

Project Update: May 2019

Syngnathid Experimental Protocol

A total of 15 pipefish (*Syngnathus temminickii*) were collected on 8th May 2019 from two selected sites along the Knysna Estuary. These individuals were utilised to determine the success of a non-destructive gut flushing method to obtain any microplastic particles potentially consumed by the fish under experimental conditions. This method has been successfully used to obtain prey items of seahorses (*Hippocampus reidi*) in the wild to assess their diet composition (Castro et al. 2008), which found that the gut flushing technique has the potential to replace destructive sampling procedures in seahorse research and has accounted for a low mortality rate in relation to the applied technique as well as short induction (< 90 sec) and recovery times (< 120 sec) using clove oil anaesthetisation (Castro et al. 2008). This is the first study of its kind that has exposed members of the syngnathidae family (i.e. pipefish) to microplastic particles under experimental conditions.

Pipefish were first allowed to acclimate for a period of 24 hours, following which they were left to empty their gut (i.e. depurate) for an additional 24 hours. The fish were then exposed to microplastic particles (1 mm- in size) in individual holding tanks to determine the effectiveness of the gut flushing protocol. The fish were observed after 2 hours using GoPro cameras. The decision was taken to further extend the exposure time to 24 hours in which footage was still recorded with cameras. The recorded footage is yet to be assessed, however, the gut flushing method did not yield any microplastic particles that may have been consumed. The way in which pipefish responded to live food being introduced into holding tanks were also recorded (using cameras) versus when plastic particles were introduced (in isolation).

There were no mortalities recorded and all fish were returned to the sites from which they were collected. All samples were collected as authorised by the South African National Parks (SANParks) research permit (Knysna section of the Garden Route National Park CLAA-L/2018-018). Appropriate ethical clearance was obtained from the SANParks Animal Care and Use Committee (AUCC) for scientific research (Reference no. 016 – 18).

NB. Please note that this experiment is an addition to the original project outline. Images are sensitive to be shared.