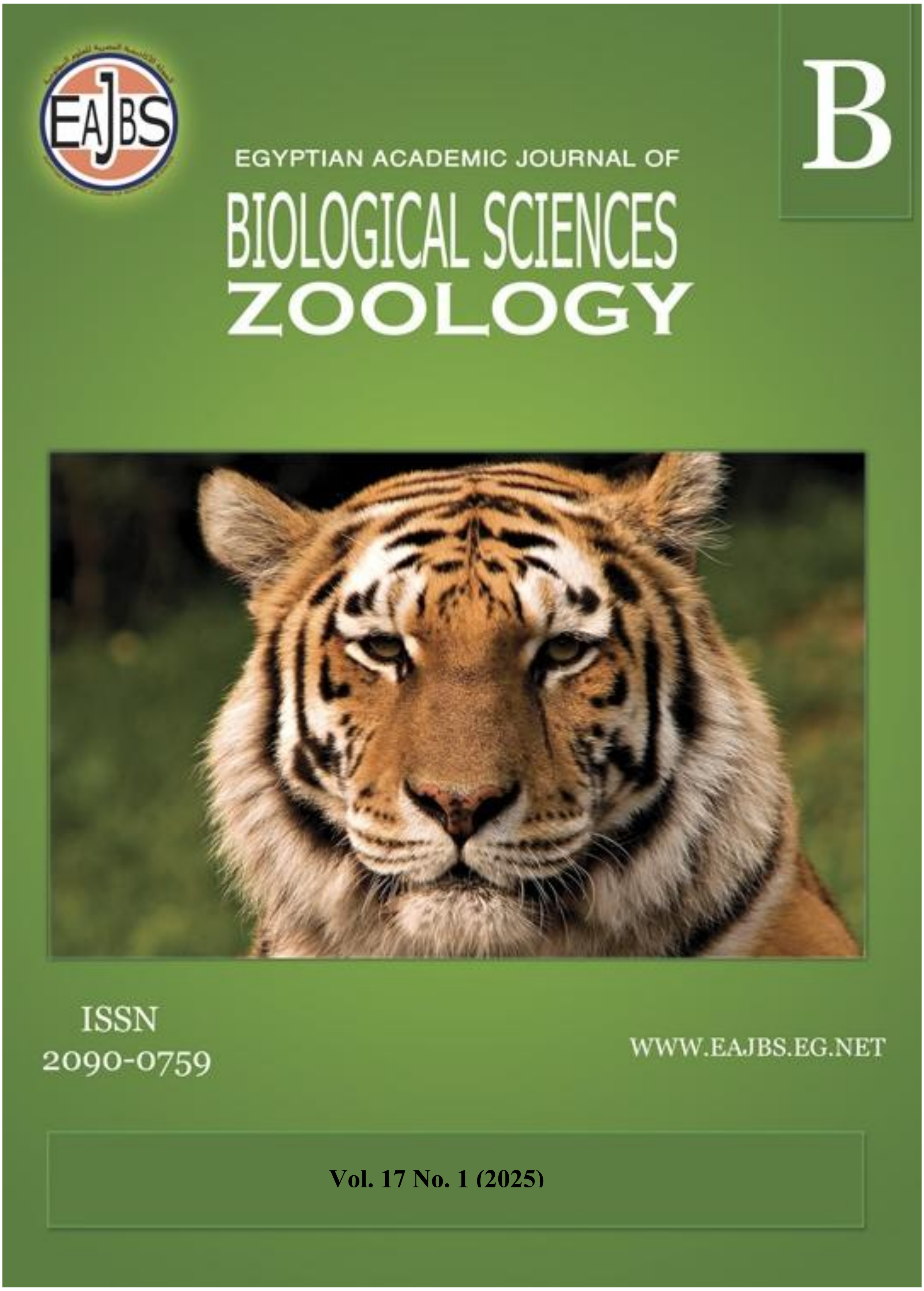

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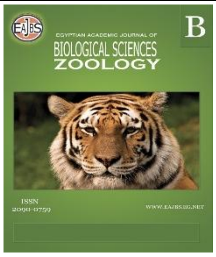

Impact of Environmentally Relevant Levels of 17α-Ethinylestradiol on Osmoregulatory Function in Yellow-Finned Seabream

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ABSTRACT

This study investigates the effects of environmental concentrations of 17α-ethinylestradiol (EE2), a synthetic estrogen and endocrine-disrupting chemical, on the osmoregulatory functions of yellowfin seabream (*Acanthopagrus latus*). Endocrine disruptors like EE2 interfere with hormonal balance in aquatic organisms, potentially affecting physiological processes such as growth, reproduction, and osmoregulation. To assess the potential impact of EE2, a semi-static experimental setup was used in which yellowfin seabreams were exposed to a concentration of 10 ng/L EE2 over two weeks. Calcium carbonate ($CaCO_3$) excretion rates were measured as a primary indicator of osmoregulatory function. The results showed no significant changes in $CaCO_3$ excretion rates, suggesting that short-term exposure to this concentration does not induce measurable osmoregulatory disruptions in this species. However, given the bioaccumulative nature of EE2 and its persistence in aquatic environments, further research is needed to evaluate the long-term effects and potential risks associated with prolonged exposure and higher concentrations. Understanding these impacts is crucial for assessing the ecological risks of pharmaceutical contaminants in marine environments and developing appropriate regulatory measures to mitigate potential harm to marine biodiversity.

INTRODUCTION

Due to the difference in salt concentration between the body fluids of aquatic vertebrates and those of their environment, they are subject to a net influx or efflux of water and salt through their permeable skin or membranes (Kültz & Gilmour, 2020; Lillywhite & Evans, 2021). Marine fish live in hyperosmotic environment and to avoid dehydration, they drink seawater where the absorption of sodium chloride (NaCl) is facilitated by Na+/K+/2Cl- (NKCC) cotransporter in the gills through phosphorylation. Calcium (Ca^{2+}) absorption occur via gills, intestinal and renal regulation.

Marine fish maintain their hypotonic body fluids by drinking seawater to make up for a water deficit and secrete excess salt across the gills (Lillywhite & Evans, 2021). Ingestion of seawater by marine fish results in NaCl being absorbed by the oesophagus, and the intestinal epithelium absorbs the remainder. Intestinal fluids are rich in magnesium (120-150 mg/L) and sulfate (100-120 mg/L) (Grosell, 2019; Grosell & Oehlert, 2023). In marine fish, studies showed that Ca^{2+} concentrations in intestinal fluid can reach up to 400 mg/L under certain conditions.

To maintain Ca^{2+} homeostasis, calcium in seawater precipitates in the gut as calcium carbonate ($CaCO_3$), restricting Ca^{2+} absorption from the ingested water. This process is controlled by the endocrine system (Whittamore *et al.,* 2010).

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The aquatic environment has been described as a sink for both natural and man-made chemicals (Sumpter, 1998). According to Sumpter (1998), the aquatic environment is a sink for natural and man-made chemicals. Several chemicals humans introduce into the environment can mimic or antagonize wildlife's natural (endogenous) hormones and affect their endocrine systems. Chemicals that disrupt the endocrine system are called endocrine-disrupting chemicals (EDCs) (Kaminuma *et al.,* 2000). It has been demonstrated that EDCs interact with native hormone receptors, disrupting hormone signalling essential for various developmental processes (Stewart *et al.,* 2023).

The effects of EDCs on the environment have received increasing attention in recent decades. There is a wide variety of EDCs, which are now commonly classified according to their biological effects. Natural and industrial EDCs are available in a wide range of forms (Darbre, 2019). Pesticides, polycyclic aromatic hydrocarbons, plasticizers containing phthalates, alkylphenols, synthetic steroids, and natural ingredients are among the agricultural chemicals (Afzaal *et al.,* 2022; Anwer *et al.,* 2016). Some synthetic steroid hormones, particularly one estrogen (ethinyl estradiol) and some progestogens (e.g. levonorgestrel) are the most concerning human pharmaceuticals (Gunnarsson *et al.,* 2019), at least in aquatic environments.

The synthetic oestrogen 17α-ethinylestradiol (EE2) is an oestrogenic EDC that threatens wildlife. EE2 a widely used oral contraceptive pill in many parts of the world, and it is a component of the oral contraceptive pill (Weerasinghe *et al.,* 2022), as well as a necessary component for livestock supplementation and agricultural applications. In addition to its widespread presence, EE2 is also resistant to degradation and can accumulate in sediments and biota (Aris *et al.,* 2014). sewage treatment works commonly introduce compounds that cause estrogenic activity into water, such as 17-estradiol (E2) and its synthetic derivative EE2 (Desbrow *et al.,* 1998; Williams *et al.,* 2003). Insufficient removal of EE2 in wastewater treatment plants contributes to the presence of EE2 in aquatic environments (Clouzot *et al.,* 2008). Osmoregulatory disruptions have been reported in fish exposed to EDCs (McCormick *et al.,* 2005; Wang *et al.,* 2019). In fish, steroid estrogens induce a variety of feminizing effects, including the induction of intersex and vitellogenin production (Azizi-Lalabadi & Pirsaheb, 2021; Islam *et al.,* 2024; Jobling *et al.,* 2006). Several laboratory and field studies have demonstrated that low concentrations of EE2 present in the aquatic environment can adversely affect fish reproduction (Aris *et al.,* 2014).

Seawater consumption provides the intestine with a substantial amount of calcium (Ca^{2+}) for absorption. Keeping Ca^{2+} homeostasis requires tight regulation of intracellular and extracellular fluid levels within the intestine. According to (Al-Jandal *et al.,* 2011), intestinal precipitation helps marine fish maintain calcium homeostasis by limiting the absorption of calcium from ingested seawater. As part of the physiological processes associated with fluid and salt transport, estrogen E2 plays a significant role in fish (Carrera *et al.,* 2007; Mancera *et al.,* 2004).

This study examined the impact of EE2 which interferes with hormone systems, on osmoregulation in seabream. We proposed in this study that EE2 may affect how seawater fish process ingested Ca^{2+} and how much $CaCO_3$ is precipitated and excreted. In turn, this will also affect overall osmoregulation.

MATERIALS AND METHODS

Yellowfin seabream (*Acanthopagrus latus*) was examined for its osmoregulation following exposure to waterborne EE2. Ethinylestradiol was administered to the fish in two treatment groups: a control group and a group exposed to 10 ng/L of environmentally relevant concentrations. In a semi-static system made of six tanks, EE2 levels were maintained in each tank for two weeks. A total of 24 fish were used in this study, with 12 fish being randomly assigned to each treatment group to ensure a random distribution, validity of the results, enhance comparability between groups, and reduce bias in the results (Fig. 1).

To maintain water quality and the target EE2 concentration, 50% of the volume of water in each exposure tank was replaced every 48 hours. To avoid contaminating the environment, water from each tank was filtered through a charcoal carbon filter before discharge. This filtration step was essential to remove EE2 from the water before disposal, minimizing the potential impact on the environment.

Fig. 1: Semi-static exposure tanks.

To avoid food waste interfering with the analysis of osmoregulatory effects, the fish were not fed for three days before and during the experiment. Fasting helped prevent food-derived faecal material from mixing with calcium carbonate ($CaCO_3$) precipitates, which could complicate measurements. The calcium carbonate precipitates were collected twice a day during the experiment. Monitoring $CaCO_3$ excretion rates was key to assessing EE2's osmoregulation impact with this collection. EE2 concentrations were also measured regularly from each tank to ensure the exposure conditions stayed within the parameters.

Every fish had its intestinal precipitate of $CaCO_3$ excreted into the chamber twice daily (Fig. 2). The following formula was used to calculate the excretion rate of $CaCO_3$ from dry weight:

Dry weight = Dry wt of ppt (g) ⁄ Fish mass (kg) / Time (h)

A dry $CaCO_3$ sample was analyzed using an Energy-Dispersive X-ray Spectroscopy (EDX) for the determination of Ca^{2+}% as part per thousand (ppt) of $CaCO_3$.

Fig. 2: Calcium carbonate cleaning and drying process.

RESULTS

The results indicated that a two-week exposure to 10 ng/L of EE2 did not alter osmoregulation processes in yellowfin sea bream. Specifically, no significant changes were observed in $CaCO_3$ excretion rates, suggesting that this exposure duration and concentration were insufficient to disrupt osmoregulation (Fig. 3). After two weeks of exposure to EE2, no significant difference was found between treatment groups. There was an excretion rate of 2.36 mg/kg/h for the control group and 2.21 mg/kg/h for the treatment group.

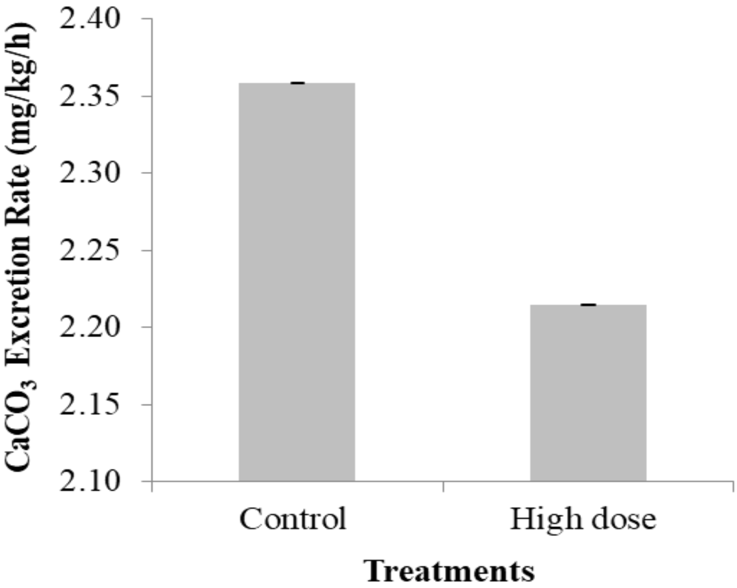

Fig. 3: Mean of Excretion rate of $CaCO_3$ in yellowfin seabream exposed to EE2 for two weeks.

DISCUSSION

The semi-static system used for fish exposure in this study has multiple advantages, such as collecting $CaCO_3$ daily without worrying about flushing it out when using the flow-through system, and this system is very useful when a limited amount of test materials is available and a prolonged test is required.

This study dealt with the effect of exposure to synthetic estrogen (EE2) on osmoregulation. The findings of the study showed no effect on osmoregulation after the exposure to EE2, which could be due to several reasons, such as the difficulty in EE2 concentration recovery in the exposure tanks after 48h, the sensitivity of the species used, and the exposure period. The EE2 exposure exerted no effect on the $CaCO_3$ excretion rate, indicating that this concentration is not enough to induce any effect on osmoregulation. The results obtained followed the trend observed in one of the studies (Al-Jandal *et al.,* 2011), in which elevated levels of circulating E2 were related to enhanced Ca^{2+} uptake via the gut and simultaneously reduced the $CaCO_3$ formation and excretion. Another study by Al Jandal (2022) examined the effects of EE2 on the osmoregulation of yellowfin sea bream (*Acanthopagrus latus*), specifically looking at the intestinal $CaCO_3$ excretion rate. The fish were exposed to EE2 at 5 and 10 ng/L for two weeks, and the results indicated no significant difference in $CaCO_3$ excretion rate between treatments. This suggests that EE2 did not impact osmoregulation in this species *(Al-Jandal et al.,* 2022).

Conclusion:

This study demonstrates that exposure to environmentally relevant concentrations of EE2 over two weeks does not disrupt osmoregulatory processes in yellowfin seabream, as evidenced by unchanged $CaCO_3$ excretion rates. It appears that short-term exposure to low levels of EE2 may not pose immediate risks to osmoregulatory functions in this species.

Further studies with higher concentrations or longer exposure periods may be necessary to observe potential effects on fish osmoregulation.

The EE2 exposure for two weeks at the concentration levels used in this study exerted no effect on the $CaCO_3$ excretion rate, indicating that these concentrations were not enough to induce any effect on the osmoregulation of fish. The present study showed that nominal concentrations of 10 ng EE2/l were not enough to exert any effects on the osmoregulation process of fish. EDCs have a wide range of ecological and physiological effects, which need to be explored in future research. Impact on fertility intersex condition, hormonal imbalances, behavioral changes, and growth rate are few examples of research areas to help in understanding the full scope of EDCs effects. To comprehensively assess the risks posed by EE2 and similar contaminants to aquatic ecosystems, further studies incorporating longer durations, varied exposure levels, and their interactions with environmental stressors are necessary. This insight will be useful in developing mitigation strategies and informing policies regarding the management of chemical pollutants in marine environments. The study paves the way for further experimental research on the ever-growing problem of endocrine disrupters in wastewater in assessing their impact on resident biota.

Declarations:

Ethical Approval: This study was granted by the research committee.

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions: Mashael Al-Mishaal, Methodology, Writing- Original draft preparation, Investigation, Validation; Shatha Albaghli, investigation and methodology of fish exposure experiment; Hiba Alfilakawi, methodology, investigation and Editing

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