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Short Communication

New findings from the first fish toxicity test conducted following the algal bloom of *Polykrikos hartmannii* (Dinophyceae) in the Bay of Izmir, Aegean Sea

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Abstract: The excessive proliferation of harmful algae can lead to fish mortality in coastal ecosystems. This study presents the results of a toxicity test conducted in response to the excessive proliferation of the species *Polykrikos hartmannii* in the Inner Bay of Izmir. The experiment used *Sparus aurata* that were exposed for 120 hours to a 100% concentration (2.4 x 10^3 ± 0.090 cells·mL^{-1}) determined based on the cell density in nature, and to dilutions of this concentration at 50%, 25% and 12.5%. A control group was also included in the study. In addition to mortality, fish behaviour was observed. Based on the results, no mortality was recorded at any concentration, and behaviour remained normal. Although *P. hartmannii* has been reported as ichthyotoxic in the literature, the current findings suggest that the toxic effect of the species may be associated with environmental conditions and feeding strategy (mixotrophic feeding).

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Introduction

Coastal ecosystems are important habitats for biodiversity, providing areas for reproduction, feeding, and shelter. With its rich biodiversity, the Bay of Izmir is one of Türkiye's important marine habitats. The bay serves as habitat for numerous micro- and macroplankton, shellfish, fish species, and migratory birds, providing breeding and living areas for different species within the ecosystem and forming an essential link in the food web (Yılmaz and Yılmaz, 2019). Marine ecosystems are under pressure from pollution resulting from increased anthropogenic activities driven by population growth in coastal areas. Factors such as domestic drainage from treatment plants, industrial waste, agricultural inputs, maritime traffic, and shipyard activities alter the marine environment chemically and biologically (Souvermezoglou and Anagnostou, 2025). These pollution sources accumulate in semi-enclosed bays with limited water circulation, threatening the long-term health of aquatic ecosystems.

The Bay of Izmir, located in the eastern Aegean Sea, is topographically divided into three areas: the inner bay, the middle bay, and the outer bay. The inner Bay of Izmir is characterized by limited water circulation and significant inflow from rivers (Sabanci et al., 2025) (Fig. 1). Factors such as increased seawater temperature due to shallowing, increased nutrient salt load, and decreased water movement combine to cause an imbalance in planktonic organisms, leading to harmful algal blooms. Dinoflagellates, in particular, which serve as indicators of water temperature and eutrophication, release toxins into the environment, causing significant impacts on the food chain and human health (Yurga, 2022).

Polykrikos hartmannii is a species belonging to the mixotrophic dinoflagellates, forming armourless, cyst-producing, single-celled zooids or two-celled pseudocolonies (Zimmermann, 1930; Hulburt, 1957; Tang et al., 2013; Lee et al., 2015). There is limited information on the phototrophic and phagotrophic activities of mixotrophic harmful algal blooms, or on how environmental conditions such as temperature, nutrients, pH, or CO_2 affect the energy-matter cycle in predator-prey interactions. All these environmental

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Figure 1. Topographical areas of Izmir Bay.

factors alter marine conditions and accelerate climate change and eutrophication by triggering harmful algal blooms even under low-nutrient, low-salinity conditions (Flynn et al., 2018). *Polykrikos hartmannii* was reported to feed on the toxic mixotrophic dinoflagellates *Cochlodinium polykrikoides* and *Gymnodinium catenatum* after immobilizing them using the nematocyst-tenniocyst complex and then ingesting them (Matsuoka et al., 2000; Tillmann and Hoppenrath, 2013; Lee et al., 2015).

Increased nutrient inputs to the Bay of Izmir are a significant factor contributing to the excessive proliferation of *P. hartmannii*. However, the mixotrophic feeding characteristic of *P. hartmannii* allows it to sustain population growth not only under high-nutrient conditions but also occasionally in nutrient-limited environments. Therefore, when both nutrient enrichment and this flexible feeding strategy are considered together, they help explain the excessive proliferation of *P. hartmannii* in the Bay of Izmir. The first record of harmful algal proliferation caused by *P. hartmannii* in the Bay of Izmir was reported by Sabanci et al. (2025). The excessive proliferation of *P. hartmannii* plankton in the Bay of Izmir poses a significant ecological risk to the food chain and may lead to toxicity. In recent years, fish deaths have been reported along the shores of the Bay of Izmir (Erdem et al., 2025). The cause of fish mortality remains unknown, and the potential effects of certain phytoplankton species, such as *P. hartmannii*, have been investigated. However, there is limited information in the literature regarding the ichthyotoxicity of *P. hartmannii*. Although *P. hartmannii* has been reported to cause ichthyotoxic effects, its toxin content has not yet been identified, and its relationship with fish mortality remains unestablished (Tang et al., 2013). This study is the first to directly assess the toxicity of *P. hartmannii* collected from the Inner Bay of Izmir to Sparus aurata and aims to contribute to a better understanding of its ecological risk and effects on aquatic life in the bay.

Materials and Methods

Phytoplankton sampling and cell counting: Sampling was conducted in early September 2025 from the surface water of the Inner Bay of Izmir (38.453389°N, 27.143000°E), where *P. hartmannii* was proliferating excessively. The surface water temperature was measured at 23°C. Surface water samples from the Bay were collected in 5 L bottles and transported to the Aegean University Aquatic Products Ecotoxicology Laboratory. The 5 L samples brought to the laboratory were combined in large containers. 1 ml was taken from the dense culture where *P. hartmannii* had proliferated excessively, and cell counting was performed in a Sedgewick-Rafter chamber. In the cell count performed in triplicate on the excessively dense water sample, *P. hartmannii* was detected at $2.4\times10^{3}\pm0.090$ cells·mL^{-1}.

Preparation of phytoplankton culture: Seawater collected from the outer bay of Izmir was filtered through coarse filters and sterilized in an autoclave. To prevent a decrease in cell count during daily water changes throughout the experiment, *P. hartmannii* was cultured in F/2 medium prepared according to Guillard (1975) in sterile glassware with aeration, under fluorescent light at a photon light intensity of approximately 100 µmol·m^{-2}· s^{-1}, with a 12:12 light:dark cycle.

Fish acclimatization: Prior to bringing in a dense phytoplankton culture from the bay, *Sparus aurata* were obtained from a farm located in the outer bay of Izmir for fish toxicity testing. The 200 fish brought to the laboratory were distributed among 12 L glass

Table 1. Cell concentrations (cells · mL^{-1}) and average lengths (cm) of fish in the experimental groups (SD: Standard Deviation).

Group (Cell concentration (%))	Cell concentrations (cells · mL^{-1})	Average fish length (cm) ± SD
Control	0	11.1±0.3
12.5	0.3x10^3	10.9±0.4
25	0.6x10^3	11±0.2
50	1.2x10^3	11.2±.3
100	2.4x10^3	10.8±0.3

aquariums (20 in total), each containing 10 L of seawater collected from the outer bay. Aeration was provided to the aquaria, and the fish were acclimated for 14 days at a laboratory temperature of 21±1 °C. During the acclimatization period, the fish were fed dry feed at 2% of their body weight three times daily. Daily maintenance was performed, including water changes. Deaths were recorded during the acclimatization period, and mortality was below 5%.

Exposure concentrations and fish toxicity test: The average cell density of 2.4 cells·mL^{-1}, obtained by counting cells in dense culture, was used as 100%. Based on this value, four different exposure groups with a final volume of 10 L were prepared: 100%, 50%, 25%, and 12% (Table 1). Dilutions were performed volumetrically with filtered sterile seawater. Cell counts were performed throughout the experiment to verify the target cell densities after each dilution. The toxicity test was initiated on the same day the dense culture was collected.

Sparus aurata, acclimatized under laboratory conditions, was used for the fish toxicity test. Three replicates were prepared for each concentration in the experimental design. 10 fish were added to each replicate, and aeration was provided. A control group consisting of three replicates of seawater without cultured cells was added. A total of 150 fish were used in the fish toxicity test. The average length of the fish in each group was between 10 and 12 cm, and there was no statistically significant difference between the control and experimental groups (Table 1) (P>0.05).

The exposure period was set at 120 hours. During this period, a complete water change was performed every 24 hours. With each renewal, the relevant concentration was prepared anew, and the fish were exposed to fresh culture. Thus, the continuity of the targeted cell densities was ensured throughout the experiment. Fish feeding was not performed during the experiment. The ambient temperature was maintained at 21±1°C, and water quality parameters, including temperature, pH, and salinity, were monitored.

Statistical analysis: No statistical difference analysis was performed, as no mortality was observed in any concentration group in the fish toxicity tests. Fish lengths were measured for each group and presented as mean ± SD. Analyses were performed using SPSS 20.0 software. Intergroup differences were tested using One-Way ANOVA, and no statistically significant differences were found (P>0.05). Phytoplankton cell densities were counted in three replicates and reported as mean ± SD; no statistical analysis was performed on these data.

Results and Discussions

In this study, fish toxicity tests conducted with *P. hartmannii* collected intensively from the surface water of the Inner Bay of Izmir in early September showed no mortality at any cell concentration during a 120-hour exposure period. Throughout the exposure period, swimming behaviour, surfacing, sinking to the bottom, and gill movements were regularly observed in the fish. No abnormal behavioural differences were observed at any concentration compared with the control group (Table 2).

The findings differ from those reported in the literature, indicating that *P. hartmannii* exhibits ichthyotoxic effects (Tang et al., 2013). One possible explanation for the discrepancy between our results and those of the previous study is that *P. hartmannii* does not produce toxins directly but instead feeds on other toxin-producing phytoplankton species. Indeed, a study examined the feeding preferences of *P. hartmannii* and reported that it fed only on two

Table 2. Results observed in the fish toxicity test.

Group (Cell Concentration (%))	Number of Fish (n)	Experiment Duration (hours)	Mortality (%)	Behavioural Observations
Control	10	120	0	Normal
12.50	10	120	0	Normal
25	10	120	0	Normal
50	10	120	0	Normal
100	10	120	0	Normal

toxin-producing mixotrophic dinoflagellates (Cochlodinium polykrikoides and Gymnodinium catenatum) among 18 phytoplankton species (Lee et al., 2015). However, there are no natural records of these two species in the Bay of Izmir. Therefore, *P.hartmannii*'s toxin production may have occurred indirectly, depending on its feeding on other toxin-producing algae in the bay.

Additionally, *P. hartmannii* exhibits mixotrophic feeding and may prefer heterotrophic feeding in nutrient-limited environments (Schenone et al., 2024). Given the high nutrient salt concentration in the Inner Bay of Izmir, this type of heterotrophic feeding may have been triggered. This situation may be one of the reasons why *P. hartmannii* does not exhibit toxic effects under laboratory conditions or in the bay. On the other hand, to clarify the fish deaths in the Inner Bay of Izmir, it is necessary to examine the species diversity and phytoplankton composition in the bay in more detail, to identify different harmful algal blooms, and to test the possible toxic effects of these species (Yurga, 2022; Şahin, 2024; Erdem et al., 2025). In this context, it is hypothesized that the periodic fish deaths observed in the Inner Bay of Izmir are attributable to phytoplankton producing diverse toxins and to changes in water quality parameters, rather than to *P. hartmannii*.

These results indicate that *P. hartmannii* does not directly cause fish mortality. However, when species diversity, environmental conditions, and food-web relationships in the bay ecosystem are considered together, more comprehensive studies are required to determine the potential for species toxicity, given existing uncertainties. Our study focused on mortality; supporting toxicity tests with studies of sublethal effects (histological examinations of gills, liver, and other tissues, enzyme activities, and molecular and cellular pathways) will contribute to a more robust evaluation of the results.

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