Environmental Research 228 (2023) 115869

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Ciguatoxin-like toxicity distribution in flesh of amberjack (*Seriola* spp.) and dusky grouper (*Epinephelus marginatus*)

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ARTICLE INFO

Handling Editor: Robert Letcher

Keywords:
Ciguatera
CTX
Canary Islands
Cell-based assay
N2a-MTT assay

ABSTRACT

Ciguatoxins (CTXs) are marine neurotoxins that cause ciguatera poisoning (CP), mainly through the consumption of fish. The distribution of CTXs in fish is known to be unequal. Studies have shown that viscera accumulate more toxins than muscle, but little has been conducted on toxicity distribution in the flesh, which is the main edible part of fish, and the caudal muscle is also most commonly targeted for the monitoring of CTXs in the Canary Islands. At present, whether this sample is representative of the toxicity of an individual is undisclosed. This study aims to assess the distribution of CTXs in fish, considering different muscle samples, the liver, and gonads. To this end, tissues from four amberjacks (*Seriola* spp.) and four dusky groupers (*Epinephelus marginatus*), over 16.5 kg and captured in the Canary Islands, were analyzed by neuroblastoma-2a cell-based assay. Flesh samples were collected from the extraocular region (EM), head (HM), and different areas from the fillet (A-D). In the amberjack, the EM was the most toxic muscle (1.510 CTX1B Eq·g^{-1}), followed by far for the caudal section of the fillet (D) (0.906 CTX1B Eq·g^{-1}). In the dusky grouper flesh samples, D and EM showed the highest toxicity (0.279 and 0.273 CTX1B Eq·g^{-1}). In both species, HM was one of the least toxic samples (0.421 and 0.166 CTX1B Eq·g^{-1}). The liver stood out for its high CTX concentration (3.643 and 2.718 CTX1B Eq·g^{-1}), as were the gonads (1.620 and 0.992 CTX1B Eq·g^{-1}). According to these results, the caudal muscle next to the tail is a reliable part for use in determining the toxicity of fish flesh to guarantee its safe consumption. Additionally, the analysis of the liver and gonads could provide further information on doubtful specimens, and be used for CTX monitoring in areas with an unknown prevalence of ciguatera.

1. Introduction

The consumption of marine organisms is strongly recommended due to their health benefits thanks to their extensive nutritional properties. However, there are some hazards associated with the intake of them, including ciguatera poisoning (CP). This foodborne disease is produced by the ingestion of fish and shellfish whose tissues contain ciguatoxins (CTXs), marine large polyether compounds responsible of cause gastrointestinal, neurological, and cardiac disorders in humans (reviewed recently by (Chinain et al., 2021).

Originally, CP was considered a tropical and subtropical illness in regions including the Caribbean Sea, and the Pacific and Indian Oceans. Nevertheless, in the last two decades, reports of CP have been recorded in other areas due to international trade and climate change, becoming a relevant food poisoning of global concern (Nishimura et al., 2013; Rhodes et al., 2017). This disease has reached European coasts, with the first case of CP in the Canary Islands in 2004 (Perez-Arellano et al., 2005). Since then, 21 additional outbreaks have been reported in this locality (Canary Government, 2022), which is currently considered a major CTX hotspot (Tudo et al., 2020). As a result, since 2011 the local government has implemented a CTX monitoring program to detect the presence of CTXs in fish and prevent cases of poisoning (DG of Fisheries of the Canary Government, 2022b).

More than 425 fish species have been linked to CP (Perez-Arellano et al., 2005). Notwithstanding, in the Canary archipelago, 13 of the 22

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https://doi.org/10.1016/j.envres.2023.115869
Received 13 February 2023; Received in revised form 3 April 2023; Accepted 8 April 2023

Abbreviations

A-D	rostral to caudal sections of the fillet
CP	ciguatera poisoning
CTXs	ciguatoxins
DEE	diethyl ether
E1-E4	*Epinephelus marginatus* individuals
EM	extraocular muscle
Eq	equivalents
HM	head muscle
IC_{20}	20% inhibition concentration
IC_{50}	50% inhibition concentration
LC-MS/MS	liquid chromatography mass spectrometry
LOD/LOQ	limits of detection and quantification
MRM	multiple reaction monitoring
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N2a	neuroblastoma-2a
O/V	ouabain/veratridine
S1–S4	*Seriola* spp. individuals
R^2	coefficient of determination
STD	standard
TE	tissue equivalents

outbreaks reported have been caused by the ingestion of amberjack (*Seriola* spp.), and four by the dusky grouper (*Epinephelus marginatus*), affecting 85 and 32 people, respectively (Perez-Arellano et al., 2005; Canary Government, 2022). These species feed on fish and crustaceans and are of great economic value to global fisheries, especially in the Canary Islands (Andrello et al., 2013; DG of Fisheries of the Canary Government, 2022a; FAO, 2022). Whereas the dusky grouper is a sedentary animal with a permanent habitat, amberjack moves to coastal regions to reproduce, and both are included in the official monitoring (DG of Fisheries of the Canary Government, 2022b). From 2017 to 2021, over 6000 individuals from both species were tested, which represents 97% of the total fish samples received (DG of Fisheries of the Canary Government, 2022b). Among these, nearly 700 specimens of the two species were found to be toxic (9% of amberjacks and 18% of dusky groupers checked). This analysis enabled over 5000 individuals with negative results being released onto the market in the last years (2017–2021), ensuring the secure consumption of risky fish.

Previous studies that have focused on this archipelago have found variations in the CTX-like activity among fish captured on the different islands. Other species examined include wahoo (*Acanthocybium solandri*), moray eels (Muraenidae), and common two-banded seabream (*Diplodus vulgaris*) (Sanchez-Henao et al., 2019, 2020; Ramos-Sosa et al., 2022). In a recently finished European project "EuroCigua", a total of 60 fish species caught in the Canary Islands were tested, the results of which showed that 17 species posed a potential CP risk in this region (Canals et al., 2021).

CTXs are not well distributed in fish. For example, the liver is considered to accumulate more of these toxins than the muscle (Li et al., 2020; Bennett and Robertson, 2021). Although research on the accumulation of CTXs in other viscera are scarce (Vernoux et al., 1985; Colman et al., 2004), several studies have focused on evaluating the affection of CTXs in the gonads and reproduction. However, most of them have been conducted under laboratory conditions of exposure (Yan et al., 2017, 2020).

In a published investigation, the distribution of CTXs throughout the muscle was examined (Oshiro et al., 2021). In this case, members of *Variola louti* were sampled, in which the eye tissue was observed to accumulate higher levels of the toxin than the flesh. The authors also found an even distribution of CTXs in the muscle. These results are in agreement with previous studies (Vernoux et al., 1982; Otero et al., 2010). Nevertheless, as highlighted by Soliño and Costa (Solino and Costa, 2020), individuals who consume the head of the fish have a more pronounced incidence of disease than those who only consume the fillet. This suggests that further research is therefore needed on how CTXs are distributed throughout the fish, especially in the flesh, for different fish species.

The Canary CTX monitoring program focuses routinely on the caudal part of the fish flesh (DG of Fisheries of the Canary Government, 2022b). To the best of our knowledge, no studies have evaluated whether this sample is representative of the entire fish muscle in these species. Several CTX research have reported on the presence of toxins in pools of fish flesh (Loeffler et al., 2019; Costa et al., 2021); albeit, few studies have attempted to determine if the distribution of the toxins in the muscle is homogeneous (Vernoux et al., 1982; Otero et al., 2010; Oshiro et al., 2021). In addition, the analysis of other tissues in doubtful specimens could provide relevant information regarding food safety.

The research of fish from the natural environment is challenging for several reasons. As fish are typically eviscerated immediately after their capture, it is common that not all tissues are available. Additionally, this experimental design requires specimens with different toxicity levels to compare distribution profiles.

This study aimed to evaluate the CTX-like toxicity distribution in the muscle of two valuable fish species caught in the waters of the Canary Islands, namely amberjack and dusky grouper, to identify the most reliable sample for the CTX monitoring. Moreover, the CTX-like toxicity in the liver and gonads were also analyzed to gain insights into the distribution of CTX within different tissues in these fish.

2. Materials and methods

Four specimens belonging to the genus *Seriola* (16.5–73.0 kg) and four to the species *E. marginatus* (21.6–26.5 kg) were captured in the waters surrounding the Canary Islands between October 2015 and January 2018. The fish were provided by the official CTX monitoring program of the Canary Islands Government and were chosen because of their different natural toxicity levels, determined during routine controls. These individuals were included in the EuroCigua framework, although the present research represents progress since project completion as different samples and objectives were considered for further investigation (Canals et al., 2021). Details of each specimen are provided in Table 1.

2.1. *Sample collection*

The complete standardized necropsies of the specimens were performed at the Institute of Animal Health and Food Safety (IUSA), following the Meyers protocol (Meyers, 2009). During the necropsy, different samples were selected for evaluation (Fig. 1): after the removal of the eye, the extraocular muscle was taken (EM). The head muscle above the nervous system was also collected (HM). Three distant flesh samples from the dorsal fillet were taken: A, rostral part of the fillet, next to the head and cranial to the dorsal fin; B, muscle sample from the mid part; D, caudal section close to the tail region. A rostral sample from the ventral fillet was collected (C). Additionally, liver and gonads were sampled from five individuals and were lacking in three specimens, which had been previously eviscerated at the origin harbor upon arrival.

2.2. *CTX extraction procedure*

The samples were kept frozen until processing. The extraction procedure used in previous studies by this research group was followed in the present research for all tissues (Sanchez-Henao et al., 2020). Briefly, 10 g of sample was extracted twice with acetone (20 mL). Then, both acetone extracts were combined and evaporated. The acetone extract was resuspended in Milli Q water (4 mL) and twice diethyl ether (DEE,

Table 1
Details of the capture (island, year, and season) and morphological features (weight, length, and sex) of the individuals examined in this study. Fish with liver and gonads available are indicated.

Individual	Species	Capture Island	Capture Year	Capture Season[a]	Weight (kg)	Length (cm)	Sex	Liver Gonads
S1	*Seriola* sp.	Tenerife	2015	Warm	73.0	190	Female	Yes
S2	*Seriola* sp.	Lanzarote	2017	Cold	42.3	141[b]	Female	Yes
S3	*Seriola rivoliana*	Tenerife	2016	Warm	28.4	124[b]	Female	Yes
S4	*Seriola* sp.	El Hierro	2017	Warm	16.5	111	–	No
E1	*E. marginatus*	El Hierro	2017	Cold	24.0	102	–	No
E2	*E. marginatus*	Lanzarote	2018	Cold	26.5	106	Male	Yes
E3	*E. marginatus*	Tenerife	2017	Cold	22.6	98	–	No
E4	*E. marginatus*	Tenerife	2017	Cold	21.6	107	Male	Yes

- Not assessable (eviscerated animals).
[a] Capture season: “Cold”: January–April; “Warm”: May–December.
[b] Standard length value of the individuals.

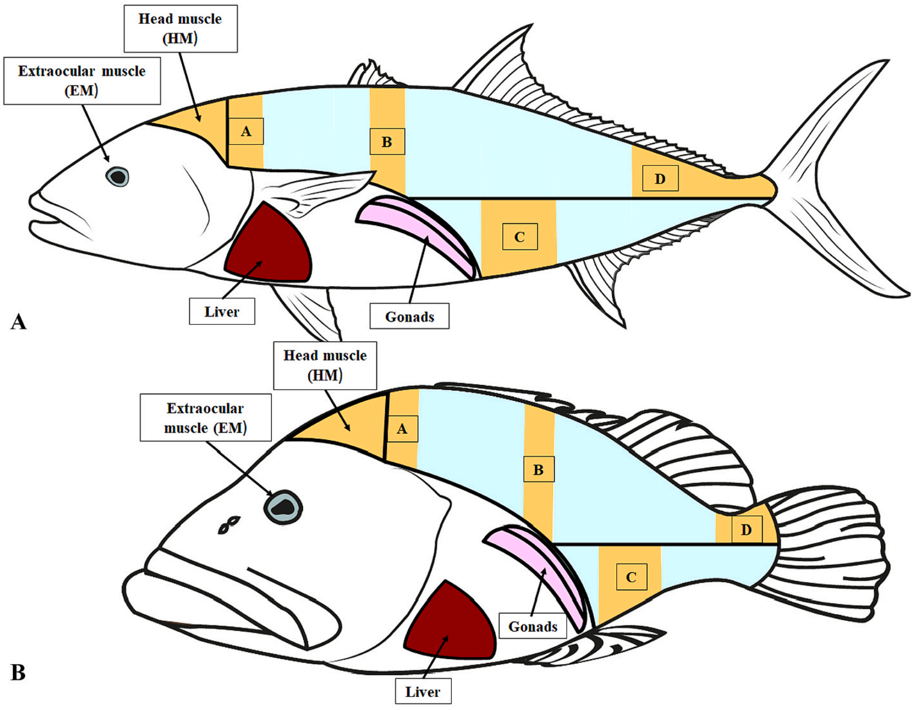

Fig. 1. Schematic representations of the areas of sample collection in amberjack (A) and dusky grouper (B) individuals.

16 mL) for a liquid-liquid partition. Both DEE fractions were pooled and evaporated for defatting with *n*-hexane (4 mL, twice) and 80% aqueous methanol (2 mL). The methanol phase was evaporated, and the final residue was dissolved in 4 mL of pure methanol and kept at −20 °C until the cytotoxicity assay.

2.3. N2a-MTT cytotoxicity assay

The toxicity of the samples was determined by Neuro-2a-MTT assay. Neuroblastoma (N2a, CCL131) cells were purchased from the American Type Culture Collection (ATCC) (LGC Standards S.L.U., Barcelona, Spain). Cells maintenance and cytotoxicity assay were performed according to the method described by (Caillaud et al., 2012). CTX1B obtained by R.J. Lewis (Queensland University, Brisbane, Queensland, Australia) was used as standard (STD) to assess CTX-like toxicity.

Extracts were exposed to a maximum concentration ranging from 25 to 100 mg tissue equivalents (TE) mL^{-1} (based on the characteristics of the sample) to avoid matrix interferences. Some matrices had low effects and allowed for the exposure of cells to 100 mg TE mL^{-1}, while others had higher interferences and the maximum concentration used was 25 mg TE mL^{-1}. Dilutions of this concentration were conducted to expose cells with and without ouabain/veratridine (O/V) pre-treatment. A calibration standard curve of CTX1B was used to calculate its 50% inhibition concentration (IC$_{50}$). The CTX1B equivalents (Eq) were calculated according to the IC$_{50}$ of the sample and the standard curve of CTX1B. To obtain a good range of concentrations of the sample for determining its IC$_{50}$, previous screening was carried out.

The limits of detection and quantification (LOD/LOQ) of the cell-based assay (CBA) was established at the level of CTX1B STD that resulted in a 20% inhibition of cell viability (IC$_{20}$), considering the concentration of extracts used for the analysis. The LOD/LOQ mean values obtained were 0.044 ± 0.021, and 0.046 ± 0.020 ng CTX1B Eq·(g tissue)$^{-1}$ in amberjacks and dusky groupers, respectively.

2.4. LC-MS/MS analysis

In the framework of the EuroCigua project, a sample of homogenized flesh from the caudal part of each of the eight individuals was analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) to identify the presence of CTX. LC-MS/MS analyses were carried out using an Agilent 1290 Infinity Liquid Chromatography system coupled to an Agilent 6495 triple quad iFunnel (Agilent Technologies, Waldbronn, Germany) according to the method described by (Estevez et al., 2019). The following CTXs were monitored in positive ionization mode using multiple reaction monitoring (MRM): CTX1B (m/z 1133.6 - > m/z 1133.6), C-CTX1 (m/z 1163.7 - > m/z 1163.7), C-CTX1-Me (m/z 1177.6 - > m/z 1177.6), 52-epi-54-deoxyCTX1B/54-deoxyCTX1B (m/z 1117.6 - > m/z 1117.6), 49-epiCTX3C/CTX3C (m/z 1045.6 - > m/z 1045.6), and CTX4A/CTX4B (m/z 1083.6 - > m/z 1083.6) (see Fig. 3 in Ramos-Sosa et al., 2022). The LOD and LOQ for CTX1B were 0.0045 and 0.015 ng g^{-1}, respectively. The characteristics of chromatographic separation are detailed in a study recently published by this research group (Ramos-Sosa et al., 2022).

3. Results and discussion

Amberjack and dusky grouper are two species in the fisheries industry and are of particular importance in the Canary Islands (DG of Fisheries of the Canary Government, 2022a). They are included in the CTX monitoring program representing most of the samples annually sent to the laboratory (DG of Fisheries of the Canary Government, 2022b).

Although flesh is known to not have the highest concentration of CTXs among the different types of tissue (Li et al., 2020; Bennett and Robertson, 2021), flesh samples are typically used when determining the presence of CTXs in an organism (FAO and WHO, 2020; Solino and Costa, 2020). This is because the majority of CP cases result from the consumption of fish meat. Additionally, the muscle is appropriate because of its great capability to accumulate toxins ant its suitable matrix characteristics for laboratory processing. Thus, the official control program for the prevention of CP intoxications in the Canary Islands evaluates the presence of CTXs in the flesh, which is the main edible part of fish (DG of Fisheries of the Canary Government, 2022b).

In particular, when collecting flesh samples for analysis, the caudal part of the dorsal muscle is often chosen in order not to depreciate the fish to be sold. However, to date, no research has sought to determine whether this part of the muscle is representative of flesh toxicity in these species, and studies on the distribution of CTXs in these fish are scarce (Oshiro et al., 2021; Ramos-Sosa et al., 2022). To elucidate this concern, four wild specimens belonging to the genus *Seriola*, and four of the species *E. marginatus*, captured in Canary Islands waters, were necropsied and evaluated, all of which had previously been declared as toxic during monitoring.

All of the specimens were evaluated by a CBA, namely the N2a-MTT cytotoxicity assay, to determine the CTX-like toxicity in different samples collected from the flesh: the extraocular (EM) and head (HM) muscles, and four samples from the fillet: (A) from the most rostral part; (B) from the mid area; (C) from the ventral fillet next to anal fins; (D) from the most caudal part, as indicated in section 2.1. The results obtained are represented in Fig. 2.

3.1. Toxicity distribution in flesh of amberjack

Four individuals of *Seriola* spp. (S1 – S4) were analyzed in this study (Fig. 2A). In three of them, the EM was found to be the most toxic sample (2.392 ng CTX1B Eq·g^{-1}, average) of all the muscle tissue studied in each animal (Table 2). However, in the least toxic specimen (S4) of this study, one sample from the fillet (B) contained a slightly higher CTX concentration (0.118 ng CTX1B Eq·g^{-1}) than its respective EM (0.109 ng CTX1B Eq·g^{-1}).

Considering the data obtained from these four amberjacks, an increasing CTX level in the flesh ("mean fillet") was associated with a relevant raise in the EM/Fillet ratio with a strong positive relationship (coefficient of determination (R^2) for linear regression of 0.917), see Supplementary Fig. 1A. Thus, the toxicity of the EM in the two most toxic amberjacks of this study was in a proportion higher than in the other two individuals with the least toxicity (Fig. 4A). On average, the EM was 2-fold more toxic than the fillet muscle, particularly relevant in the most toxic amberjacks, S1 and S2, with an EM/Fillet ratio greater than 2.7 (Table 2 and Fig. 4A). These findings are consistent with those obtained previously in *V. louti* (Oshiro et al., 2021), where the tissue surrounding the eye was found to be approximately 2.5-fold more toxic than the fillet.

As the CTX activity of the fillet increased, so did that of the HM. Nevertheless, HM toxicity remained lower that the average fillet (Table 2). The CTX levels found in the HM were comparable to those obtained in the anterior fillet muscles (A and B), with the following mean values: 0.467, 0.577, and 0.449 ng CTX1B Eq·g^{-1}, respectively.

Within the muscle fillet (A–D), the caudal samples (C and D) were the most toxic (Table 2, Fig. 3A), among which sample D displayed the highest CTX concentration in three out of the four individuals analyzed. Thus, although not significant, the difference of toxicity between fillet (D) and "mean fillet" was higher in the most toxic animals, namely S1 and S2, with a D/Fillet ratio greater than 1.4, see Table 2. Only specimen S4 exhibited a very similar CTX activity in all fillet sections (0.094–0.118 ng CTX1B Eq·g^{-1}).

In these fish species, a gradual rise in toxicity from the cranial to

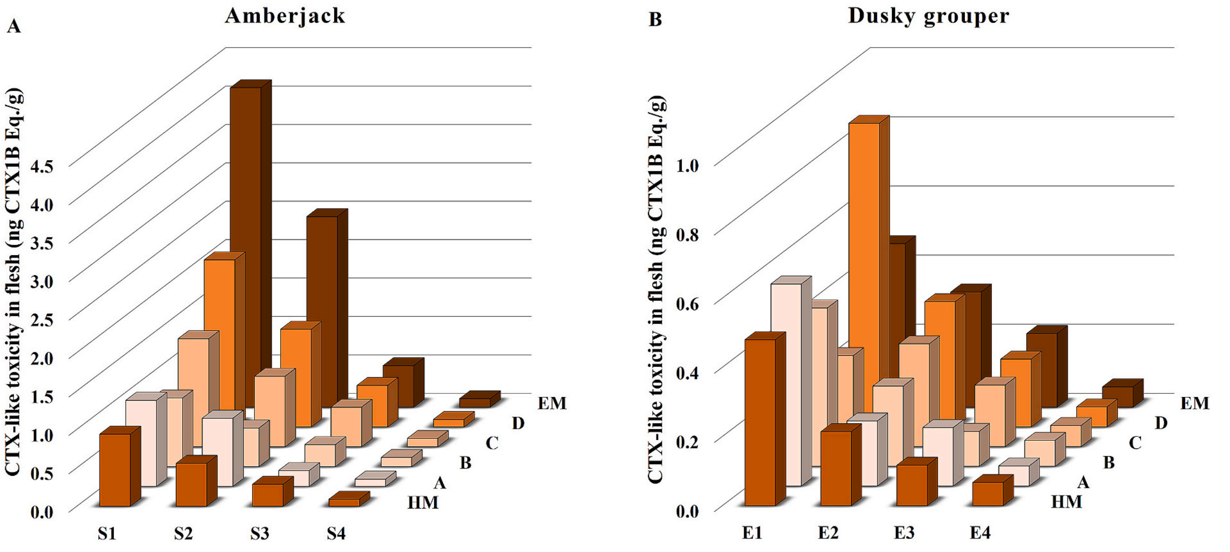

Fig. 2. CTX-like toxicity in amberjacks, S1–S4 (A) and dusky groupers, E1-E4 (B) determined by N2a-MTT cytotoxicity assay. Values are expressed as ng CTX1B Eq.·(g tissue)$^{-1}$. EM, extraocular muscle; HM, head muscle; A-D, rostral to caudal section of the fillet.

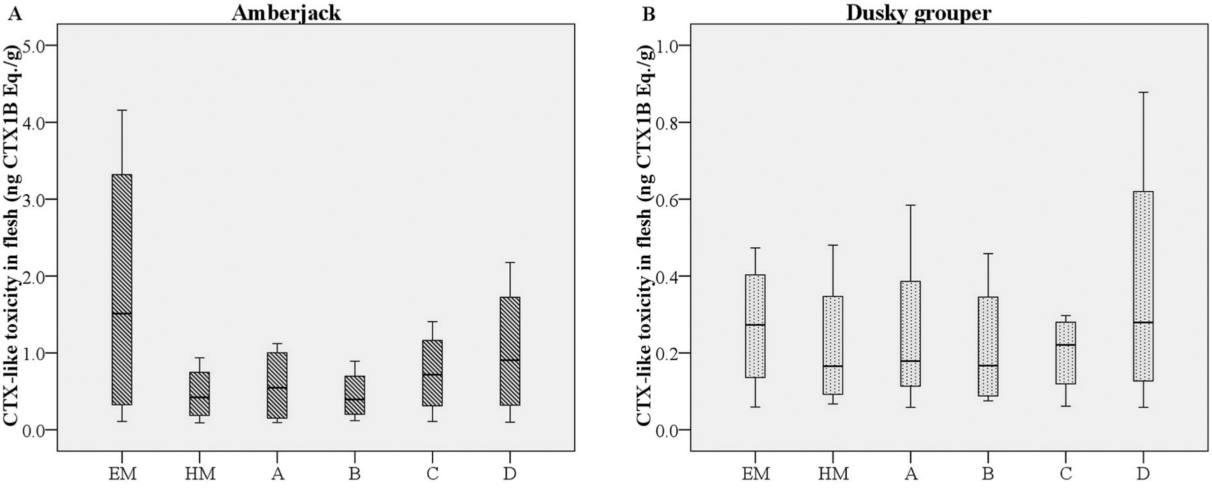

Fig. 3. Boxplot of the CTX-like toxicity determined by CBA in the different flesh samples of amberjack (A) and dusky grouper (B). The thick line indicates the median values. EM, extraocular muscle; HM, head muscle; A-D, rostral to caudal section of the fillet.

Table 2
CTX activity (CTX1B Eq·g^{-1}) in muscle samples of amberjack individuals evaluated by N2a-MTT cytotoxicity assay.

Amberjack										
Individual	EM	HM	A	B	C	D	Mean Fillet ± SD (var)	EM/Fillet Ratio	D/Fillet Ratio	LC-MS/MS
S1	4.157	0.937	1.120	0.892	1.406	2.175	1.398 ± 0.559 (0.312)	2.973	1.556	0.270
S2	2.480	0.557	0.886	0.501	0.918	1.272	0.894 ± 0.315 (0.099)	2.773	1.422	n.d.
S3	0.540	0.285	0.206	0.285	0.515	0.540	0.387 ± 0.166 (0.028)	1.397	1.397	0.050
S4	0.109	0.090	0.094	0.118	0.108	0.098	0.105 ± 0.011 (0.000)	1.043	0.938	n.d.
Mean	1.822	0.467	0.577	0.449	0.737	1.021	0.696 ± 0.263 (0.110)	2.047	1.328	0.160
Median	1.510	0.421	0.546	0.393	0.717	0.906	0.640 ± 0.241 (0.063)	2.085	1.410	0.160

Results obtained by LC-MS/MS analysis from the caudal region are also shown (ng C-CTX1·g^{-1}).
The mean and median values of each sample are shown.
EM, extraocular muscle; HM, head muscle; A-D, rostral to caudal section of the fillet.
"Mean fillet": Average toxicity of all flesh samples (A, B, C, and D), excluding HM and EM. SD: standard deviation. Var: variance.
"EM/Fillet ratio": Relationship between CTX activity observed in the EM and the "mean fillet".
"'D/Fillet ratio": Relationship between CTX activity observed in D flesh samples and the "mean fillet".
n.d.: other unknown peaks different from C-CTX1 were observed in these specimens.

caudal flesh (HM and fillet sections, A–D) was observed. Moreover, as fillet toxicity increased, flesh samples were less homogeneous, with variances ranging from 0.000 to 0.312, see Fig. 4A and Table 2. These results differ from those reported by previous studies, where CTX was found to be homogeneously distributed throughout the fillet. Vernoux et al. (1982) studied the distribution of CTXs in the fillet of eight individuals of yellow jack (*Caranx bartholomaei*), belonging to the Carangidae family as the *Seriola* spp. After separating the right and left fillets, different fractions of the flesh were collected. The results revealed that there were no major differences between the fractions. Furthermore, Otero and collaborators (Otero et al., 2010) examined different muscle samples from the head, ventral, middle, and tail of *Seriola dumerili* and found no significant differences. Nonetheless, when attempting to compare the present results with previous studies, it is essential to bear in mind the different samples, methodologies, and target species used.

The high toxicity observed in the caudal sample may be associated with the great concentration of red fiber in the muscle tissue of this area (Tsukamoto, 1984). The different types of muscle fibers in fish are not equally distributed throughout the body. Normally, in the caudal parts, the presence of red fibers increases while that of the white fibers decreases (Yanase et al., 2012). Further studies are needed to explain the possible difference in CTX accumulation in both muscle fibers.

A sample of the caudal part of flesh from each specimen was analyzed by LC-MS/MS for CTX identification; the presence of C-CTX1 was detected and quantified in two individuals, with 0.050 and 0.270 ng C-CTX1·g^{-1}, respectively (Table 2). In specimens S2 and S4, other unknown peaks were observed but C-CTX1 was not found. The potential difference between the LC-MS/MS identification of C-CTX1 and the estimation of total CTXs by the N2a assay in this study may arise from the typology of the analysis. While LC-MS/MS estimated a specific analog, the N2a assay provides a composite toxicity of all CTXs affecting the Na channels in N2a cells. Otherwise, there is a lack of standards and reference material to obtain a fulfilled LC-MS/MS analysis, reviewed in (FAO and WHO, 2020).

3.2. *Toxicity distribution in flesh of dusky grouper*

In the present study, four dusky grouper individuals were analyzed (E1–E4) (Fig. 2B). In these specimens, a similar CTX distribution among the flesh samples was observed, with variances ranging from 0.000 to 0.067 (Table 3 and Fig. 3B). On average, the ratio between EM and fillet was 1. The same trend is observed for the HM, within which toxicity increased with the CTX activity of the fillet. As the CTX level of the "mean fillet" increased, the toxicity of the fillet (D) raised as well, with a notable positive association with the corresponding ratio (D/Fillet) (R^2 for linear regression of 0.922), see Supplementary Material Fig. 1B. This indicates that the most toxic animals exhibited high CTX-activity in their caudal muscle. In fact, in individual E1, the caudal flesh sample (D) stood out for its toxicity compared to the other samples (D/Fillet ratio of 1.609). Nonetheless, in the least toxic specimen (E4) the ratio was close to 1 (Table 3 and Fig. 4B).

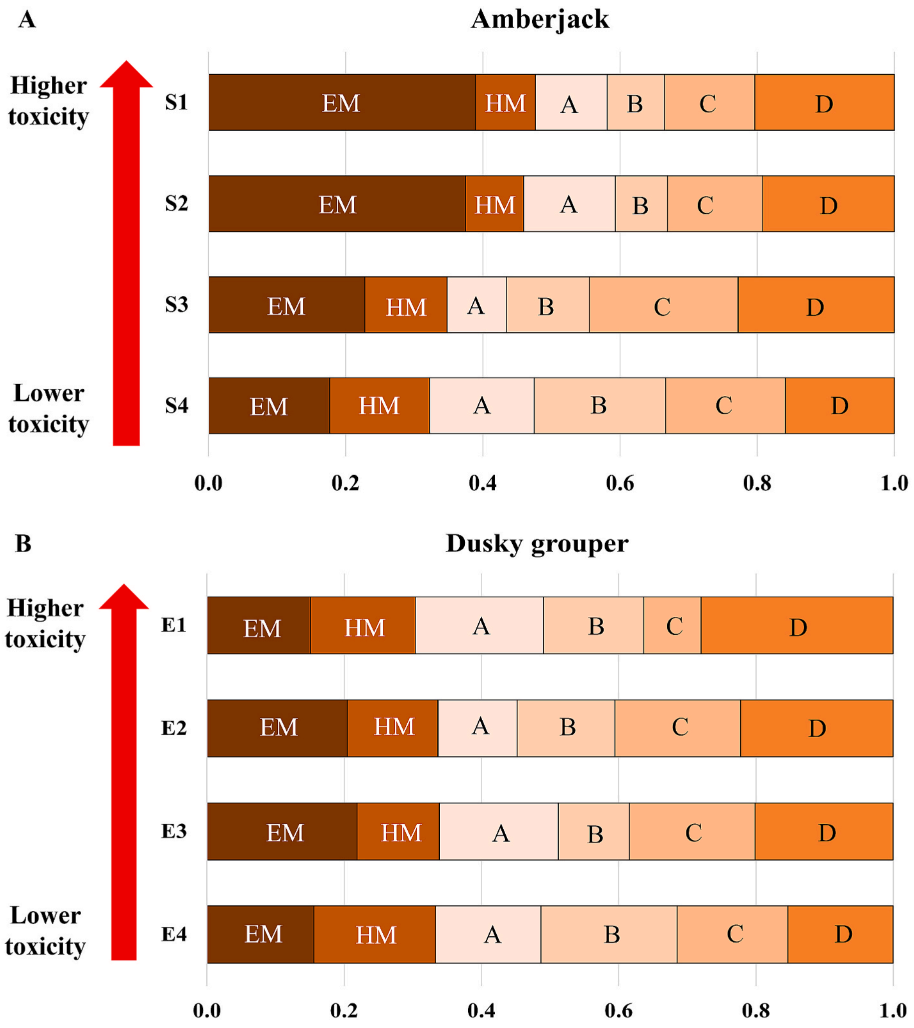

Fig. 4. Relative distribution of muscle toxicity in the specimens, from cranial to caudal, in amberjacks (A) and dusky grouper (B). The direction of the arrow indicates the animals from the lowest to the highest toxicity. EM, extraocular muscle; HM, head muscle; A-D, sections of the fillet.

Table 3
CTX activity (CTX1B Eq·g^{-1}) in muscle samples of dusky grouper individuals evaluated by N2a-MTT cytotoxicity assay.

Dusky grouper										
Individual	EM	HM	A	B	C	D	Mean Fillet ± SD (var)	EM/Fillet Ratio	D/Fillet Ratio	LC-MS/MS
E1	0.473	0.480	0.584	0.458	0.263	0.878	0.546 ± 0.258 (0.067)	0.867	1.609	0.090
E2	0.333	0.214	0.188	0.232	0.297	0.362	0.270 ± 0.076 (0.006)	1.234	1.342	0.110
E3	0.213	0.117	0.169	0.101	0.178	0.196	0.161 ± 0.042 (0.002)	1.323	1.217	0.030
E4	0.059	0.067	0.058	0.075	0.061	0.058	0.063 ± 0.008 (0.000)	0.937	0.921	0.090
Mean	0.270	0.220	0.250	0.217	0.200	0.374	0.260 ± 0.096 (0.019)	1.090	1.272	0.080
Median	0.273	0.166	0.179	0.167	0.221	0.279	0.215 ± 0.059 (0.004)	1.085	1.280	0.090

Results obtained by LC-MS/MS analysis from the caudal region are also shown (ng C-CTX1·g^{-1}).
The mean and median values of each sample are shown.
EM, extraocular muscle; HM, head muscle; A-D, rostral to caudal section of the fillet.
“Mean fillet”: Average toxicity of all flesh samples (A, B, C, and D), excluding HM and EM. SD: standard deviation. Var: variance.
“EM/Fillet ratio”: Relationship between CTX activity observed in the EM and the “mean fillet”.
‘“D/Fillet ratio”: Relationship between CTX activity observed in D flesh samples and the “mean fillet”.
n.d.: other unknown peaks different from C-CTX1 were observed in these specimens.

Oshiro et al. (2021) published research performed on the edible parts of five specimens of yellow-edged lyretail (*V. louti*); the total fillet was divided into six parts, and three muscle samples of the head were also collected. In their study, the amount of toxin and CTXs analogs were found to be equally distributed in the fillet and head samples. However, in four out of the five specimens analyzed, the most caudal samples were found to be the most toxic. The results of the present study are in accordance with these findings, as HM toxicity was comparable to that of the rostral samples of the fillet in both species.

All individuals were available to be analyzed by LC-MS/MS, and the presence of C-CTX1 was identified and quantified from the caudal region. On average, the concentration found in the flesh was 0.080 ng C-CTX1·g^{-1}, with a median value of 0.090 ng C-CTX1·g^{-1} (Table 3). As mentioned above, the differences between the results obtained by both approaches used in this study could be related to the inherent characteristics of these techniques.

3.3. Comparison of flesh toxicity between target species

The two species analyzed exhibited distinct differences in their toxicity patterns. The toxicity observed in dusky grouper was more uniform than that in amberjacks. However, in both species, as the mean fillet toxicity increased, so did the variance (Tables 2 and 3, Fig. 4). Furthermore, the EM was relevant in amberjack due to its higher concentration of toxicity, whereas this was not as significant in dusky grouper. This difference could be associated with the distinct morphology of the extraocular muscle. In amberjack, the muscle fibers are more clearly demarcated, while in dusky grouper these are embedded in the connective tissue, which may interfere with toxicity analysis.

The results indicated that the caudal sample (D) was generally the most toxic part of the fillet in the fish species studied, with an average of D/Fillet ratio of 1.3 in both species. Although this difference may not appear significant, it became more marked as the average fillet toxicity increased. For example, the CTX level of sample D ranged from an increase of 0.9- to 1.6-fold compared to the "mean fillet" between the least toxic and most toxic specimens (Tables 2 and 3). Only in two individuals (S4 and E4, the least toxic specimens of both species) was sample B the most toxic sample, with an almost 20% higher toxicity compared to sample D, see Tables 2 and 3.

3.4. CTX-like toxicity in liver and gonads

Viscera are known to be more toxic than flesh (Vernoux et al., 1985). More severe symptoms have been reported for individuals who consumed viscera compared to those who only ingested the fish meat (Chateau-Degat et al., 2007), with cases of fatalities reported for the former (Bagnis, 1970). Furthermore, intoxications have been reported after the ingestion of barracuda eggs (Hung et al., 2005). Liver toxicity has been studied in greater depth, allowing for the characterization and isolation of different CTX homologues (Yasumoto and Scheuer, 1969; Hokama et al., 1977). However, similar studies on the gonads of wild-caught fish remain scarce.

In the present research, the CTX-like toxicity in the liver and gonads of the individuals studied were also analyzed when these tissues were available (Table 4). This represents the first time that the gonads of these two species from the Canary Islands are studied for CTX toxicity. In both species, the liver samples were notable for their great CTX activity (2.021–6.439 ng CTX1B Eq·g^{-1}, in amberjacks; and 2.221–3.214 ng CTX1B Eq·g^{-1}, in dusky groupers). On average, hepatic toxicity was over four times higher than fillet toxicity ("mean fillet") in amberjacks. Similarly for dusky groupers, the ratio varied from 8 to 51 (Table 4). The liver exhibited the strongest CTX activity among all of the samples, with the exception of individual S2, which showed similar toxicity in the EM and liver (2.480 and 2.470 ng CTX1B Eq·g^{-1}, respectively) (Tables 2 and 4).

Table 4
CTX activity (CTX1B Eq·g^{-1}) of liver and gonads in amberjacks (S1, S2, and S3) and dusky groupers (E2 and E4) analyzed by N2a-MTT cytotoxicity assay.

	Individual	Liver	Gonads	Liver/ Fillet Ratio	Gonads/ Fillet Ratio	Liver/ Gonads Ratio
Amberjack	S1	6.439	2.747	4.605	1.965	2.344
	S2	2.470	0.916	2.762	1.024	2.697
	S3	2.021	1.198	5.229	3.100	1.687
Dusky grouper	E2	2.221	1.118	8.234	4.145	1.987
	E4	3.214	0.865	51.02	13.73	3.716
Amberjack	Mean	3.643	1.620	4.199	2.030	2.242
Dusky grouper	Mean	2.718	0.992	29.62	8.937	2.851

"Liver/Fillet ratio": Relationship between CTX activity of liver and fillet (average toxicity).
"Gonads/Fillet ratio": Relationship between CTX activity of gonads and fillet (average toxicity).
"Liver/Gonads ratio": Relationship between CTX activity of liver and gonads.

These results are in agreement with those observed in a recently published study (Ramos-Sosa et al., 2022), in which a total of 60 amberjacks and 26 dusky groupers were evaluated and the liver was found to be 8-fold and 15-fold more toxic than the flesh, respectively (median values). Besides, a negative correlation was observed between the CTX liver/flesh ratio and size in specimens of dusky grouper. This association could be related to that observed in the present study, which could be explained by the mechanisms developed by fish to eliminate liver toxins by increasing hepatic detoxification, which could in turn lead to a greater accumulation of CTX in the muscle. Moreover, in another recently published study (Bennett and Robertson, 2021), a different C-CTX-1 burden distribution was observed in *Lagodon rhomboides*. In the first part of the study, viscera (including the liver) were found to comprise the majority of the total measured toxicity (~92%), compared to the muscle (~7%). Nonetheless, in the second part, the muscle burden increased to 41%. These findings suggest that toxicity in tissues is not constant over time and that the amount of toxin accumulated could influence the mechanisms for its distribution.

Gonads from three female amberjacks and two male dusky groupers were available for CBA analysis. It is also worth noting that gonads from *Seriola* spp. displayed more toxicity than flesh samples (2-fold more on average). Nevertheless, in specimen S2 the CTX activity measured in the caudal flesh samples (C and D) was higher than that detected in the gonads (0.918, 1.272, and 0.916 ng CTX1B Eq·g^{-1}, respectively) (Tables 2 and 4). Additionally, in individuals S1 and S2, EM was more toxic than the gonads. The gonads presented a higher toxicity level than the rest of the flesh samples, including EM, only in the least toxic amberjack (S3) analyzed (Tables 2 and 4). Regarding dusky groupers, the toxicity activity observed in the gonads was greater than those in all of the flesh samples tested (4- and 14-fold more toxic than the fillet) (Table 4). In the present study, the gonads of both species showed higher toxicity than that observed in the fillet. However, due to the small number of specimens analyzed, further investigations in both sexes at different stages of gonadal development will be required to draw conclusions from these findings.

The difference observed between both specimens of dusky grouper was remarkable. Considering the average toxicity of the fillet, the most toxic individual (E2) exhibited much lower ratios than those observed in the least toxic animal (E4). Thus, the liver and gonads of specimen E2 were 8- and 4-fold more toxic than the respective fillet, whereas in the least toxic individual (E4), the ratios reached values of 51 and 14, respectively.

In both species, the livers were on average more than twice as toxic as their respective gonads (2.242 for amberjacks and 2.851 for dusky groupers) (Table 4).

In 1985 (Vernoux et al., 1985), the CTX toxicity distribution was studied in specimens belonging to Muraenidae, Serranidae, Scombridae, Carangidae, and Sphyraenidae. Samples of flesh, liver, spleen, gonads, and kidney were collected and analyzed, and the results showed that the gonads presented the next higher toxicity after the liver and the rest of the viscera. Subsequently, in 2004 (Colman et al., 2004), CTX-like toxicity was identified in the gonads of a Caribbean great barracuda (*Spyraena barracuda*) with higher level than that found in the liver and flesh. On the other hand, other studies have been published in which the effects of CTXs have been observed in larvae and embryos after exposure by microinjection (Edmunds et al., 1999; Mak et al., 2017). Recently, Yan et al. (2020), fed CTX1B to adult medaka fish and observed toxicity in eggs and effects on the resulting embryos. In addition, sex differences were also observed, wherein the females accumulated more CTX1B than the males (accumulation rates of 24.1 ± 1.4% and 9.9 ± 0.4%, respectively).

4. Conclusions

In the tissues analyzed, the level of CTX activity (median values) from lowest to highest was HM < Fillet (A-C) < Fillet (D) < Gonads < EM < Liver in the amberjack and HM < Fillet (A-C) < EM < Fillet (D) < Gonads < Liver in the dusky grouper. This distribution may vary depending on the level of toxicity of the specimen. CTX-like toxicity was not equally distributed throughout the muscle. The caudal muscle exhibited more CTX activity than the other fractions of the fillet and could be established as a representative sample for the CBA analysis of fish in regions where CTX monitoring is conducted to prevent CP. Extraocular muscle was notable for its high toxicity, especially in amberjack and although it could be considered a suitable sample for CTX monitoring without devaluating fish to be sold, the small amount of muscle available would preclude possible duplicate analysis requirements. The liver and gonads were both characterized by their great concentrations of CTX, which were higher in individuals with lower levels of toxicity in their flesh. For this reason, these viscera are key CTX-containing tissues and represent optimum samples to monitor biotoxins in the natural environment, even in areas where the presence of microalgae is not expected.

Funding

This work was supported by the EuroCigua project, Spain (‘Risk characterization of ciguatera food poisoning in Europe’ [GP/EFSA/AFSCO/2015/03]); CIGUARISK/CIGUAFOOD (‘Food risks associated with ciguatoxins monitoring and tracking the toxins and toxin/producing organisms in marine ecosystems’ [PID 2019-108781RR-C21, PID 2019-108781RR-C22]); and RASPA (‘Red Atlántica para la vigilancia Sanitaria de los productos Pesqueros y Acuícolas’ [INTERREG MAC 2014–2020, MAC2/1.1A/305]).

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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.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the Directorate-General for Fisheries of the Canary Government as responsible of the official control program of ciguatera in the Canary Islands. MJR-S acknowledges financial support from the Spanish Ministry of Education (FPU17/04022). AS-H was funded by the research staff training aid program Catalina Ruiz from Canary Islands Research Agency (ACIISI) (APCR2022010011).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115869.

References

Andrello, M., Mouillot, D., Beuvier, J., Albouy, C., Thuiller, W., Manel, S., 2013. Low connectivity between mediterranean marine protected areas: a biophysical modeling approach for the dusky grouper Epinephelus marginatus. PLoS One 8 (7), 15. https://doi.org/10.1371/journal.pone.0068564.

Bagnis, R., 1970. Concerning a fatal case of ciguatera poisoning in the tuamotu islands. Clin. Toxicol. 3, 579–583. https://doi.org/10.3109/15563657008990130.

Bennett, C.T., Robertson, A., 2021. Depuration kinetics and growth dilution of caribbean ciguatoxin in the omnivore Lagodon rhomboides: implications for trophic transfer and ciguatera risk. Toxins 13 (11), 774. https://doi.org/10.3390/toxins13110774.

Caillaud, A., Eixarch, H., de la Iglesia, P., Rodriguez, M., Dominguez, L., Andree, K.B., et al., 2012. Towards the standardisation of the neuroblastoma (neuro-2a) cell-based assay for ciguatoxin-like toxicity detection in fish: application to fish caught in the Canary Islands. Food Addit. Contam. Part a-Chemistry Analysis Control Exposure & Risk Assessment 29 (6), 1000–1010. https://doi.org/10.1080/19440049.2012.660707.

Canals, A., Martínez, C.V., Diogène, J., Gago-Martínez, A., Cebadera-Miranda, L., de Vasconcelos, F.M., et al., 2021. Risk characterisation of ciguatera poisoning in Europe 18 (5), 6647E.

Canary Government, C.H.S., 2022. Summary of records from "El Sistema de Vigilancia Epidemiológica de la Intoxicación por Ciguatera en Canarias (SVEICC)", 2008-2022 [Online]. Available: https://www3.gobiernodecanarias.org/sanidad/scs/content/3afef0ad-5ada-11ed-8f3c-13be511c2d56/CuadroBrotes_2008-2022.pdf. (Accessed 19 December 2022). Accessed.

Chateau-Degat, M.L., Huin-Blondey, M.O., Chinain, M., Darius, T., Legrand, A.M., Nguyen, N.L., et al., 2007. Prevalence of chronic symptoms of Ciguatera disease in French polynesian adults. Am. J. Trop. Med. Hyg. 77 (5), 842–846. https://doi.org/10.4269/ajtmh.2007.77.842.

Chinain, M., Gatti, C.M.I., Darius, H.T., Quod, J.P., Tester, P.A., 2021. Ciguatera poisonings: a global review of occurrences and trends. Harmful Algae 102, 22. https://doi.org/10.1016/j.hal.2020.101873.

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.

Costa, P.R., Estevez, P., Solino, L., Castro, D., Rodrigues, S.M., Timoteo, V., et al., 2021. An update on ciguatoxins and CTX-like toxicity in fish from different trophic levels of the selvagens islands (NE atlantic, madeira, Portugal). Toxins 13 (8), 12. https://doi.org/10.3390/toxins13080580.

DG of Fisheries of the Canary Government, 2022a. First-Sale-Points Fish Catch Statistics. https://www.gobiernodecanarias.org/agp/sgt/temas/estadistica/pesca/index.html. (Accessed 23 January 2023). Accessed.

DG of Fisheries of the Canary Government, 2022b. Official control protocol for CTX detection of fish sampled at the authorized first sale points, implemented by the Canary Government. https://www.gobiernodecanarias.org/cmsgobcan/export/sites/pesca/galerias/doc/Veterinario/Guia-Protocolo-Ciguat.-y-Exoticas-Rev.2.pdf. (Accessed 23 January 2023). Accessed.

Edmunds, J.S.G., McCarthy, R.A., Ramsdell, J.S., 1999. Ciguatoxin reduces larval survivability in finfish. Toxicon 37 (12), 1827–1832. https://doi.org/10.1016/s0041-0101(99)00119-1.

Estevez, P., Castro, D., Leao, J.M., Yasumoto, T., Dickey, R., Gago-Martinez, A., 2019. Implementation of liquid chromatography tandem mass spectrometry for the analysis of ciguatera fish poisoning in contaminated fish samples from Atlantic coasts. Food Chem. 280, 8–14. https://doi.org/10.1016/j.foodchem.2018.12.038.

FAO, 2022. *Seriola dumerili. cultured aquatic species information Programme. Text by jerez herrera, S. and vassallo agius, R.* [Online]. Accessed 2022-04-22 Updated 2016-01-18 Fisheries and Aquaculture Division: Rome Available: https://www.fao.org/fishery/en/culturedspecies/seriola_dumerili/en.

FAO, WHO, 2020. Report of the expert meeting on ciguatera poisoning: Rome, 19-23 November 2018, 9. Food Safety and Quality, Rome.

Hokama, Y., Banner, A.H., Boylan, D.B., 1977. Radioimmunoassay for detection of ciguatoxin. Toxicon 15 (4), 317–&. https://doi.org/10.1016/0041-0101(77)90014-9.

Hung, Y.M., Hung, S.Y., Chou, K.J., Huang, N.C., Tung, C.N., Hwang, D.F., et al., 2005. Short report: persistent bradycardia caused by ciguatoxin poisoning after barracuda fish eggs ingestion in southern Taiwan. Am. J. Trop. Med. Hyg. 73 (6), 1026–1027. https://doi.org/10.4269/ajtmh.2005.73.1026.

Li, J., Mak, Y.L., Chang, Y.H., Xiao, C.G., Chen, Y.M., Shen, J.C., et al., 2020. Uptake and depuration kinetics of pacific ciguatoxins in orange-spotted grouper (Epinephelus coioides). Environ. Sci. Technol. 54 (7), 4475–4483. https://doi.org/10.1021/acs.est.9b07888.

Loeffler, C.R., Handy, S.M., Quintana, H.A.F., Deeds, J.R., 2019. Fish hybridization leads to uncertainty regarding ciguatera fish poisoning risk; confirmation of hybridization and ciguatoxin accumulation with implications for stakeholders. J. Mar. Sci. Eng. 7 (4), 12. https://doi.org/10.3390/jmse7040105.

Mak, Y.L., Li, J., Liu, C.N., Cheng, S.H., Lam, P., Cheng, J.P., et al., 2017. Physiological and behavioural impacts of Pacific ciguatoxin-1 (P-CTX-1) on marine medaka (Oryzias melastigma). Abstr. Pap. Am. Chem. Soc. 253, 1.

Meyers, T.R., 2009. Standard necropsy procedures for finfish. In: NWFHS Laboratory Procedures Manual, 5 ed. United States Fish and Wildlife Service), Washington, pp. 64–74.

Nishimura, T., Sato, S., Tawong, W., Sakanari, H., Uehara, K., Shah, M.M.R., et al., 2013. Genetic diversity and distribution of the ciguatera-causing dinoflagellate gambierdiscus spp. (dinophyceae) in coastal areas of Japan. PLoS One 8 (4), 14. https://doi.org/10.1371/journal.pone.0060882.

Oshiro, N., Nagasawa, H., Kuniyoshi, K., Kobayashi, N., Sugita-Konishi, Y., Asakura, H., et al., 2021. Characteristic distribution of ciguatoxins in the edible parts of a grouper, Variola louti. Toxins 13 (3), 12. https://doi.org/10.3390/toxins13030218.

Otero, P., Perez, S., Alfonso, A., Vale, C., Rodriguez, P., Gouveia, N.N., et al., 2010. First toxin profile of ciguateric fish in madeira arquipelago (Europe). Anal. Chem. 82 (14), 6032–6039. https://doi.org/10.1021/ac100516q.

Perez-Arellano, J.L., Luzardo, O.P., Brito, A.P., Cabrera, M.H., Zumbado, M., Carranza, C., et al., 2005. Ciguatera fish poisoning, canary islands. Emerg. Infect. Dis. 11 (12), 1981–1982. https://doi.org/10.3201/eid1112.050393.

Ramos-Sosa, M.J., Garcia-Alvarez, N., Sanchez-Henao, A., Sergent, F.S., Padilla, D., Estevez, P., et al., 2022. Ciguatoxin detection in flesh and liver of relevant fish species from the canary islands. Toxins 14 (1), 17. https://doi.org/10.3390/toxins14010046.

Rhodes, L.L., Smith, K.F., Murray, S., Harwood, D.T., Trnski, T., Munday, R., 2017. The epiphytic genus gambierdiscus (dinophyceae) in the kermadec islands and zealandia regions of the southwestern pacific and the associated risk of ciguatera fish poisoning. Mar. Drugs 15 (7), 10. https://doi.org/10.3390/md15070219.

Sanchez-Henao, J.A., Garcia-Alvarez, N., Fernandez, A., Saavedra, P., Sergent, F.S., Padilla, D., et al., 2019. Predictive score and probability of CTX-like toxicity in fish samples from the official control of ciguatera in the Canary Islands. Sci. Total Environ. 673, 576–584. https://doi.org/10.1016/j.scitotenv.2019.03.445.

Sanchez-Henao, A., Garcia-Alvarez, N., Sergent, F.S., Estevez, P., Gago-Martinez, A., Martin, F., et al., 2020. Presence of CTXs in moray eels and dusky groupers in the marine environment of the Canary Islands. Aquat. Toxicol. 221, 9. https://doi.org/10.1016/j.aquatox.2020.105427.

Solino, L., Costa, P.R., 2020. Global impact of ciguatoxins and ciguatera fish poisoning on fish, fisheries and consumers. Environ. Res. 182, 16. https://doi.org/10.1016/j.envres.2020.109111.

Tsukamoto, K., 1984. The role of the red and white muscles during swimming of the yellowtail. Bull. Jpn. Soc. Sci. Fish. 50 (12), 2025–2030.

Tudo, A., Gaiani, G., Varela, M.R., Tsumuraya, T., Andree, K.B., Fernandez-Tejedor, M., et al., 2020. Further advance of gambierdiscus species in the canary islands, with the first report of gambierdiscus belizeanus. Toxins 12 (11), 23. https://doi.org/10.3390/toxins12110692.

Vernoux, J.P., Gaign, M., Riyeche, N., Tagmouti, F., Magras, L.P., Nolen, J., 1982. Evidence for A liposoluble ciguateric toxin carried by A poisonous fish, caranx-bartholomaei, in the French west-indies. Biochimie 64 (10), 933–939. https://doi.org/10.1016/s0300-9084(82)80356-8.

Vernoux, J.P., Lahlou, N., Elandaloussi, S.A., Riyeche, N., Magras, L.P., 1985. A study of the distribution of ciguatoxin in individual caribbean FISH. Acta Trop. 42 (3), 225–233.

Yan, M., Leung, P.T.Y., Ip, J.C.H., Cheng, J.P., Wu, J.J., Gu, J.R., et al., 2017. Developmental toxicity and molecular responses of marine medaka (Oryzias melastigma) embryos to ciguatoxin P-CTX-1 exposure. Aquat. Toxicol. 185, 149–159. https://doi.org/10.1016/j.aquatox.2017.02.006.

Yan, M., Mak, M.Y.L., Cheng, J.P., Li, J., Gu, J.R., Leung, P.T.Y., et al., 2020. Effects of dietary exposure to ciguatoxin P-CTX-1 on the reproductive performance in marine medaka (Oryzias melastigma). Mar. Pollut. Bull. 152, 9. https://doi.org/10.1016/j.marpolbul.2019.110837.

Yanase, K., Herbert, N.A., Montgomery, J.C., 2012. Disrupted flow sensing impairs hydrodynamic performance and increases the metabolic cost of swimming in the yellowtail kingfish, Seriola lalandi. J. Exp. Biol. 215 (22), 3944–3954. https://doi.org/10.1242/jeb.073437.

Yasumoto, T., Scheuer, P., 1969. Marine toxins of the pacific Part 8 cigua toxin from moray eel livers. Toxicon 7, 273–276.