

Research Article

Toxicity of Dinoflagellate (*Gonyaulax verior*) From Makassar Strait Waters on Medaka Fish (*Oryzias javanicus*) Embryo Development

Nita Rukminasari, Khusnul Yaqin, and Jamaluddin Fitrah Alam

Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia

Article history

Received: 08-10-2024

Revised: 03-05-2025

Accepted: 28-04-2025

Corresponding Author:

Nita Rukminasari

Faculty of Marine Science and
Fisheries, Universitas Hasanuddin,
Makassar, South Sulawesi, Indonesia
Email: nita.r@unhas.ac.id

Abstract: This study investigates the toxic effects of the dinoflagellate *Gonyaulax verior* on the embryonic development of medaka fish (*Oryzias javanicus*) sourced from four distinct aquatic environments in the Makassar Strait. Given the increasing presence of harmful algal blooms in marine ecosystems, understanding the impact of toxic dinoflagellates is crucial for assessing risks to fish populations. Embryos were exposed to varying concentrations of *Gonyaulax verior* isolated from four locations: Jeneberang Estuary, Maros Estuary, Pangkep Estuary, and Paotere Port. The effects on embryonic mortality, morphological abnormalities, and developmental rates were systematically recorded. The findings revealed significant variations in embryonic development across locations; notably, embryos exposed to dinoflagellate toxins demonstrated increased mortality rates and higher incidences of morphological deformities compared to control groups. The data reveal a significant trend: embryos exposed to dinoflagellate cysts exhibit a higher yolk absorption rate than control groups. This trend is most pronounced in the Jeneberang Estuary, where dinoflagellate-exposed embryos demonstrate the highest recorded yolk absorption rate of 0.0031 mm³/hour, contrasted with a control rate of 0.0027 mm³/hour. The Jeneberang Estuary exhibited the highest survival rates, whereas the Maros Estuary and Pangkep Estuary showed alarming levels of toxicity, leading to substantial developmental impairments. These results underscore the ecological implications of *Gonyaulax verior* proliferation and highlight the need for monitoring harmful algal species in the Makassar Strait. This research contributes to understanding the impact of marine toxins on fish embryonic development. It emphasizes the need for effective management strategies to mitigate the effects of dinoflagellate blooms on local aquatic ecosystems.

Keywords: Toxicity, Dinoflagellate (*Gonyaulax verior*), Medaka Fish (*Oryzias javanicus*), and Embryo Development

Introduction

Over the past few decades, reports of harmful algal blooms (HABs) have become increasingly common worldwide (Anderson *et al.*, 2012; Hallegraeff, 1993; Heisler *et al.*, 2008; Smayda, 1997). Dinoflagellates are responsible for 75% of HABs linked to microalgae (Smayda, 1997), and many of these dinoflagellates produce various biotoxins that pose serious risks to human

health, tourism, fisheries, and ecosystems (Hallegraeff, 1993; Nair *et al.*, 2013; Van Dolah, 2000). Although only a limited number of toxins have been identified—such as Paralysis Shellfish Toxins (PSTs), Amnesic Shellfish Toxins (ASTs), Diarrhetic Shellfish Toxins (DSTs), Neurotoxic Shellfish Toxins (NSTs), Ciguatera Finfish Toxins (CFTs), Azaprocins (AZTs), and karlotoxins (Botelho *et al.*, 2019; Farabegoli *et al.*, 2018; Twiner, 2008; Van Wagoner *et al.*, 2010)—many

ichthyotoxic (fish-killing) toxins produced by dinoflagellates remain unidentified or uncharacterized. These include those from *Margalefidinium polykrikoides*, *M. fulvescens*, *Amphidinium carteria*, *Akashiwo sanguinea*, and *Pheopolykrikos hartmannii* (Iwataki *et al.*, 2007; Murray *et al.*, 2015; Shin *et al.*, 2019; Tang *et al.*, 2013; Tang & Gobler, 2009; Xu *et al.*, 2017). In addition to generating toxins harmful to marine animals and humans, many dinoflagellate species also produce allelochemicals that adversely affect co-occurring phytoplankton, a process known as allelopathy (Fistarol *et al.*, 2004; Hakanen *et al.*, 2014; Long *et al.*, 2021; Tillmann *et al.*, 2007; Yang *et al.*, 2010).

Harmful Algal Blooms (HABs) are triggered by various species of marine phytoplankton, many of which generate potent biotoxins (Landsberg, 2002). These blooms can have significant ecological and economic repercussions, leading to mass die-offs of both wild and farmed fish and shellfish (Anton *et al.*, 2008; Gobler *et al.*, 2008; Imai *et al.*, 2006; Richlen *et al.*, 2010; Shumway, 1990; Taylor & Trainer, 2002; Whyte *et al.*, 2001). Despite these notable effects, there is limited knowledge about the wider ecological consequences of numerous HABs (Gobler *et al.*, 2008). Forage fish are vital in marine ecosystems, as they consume algae and other plankton, facilitating energy transfer to higher trophic levels (Anton *et al.*, 2008; Gobler *et al.*, 2008; Imai *et al.*, 2006; Richlen *et al.*, 2010; Taylor & Trainer, 2002). Given their importance, these fish may be especially vulnerable to HABs. Consequently, the effects on this group could lead to ripple effects throughout aquatic food webs and have significant economic ramifications (Pikitch *et al.*, 2014).

Blooms of the toxic dinoflagellate have expanded in their geographic reach, occurrence, and duration across many coastal ecosystems globally (Kudela & Gobler, 2012). These blooms in various parts of Asia, Europe, and North America have become annual occurrences (Gobler *et al.*, 2008; Kudela & Gobler, 2012; Richlen *et al.*, 2010; Taylor & Trainer, 2002; Tomas & Smayda, 2008), posing significant challenges for managing and conserving coastal living resources (Anderson, 2009; Kim, 2010; Landsberg, 2002).

Fish have commonly been utilized as animal models in toxicological research to study harmful metabolites produced by dinoflagellates. These metabolites include ciguatoxins (Argyle *et al.*, 2016; Colman *et al.*, 2004; Yan *et al.*, 2017, 2020), maitotoxin-3 (Argyle *et al.*, 2016), brevisulcatic acids (Shi *et al.*, 2012), domoic acid (Mincarelli *et al.*, 2018; Tiedeken *et al.*, 2005), okadaic acid (Escoffier *et al.*, 2007), and saxitoxin (G. Chen *et al.*, 2020; Lefebvre *et al.*, 2004). The marine medaka is an effective model for exploring the impacts of toxic metabolites on physiological responses, embryonic

development, and associated molecular biology (Dong *et al.*, 2014). Marine medaka has been successfully employed to study the adverse effects of algal extracts and toxins by analysing pathological, physiological, and molecular characteristics. For instance, multiple studies have demonstrated the toxic consequences of exposure to pacific ciguatoxin-1 (P-CTX-1), which include teratogenic effects, reduced reproductive capabilities, abnormal swimming patterns, lower hatching rates, and changes in stress-related gene expression (Mak *et al.*, 2017; Yan *et al.*, 2017, 2020). Additionally, research has shown early developmental toxicity, anti-estrogenic effects, and proteomic changes induced by saxitoxin (Tian *et al.*, 2014), along with neurotoxicity caused by brevetoxin PbTx-1 (Yau *et al.*, 2019).

The Japanese Medaka (*Oryzias latipes*), a small freshwater fish measuring 2 to 4 cm, has been utilized in toxicity testing for over 50 years. They are exceptionally resilient, have a low incidence of diseases, can withstand a broad range of salinities and temperatures, and can be easily bred in laboratory conditions. In various toxicity assessments, small fish serve as effective vertebrate models, acting as ecological sentinels (providing quick assessments to predict toxicity in mammals) or as detailed tools for mechanistic research (Hinton *et al.*, 2005; Law, 2001; Shima & Mitani, 2004). Testing protocols may involve multi-generational exposures and acute or chronic exposure of adults or developing embryos to individual compounds or mixtures. Advanced experimental techniques are also available, including stable transgenic lines (Fu *et al.*, 2000; Wayne *et al.*, 2005) and morpholino knock-down methods (Carl *et al.*, 2002; Paul-Prasanth *et al.*, 2006).

The fish embryo test (FET) has gained recognition over the past decade as a potentially useful method for evaluating the acute toxicity of chemicals and effluents to fish (Braunbeck *et al.*, 2005; Embry *et al.*, 2010; Lammer *et al.*, 2009; Schulte & Nagel, 1994). Originally, the FET was designed as a 48-hour assay where recently fertilized embryos (under 1 hour old) are exposed to various chemical concentrations, with lethality being measured throughout the study (Schulte & Nagel, 1994). Signs of lethality include embryo coagulation, absence of somites, failure of the tail to detach, or lack of heartbeat, all of which can result in death. In a prior study (Liu *et al.*, 2007), an effective assay using larval medaka (*Oryzias latipes*) was developed. Organic toxicants were concentrated 10 to 100 times from 4 litres of river water using disposable commercial adsorption cartridges (Liu *et al.*, 2007). The Japanese medaka can be easily raised in a small laboratory setting (Marsh *et al.*, 2010). It has been utilized as a model organism in various studies within environmental toxicology and developmental biology due to its transparent chorion and relatively large size, which

enhance observation (Chen *et al.*, 2001). This species is believed to maintain a consistent reproductive capacity throughout the year. Medaka embryos have been effectively utilized to investigate the teratogenic impacts of azaspiracid, a newly identified marine phycotoxin, as well as the developmental toxicity of okadaic acid, which is associated with diarrhetic shellfish poisoning (Colman *et al.*, 2005; Escoffier *et al.*, 2007).

Using *Oryzias javanicus* (commonly known as the Javanese medaka) for toxicity testing in this study offers several notable advantages, particularly in the context of assessing the impacts of harmful dinoflagellates like *Gonyaulax verior*. *Oryzias javanicus* is known for its sensitivity to a wide range of environmental pollutants, including heavy metals, pharmaceuticals, and toxic algal blooms. This sensitivity makes it an effective bioindicator for evaluating the health of aquatic ecosystems (Hwang *et al.*, 2011). The medaka has a short embryonic development period, typically hatching within 14 days under optimal conditions. This rapid development allows for efficient evaluation of toxicological effects on embryonic and larval stages, making it suitable for time-sensitive studies (Iwamatsu, 2004). *Oryzias javanicus* can be easily bred and raised in laboratory conditions, ensuring consistent availability for experiments. They can thrive in freshwater and brackish water environments, which is beneficial for research focused on a variety of aquatic habitats (Sakamoto, 2018).

There were several previous studies to test the toxicity of several dinoflagellates toxins to the embryo of *Oryzias melastigma*. However, there were not too many studies to test the toxicity of dinoflagellates toxins to *Oryzias javanicus* embryos. This study investigates the toxicity of dinoflagellates collected from four different locations in the Makassar Strait on the embryos of *Oryzias javanicus*. *Oryzias javanicus* serves as an ideal model organism for toxicity testing due to its sensitivity to contaminants, rapid development, ease of laboratory culture, and relevance to broader ecological and economic contexts. The findings from studies using this species can significantly contribute to understanding the impacts of toxins like those produced by *Gonyaulax verior* on aquatic ecosystems.

Materials and Methods

The materials used in this study were *O. javanicus* fish eggs as an object of observation, ERM as an embryo maintenance medium and used as a dilution material for dinoflagellates microalgae toxins. Fengli 0 and nauplii *Artemia* sp. as fish feed for *O. javanicus* and dinoflagellate microalgae poison solution as a poison to be exposed to *O. javanicus* fish embryos.

Oryzias javanicus Fish Rearing

Oryzias javanicus fish were obtained from the Laboratory of Aquatic Animal Physiology, Department of

Fisheries, Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar. During the maintenance period, the fish are fed Fengli 0 trademark commercial feed in powder and naupli *Artemia* sp. Fengli 0 feed is given with a frequency of twice a day, and naupli *Artemia* sp. is given once a day. Cleaning of the aquarium is carried out when there was leftover feed at the bottom of the aquarium. Cleaning was done by disposing of water in the aquarium using a siphon cleaner; the aquarium is refilled with water.

Fertilization and Harvesting of *Oryzias javanicus* Fish Eggs

Eggs are obtained from brood stock of *O. javanicus*. Fertilization in *O. javanicus* broodstock occurs naturally in the aquarium. After fertilization, the mother attaches her eggs to the abdomen, and the eggs are collected like grapes bound by threads (Herjayanto *et al.*, 2019; Yaqin, 2021). *O. javanicus* can produce 30–80 eggs (Yaqin *et al.*, 2021).

The mother who has laid eggs is removed using a shovel (small net), then the eggs are harvested, and the mother is returned to the aquarium. The harvested eggs are put into a petri dish, first filled with ERM (Embryo Rearing Media) embryo rearing medium. To clean and separate the egg from its fine threads, the egg is gently rubbed using the index finger (Yaqin, 2021; Yaqin *et al.*, 2021).

Preparation of *Oryzias javanicus* Embryos in the Laboratory

Eggs separated from the broodstock and cleaned of fine threads were then observed under a microscope using 40x magnification to select unfertilized eggs (see Yaqin *et al.*, 2021 for criteria). Fertilized eggs (those with a separated chorion and yolk) were retained for the study. Unfertilized eggs were marked with a chorion, and the yolk was not separated by a perivitelline chamber, meaning the eggs could not be used in this study. The egg has been fertilized if the perivitelline space has separated the chorion and yolk. These eggs were used in this study.

Retrieval and Hatching of Dinoflagellate Cysts

Dinoflagellates were taken from Pangkep River, Jeneberang River, Maros River, and Paotere Port, which were still cysts. They were then cultured until the cysts were hatched at the laboratory. The dinoflagellates culture is impure. The solution containing the species from the hatched cyst was then put into a sample culture bottle and stored in the Aquatic Animal Physiology laboratory for exposure to the embryos of the medaka *O. javanicus*. The species of hatching cysts were identified as consisting of 3 types of species, namely *Scropsisiella cf. rotunda*, *Scrippsiella crystallina* (non-toxic) (Rukminasari &

Tahir, 2021) and dominated by *Gonyaulax verior* (toxic) (Khokhar *et al.*, 2018; Mohamed, 2018).

Dilution of Dinoflagellates

The dilution of the dinoflagellate microalgae solution was based on the culture medium, knowing that the dominant species in the culture bottle was the toxic species of *Gonyaulax verior*. So, in the sample vial, it was assumed to be a solution with a concentration percentage of 100%. Then, it was diluted twice to be made into a solution with a concentration percentage of 50% and 75%.

The first dilution was done by inserting 50 mL of ERM solution into a 100 mL measuring cup. Then, as much as 50 mL from the 100% concentration was added to a solution of dinoflagellate microalgae. After that, it was put into a sample vial and stirred to fuse the solution. So, it becomes a solution of microalgae poison with a concentration of 50%. The second dilution was done by inserting 25 mL of ERM solution into a 100 mL measuring cup. Then, a solution of microalgae dinoflagellates was added, as much as 75 mL from the 100% concentration solution, into the sample bottle and stirred so that the solution fused. So, it becomes a solution of dinoflagellates microalgae poison with a concentration of 75%. While embryos that are only given ERM act as controls.

Experimental Design

This study used a quasi-experimental method. A quasi-experiment was a research method that, in its application, does not use random assignments (random assignment) but uses existing groups. The study was conducted with one control group with three treatments of dinoflagellate toxin concentrations, with ten replicates (1 embryo each). The number of replicate per group was ten due to usual number of replicate for a quasi-experimental method. Dinoflagellate toxin solutions were prepared with different concentrations with a total of three treatments with one control; the details of each treatment were on medium B (50%), medium C (75%), medium D (100%), and medium A as a control.

Embryos were inserted into each of the 24-well microplates using a dropper pipette. Then, each well from the embryo's micro-plate was exposed to a pre-made dinoflagellate toxin concentration of 2 mL. The embryos were exposed to the toxin at phase 19, which is the organogenesis phase. The exposure in phase 19 was carried out to avoid the suspicion that the embryo underwent pseudo-fertilization. The number of eggs used for each treatment was as many as 10 embryos, with a total of 40 embryos obtained from the spawning of female mothers in the aquarium.

Parameters tested

Egg Diameter

The diameter of the egg was measured by drawing the arrow line horizontally and vertically using the Image Raster 3.0 application; then a formula was used to obtain the diameter of the egg (Rodriguez *et al.*, 1995)

(1)

$$D_s = \sqrt{D \times d}$$

Where:

Ds = Actual diameter (mm)

D = Horizontal diameter (mm)

d = Vertical diameter (mm).

Egg Yolk Volume and Egg Yolk Absorption Rate

Measurement of yolk volume is a method used in evaluating embryo growth and development (Wang *et al.*, 2020). The volume of the yolk was calculated by drawing the arrow lines horizontally and vertically on the yolk ball using the Image Raster 3.0 application. then calculated using the following formula (Hardiana *et al.*, 2024).

(2)

$$V = \left(\frac{\pi}{6}\right) \times LH2$$

Where:

V = Volume of the yolk (mm)

L = Length of the yolk diameter (mm)

H = Height of the yolk diameter (mm).

After obtaining the value of the yolk volume, the yolk absorption rate was calculated using the following formula (Hardiana *et al.*, 2024).

(3)

$$LKT = \frac{(V_o - V_t)}{(t - t_o)}$$

Where:

LKT = Egg yolk absorption rate (mm³/h)

V0 = Initial volume (mm³)

Vt. = Volume end (mm³)

t = Start time (hours)

t_o = End time (hours).

Observation of Embryo Survival Rate (SRE)

Survival is the percentage of the ratio between the number of embryos that survive early maintenance and the number of embryos that survive late maintenance. Calculated by formula (Gebreab, 2022).

(4)

$$SRE = \frac{\text{Final number of maintenance embryos}}{\text{Initial number of maintenance embryos}} \times 100\%$$

A living embryo is characterized by the appearance of a heartbeat in the heart and the presence of embryo movement in the egg. In contrast, dead embryos were not found to have a heartbeat (Wang *et al.*, 2020).

Early Hatching Larval Length

Larval observation was carried out using a stereo microscope with a magnification of 1.5x. The larval images were then documented using the Optilab viewer application. The Image Raster 3.0 application measures the larval body's total length in each hatched embryo. The total body length of *Oryzias javanicus* larvae was measured from the tip of the lower jaw to the tip of the tail fin (Wang *et al.*, 2020). The results of the measurements were then recorded.

Hatching Time

Hatching was characterized by the chorion membrane having ruptured and the larvae actively moving (Yaqin *et al.*, 2022). The hatched eggs are recorded every day from the first hatching until all the embryos in each hatching medium are hatched. Embryos that have fully emerged from the chorion were considered to have hatched and are considered not to hatch if the opposite occurs (Wang *et al.*, 2020).

Data Analysis

The data analysis was carried out using statistical analysis using GraphPad Prism 5 software, which conducted a non-parametric Kruskal-Wallis test followed by a Dunn test analysis. The Kruskal-Wallis test provided a robust and appropriate statistical framework for analyzing this study's toxicity data, considering the non-normal distribution, independent group comparisons, and the need for a method that can handle potential outliers effectively. Statistical analysis was carried out on embryogenesis observation parameters such as egg diameter, yolk absorption rate, heart rate, hatching time, and length of the initial hatching larvae and embryo survival. The results of the abnormality observation will be presented in a descriptive and image form. The results of statistical data analysis are presented descriptively and graphically.

Results and Discussion

Embryonic Parameters

This analysis investigates the rate of yolk consumption by developing embryos, a vital physiological process influencing their growth and survival as shown in Fig. 1a. The data reveals a significant trend: embryos exposed to dinoflagellate cysts exhibit a higher yolk absorption rate than control groups. This trend is most pronounced in the Jeneberang Estuary, where dinoflagellate-exposed embryos demonstrate the highest recorded yolk absorption rate of 0.0031 mm³/hour, contrasted with a control rate of 0.0027 mm³/hour. Statistical analysis indicates that the differences in yolk absorption rates

between the groups are significant in both the Jeneberang and Maros Estuaries—as denoted by the differentiation of letters 'a' and 'b' above their respective bars—suggesting a robust effect of dinoflagellate exposure on yolk consumption.

This analysis underscores the importance of yolk consumption rates in developing embryos and highlights the significant impact that dinoflagellate cysts may have on this vital physiological process. The study indicates that embryos exposed to dinoflagellate cysts show enhanced yolk absorption compared to controls, with the most pronounced effects observed in the Jeneberang Estuary. The recorded yolk absorption rate of 0.0031 mm³/hour for dinoflagellate-exposed embryos, compared to 0.0027 mm³/hour for controls, suggests a meaningful interaction between dinoflagellates and the embryonic development process.

The implications of these findings extend to understanding the ecological dynamics within marine environments. Elevated yolk absorption rates may indicate that dinoflagellate exposure could enhance metabolic activity in developing embryos, potentially leading to faster growth rates. However, this accelerated growth requires further investigation to determine its benefits and potential drawbacks, such as the risk of hatching emerging with reduced energy reserves due to rapid yolk depletion (Ceballos-Osuna *et al.*, 2014). In trophic terms, the increased metabolic demands and subsequent yolk utilization can influence survival rates, especially if post-hatch food availability is inconsistent (Lentz *et al.*, 2020).

Moreover, the statistical significance of the differences in yolk absorption rates across the Jeneberang and Maros Estuaries indicates that the presence of dinoflagellates can influence not only individual development but also community dynamics and population health (Thompson *et al.*, 2022). Therefore, these findings invite further exploration into the ecological roles of dinoflagellates and their potential impact on larval health and survival in various ecosystems.

This analysis aims to elucidate the relationship between larval size at hatching and exposure to dinoflagellate cysts, which is a crucial indicator of developmental progress and subsequent survival potential as shown in Fig. 1b. The data consistently demonstrate that larvae subjected to dinoflagellate cysts exhibit reduced size at hatching compared to control groups across all four study locations. Notably, this trend is most pronounced in the Jeneberang Estuary, where control larvae attain an average length of 4.8 mm, in contrast to dinoflagellate-exposed larvae, which measure only 4.1 mm. The statistical significance of this difference is

consistent across all locations, underscoring the widespread and substantial impact of dinoflagellate exposure on larval growth. The shortest larvae recorded at hatching, measuring 3.9 mm, are found within the dinoflagellate treatment group at Paotere Port.

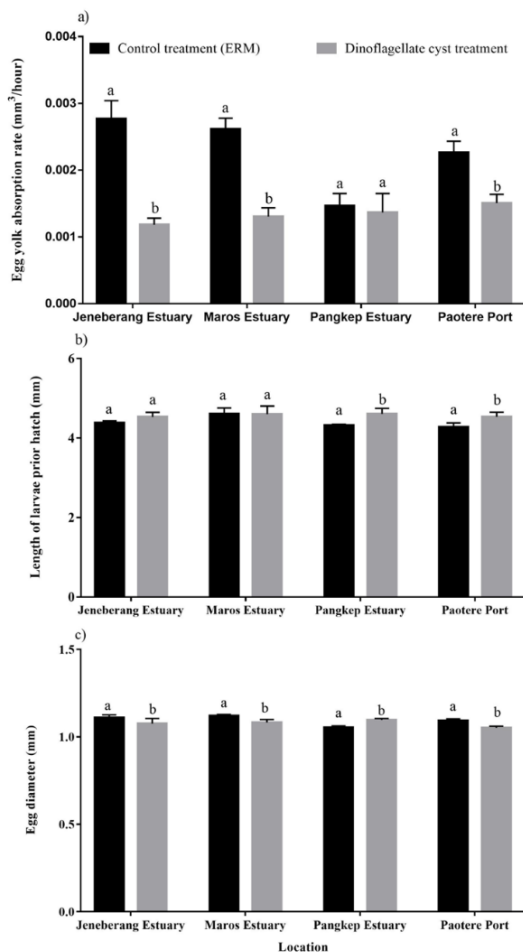


Fig. 1: a. Eggs yolk absorption rate (mm³/hour), b. length of larvae prior to hatch (mm), and c. egg diameter (mm) during embryonic development of medaka embryos exposed or not to various concentrations of dinoflagellate cyst. Note different lowercase letters indicate a significant difference (P<0.05).

The implications of reduced larval size at hatching are multifaceted. Smaller larvae are generally more vulnerable to predation and may have diminished foraging capabilities, which could contribute to lower survival rates (Houde, 2008). This phenomenon has far-reaching consequences for population dynamics and community structure in marine ecosystems, as smaller, less robust larvae are less likely to recruit successfully into adult populations (Wilson *et al.*, 2016). Furthermore, the finding that the shortest larvae, measuring 3.9 mm, are

from the dinoflagellate treatment group at Paotere Port underscores the potential local variability in the effects of environmental stressors, suggesting that certain locations may be more susceptible to detrimental impacts from dinoflagellates.

Additionally, underlying mechanisms may be driving the observed reduction in larval size. For instance, exposure to harmful compounds released by dinoflagellates could trigger physiological stress responses that lead to premature hatching or suboptimal growth conditions (Peck *et al.*, 2004). Such stressors may result in larval energy being diverted away from growth and development, ultimately impacting their fitness and survival in a competitive marine environment.

Overall, this analysis underscores the critical importance of understanding the effects of dinoflagellate exposure on larval development. As marine ecosystems face increasing pressures from harmful algal blooms, such research is essential for predicting shifts in population dynamics and ensuring the sustainability of marine resources.

This investigation elucidates the significant relationship between egg size and maternal investment, highlighting how exposure to dinoflagellate cysts can adversely affect this vital reproductive metric. The findings indicate a clear trend of reduced egg diameter in the presence of dinoflagellates, particularly within the Jeneberang Estuary, where control eggs average 1.3 mm in diameter, while eggs exposed to dinoflagellate cysts measure only 1.0 mm as shown in Fig. 1c. This substantial difference in egg size evidenced by statistical significance across the Jeneberang and Maros Estuaries—suggests a deleterious effect of dinoflagellate exposure on egg development and maternal resource allocation.

The reduction in egg size associated with dinoflagellate exposure may be attributed to several potential mechanisms. One primary explanation is that the physiological stress induced by environmental factors associated with dinoflagellates may lead to decreased energy allocation for oocyte production in adult females (Baker *et al.*, 2017). This stress can result in smaller eggs that may not provide adequate energy for developing embryos, potentially affecting their survival rates post-hatching. Additionally, the compounds released by dinoflagellates could disrupt the normal processes of oogenesis, leading to the production of smaller, less viable eggs (Llanos *et al.*, 2021).

Moreover, observing the smallest eggs recorded in the dinoflagellate treatment group at Paotere Port highlights a potential geographical variability in maternal investment influenced by local environmental conditions, which warrants further investigation into the interactions between dinoflagellates and reproductive success in marine organisms.

Ecosystem-level implications are significant; smaller egg size can result in lower energy reserves for developing larvae, influencing larval growth, resilience to stressors,

and ultimately their survival in the wild. Understanding the impacts of dinoflagellate exposure on egg size and maternal investment is vital for predicting population dynamics and ecological balance in affected habitats.

Growth Rate, Absolute Growth And Survival Rate

Fig. 2a. illustrated the daily growth rate of the organism, likely a marine species, in millimeters per day. The experiment compares a control environment (ERM) against a treatment where dinoflagellate cysts are introduced. Across all four locations (Jeneberang Estuary, Maros Estuary, Pangkep Estuary, and Paotere Port), we observe negative growth rates, meaning the organisms are, on average, decreasing in size each day as shown in Fig. 2a.

The overarching trend is negative growth, suggesting unfavorable conditions across all locations. However, the degree of negative growth varies. Jeneberang Estuary and Paotere Port show the least negative growth rates in both treatments, hovering around 0 mm/day. In contrast, Maros Estuary and Pangkep Estuary exhibit the most substantial decline, with growth rates plummeting to approximately -0.8 mm/day. This provides valuable insights into the daily growth rates of a marine organism, revealing a concerning trend of negative growth across all study locations when comparing a control environment (ERM) with a treatment involving the introduction of dinoflagellate cysts. The data indicate that organisms are, on average, decreasing in size, which signifies unfavorable growth conditions prevailing in these environments. Notably, while all four locations Jeneberang Estuary, Maros Estuary, Pangkep Estuary, and Paotere Port show negative growth, the severity of this decline varies by location.

The relatively less negative growth rates observed in Jeneberang Estuary and Paotere Port, which stabilize around 0 mm/day, indicate that these environments may be less harsh compared to Maros Estuary and Pangkep Estuary, where growth rates significantly decline to approximately -0.8 mm/day. This disparity highlights the potential influence of local environmental factors, including water quality, availability of food resources, and the presence of competing or predatory species, which affect organism growth.

Statistical designations—represented by letters 'a' and 'b' above the bars on the graph—are pivotal for understanding the significance of the results. Bars sharing the same letter within individual locations indicate no statistically significant differences in growth rates between the control and treatment groups at those sites. However, the differing letters suggest considerable variations in growth rates across locations, pointing to the conclusion that geographic variability has a more substantial impact on growth dynamics than the introduction of dinoflagellate cysts alone.

These findings underscore the complexity of marine ecosystems and emphasize the necessity of factoring in local environmental conditions when assessing the influence of specific biotic factors, such as dinoflagellates, on growth rates. The persistent negative growth trend raises concerns about the overall health and viability of the studied organisms, potentially implicating broader ecological consequences in affected areas. Dinoflagellate cysts do not significantly impact the growth rate within each location. However, regardless of treatment, the 'a' and 'b' designations reveal significant differences between locations. This suggests that location plays a more critical role in influencing growth rate than the presence of dinoflagellate cysts.

Fig. 2b. shifts the focus from daily growth rate to the overall change in size (in millimeters) over the experimental period. Again, negative values dominate, reinforcing the observation of size decrease across all locations and treatments. Mirroring the growth rate trend, absolute growth is most negative in Maros Estuary and Pangkep Estuary, reaching around -4 mm (Fig. 2b). This significantly reduces organism size. Jeneberang Estuary and Paotere Port show less severe size reduction, with values closer to -1 mm, indicating relatively less harsh conditions. As indicated by the letters, the statistical analysis reinforces the findings from the graph (a). No statistically significant difference exists between the control and dinoflagellate cyst treatment within each location. However, significant differences are apparent between locations ('a' vs. 'b'), highlighting the dominant influence of location on absolute growth.

This underscores a concerning trend regarding the overall change in size (in millimeters) of the marine organisms over the experimental period, highlighting a predominance of negative values that indicate a reduction in size across all locations and treatments. The consistent observation of negative growth aligns with previous findings that indicate unfavorable environmental conditions affecting organism growth and development.

Maros Estuary and Pangkep Estuary are identified as the locations experiencing the most substantial size reductions, with organisms showing a decrease of approximately -4 mm. This magnitude of size reduction is indicative of severe environmental stressors that may be detrimental to the overall health of populations in these estuaries (Peck *et al.*, 2004).

Such significant decreases can have dire implications for the entities involved, as reduced body size in aquatic organisms is often associated with decreased fitness, impairments in reproductive success, and heightened vulnerability to predation.

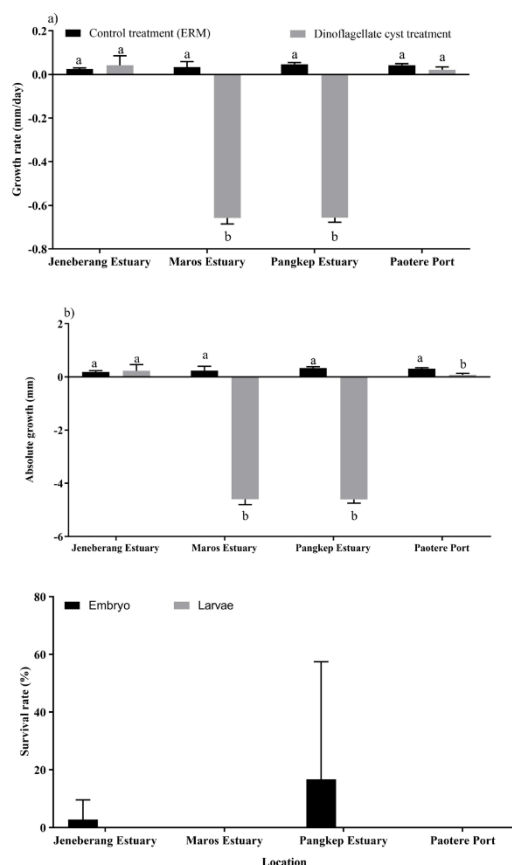


Fig. 2: a. Growth rate (mg/day), b. absolute growth (mm), and c. survival rate of medaka fish larvae exposed to various concentrations of dinoflagellate cysts. Note different small case letters indicated a significant difference ($P < 0.05$).

The lack of statistically significant differences between the control and treatment groups at each location reinforces the notion that dinoflagellate cysts, while potentially harmful, may not impose additional stress compared to the challenging environmental conditions already present. However, the marked statistical differences between locations, indicated by designations of 'a' versus 'b', emphasize the predominant role that geographic variability plays in influencing absolute growth rates (Ôkubo, 1986). This highlights the importance of integrating ecological considerations when assessing the effects of specific stressors like dinoflagellates on marine organisms.

Fig. 2c examined the survival rates of the organism at two developmental stages: embryo and larvae. It provides insights into how well the organism survives across the four locations. Survival rates are generally low, especially for larvae. Jeneberang Estuary stands out with the highest survival rates, approximately 20% for embryos and a more promising 60% for larvae. In stark contrast, Maros

Estuary, Pangkep Estuary, and Paotere Port show alarmingly low survival, nearing 0%, particularly for the larval stage. Although no specific statistical tests are cited, the prominent error bars, especially for larvae, hint at high variability in the data. This variability could make it statistically challenging to discern definitive differences between locations. However, the visual trend strongly suggests that Jeneberang Estuary provides a significantly more hospitable survival environment than the other locations.

This highlights the concerning issue of low survival rates among early life stages of marine organisms, particularly larvae, which are critical for the continuation of populations. The Jeneberang Estuary emerges as a remarkable exception, boasting higher survival rates with approximately 20% for embryos and a more encouraging 60% for larvae. This suggests that environmental conditions within the Jeneberang Estuary may be comparatively more favorable for the development and survival of these organisms, potentially due to a combination of suitable habitat features, availability of food resources, and reduced predation pressure.

In contrast, the survival rates observed in Maros Estuary, Pangkep Estuary, and Paotere Port are alarmingly low, particularly for larvae, which approach levels of 0%. Such stark reductions in survival rates indicate extremely challenging conditions for these organisms' early life stages in these locations. Factors contributing to this high mortality could include poor water quality, low food availability, habitat degradation, or increased levels of predation, all of which can significantly impact larval and embryo survival.

Large error bars in the data, particularly for larvae, suggest high variability in survival rates, which may reflect the heterogeneous nature of environmental conditions across these estuaries. This variability could make applying statistical tests to discern clear differences between locations challenging, leading to possible underestimations of location-specific challenges facing early life stages (Morris *et al.*, 2014). High variability in survival data is often characteristic of systems experiencing significant environmental stressors, where factors such as food patchiness or predatory dynamics can fluctuate dramatically over short timescales (Thorson, 1950).

Conclusion

This study highlights the significant toxic effects of the dinoflagellate *Gonyaulax verior* on the embryonic development of medaka fish (*Oryzias javanicus*) across various locations in the Makassar Strait. The analysis demonstrates that exposure to *Gonyaulax verior* not only increases embryonic mortality rates but also leads to a

higher prevalence of morphological abnormalities and developmental delays. Notably, the varying toxicity levels from different water sources indicate that environmental factors may exacerbate the deleterious effects seen in embryos, with the Maros Estuary and Pangkep Estuary exhibiting the most severe impacts on development. In contrast, the Jeneberang Estuary presented relatively better outcomes, suggesting that localized ecological conditions may play a critical role in modulating the effects of dinoflagellate toxicity.

These findings underscore the urgent need for continuous monitoring of harmful algal blooms and their potential implications for aquatic ecosystems, particularly in regions where fish populations rely on the health of embryonic stages for their recruitment. Additionally, this research informs conservation and management strategies to mitigate the impacts of dinoflagellate exposure on marine biodiversity. Further investigations into the mechanisms behind the toxicity of *Gonyaulax verior* will be essential for developing preventive measures and ensuring the sustainability of fish populations within the influenced habitats of the Makassar Strait.

Acknowledgement

This research was funded by Professor Research Scheme of Hasanuddin University with the contract number: 11151/UN4.1.2/HK.07.00/2024, date 18 March 2024. The authors thank the students who assisted with running the toxicity experiment of our research.

Funding information

This research was a part of the project titled “Toxicity test of dinoflagellate cysts using medaka fish”, funded by Hasanuddin University under Professorship Research Scheme. The funders had no role in study design, data collection and analysis, publication decision, or manuscript preparation.

Author Contributions

Nita Rukminasari conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed paper drafts, sample collection, and approved the final draft.

Khusnul Yaqin conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Jamaluddin Fitrah Alam conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Ethics

We are committed to ensuring the humane and ethical treatment of medaka fish (*Oryzias javanicus*) used in our research. All procedures involving these animals comply with established ethical guidelines and regulations to minimize discomfort, stress, and suffering. Our team is dedicated to promoting the welfare of the fish through proper housing, care, and handling practices. We continuously monitor their health and wellbeing, and adopt the 3Rs principles—Replacement, Reduction, and Refinement—to ensure that the use of medaka fish is justified, minimized, and conducted in the most humane manner possible. Any research protocol that involves animal subjects has undergone rigorous ethical review and approval prior to implementation.

Conflict of Interest

The authors declare there are no competing interests.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All relevant data, including raw observations, experimental results, and analysis datasets, are contained within the manuscript and its supplementary materials. Due to confidentiality or data sharing agreements, some data may be restricted; however, access can be granted on a case-by-case basis to qualified researchers.

References

- Anderson, D. M. (2009). Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management*, 52(7), 342–347.
<https://doi.org/10.1016/j.ocecoaman.2009.04.006>
- Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in Understanding Harmful Algal Blooms: Paradigm Shifts and New Technologies for Research, Monitoring, and Management. *Annual Review of Marine Science*, 4(1), 143–176.
<https://doi.org/10.1146/annurev-marine-120308-081121>
- Anton, A., Teoh, P. L., Mohd-Shaleh, S. R., & Mohammad-Noor, N. (2008). First occurrence of *Cochlodinium* blooms in Sabah, Malaysia. *Harmful Algae*, 7(3), 331–336.
<https://doi.org/10.1016/j.hal.2007.12.013>
- Argyle, P., Harwood, D., Rhodes, L., Champeau, O., & Tremblay, L. (2016). Toxicity assessment of New Zealand and Pacific dinoflagellates *Ostreopsis* and *Gambierdiscus* (Dinophyceae) extracts using bioassays. *New Zealand Journal of Marine and*

- Freshwater Research*, 50(3), 444–456. <https://doi.org/10.1080/00288330.2016.1159581>
- Botelho, M. J., Vale, C., & Ferreira, J. G. (2019). Seasonal and multi-annual trends of bivalve toxicity by PSTs in Portuguese marine waters. *Science of The Total Environment*, 664, 1095–1106. <https://doi.org/10.1016/j.scitotenv.2019.01.314>
- Braunbeck, T., Boettcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., Rudolf, M., & Seitz, N. (2005). Towards an alternative for the acute fish LC50 test in chemical assessment: The fish embryo toxicity test goes multi-species update. *Altex*, 22(2), 87–102.
- Carl, M., Loosli, F., & Wittbrodt, J. (2002). *Six3* inactivation reveals its essential role for the formation and patterning of the vertebrate eye. *Development*, 129(17), 4057–4063. <https://doi.org/10.1242/dev.129.17.4057>
- Chen, C. M., Yu, S. C., & Liu, M. C. (2001). Use of Japanese medaka (*Oryzias latipes*) and tilapia (*Oreochromis mossambicus*) in Toxicity Tests on Different Industrial Effluents in Taiwan. *Archives of Environmental Contamination and Toxicology*, 40(3), 363–370. <https://doi.org/10.1007/s002440010184>
- Chen, G., Jia, Z., Wang, L., & Hu, T. (2020). Effect of acute exposure of saxitoxin on development of zebrafish embryos (*Danio rerio*). *Environmental Research*, 185, 109432. <https://doi.org/10.1016/j.envres.2020.109432>
- Colman, J. R., Dechraoui, M.-Y. B., Dickey, R. W., & Ramsdell, J. S. (2004). Characterization of the developmental toxicity of Caribbean ciguatoxins in finfish embryos. *Toxicon*, 44(1), 59–66. <https://doi.org/10.1016/j.toxicon.2004.04.007>
- Dong, S., Kang, M., Wu, X., & Ye, T. (2014). Development of a Promising Fish Model (*Oryzias melastigma*) for Assessing Multiple Responses to Stresses in the Marine Environment. *BioMed Research International*, 2014, 1–17. <https://doi.org/10.1155/2014/563131>
- Embry, M. R., Belanger, S. E., Braunbeck, T. A., Galay-Burgos, M., Halder, M., Hinton, D. E., Léonard, M. A., Lillicrap, A., Norberg-King, T., & Whale, G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. *Aquatic Toxicology*, 97(2), 79–87. <https://doi.org/10.1016/j.aquatox.2009.12.008>
- Escoffier, N., Gaudin, J., Mezhoud, K., Huet, H., Chateau-Joubert, S., Turquet, J., Crespeau, F., & Edery, M. (2007). Toxicity to medaka fish embryo development of okadaic acid and crude extracts of *Prorocentrum* dinoflagellates. *Toxicon*, 49(8), 1182–1192. <https://doi.org/10.1016/j.toxicon.2007.02.008>
- Farabegoli, F., Blanco, L., Rodríguez, L. P., Vieites, J. M., & Cabado, A. G. (2018). Phycotoxins in Marine Shellfish: Origin, Occurrence and Effects on Humans. *Marine Drugs*, 16(6), 188. <https://doi.org/10.3390/md16060188>
- Fistarol, G., Legrand, C., Selander, E., Hummert, C., Stolte, W., & Granéli, E. (2004). Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. *Aquatic Microbial Ecology*, 35, 45–56. <https://doi.org/10.3354/ame035045>
- Fu, L., Mambrini, M., Perrot, E., & Chourrout, D. (2000). Stable and full rescue of the pigmentation in a medaka albino mutant by transfer of a 17 kb genomic clone containing the medaka tyrosinase gene. *Gene*, 241(2), 205–211. [https://doi.org/10.1016/s0378-1119\(99\)00473-4](https://doi.org/10.1016/s0378-1119(99)00473-4)
- Gebreab, Kiflom, "Assessment of the Toxicity of Legacy and Next-Generation Perfluoroalkyl Substances (PFAS) in Early-Life Stages of Freshwater and Marine Fish" (2022). FIU Electronic Theses and Dissertations. 5029. <https://digitalcommons.fiu.edu/etd/5029>
- Gobler, C. J., Berry, D. L., Anderson, O. R., Burson, A., Koch, F., Rodgers, B. S., Moore, L. K., Goleski, J. A., Allam, B., Bowser, P., Tang, Y., & Nuzzi, R. (2008). Characterization, dynamics, and ecological impacts of harmful *Cochlodinium polykrikoides* blooms on eastern Long Island, NY, USA. *Harmful Algae*, 7(3), 293–307. <https://doi.org/10.1016/j.hal.2007.12.006>
- Hakanen, P., Suikkanen, S., & Kremp, A. (2014). Allelopathic activity of the toxic dinoflagellate *Alexandrium ostenfeldii*: Intra-population variability and response of co-occurring dinoflagellates. *Harmful Algae*, 39, 287–294. <https://doi.org/10.1016/j.hal.2014.08.005>
- Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia*, 32(2), 79–99. <https://doi.org/10.2216/i0031-8884-32-2-79.1>
- Hardiana, A. D., Yaqin, K., & Rahim, S. W. (2024). Detection of Triclosan Pollutants Adsorbed on Polypropylene Microplastics Using Simple Biomarkers in the Embryos of *Oryzias Javanicus* (Bleeker, 1854). *Journal of Sustainability Science and Management*, 19(10), 115–132. <https://doi.org/10.46754/jssm.2024.10.010>
- Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. A., Stoecker, D. K., & Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, 8(1), 3–13. <https://doi.org/10.1016/j.hal.2008.08.006>

- Hinton, D. E., Kullman, S. W., Hardman, R. C., Volz, D. C., Chen, P.-J., Carney, M., & Bencic, D. C. (2005). Resolving mechanisms of toxicity while pursuing ecotoxicological relevance? *Marine Pollution Bulletin*, 51(8–12), 635–648. <https://doi.org/10.1016/j.marpolbul.2005.07.020>
- Hwang, P. P., Lee, K. J., & Lee, Y. H. (2011). The use of medaka (*Oryzias latipes*) as a model organism in ecotoxicology and environmental studies. *Aquatic Toxicology*, 99(2), 534–544.
- Imai, I., Yamaguchi, M., & Hori, Y. (2006). Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankton and Benthos Research*, 1(2), 71–84. <https://doi.org/10.3800/pbr.1.71>
- Iwamatsu, T. (2004). Stages of normal development in the medaka *Oryzias latipes*. *Mechanisms of Development*, 121(7–8), 605–618. <https://doi.org/10.1016/j.mod.2004.03.012>
- Iwataki, M., Kawami, H., & Matsuoka, K. (2007). *Cochlodinium fulvescens* sp. nov. (Gymnodiniales, Dinophyceae), a new chain-forming unarmored dinoflagellate from Asian coasts. *Phycological Research*, 55(3), 231–239. <https://doi.org/10.1111/j.1440-1835.2007.00466.x>
- Kim, H. (2010). An Overview on the Occurrences of Harmful Algal Blooms (HABs) and Migration Strategies in Korean Coastal Waters. *Coastal Environmental and Ecosystem Issues of the East China Sea*, 121–131.
- Kudela, R. M., & Gobler, C. J. (2012). Harmful dinoflagellate blooms caused by *Cochlodinium* sp.: Global expansion and ecological strategies facilitating bloom formation. *Harmful Algae*, 14, 71–86. <https://doi.org/10.1016/j.hal.2011.10.015>
- Lammer, E., Carr, G. J., Wendler, K., Rawlings, J. M., Belanger, S. E., & Braunbeck, Th. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 149(2), 196–209. <https://doi.org/10.1016/j.cbpc.2008.11.006>
- Landsberg, J. H. (2002). The Effects of Harmful Algal Blooms on Aquatic Organisms. *Reviews in Fisheries Science*, 10(2), 113–390. <https://doi.org/10.1080/20026491051695>
- Law, J. M. (2001). Mechanistic Considerations in Small Fish Carcinogenicity Testing. *ILAR Journal*, 42(4), 274–284. <https://doi.org/10.1093/ilar.42.4.274>
- Lefebvre, K. A., Trainer, V. L., & Scholz, N. L. (2004). Morphological abnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin. *Aquatic Toxicology*, 66(2), 159–170. <https://doi.org/10.1016/j.aquatox.2003.08.006>
- Liu, R., Kameya, T., Kobayashi, T., Sugimura, Y., Kubo, T., Sawai, A., & Urano, K. (2007). Evaluating the fish safety level of river water and wastewater with a larval medaka assay. *Chemosphere*, 66(3), 452–459. <https://doi.org/10.1016/j.chemosphere.2006.06.014>
- Long, M., Peltekis, A., González-Fernández, C., Hégaret, H., & Bailleul, B. (2021). Allelochemicals of *Alexandrium minutum*: Kinetics of membrane disruption and photosynthesis inhibition in a co-occurring diatom. *Harmful Algae*, 103, 101997. <https://doi.org/10.1016/j.hal.2021.101997>
- Mak, Y. L., Li, J., Liu, C.-N., Cheng, S. H., Lam, P. K. S., Cheng, J., & Chan, L. L. (2017). Physiological and behavioural impacts of Pacific ciguatera toxin-1 (P-CTX-1) on marine medaka (*Oryzias melastigma*). *Journal of Hazardous Materials*, 321, 782–790. <https://doi.org/10.1016/j.jhazmat.2016.09.066>
- Marsh, K. E., Paterson, G., Foran, C. M., & Bennett, E. R. (2010). Variable Vitellogenin Response of Japanese Medaka (*Oryzias latipes*) to Weekly Estrogen Exposure. *Archives of Environmental Contamination and Toxicology*, 58(3), 793–799. <https://doi.org/10.1007/s00244-010-9468-7>
- Mincarelli, L. F., Paula, J. R., Pousão-Ferreira, P., Rosa, R., & Costa, P. R. (2018). Effects of acute waterborne exposure to harmful algal toxin domoic acid on foraging and swimming behaviours of fish early stages. *Toxicon*, 156, 66–71. <https://doi.org/10.1016/j.toxicon.2018.11.297>
- Murray, S. A., Kohli, G. S., Farrell, H., Spiers, Z. B., Place, A. R., Dorantes-Aranda, J. J., & Ruszczyk, J. (2015). A fish kill associated with a bloom of *Amphidinium carterae* in a coastal lagoon in Sydney, Australia. *Harmful Algae*, 49, 19–28. <https://doi.org/10.1016/j.hal.2015.08.003>
- Nair, A., Thomas, A. C., & Borsuk, M. E. (2013). Interannual variability in the timing of New England shellfish toxicity and relationships to environmental forcing. *Science of The Total Environment*, 447, 255–266. <https://doi.org/10.1016/j.scitotenv.2013.01.023>
- Paul-Prasanth, B., Matsuda, M., Lau, E.-L., Suzuki, A., Sakai, F., Kobayashi, T., & Nagahama, Y. (2006). Knock-down of DMY initiates female pathway in the genetic male medaka, *Oryzias latipes*. *Biochemical and Biophysical Research Communications*, 351(4), 815–819. <https://doi.org/10.1016/j.bbrc.2006.10.095>
- Pikitch, E. K., Rountos, K. J., Essington, T. E., Santora, C., Pauly, D., Watson, R., Sumaila, U. R., Boersma, P. D., Boyd, I. L., Conover, D. O., Cury, P., Heppell, S. S., Houde, E. D., Mangel, M., Plagányi, É., Sainsbury, K., Steneck, R. S., Geers, T. M.,

- Gownaris, N., & Munch, S. B. (2014). The global contribution of forage fish to marine fisheries and ecosystems. *Fish and Fisheries*, 15(1), 43–64. <https://doi.org/10.1111/faf.12004>
- Richlen, M. L., Morton, S. L., Jamali, E. A., Rajan, A., & Anderson, D. M. (2010). The catastrophic 2008–2009 red tide in the Arabian gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*. *Harmful Algae*, 9(2), 163–172. <https://doi.org/10.1016/j.hal.2009.08.013>
- Rukminasari, N., & Tahir, A. (2021). Pattern and germination rate of dinoflagellate cyst from three river estuaries (Jeneberang, Maros and Pangkep Estuary) of Makassar Strait. *IOP Conference Series: Earth and Environmental Science*, 860(1), 012010. <https://doi.org/10.1088/1755-1315/860/1/012010>
- Sakamoto, T. (2018). Medaka: A model organism for aquatic ecotoxicology. *Environmental Toxicology and Chemistry*, 37(10), 2857–2864. <https://doi.org/10.1002/etc.4267>
- Schulte, C., & Nagel, R. (1994). Testing Acute Toxicity in the Embryo of Zebrafish, *Brachydanio rerio*, as an Alternative to the Acute Fish Test: Preliminary Results. *Alternatives to Laboratory Animals*, 22(1), 12–19. <https://doi.org/10.1177/026119299402200104>
- Shi, F., McNabb, P., Rhodes, L., Holland, P., Webb, S., Adamson, J., Immers, A., Gooneratne, R., & Holland, J. (2012). The toxic effects of three dinoflagellate species from the genus *Karenia* on invertebrate larvae and finfish. *New Zealand Journal of Marine and Freshwater Research*, 46(2), 149–165. <https://doi.org/10.1080/00288330.2011.616210>
- Shima, A., & Mitani, H. (2004). Medaka as a research organism: past, present and future. *Mechanisms of Development*, 121(7–8), 599–604. <https://doi.org/10.1016/j.mod.2004.03.011>
- Shin, Y. K., Nam, S.-E., Kim, W. J., Seo, D. Y., Kim, Y.-J., & Rhee, J.-S. (2019). Red tide dinoflagellate *Cochlodinium polykrikoides* induces significant oxidative stress and DNA damage in the gill tissue of the red seabream *Pagrus major*. *Harmful Algae*, 86, 37–45 <https://doi.org/10.1016/j.hal.2019.04.008>
- Shumway, S. E. (1990). A Review of the Effects of Algal Blooms on Shellfish and Aquaculture. *Journal of the World Aquaculture Society*, 21(2), 65–104. <https://doi.org/10.1111/j.1749-7345.1990.tb00529.x>
- Smayda, T. J. (1997). Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography*, 42(5part2), 1137–1153. https://doi.org/10.4319/lo.1997.42.5_part_2.1137
- Tang, Y. Z., & Gobler, C. J. (2009). Characterization of the toxicity of *Cochlodinium polykrikoides* isolates from Northeast US estuaries to finfish and shellfish. *Harmful Algae*, 8(3), 454–462. <https://doi.org/10.1016/j.hal.2008.10.001>
- Tang, Y. Z., Harke, M. J., & Gobler, C. J. (2013). Morphology, phylogeny, dynamics, and ichthyotoxicity of *Pheopolykrikos hartmannii* (Dinophyceae) isolates and blooms from New York, USA. *Journal of Phycology*, 49(6), 1084–1094. <https://doi.org/10.1111/jpy.12114>
- Taylor, F. J. R., & Trainer, V. L. (2002). *Harmful algal blooms in the PICES region of the North Pacific (PICES Scientific Report No. 23)*. North Pacific Marine Science Organization.
- Tian, L., Cheng, J., Chen, X., Cheng, S. H., Mak, Y. L., Lam, P. K. S., Chan, L. L., & Wang, M. (2014). Early developmental toxicity of saxitoxin on medaka (*Oryzias latipes*) embryos. *Toxicology*, 77, 16–25. <https://doi.org/10.1016/j.toxicology.2013.10.022>
- Tiedeken, J. A., Ramsdell, J. S., & Ramsdell, A. F. (2005). Developmental toxicity of domoic acid in zebrafish (*Danio rerio*). *Neurotoxicology and Teratology*, 27(5), 711–717. <https://doi.org/10.1016/j.ntt.2005.06.013>
- Tillmann, U., John, U., & Cembella, A. (2007). On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. *Journal of Plankton Research*, 29(6), 527–543. <https://doi.org/10.1093/plankt/fbm034>
- Tomas, C. R., & Smayda, T. J. (2008). Red tide blooms of *Cochlodinium polykrikoides* in a coastal cove. *Harmful Algae*, 7(3), 308–317. <https://doi.org/10.1016/j.hal.2007.12.005>
- Twiner, M. (2008). Azaspiracid Shellfish Poisoning: A Review on the Chemistry, Ecology, and Toxicology with an Emphasis on Human Health Impacts. *Marine Drugs*, 6(2), 39–72. <https://doi.org/10.3390/md20080004>
- Van Dolah, F. M. (2000). Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, 108(suppl 1), 133–141. <https://doi.org/10.1289/ehp.00108s1133>
- Van Wagoner, R. M., Deeds, J. R., Tatters, A. O., Place, A. R., Tomas, C. R., & Wright, J. L. C. (2010). Structure and Relative Potency of Several Karlotoxins from *Cochlodinium veneticum*. *Journal of Natural Products*, 73(8), 1360–1365. <https://doi.org/10.1021/np100158r>

- Wang, H., Hu, Z., Shang, L., Leaw, C. P., Lim, P. T., & Tang, Y. Z. (2020). Toxicity comparison among four strains of *Margalefidinium polykrikoides* from China, Malaysia, and USA (belonging to two ribotypes) and possible implications. *Journal of Experimental Marine Biology and Ecology*, 524, 151293.
<https://doi.org/10.1016/j.jembe.2019.151293>
- Wayne, N. L., Kuwahara, K., Aida, K., Nagahama, Y., & Okubo, K. (2005). Whole-Cell Electrophysiology of Gonadotropin-Releasing Hormone Neurons that Express Green Fluorescent Protein in the Terminal Nerve of Transgenic Medaka (*Oryzias latipes*)1. *Biology of Reproduction*, 73(6), 1228–1234.
<https://doi.org/10.1095/biolreprod.105.042721>
- Whyte, J. N. C. (Ian), Haigh, N., Ginther, N. G., & Keddy, L. J. (2001). First record of blooms of *Cochlodinium* sp. (Gymnodiniales, Dinophyceae) causing mortality to aquacultured salmon on the west coast of Canada. *Phycologia*, 40(3), 298–304.
<https://doi.org/10.2216/i0031-8884-40-3-298.1>
- Xu, N., Wang, M., Tang, Y., Zhang, Q., Duan, S., & Gobler, C. (2017). Acute toxicity of the cosmopolitan bloom-forming dinoflagellate *Akashiwo sanguinea* to finfish, shellfish, and zooplankton. *Aquatic Microbial Ecology*, 80(3), 209–222.
<https://doi.org/10.3354/ame01846>
- Yan, M., Leung, P. T. Y., Ip, J. C. H., Cheng, J., Wu, J.-J., Gu, J.-R., & Lam, P. K. S. (2017). Developmental toxicity and molecular responses of marine medaka (*Oryzias melastigma*) embryos to ciguatoxin P-CTX-1 exposure. *Aquatic Toxicology*, 185, 149–159.
<https://doi.org/10.1016/j.aquatox.2017.02.006>
- Yan, M., Mak, M. Y. L., Cheng, J., Li, J., Gu, J. R., Leung, P. T. Y., & Lam, P. K. S. (2020). Effects of dietary exposure to ciguatoxin P-CTX-1 on the reproductive performance in marine medaka (*Oryzias melastigma*). *Marine Pollution Bulletin*, 152, 110837.
<https://doi.org/10.1016/j.marpolbul.2019.110837>
- Yang, W.-D., Xie, J., van Rijssel, M., Li, H.-Y., & Liu, J.-S. (2010). Allelopathic effects of *Alexandrium* spp. on *Prorocentrum donghaiense*. *Harmful Algae*, 10(1), 116–120. <https://doi.org/10.1016/j.hal.2010.08.001>
- Yaqin, K., Rahim, S. W., & Sari, D. kesuma. (2021). Dry transportation of *Oryzias wolasi* embryo for ecotoxicological studies. *IOP Conference Series: Earth and Environmental Science*, 860(1), 012102.
<https://doi.org/10.1088/1755-1315/860/1/012102>
- Yau, M.-S., Lei, E. N.-Y., Ng, I. H.-M., Yuen, C. K.-K., Lam, J. C.-W., & Lam, M. H.-W. (2019). Changes in the neurotransmitter profile in the central nervous system of marine medaka (*Oryzias melastigma*) after exposure to brevetoxin PbTx-1 – A multivariate approach to establish exposure biomarkers. *Science of The Total Environment*, 673, 327–336.
<https://doi.org/10.1016/j.scitotenv.2019.03.193>