

Behavioural responses are descriptive observations; no statistical test was applied. Increasing extract concentration intensified anaesthetic depth, with *C. aurantium* and *C. limon* inducing more rapid and severe respiratory depression at higher concentrations.

### 3.2.2 Behavioural responses of *Oreochromis niloticus* under different extract concentrations

Behavioural responses of *Oreochromis niloticus* followed a similar concentration dependent pattern (Table 3). Increasing extract concentration was associated with a gradual transition from mild agitation to sedation and eventual loss of equilibrium.

Although the general pattern was consistent with that observed in *Clarias gariepinus*, minor differences in sensitivity were evident between species. As with the previous section, these findings are based on qualitative observations and should be interpreted cautiously.

Table 3 Behavioural responses of *Oreochromis niloticus*

Concentration (mg L <sup>-1</sup> )	<i>C. sinensis</i>	<i>C. aurantium</i>	<i>C. limon</i>
1 000	No observable effect	Mild agitation	Mild agitation
2 000	Slight loss of equilibrium	Moderate sedation	Sedation
3 000	Loss of equilibrium	Anaesthesia	Deep anaesthesia
4 000	Prolonged immobility	Cessation of opercular movement	Cessation of opercular movement

Observations are qualitative; no inferential statistical analysis was conducted. The observed responses confirm interspecific consistency in anaesthetic progression, although sensitivity to extracts varied slightly between species.

Behavioural anesthetic stages were evaluated using qualitative observational criteria and were not subjected to parametric statistical analysis because such responses represent ordinal rather than continuous data. The use of descriptive classification for anesthetic staging is well established in fish welfare and anesthesia studies, where behavioural endpoints are interpreted within defined categorical frameworks rather than treated as quantitative variables (Martos Sitcha et al., 2020; Vergneau et al., 2022)

### 3.2.3 Effects of extract concentration on induction and recovery time

Induction time decreased with increasing extract concentration, while recovery time increased correspondingly in both species.

For *Clarias gariepinus*, one way analysis of variance indicated a statistically significant effect of concentration on induction time ( $F(3,8) = 6.42$ ,  $p = 0.016$ ) and recovery time ( $F(3,8) = 7.85$ ,  $p = 0.009$ ). Similarly, *Oreochromis niloticus* exhibited significant variation in induction time ( $F(3,8) = 5.97$ ,  $p = 0.019$ ) and recovery time ( $F(3,8) = 7.21$ ,  $p = 0.011$ ).

These analyses were restricted to treatments in which recovery occurred, as induction and recovery endpoints could not be defined in cases of complete mortality. While this approach ensures analytical consistency, it limits comparison across the full range of concentrations. In addition, given the limited replication, these statistical outcomes should be interpreted as indicative of general trends rather than definitive effects.

## 3.3 Mortality responses at the highest concentration

Mortality outcomes at the highest concentration (4 000 mg L<sup>-1</sup>) are presented (Table 4). No mortality was recorded in either species exposed to *Citrus sinensis*. In contrast, exposure to *Citrus aurantium* and *Citrus limon* resulted in complete mortality in both species.

These results indicate substantial differences in safety margins among the extracts. As mortality represents a definitive biological endpoint, the findings are presented descriptively without inferential statistical analysis.