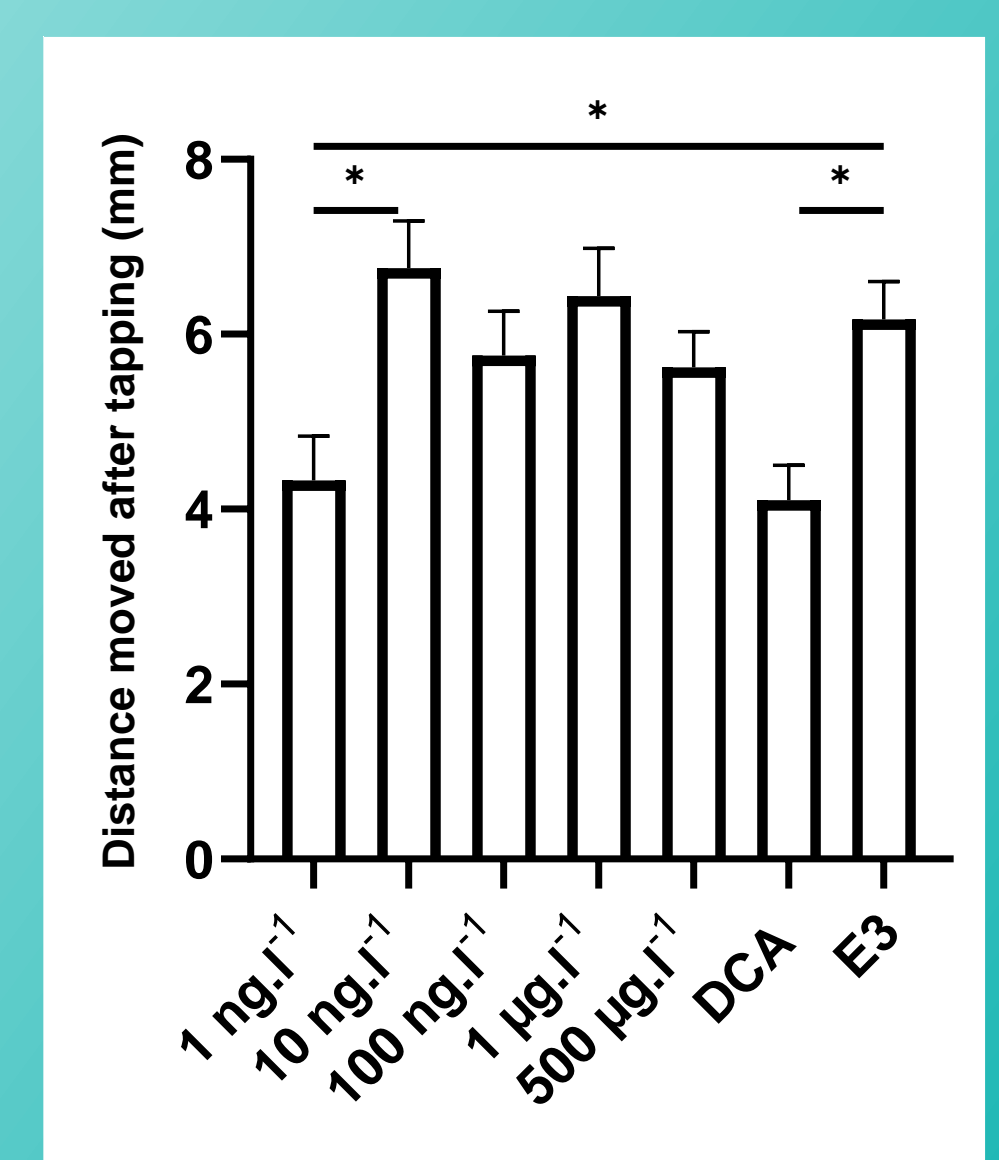


Fig 4. Effect of different concentrations of BMAA on *Danio rerio* behaviour 1 second after a force 8 tap performed after 72h of exposure and 10 min of adaptation (n=80). Mean \pm SEM (P<0.05, Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks)

Conclusions

- Environmental concentrations of BMAA showed some toxicity even at the lowest concentration tested (1ng l⁻¹) and, notably, a possible non-linear response;
- The morphological analysis showed results that are not statistically significant. Further investigations are needed (e.g. Real Time-qPCR studies);
- The behavioural analysis showed that an effect of BMAA was present at the lowest concentration, with or without tapping stimuli. The results are statistically significant;
- The survival test on *Artemia* nauplii seems to confirm a non-linear response to BMAA;
- The morphological analysis on zebrafish larvae evidenced the importance of the observation time. A possible remission of the effects (mainly edema) has been noticed.



References

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