

Using Automated Size Measurement Software as a Possible New Approach Methodology to Support Adverse Outcome Pathway

Background

Oxidative phosphorylation (OXPHOS) is the main metabolic pathway, taking place in the mitochondria, that produces energy in the form of ATP. Chemicals that act as mitochondria uncouplers can disrupt the OXPHOS process and can lead to adverse outcomes of regulatory concern such as growth inhibition. Chemical regulatory frameworks (e.g. REACH), aims to reduce traditional animal toxicity tests in favor of alternative testing methods, for hazard assessment of chemicals. Under the adverse pathway (AOP) concept, an AOP has been published linking the uncoupling of OXPHOS to growth inhibition, via a reduction of ATP pool and cell proliferation (OECD project #1.92, AOPWiki, AOP #263). This study aims to utilize new approach methodologies to investigate the sublethal effects of mitochondrial uncouplers on the growth as well as developmental inhibition of zebrafish (*Danio rerio*) larvae.

Approach

- Exposure of **zebrafish embryos** (0 – 96 hpf) to known mitochondria uncoupler CCCP
- Lateral images of zebrafish larvae at the end of the exposure period (96hpf)
- FishInspector software (version 1.69), was utilised to automatically annotate features on the acquired images of zebrafish larvae (Fig. 1)

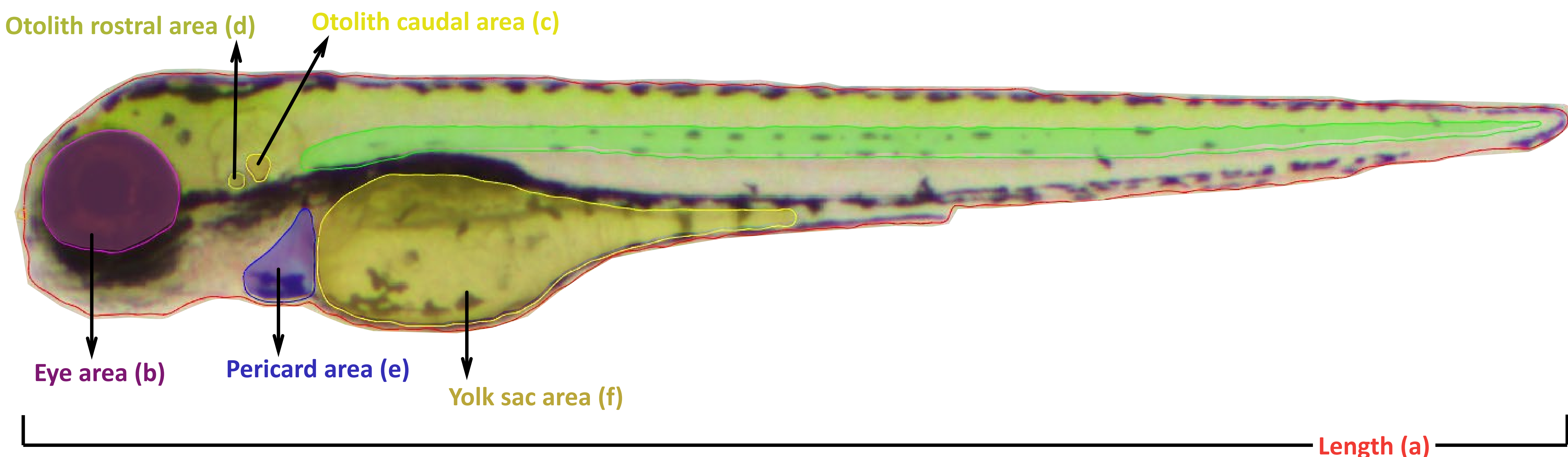


Fig. 1. Annotation of zebrafish larvae features using FishInspector (version 1.69). Letters in brackets indicate each sub-figure in the results .

Results & Conclusions

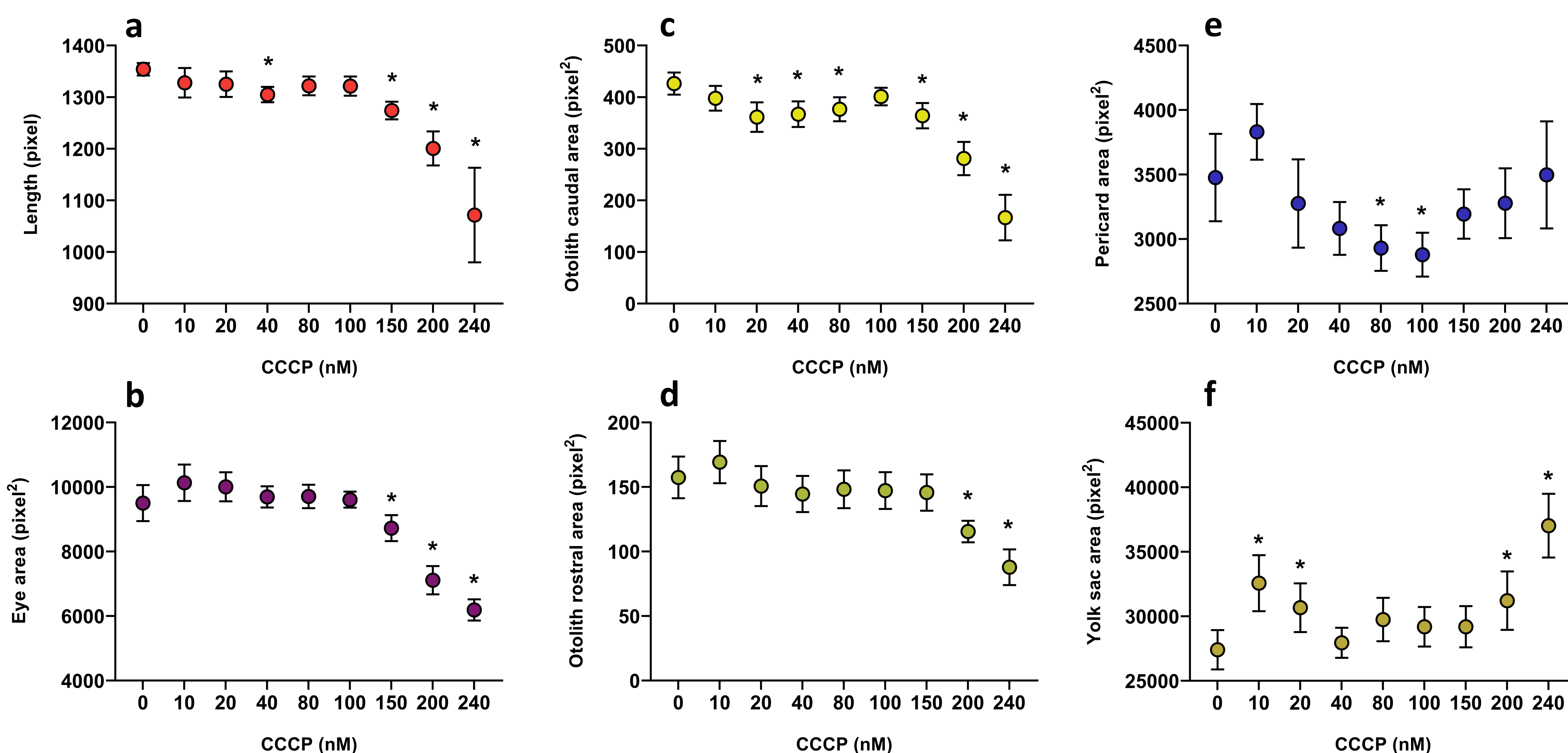


Fig. 2. Effects of exposure to CCCP for 96h on a) length, b) eye area, c) otolith caudal area, d) otolith rostral area, e) pericard area and f) yolk sac area of zebrafish larvae at 96hpf. Asterisks indicate significant differences compared to control ($p < 0.05$). Data shown are means \pm 95% CI

Exposure to the mitochondria uncoupler CCCP has caused growth inhibition in terms of length in previous experiments. Using the FishInspector software, we have observed that exposure to CCCP also causes developmental inhibition in all features examined in a monotonic (Fig 2 a-d) or non-monotonic manner (Fig 2 e-f). The results will be used to expand the current adverse outcome of AOP263 as well as to construct a quantitative AOP (qAOP).

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