

relationship is supported by the statistical trends reported for both species, although the limited replication requires cautious interpretation.

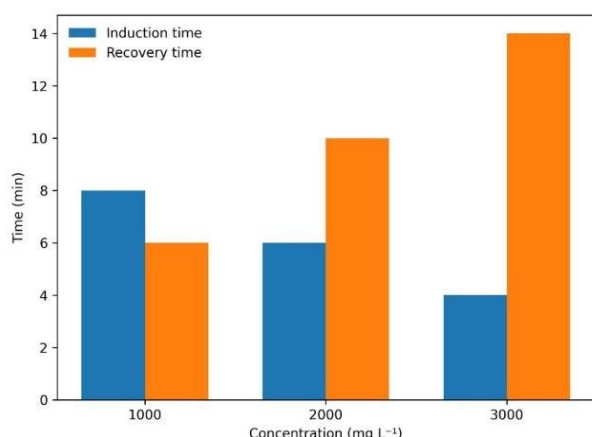


Figure 3 Induction and recovery times of fish exposed to citrus leaf extracts

Rapid induction is widely recognised as a consequence of efficient absorption of anaesthetic agents across the gill epithelium, leading to swift central nervous system depression (Neiffer, 2021). However, excessive accumulation can disrupt metabolic and respiratory processes, resulting in delayed recovery or mortality (Soldatov, 2021). Similar concentration dependent effects have been documented for plant derived anaesthetics such as citronellal and chamomile oil, where increased potency must be balanced against safety considerations (Ak et al., 2022; Hoseini et al., 2022).

The recovery patterns presented (Table 5, Figure 3) further support this interpretation. Extracts associated with shorter recovery times indicate efficient elimination and minimal physiological disruption, which are essential characteristics of suitable anaesthetic agents for routine aquaculture operations (Neiffer, 2021). Conversely, prolonged recovery or absence of recovery reflects deeper physiological disturbance and reduced suitability for practical use.

The level of replication employed in this study is consistent with established protocols in fish anesthesia research, where treatment groups are replicated at the tank level to capture variability in collective behavioural and physiological responses under controlled immersion conditions. Such designs have been demonstrated to provide sufficient statistical power for detecting treatment effects in induction and recovery parameters without compromising experimental feasibility (Neiffer, 2021; Vergneau Grosset and Benedetti, 2022)

#### 4.4 Mortality patterns and physiological implications

The mortality outcomes presented in Table 4 and Figure 2 provide a critical indication of the physiological limits of the tested extracts. The absence of mortality in treatments involving *Citrus sinensis* suggests that its effects are reversible and do not compromise vital physiological functions. In contrast, complete mortality observed with *Citrus aurantium* and *Citrus limon* at higher concentrations indicates severe disruption of respiratory and metabolic processes.

In fish, mortality during anaesthetic exposure is commonly associated with respiratory depression, impaired ion regulation, and metabolic imbalance (Soldatov, 2021). The behavioural signs preceding mortality, including reduced opercular activity observed (Table 2, Table 3), are consistent with compromised oxygen uptake. Comparable findings have been reported in studies of essential oil based anaesthetics, where increased potency is associated with reduced safety margins (Hoseini et al., 2022).

These results highlight that the evaluation of anaesthetic suitability must consider both survival and recovery outcomes, as survival alone does not fully reflect physiological integrity.