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## MORPHOLOGICAL AND BEHAVIOURAL ANALYSIS OF BMAA IN ZEBRAFISH LARVAE

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► **To cite this version:**

Jeanne Lichtfouse, Audrey Courtier, Annette C Vergunst, Patrizia Giannoni. MORPHOLOGICAL AND BEHAVIOURAL ANALYSIS OF BMAA IN ZEBRAFISH LARVAE. First French Zebrafish Meeting, Oct 2023, Montpellier, France. hal-04263376

**HAL Id: hal-04263376**

**<https://hal.science/hal-04263376v1>**

Submitted on 28 Oct 2023

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BMAA ( $\beta$ -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 ( $\text{ng.l}^{-1}$  to  $\mu\text{g.l}^{-1}$ )

Concentrations tested in our study:

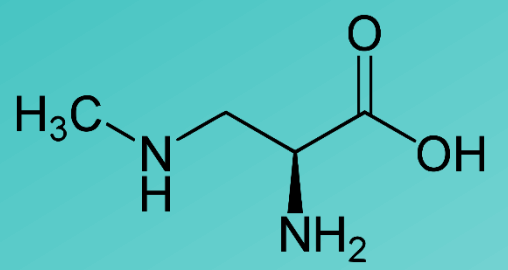
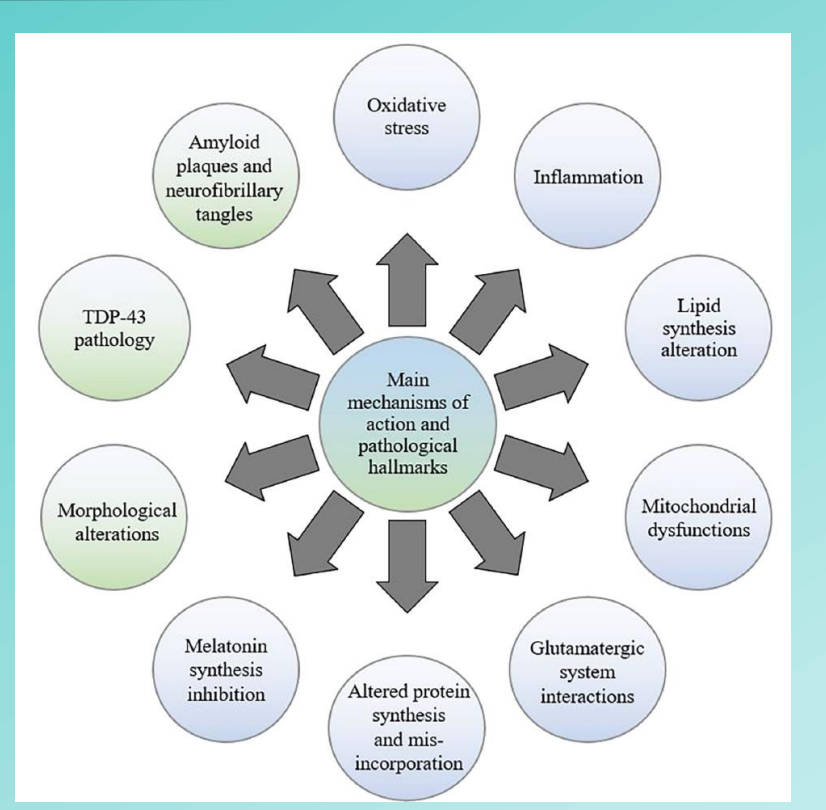



Fig 1. From Courtier *et al.*, 2022


**2 Morphological effects**

**Method**

- OCDE 236; 20 larvae per condition; all experiments were repeated 4 times;
- Dechorionated larvae at 28 hpf (hours post-fertilization);
- Exposure 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.

**5 Artemia salina**

Objective: Comparison of results with another model organism

**Method**

- Artemia survival test (nauplii);
- Exposure 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.

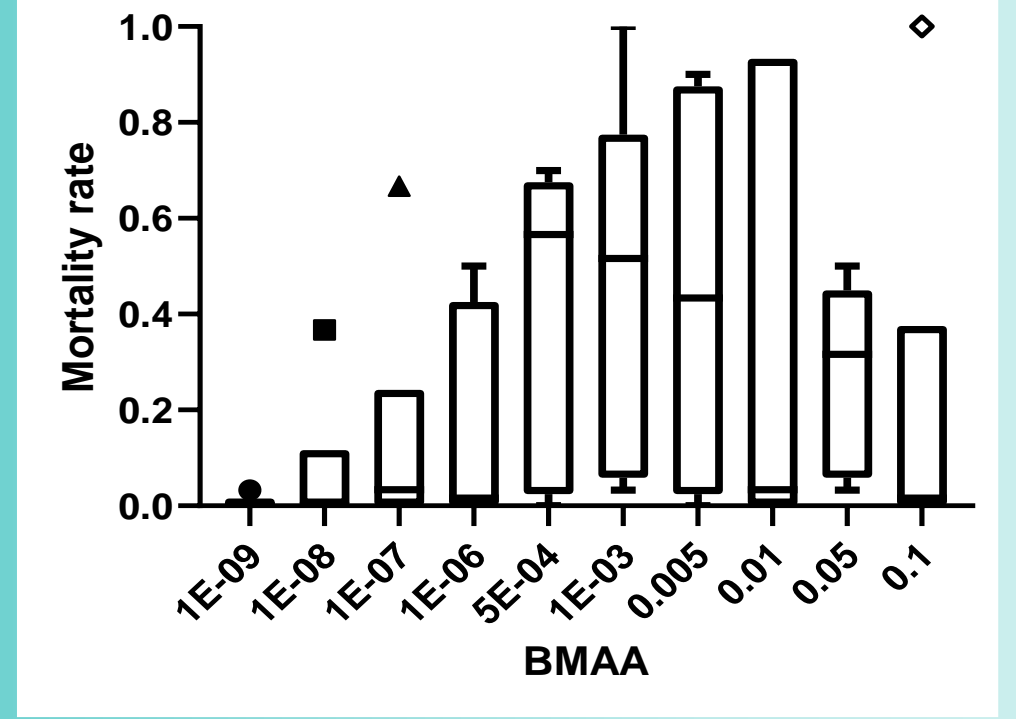
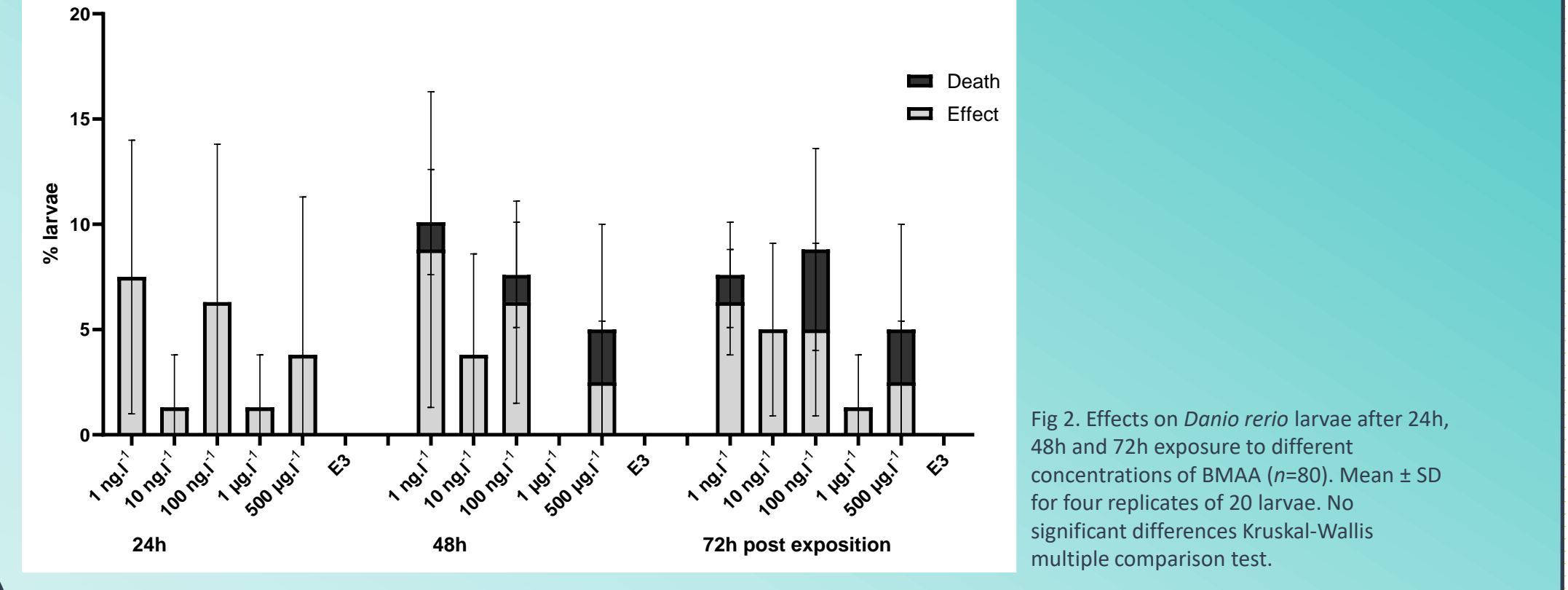


Fig 7. Mortality rate of *Artemia* nauplii after 24h exposure to different concentrations of BMAA. ( $n=60$ ). Tukey representation. No significant differences. Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks.



**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**

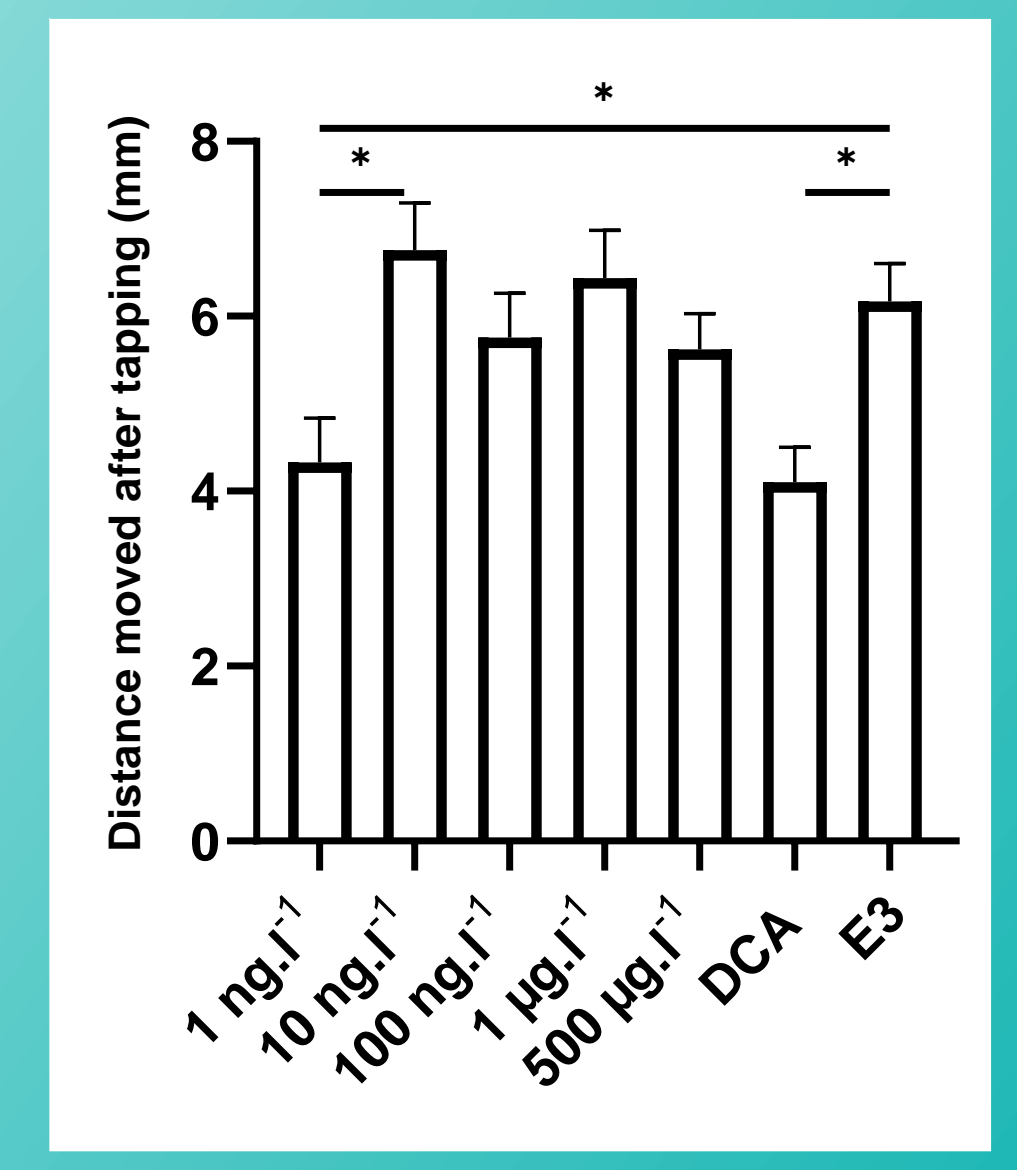
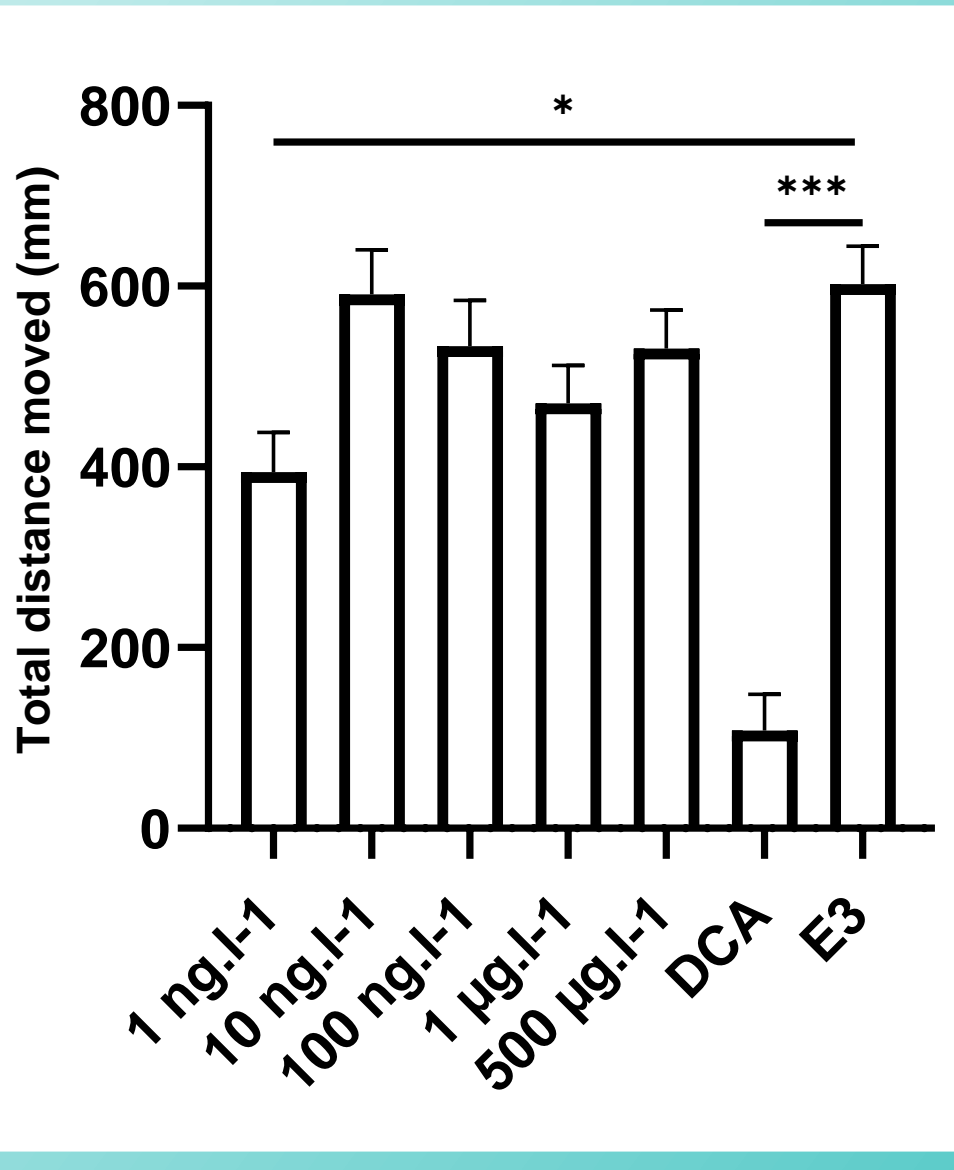
Adaptation period: 10 min

before | after: 5 min

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

**Results**

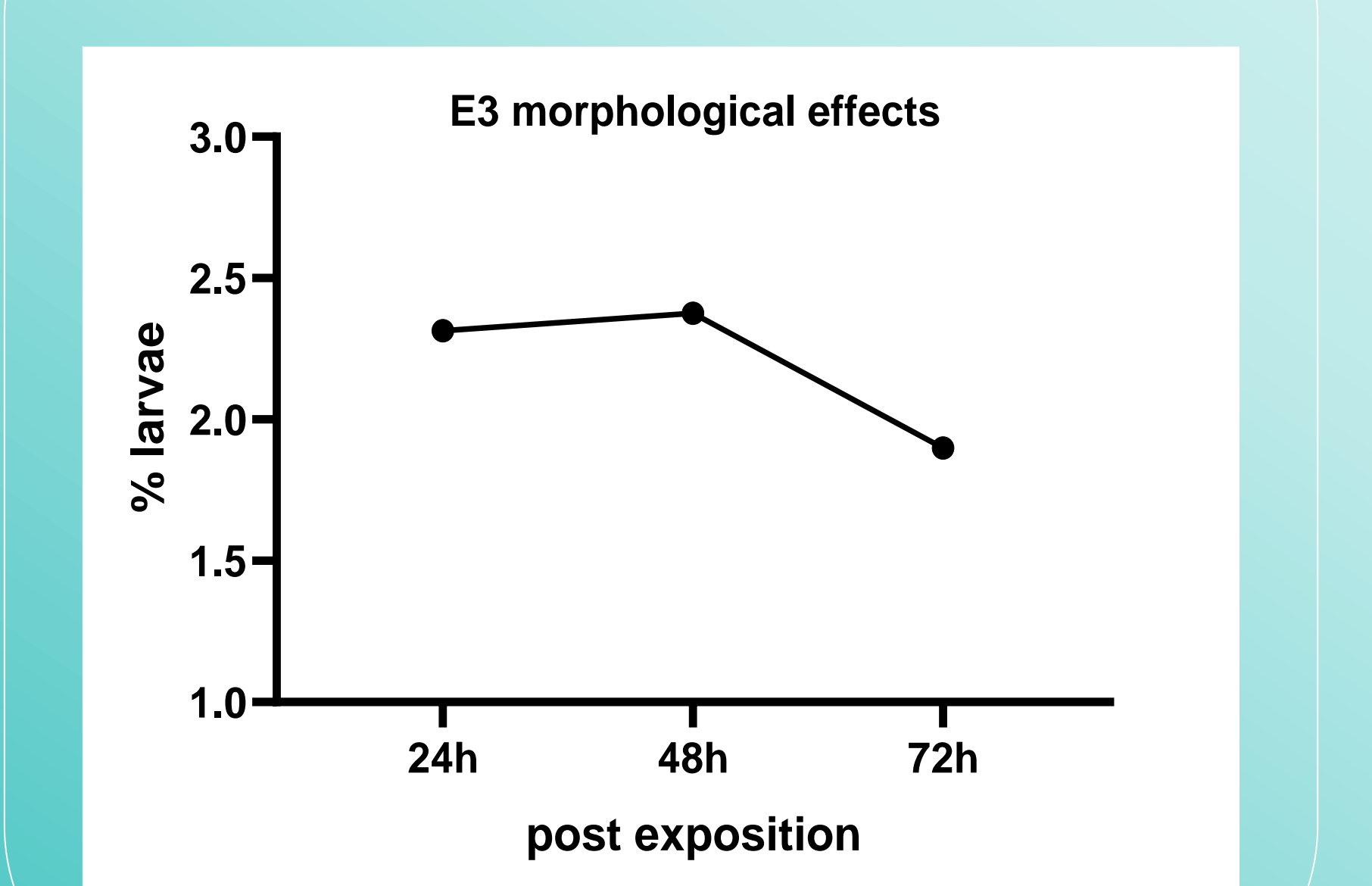
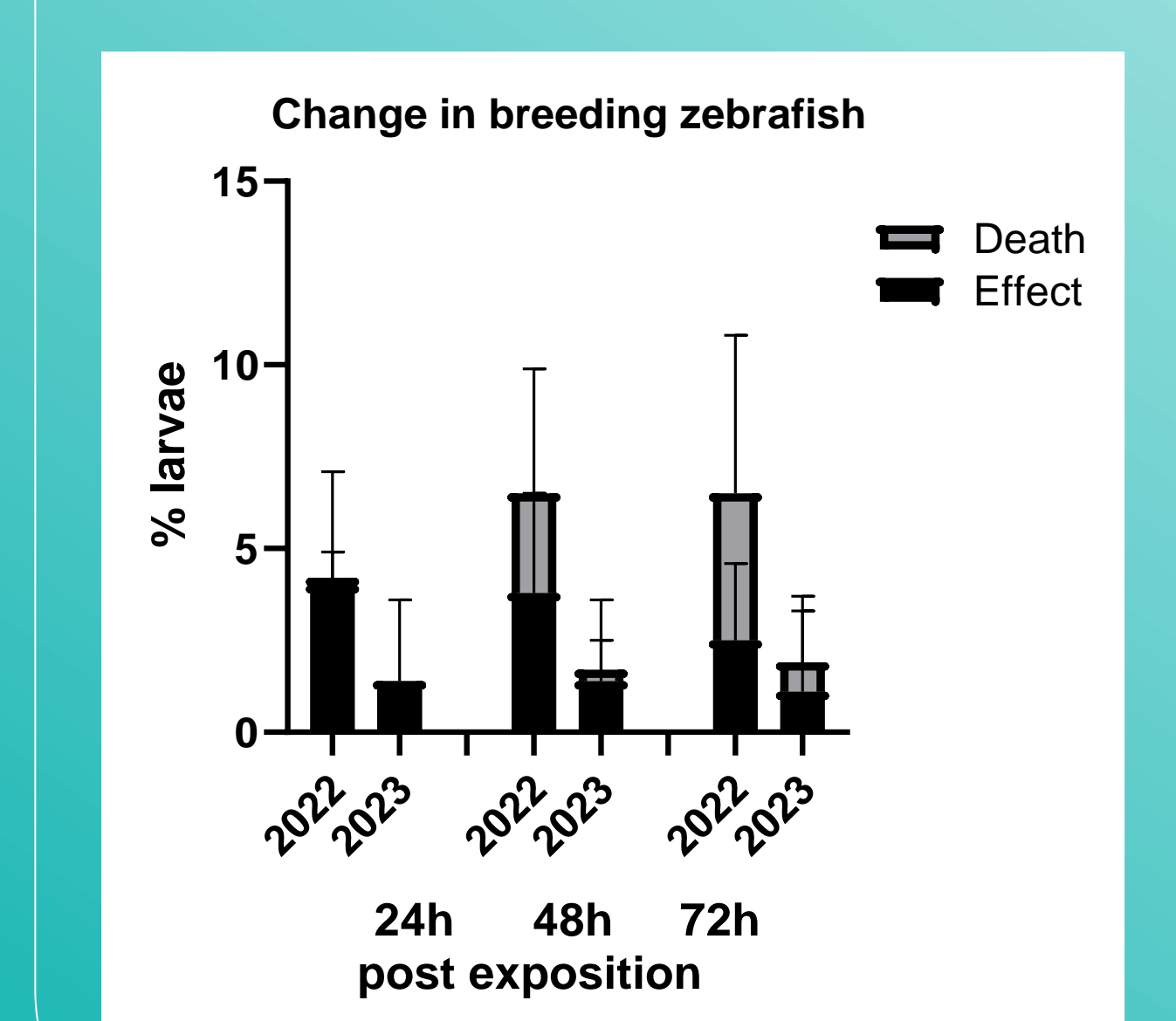
Larvae exposed at the lowest concentration studied (1  $\text{ng.l}^{-1}$ ) covered a significantly smaller distance if compared to the negative control (E3). Results show the same trend when analysing both the total distance (Fig 3) and the startle response (Fig 4).



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



**Conclusions**

- Environmental concentrations of BMAA showed some toxicity even at the lowest concentration tested (1 $\text{ng l}^{-1}$ ) 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.