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Modelling spatiotemporal distributions of *Vulcanodinium rugosum* and pinnatoxin G in French Mediterranean lagoons: Application to human health risk characterisation

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Abstract :

Consumption of seafood contaminated by phycotoxins produced by harmful algae is a major issue in human public health. Harmful algal blooms are driven by a multitude of environmental variables; therefore predicting human dietary exposure to phycotoxins based on these variables is a promising approach in health risk management. In this study, we attempted to predict the human health risks associated with Vulcanodinium rugosum and its neurotoxins, pinnatoxins (PnTXs), which have been regularly found in Mediterranean lagoons since their identification in 2011. Based on environmental variables collected over 1 year in four Mediterranean lagoons, we developed linear mixed models to predict the presence of V. rugosum and PnTX G contamination of mussels. We found that the occurrence of V. rugosum was significantly associated with seawater temperature. PnTX G contamination of mussels was highest in summer but persisted throughout the year. This contamination was significantly associated with seawater temperature and the presence of V. rugosum with a time lag, but not with dissolved PnTX G in seawater. By using the contamination model predictions and their potential variability/uncertainty, we calculated the human acute dietary exposures throughout the year and predicted that 25% of people who consume mussels could exceed the provisional acute benchmark value during the warmest periods. We suggest specific recommendations to monitor V. rugosum and PnTX G.

Highlights

► *V. rugosum* presence was associated with seawater temperature. ► PnTX G concentration in mussels was associated with seawater temperature and *V. rugosum* presence. ► PnTX G persisted for several months in mussels and in seawater. ► Predicted human health risk associated with PnTX G in mussels was maximum in the warmest periods of the year.

Keywords : Vulcanodinium rugosum, Pinnatoxins, Environmental determinism, Toxin persistence, Risk assessment, Modelling

Abbreviations

ANSES	French Agency For Food Safety
PnTX	pinnatoxin
Prtn	portimine
PABV	provisional acute benchmark value
SPATT	solid phase adsorption toxin tracking

1. Introduction

In recent decades, harmful algal blooms (HABs) have caused increased socio-economic impacts (Hallegraeff et al., 2021). HABs are related to climate change and environmental variations (Granéli and Turner, 2006; Hallegraeff, 2010; Pulido, 2016; Glibert, 2020; Ralston and Moore, 2020). Their development and the resulting marine organism contamination are driven by many environmental factors including temperature, salinity, light intensity, wind speed, tides, nutrient availability or the synergic combination of several of these factors (Anderson and Keafer, 1987; Granéli and Turner, 2006; Collos et al., 2007, 2009; Ninčević Gladan et al., 2008; Davidson et al., 2009; Genovesi et al., 2009; Xu et al., 2010; Laabir et al., 2013; Ishikawa et al., 2014; Paterson et al., 2017; Figueroa et al., 2018).

Vulcanodinium rugosum is a benthopelagic neurotoxic dinoflagellate species identified in 2011 as the producer of two types of toxins, pinnatoxins (PnTXs) and portimines (Prtns) (Nézan and Chomérat, 2011; Selwood et al., 2013). This species has been found in many marine areas around the world including China, Japan, New Zealand, Australia, Hawai, Qatar, Cuba, Mexico, Spain and France (Nézan and Chomérat, 2011; Rhodes et al., 2011; Smith et al., 2011; Zeng et al., 2012; Hernandez-Becerril et al., 2013; Satta et al., 2013; Al-Mufta and Yilmaz, 2015; Moreira-González et al., 2016; Abadie et al., 2018; REPHY, 2022). PnTXs belong to the marine biotoxin group of cyclic imines (Efsa, 2010) and are acetylcholine competitive antagonists. They cause death after oral or intra-peritoneal administration in mice (Munday et al., 2012). PnTXs have been shown to accumulate in bivalve molluscs (mussels, oysters and clams) and fish (mullets) (Abadie, 2015; Bouquet et al., 2022). Prtns can also cause death in mice but show considerably lower toxicity and are thus not be considered in this study (Selwood et al., 2013). The French Agency for Food Safety (ANSES) recently extrapolated potential human neurological symptoms and proposed a provisional acute benchmark value (PABV) for PnTX G of 0.13 µg/kg body weight per day (Arnich et al.,

2020). A risk assessment was performed in French Mediterranean lagoons where *V. rugosum* has been causing recurrent shellfish contaminations (Abadie et al., 2018; Arnich et al., 2020). However, the risk was calculated in the worst-case scenario (highest observed contamination). The calculus did not take into account the seasonal variability of PnTX G concentrations in shellfish during the considered years.

Studies have shown that environmental factors influence the development and toxin production of *V. rugosum* (Abadie et al., 2015, 2016). Algal growth and the toxin cell contents are maximum for a temperature of 25 °C and a salinity of 30-40. The growth rate is similar when the dinoflagellate is cultivated with ammonium (NH_4^+), urea and nitrate (NO_3^-). *In situ* densities in Ingril, a French Mediterranean lagoon, were shown to be positively correlated with seawater temperature, salinity and phosphate (PO_4^{3-}), and negatively correlated with nitrogenous nutrients (Abadie et al., 2018). Thus, the risks associated with shellfish contamination by PnTX could depend on those environmental parameters.

Given the complexity of HAB dynamics and their various environmental drivers, empirical statistical strategies have generally shown better performances than mechanistic models (McGillicuddy et al., 2011; Lima et al., 2022). Several studies have used environmental variables as predictors for near-term HAB forecasting (González Vilas et al., 2014; Rossini, 2014; Davidson et al., 2016; Franks, 2018; Derot et al., 2020; Ralston and Moore, 2020; Cruz et al., 2021). Statistical models can integrate a large number of predictors and be adapted to any data distributions (Davidson et al., 2016). They have been used previously to predict the dynamics of harmful algae such as *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis* as well as the contamination of marine organisms by their toxins (Lane et al., 2009; Anderson et al., 2010; Seubert et al., 2013; Bouquet et al., 2022).

An interesting predictor that could be implemented in statistical models is the determination of dissolved toxins in seawater using solid phase adsorption toxin trackers (SPATT)

(MacKenzie et al., 2004; Kudela, 2011, 2017; Howard et al., 2018). These devices have been used to forecast shellfish contamination by various toxins including okadaic acid, domoic acid, pectenotoxins and saxitoxins (MacKenzie et al., 2004; Lane et al., 2010; Li et al., 2016; Roue et al., 2018).

We aimed to characterise, using environmental predictors, the human health risk associated with *V. rugosum* and PnTX G in four French Mediterranean lagoons. To this end, we studied variations in water physicochemistry and PnTX G concentrations in seawater and mussels and their relationship with the occurrence of *V. rugosum* described in our previous study (Bouquet et al., 2023). Then, we developed statistical models based on environmental predictors to predict the presence of *V. rugosum* and PnTX G contamination of mussels. Finally, we predicted the human acute dietary exposure to PnTX G through the year and characterised the human health risk based on these predictions.

2. Materials and methods

2.1. Area and period

Samples were collected in four French Mediterranean lagoons (Figure 1). Thau lagoon (43°27′02.8″N 3°40′01.4″E, 7.00 × 10^7 m², mean depth 4 m) (Le Fur et al., 2018) is the fourth largest oyster production area in France: it represents 10% of the national production and has an average mussel production of 14,000 tonnes/year. It is adjacent to Ingril lagoon (43°26′09.6″N 3°46′39.8″E, 6.85 × 10^6 m², mean depth 0.6 m) (Le Fur et al., 2018), where shellfish harvesting and farming have been banned since 1 January 2020 because of the high levels of PnTX G found in mussels. To the east of Ingril lagoon are Vic (43°30′32.8″N 3°49′20.7″E, 1.34 × 10^7 m², mean depth 1m) and Prevost (43°30′58.7″N 3°54′06.7″E, 2.45×10^6 m², mean depth 0.8 m (Le Fur et al., 2018) lagoons. No shellfish farming is implemented in these lagoons but shellfish harvesting is still common. The four lagoons connect through the 'Canal from the Rhône to Sète'. The sampling locations are the same as those described in previous studies on *V. rugosum* (Abadie, 2015; Bouquet et al., 2023). Samples were collected from April 2021 to April 2022, at the same time as *V. rugosum* monitoring described by Bouquet et al. (2023).

2.2. Abiotic parameters

Hobo recorders were hung on metallic peaks at around 0.40 m below the water surface. They recorded seawater temperature and visible light intensity every 15 min. They were collected and replaced every 2 weeks. Mean values were calculated between each collection date. Salinity was recorded every 2 weeks using a WTW probe (LF197-S). Two litres of seawater was sampled every 4 weeks in plastic bottles for nutrient measurements. At the laboratory, water was filtered on a GF/F Whatman membrane filter (25 mm diameter) before nutrient analyses. Nutrient concentrations were measured by continuous segmented flow analysis

using SEAL analytical autoanalysers according to the protocol by Aminot and Kerouel (2007). PO_4^{3-} and the sum of nitrites and nitrate (NO_2^- + NO_3^-) were detected with colourimetric detection, and NH_4^+ was evaluated with fluorimetric detection. Wind speed data, recorded every 3 hours at Sète (43°23′50″N 3°41′32″E) and Mauguio (Montpellier Airport) (43°34′34″ N 3°57′53″E), was collected from the Météo France database (Météo France, 2023) (Figure 1). The wind speed at each lagoon was estimated as the value at the closest station: Sète for Thau and Ingril lagoons and Mauguio for Vic and Prevost lagoons. Mean values were calculated every 2 weeks.

2.3. Biotic parameters

Two litres of seawater was sampled every 4 weeks in plastic bottles for chlorophyll *a* measurement. Chlorophyll *a* analysis was carried out following the protocol described by Abadie et al. (2015). A least 500 mL of seawater was filtered on a GF/F Whatman membrane filter (25 mm diameter). Filters were put in polypropylene tubes and stored at -20 °C. Extraction was performed by crushing filters with an ultrasonic rod in 5 mL of 90% acetone (Vibra cell™ 7518, 130 W and 50 kHz). The mix was centrifuged at 2750 *g* for 30 minutes at 4 °C and the supernatant was analysed by spectrofluorimetry (Perkin Elmer, LS50B) according to the protocol by Neveux and Lantoine (1993).

2.4. PnTX G measurements

2.4.1. Samplings and extraction in seawater

SPATT were set up at each station and were collected and replaced every 2 weeks. They consisted of 3 g of Diaion HP20 porous synthetic resin encased within a nylon mesh and two circular polyvinyl chloride frames. Triplicates were inserted in a protection Exposmeter inox canister. They were stored at -20 °C until PnTX G analysis in resin.

PnTX G stability in seawater was studied in laboratory conditions. *V. rugosum* strain IFR-VRU-01 was used to prepare PnTX G solution. It was cultivated in 250-mL Nunc^{TM} non-treated culture flasks in enriched natural sea water, composed of Thau seawater (kept at obscurity for several months, filtered at 0.2 µm, and autoclaved), sodium nitrate, ferric EDTA, monosodium phosphate, vitamins and other oligo-elements (Harrison et al., 1980). One litre of culture was sonicated for three cycles of 2 minutes each with an ultrasonic cleaner to lyse microalgal cells. The solution was then filtered on a GF/F Whatman filter membrane (25 mm diameter). The absence of microalgal cells in the filtered solution was checked under a light microscope. The solution was then divided in 10-mL aliquots in low-retention Falcon tubes, which were maintained at 18 °C with a photon flux density of 100 µM m^{-2} s^{-1} and a 16-hour photoperiod. Three Falcon tubes were randomly selected for PnTX G analysis just after aliquot preparation, then twice a week for a month, and finally once a week for 3 months. Each time, 1 g of Diaion HP20 porous synthetic resin was poured in the three Falcon tubes. They were agitated for 24 hours at 18 °C using a rotator mixer. Solutions were then filtered using 20 µm nylon mesh and resin was stored at -20 °C until PnTX G analysis.

PnTX G in resin was extracted using a modified version of the extraction protocol developed by Fux et al. (2008). The resin was rinsed using approximately 500 mL of Mili-Q water. It was transferred using Mili-Q water into polypropylene reservoirs with two frits (Agilent)

placed on a manifold; the remaining water was eliminated by vacuum. The resin was eluted with 25 mL of methanol added dropwise. The extracts were evaporated to dryness under a nitrogen stream at 45 °C and then reconstituted with 750 µL of methanol. It was finally filtered on Nanosep MF centrifugal 0.2 µm filters (Pall) and transferred into liquid chromatography vials for analysis.

2.4.2. Samplings and extraction in mussels

Wild Mediterranean mussels *Mytilus galloprovincialis* initially taken from Prevost lagoon were grown one year in Thau lagoon. Two pouches each containing around 150 mussels (mean size 4.5cm) were laid down on metal structures at Thau, Ingril and Vic lagoons (Figure 1). No pouch was set up at Prevost lagoon because many wild mussels from the same species are found on nearby rocks. Every 2 weeks, 10 mussels were randomly selected and sampled. They were stored at 4 °C until toxin extraction.

PnTX G was extracted from mussels according to Hess et al. (2013). For each station, mussels were pooled and PnTX G was extracted in methanol (9 mL) with a high-speed homogeniser (Polytron) at 15000 rpm for 2 minutes before centrifugation at 3700 *g* for 10 min. This extraction was repeated twice and supernatants were transferred to volumetric flasks; the volume was adjusted to 20 mL with methanol. Five hundred microlitres aliquots of extracts were filtered on a 0.2 µm membrane and stored at -20 °C until toxin analyses.

2.4.3. HPLC MS-MS analysis

PnTX G extracts from resin and mussels were analysed according to Hess et al. (2013) using liquid chromatography (Shimadzu, UFLC Nexera) coupled to tandem mass spectrometry (API–5500 Q-Trap and API-4000 Q-Trap). Calibration was performed with a certified standard solution of PnTX G (1.92 ± 0.09 µM/L) obtained from the National Research Council in Halifax, Canada. Chromatographic separation was achieved on a Phenomenex

Kinetex C18 column (100 × 2.1 mm, 2.6 µm) at 40 °C for analysis (injection volume of 5 µL). The limits of detection were 0.8 µg/kg total flesh and 0.02 ng/g of resin. The limits of quantification were 2.5 µg/kg total flesh and 0.0625 ng/g of resin.

2.5. Statistical analysis

2.5.1 Spatiotemporal data distribution

All analyses were performed using R version 4.2.2 (R Core Team, 2023). The data were represented graphically using ggplot from the ggplot2 package version 3.4.0 (Wickham, 2009). The Kruskal–Wallis one way analysis of variance on ranks tests (KW test) was performed to compare values between lagoons. The presence of *V. rugosum* (Bouquet et al., 2023) was represented graphically. It was encoded as a binary variable (0 = no detection and 1 = presence) as it was most of the time below the limit of quantification.

2.5.2. Modelling the presence of V. rugosum in seawater and PnTX G concentrations in mussels

A binomial generalised linear mixed model was developed to describe the probability of *V. rugosum* being present using glmer from the lme4 R package version 1.1.31 (Bates et al., 2015). The probability of presence (P) was determined from environmental predictors:

$$P = \frac{\exp\left(\beta_0 + \sum \lambda_{Xi} X_i + \sum u_{zj} Z_j\right)}{1 + \exp\left(\beta_0 + \sum \lambda_{Xi} Xi + \sum u_{zj} Z_j\right)}$$

where β_0 is the intercept, λ_{Xi} quantifies the influence of each environmental factor X_i and u_{Zj} quantifies the random-intercept effect for each site Z_j.

The environmental predictors tested in this model were seawater temperature, salinity, light intensity, wind speed, PO_4^{3-}, NH_4^+, NO_2^-+NO_3^-, the sampling site and dissolved PnTX G in water. Numerical predictor data were scaled before being included in the model.

A linear mixed model was developed to describe the concentration of PnTX G in mussels using lmer from the lme4 R package. The log of the concentration C was determined using environmental predictors as follows:

$$\log C = \beta_0 + \sum \lambda i Xi + \sum uj Zj$$

where β_0 is the intercept, λ_{Xi} quantifies the influence of each environmental factor X_i and u_{Zj} quantifies the random-intercept effect for each site Z_j.

The environmental factors tested in this model were the presence of *V. rugosum*, temperature, salinity, light intensity, wind speed, PO_4^{3-}, NH_4^+, NO_2^-+NO_3^-, the sampling site and dissolved toxins in water. Numerical predictor data were scaled before being included in the model.

Mixed models were fitted with glmer from the *lme4* R package to account for random/cluster effects in the dataset inside each lagoon (Bates et al., 2015). The percentage of variability explained by fixed factors and the percentage explained by both fixed and random factors (i.e. the entire models) were estimated respectively using marginal and conditional R^2 of Nakagawa and Schielzeth (2013).

No interaction and no non-linear relationship were considered. Indeed, given the large number of predictors used, testing non-linear relationships or interactions would have led to a very large number of models. Considering the short duration of the study (1 year) and the relatively infrequent number of samplings (every 15 days), it could result in artificial relationships and/or overfitting results. The aim of the mixed linear model was to highlight the principal predictors explaining microalgal growth or inhibition and PnTX G concentrations changes, without necessarily explaining all the variability.

For all analyses, the complete model was adjusted and the absence of correlations between explanatory variables was ensured based on the variance inflation factor (VIF). When there is a correlation between predictors, the variance of predictor coefficients increases; the VIF

quantifies how much the variance is inflated. Multicollinearity is present if the VIF is > 5 (Kim, 2019; Shrestha, 2020).

For all analyses, after adjusting the complete model, all possible sub-models were selected by using the dredge function from the MuMIn R package version 1.47.1 (Bartoń, 2022). Model selection was based on the Akaike Information Criterion corrected for a small sample size (AICc), keeping models for which the difference between their AICc value and the lowest AICc value (ΔAICc) was < 2 (Burnham and Anderson, 2002). The Akaike weights, which are relative model likelihoods normalised based on the likelihoods of all possible sub-models, were examined for each candidate. A weight can be interpreted as the probability of a candidate being the best model given the data and the set of possible sub-models (Wagenmakers and Farrell, 2004). The sum of model weights including each predictor was calculated using the sw function from the MuMIn package version 1.47.1. The final model was chosen following the principle of parsimony, that is, the model with the fewest parameters from the set of 'best models' (Bentler and Mooijaart, 1989). For the predictions, within these models, the model with the fewest predictors was selected, following the principle of parsimony (Bentler and Mooijaart, 1989).

$$\mathrm{AICc} = p.Ln\left(\frac{RSS}{p}\right) + 2 + 2k\frac{k+1}{p-k-1}$$

where RSS is the residual sum of squares, p is the number of experimental points and k is the number of predictors in the model. In the final model, the random effects were normally distributed and the adequacy of the model was checked with residual plots. Every model with $\Delta AIC_c < 2$ was also kept in the results to show alternative plausible biological models.

The observed and predicted contamination data were represented graphically and compared with the concentration that should not be exceeded in shellfish, namely 23 µg PnTX G/kg of total flesh proposed by ANSES (Arnich et al., 2020).

2.5.3. Modelling the kinetics of PnTX G reduction in seawater and mussels

Using the results of the dissolved PnTX G stability in seawater experiment, a simple linear model was applied to evaluate the decrease in dissolved PnTX G in seawater. The formula is:

$$C_t = A \times \beta \times t$$

where C_t is the concentration of PnTX G at time t (in µg/kg body weight), A is the initial value of C_t and β is the decay rate to be estimated. The decay rate was fitted with the lm function from the R stats package.

Using the PnTX G values observed in mussels in each lagoon, exponential decay models were applied to evaluate the elimination kinetics of PnTX G in mussels. The period of elimination after a toxin peak was selected based on visual examination of the toxin distribution. The proposed models followed the formula:

$$\log(C_t) = \log(A) \times \exp(\beta \times t)$$

The decay rates were fitted with the nls() R function (non-linear least-squares estimates using Gauss–Newton algorithm). The confidence intervals of the fitted parameters were assessed by bootstrapping using Boot function from the car package version 3.1.1 (Fox and Weisberg, 2019).

The time needed to obtain a 50% and 90% decrease of the initial PnTX G value in seawater and mussels was calculated. Predictions and observations were displayed graphically.

2.6. Human exposure assessment

2.6.1. Mussel consumption patterns and consumer body weight

A consumption survey concerning seafood products in France (Consomer) was conducted among Mediterranean adults in 2016–2017 with an online self-reported Frequency Food

Questionnaire (FFQ) (Arnich et al., 2020). Data from a representative sampling of coastal Mediterranean adults were used in this study, including 821 individuals of whom 588 consumed mussels. Consumption data included usual portion sizes and the frequency of consumption during a 6-month period. The statistical weight of each individual was estimated to assess the representativeness of coastal Mediterranean areas. Only mussel consumer data were used in this study. Mussel consumers represent around 71.96% of the Mediterranean population studied in Consomer.

2.6.2. Contamination data

The predictions of contamination at each time (on a log scale) were obtained using the best model for each lagoon. For each predicted value, the 90% and 95% confidence intervals were calculated including random effects, using predictInterval from the merTools R package version 0.5.2 (Knowles and Frederick, 2020). Then, the contamination prediction was fitted with a normal distribution at each time (on a log scale). The fitted values were back-transformed into a log-normal distribution (raw scale) to estimate exposure.

2.6.3. Human exposure calculation

Acute exposure at each time was calculated for each lagoon, using the P95 of the predicted PnTX G contamination. For each lagoon, sampling time and individual, acute exposure $AE_{k,t,i}$ (µg/kg body weight) was calculated with the following formula:

$$AE_{k,t,i} = \frac{P_i \times C_{k,t}}{Bw_i}$$

where P_i is the consumption of mussels by individual i (g/ by meal) (usual portion size), $C_{k,t}$ is P95 of the prediction of PnTX G contamination of mussels in lagoon k at time t (µg/kg of fresh weight) and B_{Wi} is the body weight of individual i (kg). Using individual statistical weight, the 5th, 50th and 95th percentiles (P5, mean and P95 values, respectively) of

individual exposures were calculated using syvquantile from the survey R package version 4.0 (Lumley, 2020) and were represented graphically for each time and lagoon.

2.7. Human health risk characterisation

The data collected in this study were compared with the PABV (0.13 µg PnTX G/kg body weight) (Arnich et al., 2020). The percentage of the population exceeding the PABV was calculated considering the 95th percentile of the annual observed contamination data in each lagoon as described by Arnich et al. (2020). This method considers the variability of the population but not the variability of the contamination.

Then, the predicted exposure was compared with the PABV. To consider both the variability of the contamination model and the portion size of a population of mussel consumers, 10,000 exposure simulations were performed based on the model contamination prediction for each day and individual. For each day, simulation and consumer, we calculated whether the individual exceeded the PABV. At the population level, for each simulation, the percentage of the population exceeding the PABV was calculated considering the statistical weights. For each day, the results are summarised with the median and 90% and 95% confidence intervals of the simulations.

3. Results

3.1. Spatiotemporal data distribution

3.1.1. Abiotic and biotic parameters

The parameters varied according to the locations. Overall, light intensity and salinity were higher in Thau than the other lagoons (KW test Thau vs Ingril/Prevost/Vic, $p = 2.2 \times 10^{-16}$ and 1.3×10^{-2}, respectively) while chlorophyll *a* was higher in Vic and Prevost than in Ingril and Thau (KW test Vic/Prevost vs Ingril/Thau, $p = 4.1 \times 10^{-9}$) (Figure 2 and Table 1). The NO_2^- +

NO_3^- and NH_4^+ values were higher in Vic and Prevost than in Ingril and Thau (KW test, $p = 6.8 \times 10^{-8}$ and 6.9×10^{-11}, respectively) (Figure 3 and Table 1). The parameters also varied widely through the year. Seawater temperature, light intensity and salinity were higher in summer and early autumn (June–October) than during the rest of the year (KW test, $p < 2.2 \times 10^{-16}$, 1.7×10^{-7} and 3.6×10^{-8}, respectively) while the wind in Sète was stronger in winter and spring (KW test, $p = 5.0 \times 10^{-5}$).

3.1.2. Dissolved PnTX G in the seawater

The SPATT measured PnTX G in all four lagoons (Figure 4A). The mean values were 4.3 ± 6.0, 1.2 ± 0.8, 2.3 ± 4.1 and 1.1 ± 0.9 ng/g in Ingril, Prevost, Thau and Vic, respectively. There was a slight increase in Ingril, Prevost and Vic in summer which corresponds to periods where *V. rugosum* cells were detected. However, we observed the highest concentrations during winter and spring in Ingril and Thau. We measured the stability of PnTX G in seawater; it had a particularly long half-time life of 91 days (Figure S1).

3.1.3. PnTX G concentrations in mussels

We found PnTX G in mussels from all four lagoons (Figure 4B). The mean values were 60.4, 50.8, 32.4 and 69.2 µg/kg total flesh for Ingril, Prevost, Thau and Vic, respectively (Table 1). Thau showed a significantly lower level than the other lagoons (KW test Thau vs Ingril/Vic/Prevost, $p = 0.019$). We observed the highest values during summer and early autumn, which correspond to periods when *V. rugosum* cells were detected in the same year and the same lagoons (Bouquet et al., 2023). We observed a time lag between the first occurrence of *V. rugosum* and the first PnTX G concentration over 100 µg/kg total flesh in mussels (6 weeks in Ingril and Vic and 8 weeks in Prevost). Similarly, there was a time lag between the last occurrence of *V. rugosum* and the first value below 100 µg/kg in mussels in the same lagoons in autumn (6 weeks in Ingril, 8 weeks in Prevost and 4 weeks in Vic). In

Thau, PnTX G values never exceeded 100 µg/kg total flesh in mussels. Outside these periods, the PnTX G concentrations were lower but always quantifiable (limit of quantification 2.5 µg/kg total flesh). We calculated the time needed to eliminate 50% and 90% of the PnTX G in mussels based on *in situ* values: there was a long half-life in all lagoons except Prevost (47, 8, 33 and 20 days for Ingril, Prevost, Thau and Vic, respectively) (Figure S2).

3.2. Models and predictions

3.2.2. Predicted probability of the presence of *V. rugosum* in seawater

We fitted mixed logistic models including environmental variables to predict the probability of the presence of *V. rugosum*. The models with ΔAICc < 2 compared with the best fitting model showed that the probability of the presence of *V. rugosum* was enhanced by an increase in seawater temperature, wind speed, NO_2^- + NO_3^-, PO_4^{3-} and dissolved PnTX G in SPATT, (odds ratio > 1). The probability was reduced by an increase in light intensity and NH_4^+ (odds ratio < 1) and it was influenced by the sampling site (Table 2). The weights of the predictors showed that seawater temperature, NH_4^+ and the site effect were the most important predictors to predict the presence of *V. rugosum* (respective total weights 1, 0.60 and 0.50), followed by dissolved PnTX G in seawater; wind speed; and, to a lesser extent, light intensity, NO_2^- + NO_3^- and PO_4^{3-} (respective total weights 0.18, 0.17, 0.08, 0.08 and 0.07).

Using the parsimony criterion, the model retained to predict the presence of *V. rugosum* only included seawater temperature as a significant predictor (Wald test, $p = 1.41 \times 10^{-3}$) (Figure 5A). The fixed factors (marginal R^2) and the sum of the fixed and random factors (conditional R^2) explained 19.6% and 20.7%, respectively, of the variability in the presence of *V. rugosum*. The probability of *V. rugosum* being present increased during summer, in particular from June to October (Figure 5B). Maximum probabilities occurred in June, reaching 0.48, 0.43, 0.49

and 0.55 in Ingril, Prevost, Thau and Vic, respectively. It was below 0.15 from December to February in each lagoon.

3.2.3. Predicted PnTX G contamination of mussels

We fitted log-transformed PnTX G concentrations in mussels with environmental predictors and detection of *V. rugosum* with models. Given the time lag between the detection of *V. rugosum* and increased PnTX G concentrations in mussels, we tested different time lags from 0 to 12 weeks. The best models, based on the AICc, included time lags of 6, 8 or 10 weeks (no significant difference between these models). We chose the model with the shortest time lag (6 weeks) because it was the most biologically plausible. The models with $\Delta AICc < 2$ compared with the best fitting model showed that the PnTX G concentration in mussels increased with seawater temperature, salinity and PO_4^{3-} (estimates > 0) and was enhanced by the presence of *V. rugosum* (Table 3). The weights of the predictors showed that seawater temperature and the presence of *V. rugosum* were the most important factors influencing the PnTX G concentration, followed by PO_4^{3-} and to a lesser extent salinity (respective weights 1, 1, 0.74 and 0.28).

We established the model to predict the PnTX G concentration in shellfish by using the parsimony criterion. It included seawater temperature and the presence of *V. rugosum* as predictors (likelihood ratio test, $p = 9.46\times10^{-6}$ and 6.13×10^{-3}, respectively) (Figure 5C). The fixed factors (marginal R^2) explained 33.8% of the PnTX G concentration variability, while the sum of the fixed and random factors (conditional R^2) explained 39.3% of this variability. The highest values predicted by this model corresponded to the highest contamination of mussels observed from July to October. (Figure 5D). Overall, this model provided a good fit for the observed contamination as only extreme observed values were outside the 90% confidence interval of the predictions. We compared the observed and average predicted values with the maximum value not expected to result in adverse effects in humans (23 µg/kg

total flesh) (Arnich et al., 2020). There were predicted concentrations above this value throughout the year in the four lagoons, except for the short winter periods in Thau, Prevost and Vic (Figure 5D).

3.3. Predicted human acute dietary exposure

Based on the predicted contamination, we predicted dietary acute exposure to PnTX G associated with the consumption of mussels by the French Mediterranean population (Figure 6). These values represent an estimate of the quantity of PnTX G ingested for a portion of mussels throughout the year. The highest exposure values occurred in Ingril and Vic (maximum values 0.28, 0.22, 0.19 and 0.29 µg/kg body weight per day in Ingril [June], Prevost [August], Thau [September] and Vic [August], respectively). These predicted exposure values provide the first view of the human health risk: the PABV (0.13 µg PnTX G/kg body weight per day) was exceeded in all of the four lagoons, from June to October in Prevost, Thau and Vic, and from May to November in Ingril and Vic.

3.4. Human health risk characterisation

3.4.1. Risk characterized based on the observed data

We characterised the human health risk by predicting the percentage of the French Mediterranean population exceeding the PABV. We calculated the risk by first considering the 95th percentile of the observed data and only the variability of mussel consumption (Table 4). Consumers of mussels were exposed on average to 0.137, 0.133, 0.054 and 0.139 µg/kg body weight per day in Ingril, Prevost, Thau and Vic, respectively, and 54.6%, 52.1%, 6.1% and 56.8% exceeded the benchmark value in these respective areas.

3.4.2. Risk characterised from predicted data

We employed a second approach to characterise the risk based on predicted exposures: we calculated the probability of exceeding the PABV considering both the variability of the model predictions at each sampling time and the variability of mussel consumption (Figure 7). The probability exceeded 25% many times and often reached 20% in Ingril and Vic from June to October. This means that at least 20% of mussels consumer were expected to exceed the PABV in summer 2021 if they ate mussels from these areas at this time. In Prevost, the probability occasionally reached 15% in summer. In Thau, the probability remained low apart from September, when it reached 12%. The probability was below 5% in all lagoons from November to May, but the confidence intervals still highlighted a slight human health risk during these periods.

4. Discussion

We have described the environmental drivers of *V. rugosum* and PnTX G spatiotemporal distributions in four French Mediterranean lagoons. We have also characterised the human health risks associated with the consumption of mussels according to environmental predictors. To our knowledge, this is the first study to predict human health risks related to harmful algae based on environmental variables.

4.1. Impacts of environmental variables on the probability of the presence of *V. rugosum*

Our models showed relationships between the presence of *V. rugosum* and environmental variables congruent with previous experimental and *in situ* studies (Abadie et al., 2015, 2016, 2018). Based on the model selected to predict the presence of *V. rugosum*, seawater temperature is the main predictor of this species *in situ* development. *V. rugosum* shows a maximum growth at a seawater temperature of 25 °C (Abadie et al., 2016), which corresponds to seawater temperatures recorded where it was mainly predicted to be present (43%–55%

probability of occurrence for a temperature of 22.3–27.3 °C). NH_4^+ and wind speed were not selected to predict the presence of *V. rugosum* (following the parsimony criterion) but still influenced the probability that *V. rugosum* would be present. NH_4^+ negatively impacted the presence of *V. rugosum*. This nutrient is involved in blooms of many other dinoflagellates and cyanobacteria species (Glibert and Burford, 2017; Glibert, 2020) and we hypothesise that it is associated with other microalgal proliferations that take over *V. rugosum*. However, as no impact of chlorophyll *a* on the presence of *V. rugosum* was shown, additional studies focusing on the impacts of microalgae total count and of other pigments are needed to test this hypothesis. Wind speed played a moderate role: this factor is known to be crucial for benthic species because it resuspends cells living on the substrate in the water column (Cao et al., 2006; Bouimetarhan et al., 2009; Laanaia et al., 2013; Kubryakov et al., 2019; Hu et al., 2021). We also observed a site effect, as Thau was less contaminated than the other lagoons. This lagoon shows high light intensity but low nutrient concentrations and wind during summer. These conditions could be indicative of low water turbulence and thus low cell resuspension in the water column. Other hydrodynamic, geomorphological and/or biological factors could also be involved, such as the high exchange rate with the sea, the depth (up to 10 m in Thau lagoon), and the composition of macrophyte communities, which can be substrates for *V. rugosum* cells (Abadie et al., 2018; Le Fur et al., 2018). Overall, these results show that we can efficiently predict *V. rugosum* development through environmental conditions in French Mediterranean lagoons.

4.2. Dynamics of PnTX G in seawater

Dissolved PnTX G in seawater showed different intra-annual patterns within the lagoons. A slight increase was observed in summer in Ingril, Prevost and Vic (up to 4.4, 3.1 and 3.4 ng/g of resin, respectively) corresponding to the presence of *V. rugosum*. However, we observed the highest concentrations in winter in Ingril and Thau (up to 30.7 and 14.8 ng/g of resin,

respectively). We propose two explanations for the winter increase of PnTX G in seawater in Ingril and Thau. First, wind speed was higher in winter than in summer in Sète, which could have caused *V. rugosum* resuspension, cell lysis because of unfavourable conditions and intracellular toxin release into the water. Such a phenomenon has already been described for the dinoflagellate *Karenia brevis*, which release brevetoxins (Kusek et al., 1999; Ramsdell, 2008) and is consistent with *V. rugosum* cells detected in Thau in winter. Second, the wind could also release toxins trapped in interstitial water, as observed for okadaic acid (Blanco et al., 2018). Indeed, our results suggest that PnTX G could persist in seawater for several months.

SPATTs clogging could explain why we observed lower PnTX G concentrations in seawater in Vic and Prevost despite strong winds. Indeed, the collected SPATT disks in these two lagoons were always covered by a layer of mud or organic elements, that may have prevented water flow and toxin adsorption to the resin. This view is consistent with high nutrient and chlorophyll *a* values observed in these lagoons, indicative of highly organic and sediment-laden waters. Nevertheless, other environmental parameters may also be implicated. A low salinity (13.5) is known to increase the adsorption of lipophilic diarrhoeic shellfish toxins on SPATT (Fan et al., 2014). However, the higher salinity observed in Thau and Ingril than in Prevost and Vic does not support this hypothesis. The effect of salinity and of other physicochemical and hydrodynamic parameters influencing specific PnTX G adsorption on SPATT remain to be investigated.

4.3. The relationship between PnTX G concentration patterns in mussels and environmental variables

We detected PnTX G in mussels in all studied lagoons. The PnTX G concentrations in mussels in Prevost, Thau and Vic (50.8, 69.2 and 32.4 µ/kg total flesh, respectively) were higher than the concentrations measured in the same lagoons from 2010 to 2017 (26.2, 48.4

and 10 µ/kg total flesh, respectively) (Arnich et al., 2020). The maximum concentrations in Ingril, Vic and Prevost exceeded the levels reported in shellfish from all other countries (Table 5). It must be noticed that contaminations and blooms can vary widely from year to year for harmful algae in French Mediterranean lagoons (REPHY, 2022; REPHYTOX, 2022). Thus, contamination levels from this particular year should not be interpreted as a global trend.

PnTX G accumulated in mussels mainly during summer and early autumn, seasons in which PnTX G mussel contamination has been observed in French Mediterranean lagoons (Hess et al., 2013; Amzil et al., 2021). We noted a delay between the presence of *V. rugosum* and the maximum toxin concentration. Lags between exposure to microalgae and toxin accumulation in organisms have been described in the literature (Shumway and Cembella, 1993; Tweddle et al., 2010). As there were mostly low abundances (mostly non-quantifiable) of *V. rugosum*, low but persistent exposure likely led to slow contamination, which would explain the delay before PnTX G reached its peak. A particularly long half-time life in mussels indicates a very low elimination rate. We still observed PnTX G during winter: such persistent contamination has been observed for other toxins like domoic acid (Lopes et al., 2018) and can be attributed to a low decay rate. Overall, the trends observed in PnTX G mussel contamination are consistent with the temporal distribution of *V. rugosum* and toxin kinetics in organisms.

Environmental factors showed clear impacts on PnTX G variations in mussels. The model we selected to predict PnTX G concentrations in mussels was based on the presence of *V. rugosum* and seawater temperature, supporting that this species is responsible for PnTX G contamination of mussels in French Mediterranean lagoons (Hess et al., 2013). Although they were not selected to predict PnTX G contamination of mussels, salinity and PO_4^{3-} were also significantly and positively associated with toxin concentrations in mussels. These relationships could reveal the impacts on PnTX G production by *V. rugosum* or on its

absorption/accumulation in mussels. PnTX G production by *V. rugosum* has been shown experimentally to reach a maximum for seawater temperatures of 25–30 °C and salinities of 25–40 (Abadie et al., 2016). These values correspond to summer conditions of the lagoons we evaluated, apart from slight salinity peaks in Vic. No study has focused on the impact of PO_4^{3-} on *V. rugosum* toxin production, but this nutrient is known to be involved in phycotoxin production and its availability influences the content of several toxins in dinoflagellates (Azad and Borchardt, 1970; Siu et al., 1997; Yin et al., 1997; Guisande et al., 2002; Lee et al., 2016). Besides, seawater temperature and salinity may influence the filtration rate of mussels (Bøhle, 1972; Schulte, 1975; Jørgensen et al., 1990; Sool and Seok, 1998; Denis, 1999; Loayza-Muro and Elías-Letts, 2007; Riisgård et al., 2013, 2014). Seawater temperature also has a positive impact on the uptake of gonyautoxins and a negative impact on the uptake of saxitoxins (Moroño et al., 2001; Braga et al., 2018; Tang et al., 2021). Taken together, these results indicate that environmental factors drive PnTX G contamination of mussels, in particular seawater temperature, which may be involved in every step leading to accumulation. This factor, combined with the presence of *V. rugosum*, can be used as a good predictor of the PnTX G concentration and for environmental monitoring.

4.4. Risks characterisation and sanitary recommendations

To characterise the human health risks associated with PnTX G contamination of mussels, we first used the methodology described by Arnich et al. (2020) based on the annual observed data and the worst-case contamination scenario (95th percentile). The PABV (0.13 µg PnTX G/kg body weight per day) was exceeded for around 50% of the mussel consumers in Ingril, Prevost and Vic and around 6% in Thau. Compared with a previous study (Arnich et al., 2020), the probability decreased in Ingril but increased markedly in all other lagoons (from 2010 to 2017: 100% of mussel consumers exceeded the PABV in Ingril, 10% in Prevost and

Vic and 0% in Thau). Our results indicate that the increase in the human health risk in several areas could be linked to the expansion of *V. rugosum* in French Mediterranean lagoons.

Second, we developed a probabilistic approach based on the predicted contamination at each sampling time to provide human health risks throughout the year. Mussel consumers were predicted to exceed the PABV mostly in summer (June–November in Ingril and Vic and July–October in Thau and Prevost). However, the confidence intervals showed that there was still a slight human health risk in winter. This finding is consistent with an overlap in the PnTX G concentrations in mussels with concentration not expected to result in adverse effects in humans (23 µg/kg body weight) (Arnich et al., 2023) almost year around in all four lagoons. During summer, > 25% of people who consume mussels from Ingril and Vic could exceed the PABV; thus, these lagoons should thus be considered the most at risk. However, Prevost and Thau should not be exempt from monitoring, especially considering the important shellfish activity in Thau. These results may seem surprising given that no case of human poisoning by PnTX have been reported so far. Several reasons could explain this. Poisonings may have occurred without being reported because of the nonspecific nature of the symptoms expected from PnTX (Delcourt et al., 2019). Moreover, shellfish farming and harvesting are so far prohibited in Ingril lagoon and often regulated at the other lagoons. This work emphasises the importance of the current restrictions.

The method we have developed is helpful to characterise risky periods based on environmental predictors, without preliminary toxin analysis, which is particularly interesting for monitoring purposes. Comparison of predicted and observed contamination remains imperfect because part of the variability is not explained and requires careful interpretation. Additional studies should be performed over several years with more frequent samplings. This endeavour would allow testing potential non-linear relationships and/or interactions and may help to improve the models. Experimental studies on PnTX G contamination of mussels under

different physicochemical conditions would also allow understanding the kinetics observed *in situ*. Finally, the model should be validated by comparing observations and predictions in the following years. The effect of environmental parameters could indeed evolve with climate change. Overall, we believe that the two complementary methods we used in this study represent a powerful tool for sanitary and environmental monitoring.

Based on our results, we propose surveillance recommendations that complement those proposed by Arnich et al. (2020). First, we recommend monitoring the benthic population of *V. rugosum* in risk areas at least during blooming periods (June–September). The use of AS-qPCR (Artificial Substrate coupled with quantitative Polymerase Chain Reaction) for this benthopelagic species appears to be an adapted predictive tool for such monitoring, as the first detection of cells through this tool are associated with PnTX G contamination of mussels with a delay of approximately 6 weeks. We recommend measuring seawater temperature along with cell abundance with special attention to potential contamination when high temperatures are observed. Low NH_4^+ or high PO_4^{3-} should also be considered as warning conditions during warm periods. Given the persistence of PnTX G in mussel tissues, particular attention must be given to at least 6 weeks after the last detection of *V. rugosum* cells. Finally, SPATT should be calibrated regarding physicochemical and hydrodynamic parameters prior to their use for monitoring dissolved PnTX G concentrations. These easy-to-implement proposals may help monitor *V. rugosum* and its toxins in French Mediterranean lagoons, which might be even more necessary in the future in the context of global warming.

5. Conclusion

We have described the spatiotemporal distribution of *V. rugosum* and PnTX G in seawater and mussels in relationship with environmental parameters in four French Mediterranean lagoons during one year. This allowed us to develop models for predicting *V. rugosum* presence and PnTX G concentrations in mussels and to employ an innovative approach to

characterise health risk. PnTX G contamination of mussels has been increasing in Vic, Prevost and Thau lagoons compared with previous years. Mussel contamination was the highest in summer but persisted during other periods of the year. Seawater temperature, NH_4^+ and wind speed impacted the presence of *V. rugosum*, while seawater temperature, the presence of *V. rugosum*, salinity and PO_4^{3-} influenced PnTX G concentrations in mussels. However, PnTX G in seawater was not linked with PnTX G in mussels. Using these results, we have accurately predicted the presence of *V. rugosum* using only seawater temperature as a predictor, and PnTX G concentrations in mussels could be predicted using the presence of *V. rugosum* and seawater temperature. We employed two approaches to characterise the health risk associated with consumption of mussels contaminated with PnTX G. The first considered the 95th percentile of observed data and showed a risk increase compared with previous years in Vic, Prevost, and Thau, but not in Ingril. The second considered environmental parameters and consumption variability and showed that the risk was the highest in Ingril and Vic from June to November. Thus, we have shown that a few environmental parameters could be used to predict health risks and we provided sanitary recommendations to sanitary stakeholders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1 : Sampling stations set up in Ingril, Prevost, Thau and Vic lagoons from May 2021 to April 2022 (Yellow circles) and land-based meteorological stations located in Mauggio and Sète (red crosses).

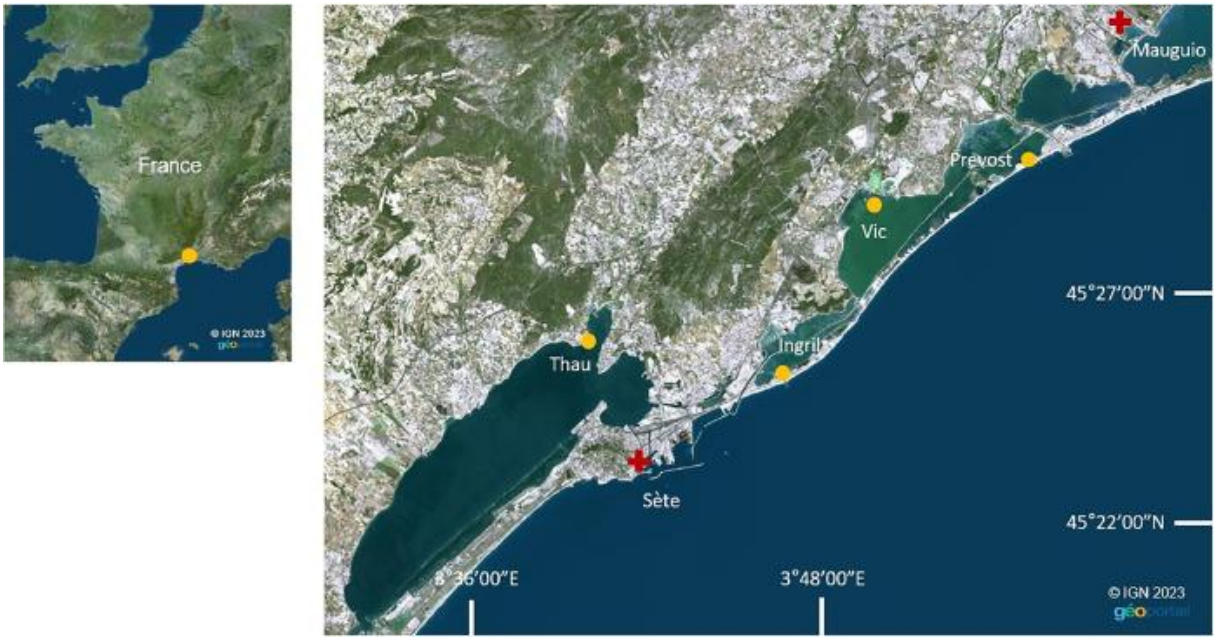


Figure 2 : Physicochemical variability in Ingril, Prevost, Thau and Vic lagoons and at Mauggio and Sète land-based meteorological stations from May 2021 to April 2022. A: Seawater temperature, B: Salinity, C: Light intensity, D: Chlorophyll a, E: Wind speed.

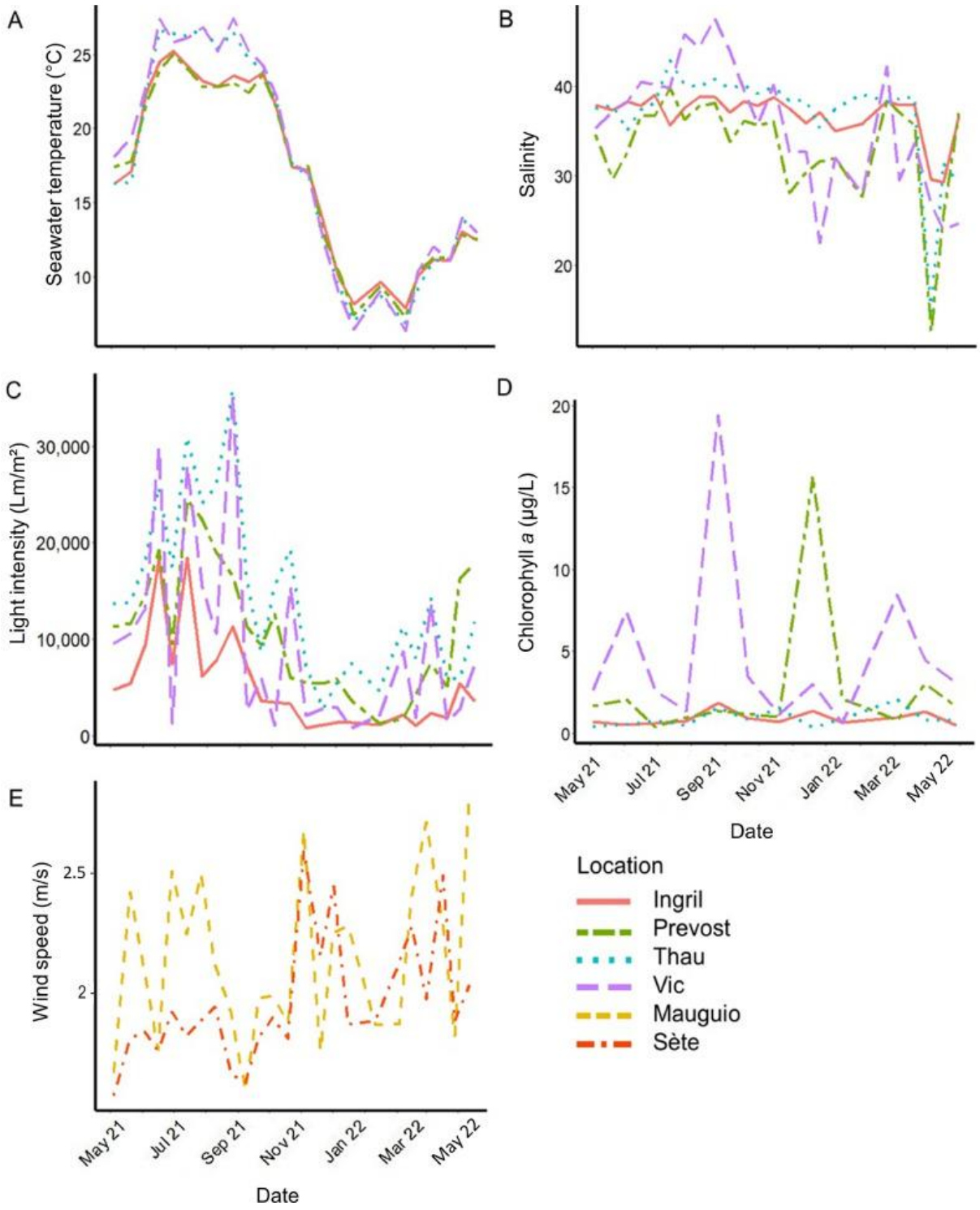


Figure 3 : Nutrients variability in Ingril, Prevost, Thau and Vic lagoons from May 2021 to April 2022. A: PO43- (Phosphate), B: NO2-+NO3- (Sum of Nitrite and Nitrate), C: NH4+ (Ammonium).

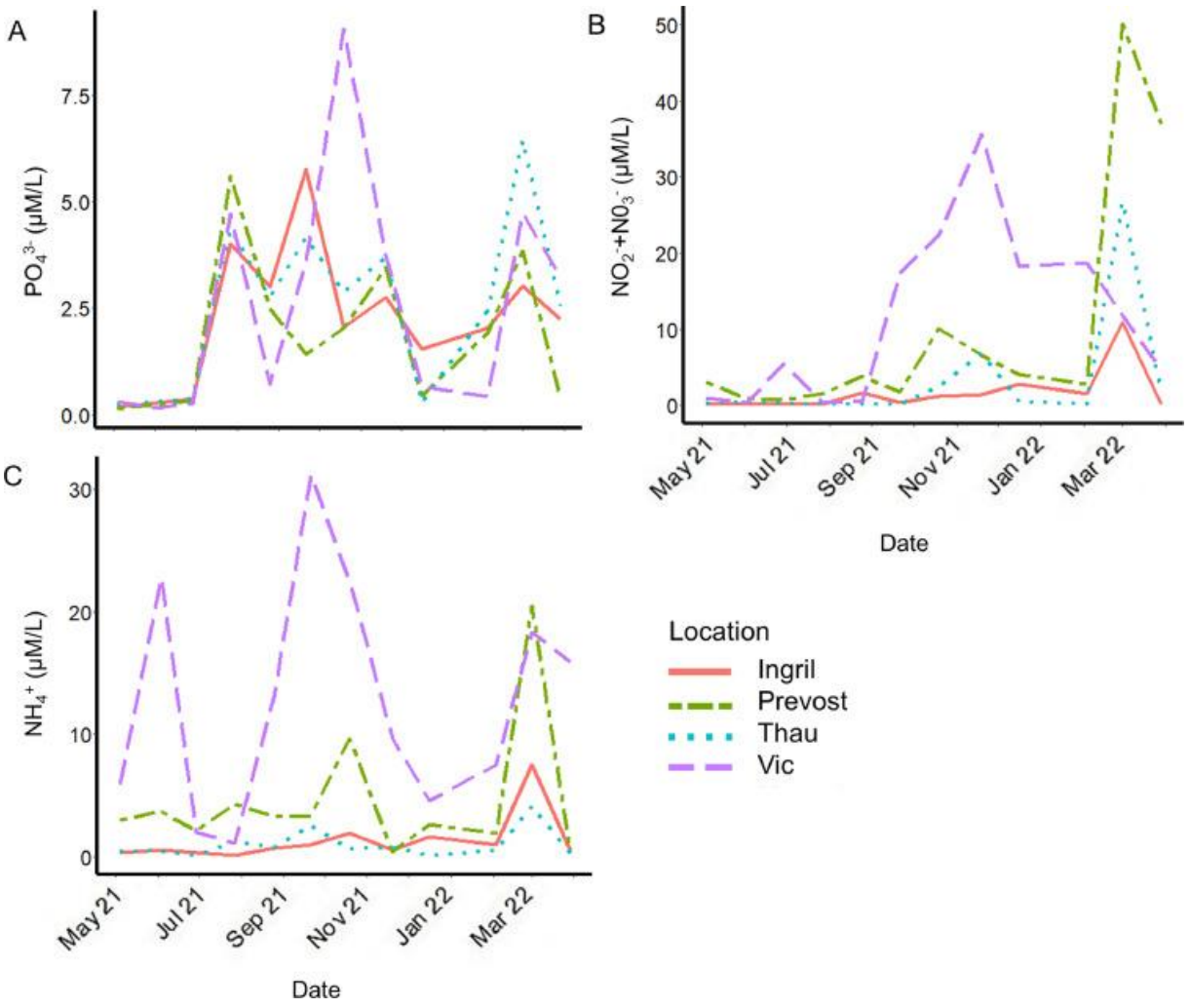


Figure 4 : Temporal variability of pinnatoxin G (PnTX G) mean concentrations in seawater using Solid Phase Adsorption Toxins tracking (SPATT) (µg/g of resin) (A) and in mussels (µg/kg total flesh) (B) in Ingril, Prevost, Thau and Vic lagoons from May 2021 to April 2022. The plus signs mean that Vulcanodinium rugosum was detected by quantitative Polymerase Chain Reaction (qPCR) at this date (Bouquet et al. 2023). The error bars represent ± standard deviation from triplicate mean values.

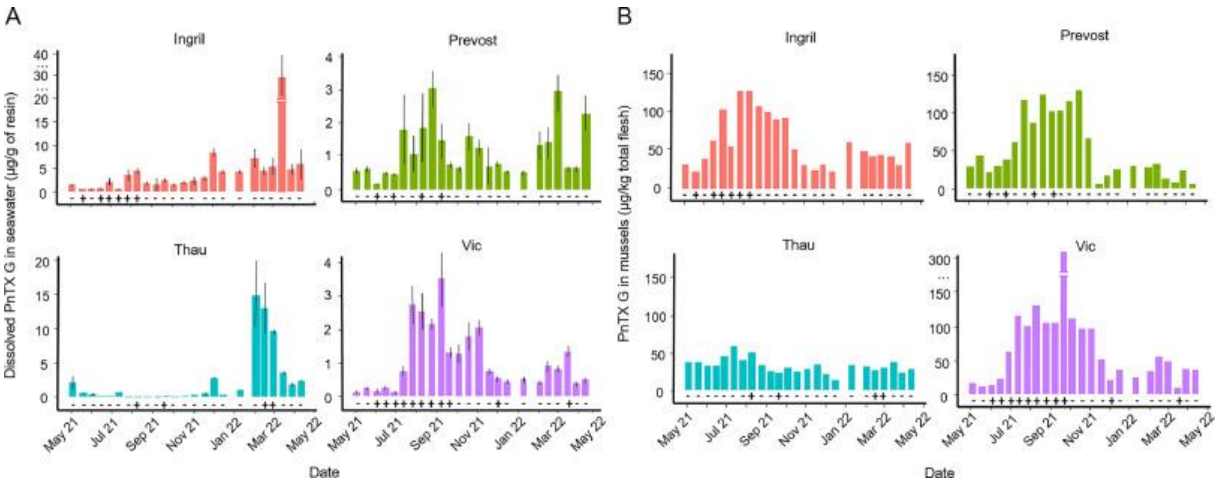


Figure 5 : Characteristics (A, B) and fitted values (C, D) of the model predicting Vulcanodinium rugosum probability of presence using scaled seawater temperature as predictor and of the model predicting pinnatoxin G (PnTX G) concentrations in mussels using Vulcanodinium rugosum (6 weeks lag) and scaled seawater temperature as predictors in the four lagoons of Ingril, Prevost, Thau and Vic from May 2021 to April 2022. The points represent observed detections of V. rugosum (C) and the log of the PnTX G observed concentrations in mussels (D). The gray areas represent the 90 % and 95 % confidence intervals of the models predictions. The dashed horizontal line represents the log of the guideline value of 23 µg PnTX G/kg total meat that should not be exceeded in shellfish proposed by ANSES (Arnich et al., 2020) (B). LRT: Likelihood ratio test.

A

Term	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.29	0.30	-4.16	3.21×10^{-5}
Seawater temperature	2.46	0.28	3.19	1.41×10^{-3}

C

Term	Estimate	Std. Error	t value	LRT p.value
Intercept	3.60	0.11	32.22	-
Seawater temperature	0.31	0.07	4.69	9.46×10^{-6}
V. rugosum presence	0.41	0.15	2.72	6.13×10^{-3}

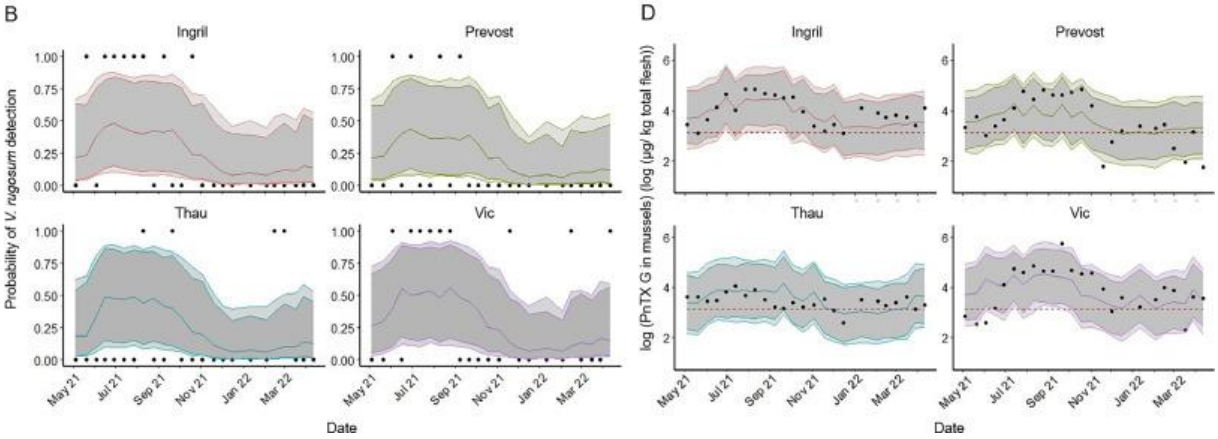


Figure 6 : Prediction of acute dietary exposure of mussels consumers (French Mediterranean population) calculated from the 95th percentile (P95) of daily predicted contamination values (middle line) by pinnatoxin G in the four lagoons of Ingril, Prevost, Thau and Vic from May 2021 to April 2022. 90% and 95% confidence intervals (gray areas) represent the consumer variability. The red dotted line represents the provisional acute benchmark value (PABV) (0.13µg/kg/d bw).

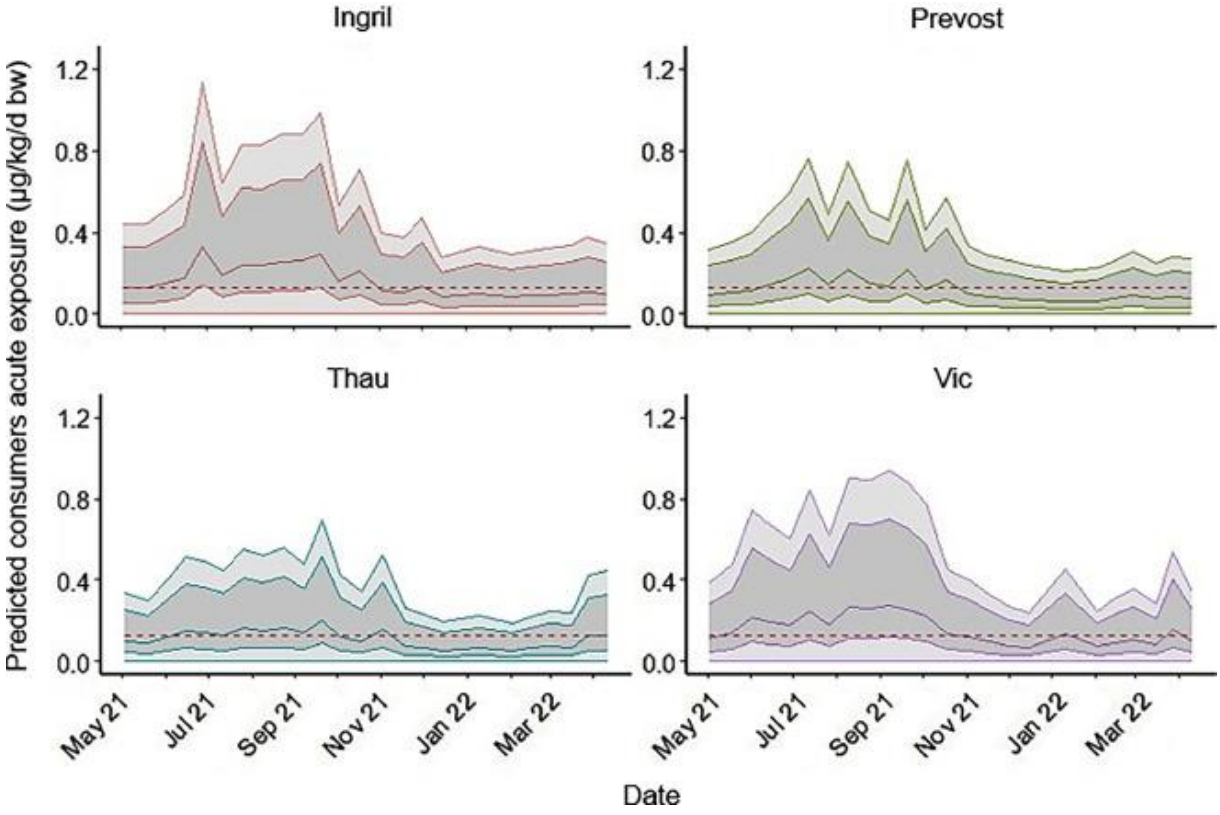
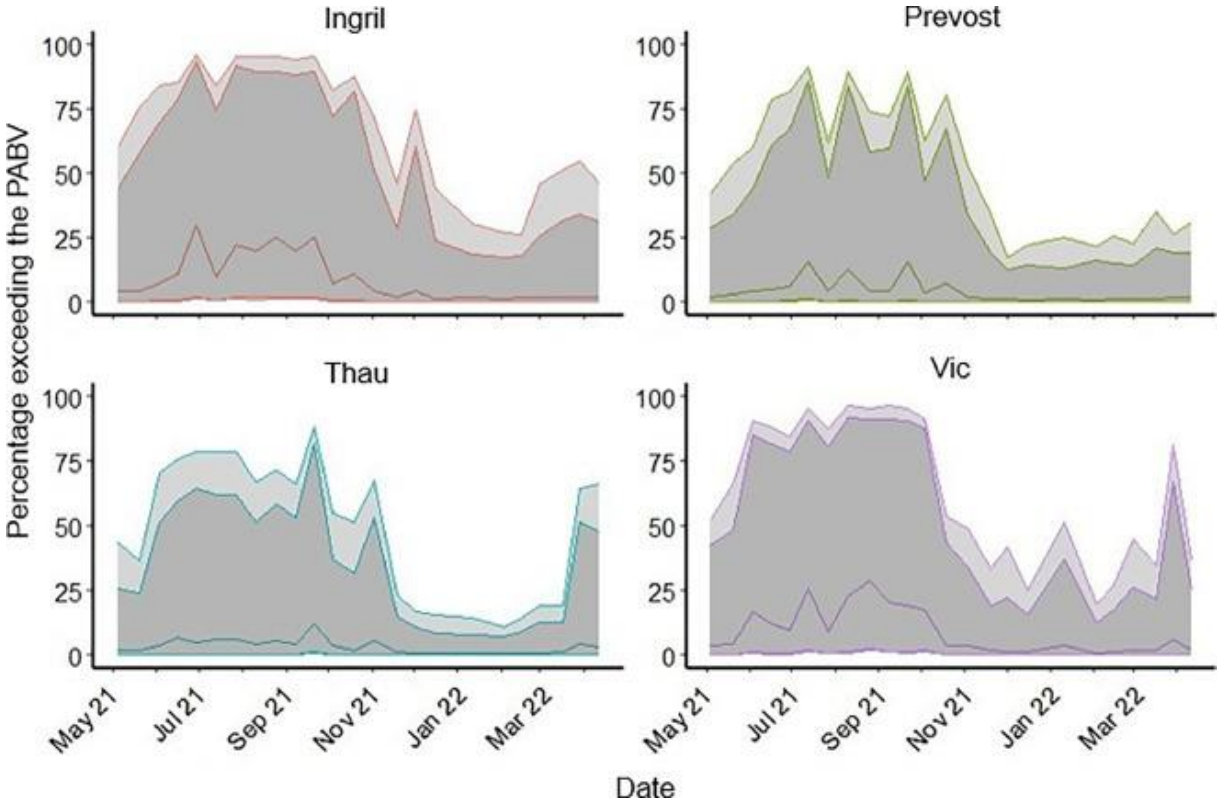


Figure 7 : Probability of exceeding the provisional acute benchmark value (PABV) for pinnatoxin G (PnTX G) for mussels consumers (middle line) in the four lagoons of Ingril, Prevost, Thau and Vic from May 2021 to April 2022. 90% and 95% confidence intervals (gray areas) represent both the variability of consumers and of the predicted contamination values.



Tables

Table 1: Summary of water physicochemical and pinnatoxin G (PnTX G) data in Ingril, Prevost, Thau and Vic lagoons and at Mauguio and Sète land-based meteorological stations from May 2021 to April 2022.

		Water physicochemistry								**PntX G**	
		Seawater temperature (°C)	**Salinity**	**Light intensity (Lm/m²)**	**Chl a (µg/L)**	**Wind speed (m/s)**	**PO_4^{3-} (µM/l)**	**NO_2^- +NO_3^- (µM/l)**	**NH_4^+ (µM/l)**	**PnTX G in seawater (ng/g of resin)**	**PnTX G in mussels (µg/kg bw)**
Ingril	Mean	17.0	36.8	5.3×10^3	0.9	-	2.3	1.7	1.4	4.3	60.4
	Min	7.9	29.3	8.0×10^2	0.5	-	0.2	0.1	0.2	0.5	22.0
	Max	25.2	39.0	1.8×10^4	1.87	-	5.8	10.9	7.5	30.8	128.6
Prevost	Mean	16.9	33.3	1.1×10^4	2.7	-	1.9	10.2	4.7	1.2	50.8
	Min	7.3	12.7	1.2×10^3	0.4	-	0.2	0.8	0.4	0.2	5.7
	Max	25.0	39.8	2.5×10^4	15.7	-	5.6	50.1	20.5	3.1	128.7
Thau	Mean	17.5	37.0	1.5×10^4	0.9	-	2.5	3.3	1.0	2.3	32.4
	Min	6.9	14.9	2.8×10^3	0.3	-	0.2	0.1	0.1	0.1	13.3
	Max	26.8	42.9	3.6×10^4	2.1	-	6.5	26.7	4.1	0.2	57.9
Vic	Mean	17.5	35.5	9.7×10^3	4.9	-	2.7	11.6	13.3	1.1	69.2
	Min	6.4	22.5	7.6×10^2	0.6	-	0.2	0.4	1.1	0.2	9.9
	Max	27.4	47.5	3.5×10^4	19.4	-	9.1	35.7	31.1	3.4	317.8
Sète	Mean	-	-	-	-	3.9	-	-	-	-	-
	Min	-	-	-	-	3.2	-	-	-	-	-
	Max	-	-	-	-	5.1	-	-	-	-	-
Mauguio	Mean	-	-	-	-	4.3	-	-	-	-	-
	Min	-	-	-	-	3.2	-	-	-	-	-
	Max	-	-	-	-	5.6	-	-	-	-	-

Chl a: Chlorophylle *a*, NH_4^+*:* Ammonium, NO_2^-+NO_3^-*:* Nitrite + Nitrate, PO_4^{3-}*:* Phosphate

Table 2: Characteristics of the models fitting the probability of presence of *Vulcanodinium rugosum* using scaled predictors.

Intercept	**Wind speed**	**Light intensity**	**$NH4^+$**	**$NO2^-$ + $NO3^-$**	**Dissolved PnTX G in water**	**PO_4^{3-}**	**Site**	**Seawater temperature**	**AICc**	**ΔAICc**	**Weight**
0.30	-	-	0.45	-	-	-	+	2.61	103.73	0.00	0.033
0.29	-	-	-	-	-	-	-	2.46	104.06	0.33	0.028
0.25	-	-	0.41	-	1.39	-	+	2.89	104.91	1.18	0.019
0.28	-	-	0.67	-	-	-	-	2.51	104.96	1.23	0.018
0.33	1.36	-	0.44	-	-	-	+	2.89	105.08	1.35	0.017
0.28	-	-	-	-	1.32	-	-	2.72	105.13	1.40	0.016
0.28	1.32	-	-	-	-	-	-	2.72	105.16	1.43	0.016
0.29	-	0.75	-	-	-	-	-	3.00	105.32	1.59	0.015
0.28	-	-	0.40	-	-	1.27	+	2.66	105.38	1.65	0.015
0.33	-	-	0.42	1.40	-	-	+	3.03	105.46	1.73	0.014
Total weight	**0.17**	**0.08**	**0.60**	**0.08**	**0.18**	**0.07**	**0.50**	**1**			

Each line represents the odds ratio, corrected Akaike Information Criterion (AICc), ΔAICc compared with the best fitting model, and Akaike weight (relative likelihood of the model) for each model. The last line represents the sum of the relative weights of all models in which the predictor appeared. Other predictors tested but not retained in models respecting the AICc included salinity and chlorophyll *a*. NH_4^+: Ammonium, NO_2^-+NO_3^-: Nitrite + Nitrate, PO_4^{3-}: Phosphate.

Table 3: Characteristics of the models fitting the concentration of pinnatoxin G (PnTX G) in mussels using scaled predictors.

Intercept	**PO_4^{3-}**	**Salinity**	**Temp.**	***V. rugosum***	**AICc**	**ΔAICc**	**Weight**
3.61	0.17	-	0.32	+	196.24	0.00	0.11
3.61	0.19	0.16	0.25	+	197.22	0.98	0.07
3.60	-	-	0.31	+	197.39	1.15	0.06
Total weight	**0.74**	**0.28**	**1**	**1**			

Each line represents the odds ratio, corrected Akaike Information Criterion (AICc), ΔAICc compared with the best fitting model, and Akaike weight (relative likelihood of the model) for each model. The last line represents the sum of the relative weight of all models in which the predictor appeared. Other predictors tested but not retained in models respecting the AICc included light intensity, dissolved PnTX G on solid phase adsorption toxins tracking (SPATT), wind speed, Nitrite + Nitrate, Ammonium and chlorophyll *a*. PO_4^{3-}: Phosphate.

Table 4: Acute dietary exposure to PnTX G associated with consumption of mussels (CONSOMER, French Mediterranean area) for mussels consumers (n=588) (in µg PnTX G/kg body weight) with observed contamination data. PABV: provisional acute benchmark value.

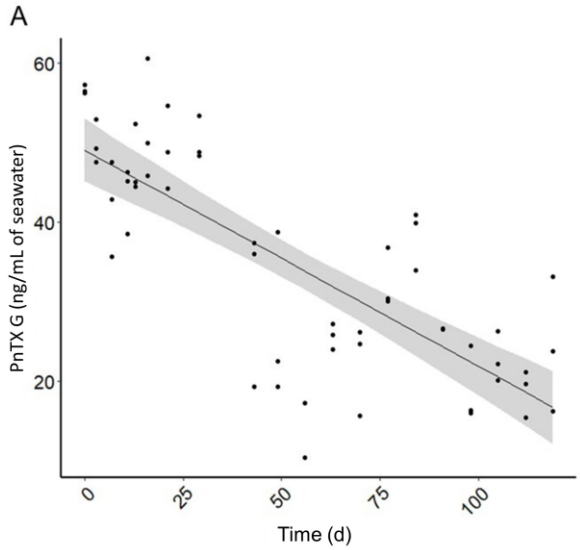
Site	**Mean exposure**	**95th percentile**	**Percentage of consumers > PABV**
Ingril	0.137	0.347	54.6
Prevost	0.133	0.337	52.1
Thau	0.054	0.137	6.1
Vic	0.139	0.353	56.8

Table 5: Maximum concentrations of pinnatoxin (PnTXs) reported in shellfish around the world

Country	**Species**	**Toxin**	**Concentration (µg/kg total flesh)**	**Reference**
Norway, Vestvågøy	Mussel	PnTX G	115	Rundberget et al., 2011
Canada, East coast	Mussel	PnTX A	2.4	McCarron et al., 2012
		PnTX G	108	
New Zealand, Northland	Oyster	PnTX D	3.9	McNabb et al., 2012
		PnTX E	126	
		PnTX F	68	
France, Ingril lagoon	Mussel	PnTX G	1244	Hess et al., 2013; Arnich et al., 2020
	Clam	PnTX G	95	
Spain, Mediterranean coast	Mussel	PnTX G	59	Garcia-Altares et al., 2014
Spain, Atlantic and Cantabrian coast	Mussel	PnTX G	15.0	Lamas et al., 2019
Scotland, Ireland, Northern Ireland, Italy, Norway, Portugal	Mussel	PnTX A	2.6	Aráoz et al., 2020
		PnTX G	45.4	
	Clam	PnTX A	1.5	
		PnTX G	2.0	
	Scallop	PnTX G	4.2	
Italy, Spain, The Netherland, Norway	Mussel	PnTX G	5.1	Rambla-Alegre et al., 2018
	Clam	PnTX G	4	
Chile	Mussel	PnTX G	100	Norambuena and Mardones, 2023

Supplementary material

Figure S1: Decrease kinetics of PnTX G in seawater evaluated experimentally (A), fitted with a simple linear model (B). The time needed to obtain a 50 % and 90 % decrease of the initial PnTX G value was calculated (C).



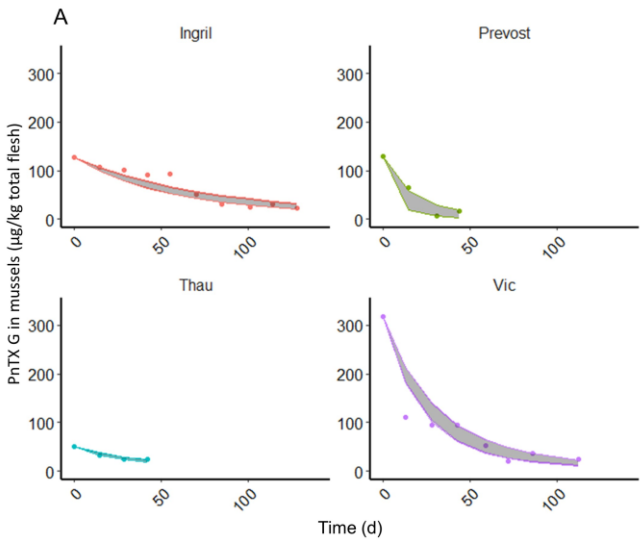
B

Parameter	Estimate	Std. Error	T value	Pr (>t)
Intercept	49.07	1.98	24.79	$<2.00\times10^{-16}$
Time	-0.27	0.03	-9.04	1.15×10^{-12}

C

Time to eliminate 50% of PnTX G (d)			Time to eliminate 90% of PnTX G (d)		
P50	P5	P95	P50	P5	P95
91	74	116	163	134	208

Figure S2 : Elimination kinetics of PnTX G in mussels in Ingril, Prevost, Thau and Vic lagoons (A), fitted with exponential decay models (B). The time needed to obtain a 50 % and 90 % decrease of the initial PnTX G value was calculated for each lagoon (C).



B

Lagoon	Estimate	Std. Error	p.value
Ingril	-3.25×10^{-3}	-2.89×10^{-4}	-1.41×10^{-6}
Prevost	-1.84×10^{-2}	-5.79×10^{-3}	-3.82×10^{-2}
Thau	-5.84×10^{-3}	-6.16×10^{-4}	-1.34×10^{-3}
Vic	-6.37×10^{-3}	-6.54×10^{-4}	-2.23×10^{-5}

C

Site	Time to eliminate 50% of PnTX G (d)			Time to eliminate 90% of PnTX G (d)		
	P50	P5	P95	P50	P5	P95
Ingril	47	42	57	198	175	239
Prevost	8	5	13	35	20	54
Thau	33	28	38	151	126	172
Vic	20	16	23	80	66	91