Environment International 101 (2017) 70–79

Contents lists available at ScienceDirect

Environment International

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

Acute and chronic dietary exposure to domoic acid in recreational harvesters: A survey of shellfish consumption behavior

Bridget E. Ferriss [a,*], David J. Marcinek [b], Daniel Ayres [c], Jerry Borchert [d], Kathi A. Lefebvre [e]

[a] Department of Radiology, University of Washington, Seattle, WA 98195, USA
[b] Department of Radiology, Pathology, and Bioengineering, University of Washington, Seattle, WA 98195, USA
[c] WA State Department of Fish and Wildlife, 48 Devonshire Road, Montesano, WA 98563, USA
[d] WA State Department of Health, 243 Israel Road SE, Tumwater, WA 98501, USA
[e] Environmental and Fisheries Science Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112, USA

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 5 January 2017
Accepted 6 January 2017
Available online 18 January 2017

Keywords:
Domoic acid
Razor clam
Siliqua patula
Consumption rate
Acute reference dose
Chronic

ABSTRACT

Domoic acid (DA) is a neurotoxin that is naturally produced by phytoplankton and accumulates in seafood during harmful algal blooms. As the prevalence of DA increases in the marine environment, there is a critical need to identify seafood consumers at risk of DA poisoning. DA exposure was estimated in recreational razor clam (*Siliqua patula*) harvesters to determine if exposures above current regulatory guidelines occur and/or if harvesters are chronically exposed to low levels of DA. Human consumption rates of razor clams were determined by distributing 1523 surveys to recreational razor clam harvesters in spring 2015 and winter 2016, in Washington, USA. These consumption rate data were combined with DA measurements in razor clams, collected by a state monitoring program, to estimate human DA exposure. Approximately 7% of total acute exposures calculated (including the same individuals at different times) exceeded the current regulatory reference dose (0.075 mg DA·kg bodyweight^{-1}·d^{-1}) due to higher than previously reported consumption rates, lower bodyweights, and/or by consumption of clams at the upper range of legal DA levels (maximum 20 mg·kg^{-1} wet weight for whole tissue). Three percent of survey respondents were potentially at risk of chronic DA exposure by consuming a minimum of 15 clams per month for at 12 consecutive months. These insights into DA consumption will provide an additional tool for razor clam fishery management.

1. Introduction

Domoic acid (DA) is a neurotoxic amino acid that is naturally produced by some species of marine diatoms in the genus *Pseudo-nitzschia* and presents a significant health threat to marine mammal and human populations via transfer of the toxin through the marine food web (Bejarano et al., 2008; Lefebvre and Robertson, 2010; Perl et al., 1990; Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – domoic acid, 2009; Trainer et al., 2012). Algal blooms of DA-producing *Pseudo-nitzschia* are increasing in frequency and size globally, placing coastal communities at risk due to high levels of shellfish consumption (Moore et al., 2008). Acute, high level DA exposure in humans causes a neurotoxic illness known as amnesic shellfish poisoning (ASP) characterized by gastrointestinal distress, confusion, disorientation, seizures, memory loss and death in the most severe cases (Perl et al., 1990; Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – domoic acid, 2009). Chronic low level DA exposure (i.e. levels below those that cause the overt signs of toxicity listed above) has been connected to increased toxin susceptibility and impaired mitochondrial function in laboratory studies, and potential memory deficits in humans (Grattan et al., 2016; Hiolski et al., 2014; Lefebvre et al., 2012).

Quantifying human exposure to seafood toxins requires knowledge of human consumption rates, which can vary between age, race/ethnicity, gender, income level, season, and marine species consumed (Burger et al., 1999; Donatuto and Harper, 2008; Toth and Brown, 1997; US Environmental Protection Agency, 2014; WA Department of Ecology, 2013). Non-standard pathways leading to excessive exposure to contaminants and toxins include high consumption rates of self-caught fish/shellfish among Native Americans, minorities, low-income populations, and recreational fishers (Burger and Gochfeld, 2011; Gochfeld and Burger, 2011; O'Neill, 2000). Current fisheries' management practices are based on consumption rates that tend to underestimate exposure levels for these populations due to a lack of population- and site-specific consumption rate data, and exclusion of the higher end of the consumption rate distribution when establishing fishing regulations (i.e., not

* Corresponding author at: University of Washington, Department of Radiology, Box 357115, Seattle, WA 98195, USA.
E-mail address: ferriss@uw.edu (B.E. Ferriss).

http://dx.doi.org/10.1016/j.envint.2017.01.006
0160-4120/

using the highest consumption rate as a basis for exposure regulations; Burger and Gochfeld, 2011). In addition, most fisheries are managed for acute (short term, high level) exposure and not chronic (long term, low level) exposure to seafood toxins, primarily due to a lack of knowledge on long-term consumption habits and associated health effects (Lefebvre and Robertson, 2010).

The management of razor clam (*Siliqua patula*) fisheries for human DA exposure in North America began after the first reported occurrence of amnesic shellfish poisoning (ASP) in eastern Canada in 1987 (Perl et al., 1990; Wekell et al., 2004). A reference dose for acute DA exposure (acute reference dose: ARfD = 0.075 mg DA·kg bodyweight^{-1}·d^{-1}) for human consumers was established by the US Food and Drug Administration based on DA concentrations in uneaten clams during the Canadian event, lowered by an order of magnitude as a safety factor for more susceptible demographics (Marien, 1996; Wekell et al., 2004). The US regulatory limit for DA levels in razor clams considered safe for harvest (<20 mg·kg^{-1} wet weight for whole tissue) was established by calculating the maximum razor clam DA levels consumed by a 70 kg person, who ate 6 clams in one meal, that would produce an exposure equivalent to the ARfD (Marien, 1996; Wekell et al., 2004). The assumed human consumption rate was based on a survey of recreational razor clam harvesters on the coast of Washington, USA, conducted over one weekend, at a single beach in 1993. The use of this consumption rate (6 clams·day^{-1}) and a single bodyweight (70 kg) for exposure calculations risks the omission of temporal, demographic, and spatial variation in toxin exposure found in other recreational fisheries (Burger and Gochfeld, 2011; Gochfeld and Burger, 2011; Marien, 1996). Consumers who eat more than six clams in a meal, weigh <70 kg, or are otherwise sensitive to DA toxicity (children, pregnant women, elderly, and those with renal dysfunction) may not be adequately protected by current DA regulations (Doucette et al., 2004; Doucette et al., 2000; Funk et al., 2014; Hesp et al., 2007; Maucher and Ramsdell, 2007; Perl et al., 1990).

Razor clam fisheries do not have regulatory limits for long-term, low-level exposure to DA (below the regulatory limit of 20 mg·kg^{-1} wet weight for whole tissue) despite the ability of razor clams to retain DA for over a year after a HAB event (Wekell et al., 1994). A current lack of knowledge of long-term razor clam consumption behaviors and of potential human health effects of repeated, low-level exposure has precluded the inclusion of chronic exposure in management policies for this fishery (Lefebvre and Robertson, 2010). Limited studies on low dose, acute DA exposure and low dose, repetitive DA exposure present the potential for human health impacts that are not currently regulated. Lower dose, short-term DA exposures in mouse and rat models have led to lower seizure thresholds, persistent changes in behavioral and molecular indicators of stress response, renal dysfunction, and effects on levels of spontaneous behavior (Funk et al., 2014; Gill et al., 2010; Gill et al., 2012; Schwarz et al., 2014). California sea lions (*Zalophus californianus*) and rats have shown chronic health effects in the form of persistent toxicity syndrome stemming from short-term, acute DA exposure (Cook et al., 2015; Goldstein et al., 2008; Muha and Ramsdell, 2011). Repetitive (chronic), low-level DA exposure experiments have shown subclinical signs of toxicity in the form of increased toxin susceptibility and impaired mitochondrial function in zebrafish (Hiolski et al., 2014; Lefebvre et al., 2012) while shorter-term repetitive low-level exposure studies in rats and cynomolgus monkeys reported no signs of toxicity (Truelove et al., 1996; Truelove et al., 1997). Grattan et al. (2016) were the first to study chronic DA exposure in human consumers (American Indians in Washington State), recording potential memory impairment associated with chronic, low level DA exposure via self-reported razor clam consumption. In response to Grattan et al. (2016), Washington State (USA) recently issued a health advisory suggesting consumers limit their razor clam consumption to 15 clams per month over 12 month period (http://www.doh.wa.gov/CommunityandEnvironment/Shellfish/BiotoxinsIllnessPrevention/Biotoxins/DomoicAcidinRazorClams, accessed October 15th, 2016). It is unknown if recreational fishers consume clams at the frequency and magnitude of subsistence harvesters, and if this chronic health advisory is applicable to this population of harvesters.

Washington razor clams are the basis for important tribal, commercial, and recreational fisheries. The recreational fishery season is worth approximately US$24 million–US$40 million in related expenditures and included 397,000 people harvesting 5.7 million clams in 2014/2015 (Ayres, 2015a, b; Dyson and Huppert, 2010). A record-setting bloom of DA-producing diatoms occurred along the US west coast from spring 2015 through early 2016, causing fisheries closures of razor clams, Dungeness crabs, sardines, and negatively impacting marine mammals along the North American west coast (McCabe et al., 2016). This bloom was considered a preview of future conditions, as increased frequency and toxicity of DA-producing phytoplankton blooms are predicted under future ocean acidification and warming scenarios (Wells, 2015). This recent occurrence of elevated DA levels in razor clams resulted in a unique opportunity to directly quantify the exposure of recreational harvesters across a range of DA levels to determine if current management thresholds adequately protect the razor clam consuming population.

The goals of this study were to determine if recreational razor clam harvesters are exposed to DA levels above the regulatory reference levels and/or chronically exposed to low levels of DA. Estimates of actual human DA exposure levels were calculated on the Washington outer coast in spring 2015 and winter 2016, based on shellfish consumption rates and clam DA levels measured during the same time period. These consumption rate data contributed to the development of a predictive model used to estimate human DA exposure under varying razor clam DA levels. These results can be used to identify potential DA exposure risks to humans that are above the established seafood safety allowable limit and determine if human long-term, chronic, low-level DA exposure occurs.

2. Materials and methods

2.1. Razor clam DA data collection

The Washington Department of health (WDOH), in partnership with the Washington Department of Fish and Wildlife (WDFW), provided a time series of razor clam DA concentrations from four razor clam recreational harvest beaches (Longbeach, Twin Harbors, Copalis, Mocrocks) on the Washington coast from 1991 to 2016 (Fig. 1 & Supplementary material A2). Long beach extends from the Columbia River to Leadbetter Point, Twin Harbors Beach extends from the mouth of Willapa Bay north to the south jetty at the mouth of Grays Harbor, Copalis beach extends from the Grays Harbor north jetty to the Copalis River, and Mocrocks beach extends from the Copalis River to the southern boundary of the Quinault Reservation near the Moclips River (Supplementary material A2). WDFW collected 12 adult clams per sample from 3 separate sections of each beach approximately every 2 weeks, or more frequently during fishery openings and when DA concentrations were elevated (Ayres, 2015b). WDOH pooled and homogenized the 12 clams, and extracted DA from homogenized razor clam tissue using a methanol: water extraction procedure. High Pressure Liquid Chromatography was used as the means of separation and quantitation of DA from the sample extracts (Quilliam et al., 1991). Final values represented average concentrations of DA in razor clams (not including epi-domoic acid), reported as fresh weight for whole tissue.

We analyzed the variation in razor clam DA levels across beaches (Supplementary material A2) to determine if consumers who harvest clams from different beaches should be analyzed separately or together. The uneven clam DA sampling frequency was addressed by using two-week averages of DA for each beach in our analysis. The razor clam DA time series data were analyzed for spatial and annual variation by conducting an ANOVA on the annual means of log transformed DA data from each of the four harvesting beaches, including the beach,

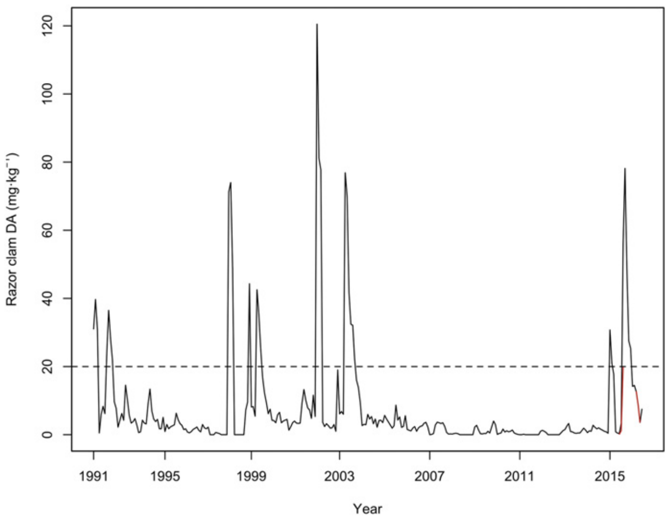

Fig. 1. Monthly averages of razor clam domoic acid (DA) levels ($mg \cdot kg^{-1}$ fresh weight of whole tissue) from January 1991–March 2016. The red lines denote the time periods in which the clam consumption surveys were conducted (March–May 2015 and December–March 2016). The horizontal dashed line represents the current regulatory limit for razor clams (20 $mg \cdot kg^{-1}$ fresh weight of whole tissue).

year, and the interaction between beach and year as factors in the analysis.

2.2. Human clam consumption data collection

To estimate the amount and frequency of clams consumed and to determine the presence of elevated acute and chronic exposure, 1523 surveys were distributed across the four recreational harvest beaches from March 20 through May 7, 2015 and December 24 through March 19, 2016 (Supplementary material A2). WDFW distributed the surveys during routine monitoring of razor clam recreational fishery openings, following the survey distribution methods used in Dyson and Huppert (2010). Clamming parties were approached, given a brief verbal description of the research project and asked to complete the survey. The survey was given to one, adult member of each clamming party. The survey was completed and mailed back to the research team anonymously. The survey distribution occurred during Washington state managed fishery openings and not during tribal fishery openings, which occur at different times. Thus the Quinault, Quileute, and other local tribes are not expected to be represented in this survey.

The survey collected data on the amount (#clams eaten$\cdot$day^{-1}) and frequency (#days eating clams$\cdot$month^{-1}) of human consumption of razor clams over the past two years (a copy of the survey is provided in Supplementary material A1). Data were also collected on harvest and consumption behaviors including the months in which clams were eaten, beaches used for harvesting, and whether clams were eaten immediately or preserved and eaten at a later date. Survey respondents could answer questions regarding clam consumption frequency and amount for up to six members of their household (including age and gender of those household members). Other questions, including race, household zipcode, and clam preserving behaviors, could only be answered by the primary survey respondent. Analysis was restricted to surveys that included age, gender, number of clams eaten per day, and number of days month per month in which clams were eaten.

2.3. Human DA exposure analysis

Actual human exposure to DA through razor clam consumption was estimated during the period of survey distribution using the consumption rate survey data and the WDOH razor clam DA time series. The consumption survey data provided average daily razor clam consumption rates for each month (Q = clams eaten$\cdot$day^{-1}). Consumption data from all household members in the survey were used in the analysis (surveys could include consumption data for up to 6 family members). Weekly average DA levels in razor clams ($mg \cdot kg^{-1}$) were calculated to correspond to the survey period from all four recreational harvest beaches. Daily human DA exposure (mg DA$\cdot$kg bodyweight$^{-1}\cdot$day^{-1}) through clam consumption was calculated following Marien (1996)

$$DA_{human} = \frac{(Q \cdot W_{clam} \cdot DA_{clam})}{W_{human}} \quad (1)$$

assuming an average clam weight of 0.045 kg (W_{clam}) (Marien, 1996). Gender- and age- specific bodyweights for consumers aged 1–20 years were used to calculate human DA exposure in spring 2015 and winter 2016 (W_{human}: Supplementary Table S1) (Fryar et al., 2012). Ages 21 and above were assigned mean weights specific to their age group (21 years–40 years, 41 years–60 years, 61 + years) and gender (W_{human}: Supplementary Table S1) (Fryar et al., 2012). When predicting human DA exposure using the statistical model, consumers under 10 years of age were not included and mean, gender specific bodyweights were used for ages 10–20 (Supplementary Table S1). Consumers under 10 years of age were not included as our sample size was too small, given the relatively large range in consumption rates and bodyweight in that age range.

A common approach to modeling these types of data is using a two stage modeling approach; first modeling consumption/no consumption and then, in cases where clams are eaten, modeling the corresponding consumption rate (Martin et al., 2005; Zeileis, 2008; Zuur, 2009). However, the focus of the study was to determine how many clams were eaten, when clams were being eaten (and the subsequent DA exposure). Therefore, the analysis was simplified by applying a mixed effect generalized linear model with a truncated negative binomial distribution to the positive count data only, using the R programming environment (R Core Team, 2013). In this way, the added complexity of modeling the consumption/no consumption component of the data was avoided, reducing overall uncertainty of our predictions. Mixed effects, generalized linear models were used to explain variation in the count data describing the number of clams eaten per day. Individual survey identifiers and individual household members were treated as nested random effects to account for the lack of independence among consumption habits of members of the same household (up to six household members could be included in one survey), and between monthly consumption rates for an individual person. The explanatory variables of the model included age group, month, and gender. Akaike Information Criterion (AIC) was used to determine the model that best explained variation in the clam consumption data. The predicted values for number of clams eaten per day can be interpreted as the average clam consumption rates and resulting DA exposures, on days when clams are being consumed.

For the purposes of this study, acute exposure was defined as exceeding the ARfD (0.075 mg DA$\cdot$kg bodyweight$^{-1}\cdot$d^{-1}) and chronic exposure was defined as consumption of a minimum of 15 clams per month for 12 consecutive months (Grattan et al., 2016). Using this chronic exposure definition, we compared DA exposures of chronic clam consumers for 12 months prior to the DA bloom (May 2015) with potential exposure after the fishery reopened (January–December 2016) to determine the range of potential monthly exposure for chronic consumers. We only used chronic consumers from the spring survey population as the fishery was closed for six months prior to the winter survey. For each individual who met the chronic consumption threshold, we calculated the number of clams eaten per month. We combined monthly average razor clam DA values from the Washington Department of Health for the pre- and post-bloom period, with the chronic consumption rates (using the same consumption rates for both time periods) to estimate pre- and post-bloom monthly DA exposure.

3. Results

Annual concentrations of DA in razor clams sampled from each of the four harvesting beaches were highly correlated ($R^2 = 0.82$–0.97) and showed no significant difference between beaches ($F(3,92) = 0.208$, $p > 0.1$). The DA values differed by year ($F(1,92) = 14.083$, $p < 0.001$) but the interaction term between beach and year was not significant ($F(3,92) = 0.08$, $p > 0.1$). As a result, razor clam DA data were combined from all four harvesting beaches into a single dataset to be used in the human exposure estimates below.

Of the 1523 consumption rate surveys distributed, 37% were returned from across all beaches (315 surveys in spring 2015, 246 surveys from winter 2016) (Table 1). The 561 surveys contained clam consumption rates (#clams eaten$\cdot$d^{-1} and #days$\cdot$month^{-1} in which clams were eaten), age, and gender data for 965 people (Table 1). The vast majority of survey responses came from Washington and to a lesser extent Oregon, with a few representatives from Alaska, Idaho, Montana, Nevada, and California (Supplementary material A2).

The surveys provided information on age, gender, race, household zipcode, and harvesting and preserving behaviors of recreational razor clam harvesters. The ages of survey respondents (including all 965 household members described in the surveys) ranged from 1 to 91 years. The age of 77% of the household members was >41 years (Table 1). Of all 965 respondents, 46% were female and 54% were male. Of all the surveys, 88% of the primary responders listed their race as "White".

Calculated exposures of recreational harvesters in spring 2015 and winter 2016 followed the increasing and decreasing trends of clam DA concentrations, and exceeded the ARfD (0.075 mg DA$\cdot$kg bodyweight$^{-1}\cdot$d^{-1}) in numerous instances (Figs. 2 and 3). Consumers (often the same consumers in multiple months) exceeded the ARfD threshold 246 times (33 in spring 2015 and 213 in winter 2016), or 7.4% of the total exposure estimates across all time periods. The rapid increase in clam DA levels in spring 2015 resulted in consumers exceeding the ARfD in the last month prior to the fishery closure (May). The slow depuration of DA in clams resulted in consumers exceeding the ARfD four months after the fishery reopened (Dec–March 2016) period. During the spring 2015 season, mean exposures for males under 21 years exceeded the ARfD in May 2015, when razor clam DA levels were at their highest legal levels (Fig. 2). Exposures within one standard error of the mean exceeded the ARfD for both genders aged 41–60 years (Fig. 2). During the winter 2016 season, mean exposures for females under 21 years exceeded the ARfD in December and January, and exposures within one standard error of the mean exceeded the ARfD in multiple other age groups and months (Fig. 3).

Table 1
A summary of the number of surveys returned, and of surveys and household members, by age (year) and gender (female/male), specifying #clams eaten per day and #days per month in which clams were eaten.

Survey category	Total	Spring 2015	Winter 2016
Total returned	561	315	246
Surveys with clam consumption data	449	261	188
Household members with clam consumption data	965	570	395
Age 1:20	30/38	23/20	7/18
Age 21:40	64/76	35/43	29/33
Age 41:60	173/191	104/116	69/75
Age 61 +	179/214	103/126	76/88

The model that best explained variation in human consumption rates of razor clams eaten per day was the full model, including month consumed, gender, age group, and season the survey was distributed as fixed variables (Table 2, ΔAIC < 2). The predicted mean consumption rate across all genders and months, on days when clams are eaten, was 6.0 ± 0.9 (mean $\pm$ SD) clams$\cdot$day^{-1}. Males ate more clams$\cdot$day^{-1} than females (6.5 clams$\cdot$day^{-1} $\pm$ 0.8 and 5.4 clams$\cdot$day^{-1} $\pm$ 0.6 respectively) and respondents from the surveys distributed in spring 2015 had higher consumption rates than the respondents from the winter 2016 distribution (6.5 clams$\cdot$day^{-1} $\pm$ 0.8 and 5.4 clams$\cdot$day^{-1} $\pm$ 0.7 respectively) (Fig. 4). Model estimated consumption rates of respondents from both survey distribution periods were highest in April and lowest in the summer months (May–September).

Average clam consumers met the ARfD threshold (0.075 mg$\cdot$kg^{-1}) while consuming clams under the regulatory limit (razor clam DA = 20 mg$\cdot$kg^{-1} wet weight for whole tissue) in 4 out of 16 subgroups (age/gender/survey season; Table 3). Consumers within one standard error of the estimated mean daily clam consumption rate met the ARfD threshold while consuming razor clams with DA values below the regulatory limit in 14 out of 16 subgroups (Table 3). Consuming clams that have the long-term mean DA levels under legal harvesting conditions (razor clam DA = 4 mg$\cdot$kg^{-1} wet weight for whole tissue) produced daily human exposures <0.025 mg DA$\cdot$kg bodyweight$^{-1}\cdot$day^{-1} for all groups.

Male respondents from the Spring 2015 survey distribution, aged 10–20 years old, had the highest DA exposure due to lower bodyweights than other age groups and higher consumption rates than females and the winter survey population (Table 3, Figs. 4 & 5). Consumers within one standard error of the mean daily clam consumption rate of this group exceeded the ARfD when consuming razor clams with DA concentrations >14.7 mg$\cdot$kg wet weight for whole tissue (Fig. 5). Predicted daily DA exposure of male consumers from the spring population, within one standard error from the mean consumption rate, ranged from 0.003 to 0.09 mg DA$\cdot$kg bodyweight$^{-1}\cdot$day^{-1} under legal harvesting scenarios (razor clam DA < 20 mg$\cdot$kg^{-1} wet weight for whole tissue) (Fig. 5). The relatively large range of bodyweights between 10 and 20 year olds probably resulted in the overestimation of exposure of the older consumers (i.e. 20 year olds) and the underestimation of exposure of the younger consumers when applying a single average bodyweight.

Of the 965 survey responses, 27 people (3%) from 20 families reached our consumption threshold of chronic exposure, eating a minimum of 15 razor clams per month for 12 consecutive months. The 27 chronically exposed consumers ate an average of 10 clams per day on days when they ate clams. Twenty-four people in this group ate six or more clams per day, on days they ate clams, for 12 consecutive months. The demographics of this consumer group were 67% male and all over the age of 40 (63% over the age of 60). Respondents from the spring survey population constituted 70% of the chronic consumers.

We calculated the mean DA exposure to chronic consumers for those meeting the threshold of 15 clams per month for 12 consecutive months, for the spring survey population (Supplementary Table S2). We only used the spring survey population as the fishery was closed for approximately seven months prior to the winter survey. The pre-bloom monthly average DA exposure for each age/gender group of the 19 chronic consumers represented in the spring survey population ranged from 0.011 (0.006) mg$\cdot$kg$^{-1}\cdot$month^{-1} to 0.014 (0.009) mg$\cdot$kg$^{-1}\cdot$month^{-1} (Fig. 6 & Supplementary Table S2). When applying the same monthly clam consumption rates to clam DA levels in the months post bloom (January 2016–December 2016) chronic exposures ranged from 0.116 (0.073)–0.145 (0.092) (mg DA$\cdot$kg bodyweight$^{-1}\cdot$month^{-1}) (Fig. 6 & Supplementary Table S2). The average ($\pm$SD) DA concentrations in razor clams were 0.9 ($\pm$0.4) mg$\cdot$kg^{-1} wet weight for whole tissue pre-bloom (May 2014–April 2015) and 9.4 ($\pm$4.7) mg$\cdot$kg^{-1} wet weight for whole tissue once the fishery had reopened post-bloom (January 2016–December 2016).

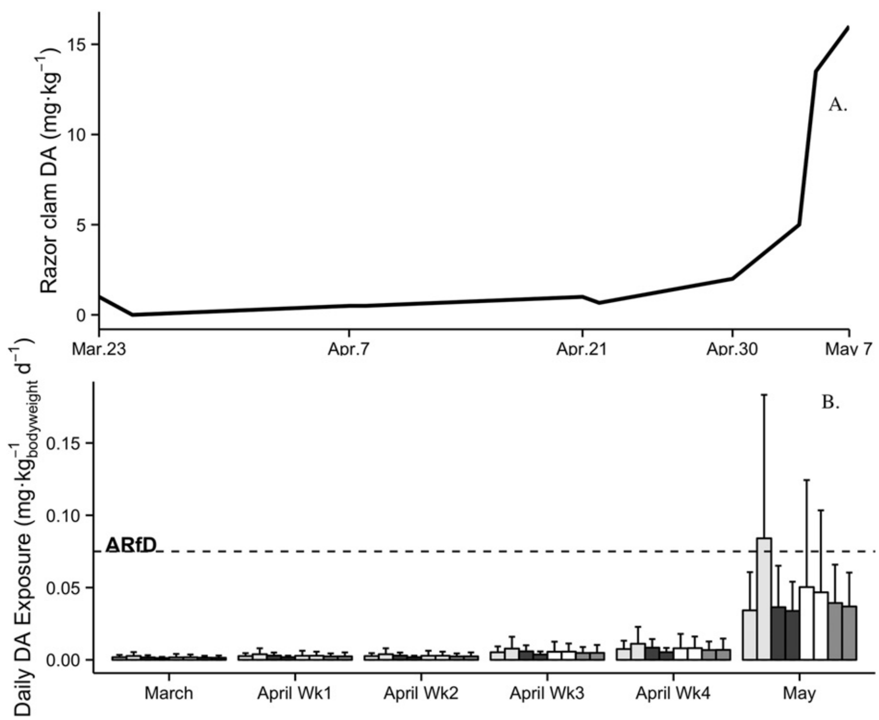

Fig. 2. Weekly mean domoic acid (DA) levels in razor clams from March 23 to May 7, 2015 (A) and corresponding weekly mean DA exposure (mg DA·kg bodyweight^{-1}·d^{-1}) for recreational harvesters from the last week in March to the first week in May 2015 (B). DA exposures are aggregated by age and gender. Colors represent four age groups of 10–20 years (light gray), 21–40 years (dark gray), 41–60 years (white), and 61+ years (medium gray), and pattern represents gender (female = solid, male = hatched). The dashed line in the lower panel represents the acute reference dose (ARfD) of 0.075 mg DA·kg bodyweight^{-1}·d^{-1} that was used to set the legal limit for DA levels in razor clams of 20 mg·kg^{-1} fresh weight of whole tissue. The error bars represent exposure calculated using DA levels one standard error above/below the mean and consumption rates one standard error above/below the mean (using Eq. (1)).

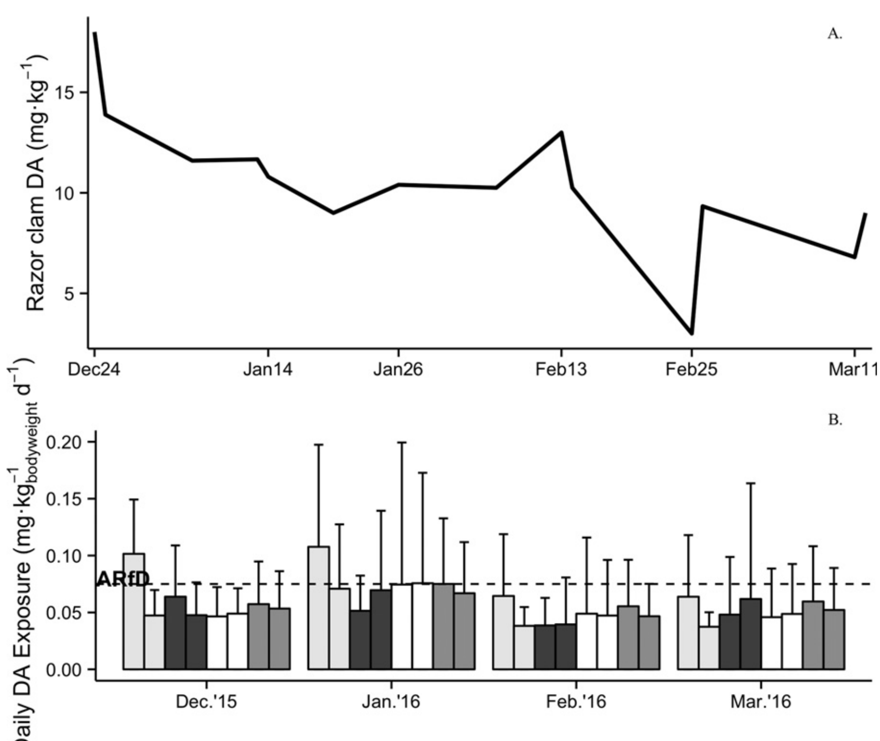

Fig. 3. Weekly mean domoic acid (DA) levels in razor clams from December 24, 2015 to March 20, 2016 (A) and monthly mean DA exposure (mg DA·kg bodyweight^{-1}·d^{-1}) for recreational harvesters from December 24, 2015 to March 20, 2016 (B). Domoic acid exposures are aggregated by age and gender. Colors represent four age groups of 10–20 years (light gray), 21–40 years (dark gray), 41–60 years (white), and 61+ years (medium gray), and pattern represents gender (female = solid, male = hatched). The dashed line in the lower panel represents the acute reference dose (ARfD) for DA exposure from razor clams (0.075 mg DA·kg bodyweight^{-1}·d^{-1}). The error bars represent exposure calculated using DA levels one standard error above/below the mean and consumption rates one standard error above/below the mean (using Eq. (1)).

Table 2
Models explaining variation in #clams eaten·day^{-1}, including degrees of freedom (df), Akaike Information Criterion (AIC) and AIC values relative to the lowest value (ΔAIC).

Model (fixed variables)	df	AIC	ΔAIC
Intercept	4	22,351.4	104.4
Month consumed	15	22,343.4	96.4
Gender	5	22,270.4	23.4
Age	7	22,343.8	96.8
Season distributed	5	22,346.6	99.6
Month consumed + gender	16	22,262.4	15.4
Gender + age	8	22,259.6	12.6
Month consumed + age	18	22,335.6	88.6
Season distributed + month consumed	16	22,338.6	91.6
Season distributed + gender	6	22,265.6	18.6
Season distributed + age	8	22,339	92
Month consumed + gender + age	19	22,251.6	4.6
Month consumed + gender + season distributed	17	22,258	11
Month consumed + gender + age + season distributed	20	22,247	0

4. Discussion

This study produced the first quantitative estimates of acute and chronic human exposure to DA from consuming razor clams in North America. Two populations of recreational shellfish harvesters were identified to be potentially at risk to DA toxicity due to elevated and/or chronic, low level exposure to DA. One group of consumers would be exposed to DA levels above the regulatory reference dose for acute exposure if they harvested clams that had DA concentrations at the upper end of the legal limit. The second group of consumers may be susceptible to potential health impacts from chronic, low level exposure to DA. The majority of consumers had DA exposures below the threshold used by regulatory agencies to determine acceptable levels in razor clams (ARfD = 0.075 mg DA·kg bodyweight^{-1}·d^{-1}) (Wekell et al., 2002).

In the Washington State razor clam fishery, the majority of harvesters who adhere to management guidelines (harvesting clams with DA < 20 mg·kg^{-1} fresh weight for whole tissue) are protected from effects of acute DA exposure, however some may be unknowingly exposed to DA levels above those deemed safe for the general public. Acute DA exposures of the at-risk consumers from our study exceeded the ARfD due to a combination of higher consumption rates, lower bodyweights than those used to create the regulations (6 clams·day^{-1} and 70 kg bodyweight) (Wekell et al., 2002), and consumption of clams with high, but legal DA levels (above approximately 14.7 mg·kg^{-1} fresh weight for whole tissue). It is assumed that most consumers are not aware that the regulations are based on this consumption rate and bodyweight, and thus are unaware when they exceed the ARfD. This study improved upon the previous understanding of razor clam consumption rates (Marien, 1996), by adding additional demographics (gender and age), spatial coverage (multiple beaches), temporal coverage, and using gender- and age-specific bodyweights in the exposure calculations. Importantly, children and young adults (10–20 years of age) had the highest predicted DA exposure due to their lower bodyweights, despite lower consumption rates than other age groups, as has been found in other exposure studies (e.g., Weirich and Miller, 2014). In addition, the survey respondents from the spring distribution had higher consumption rates than those from the winter distribution, indicating a potentially different population of razor clam harvesters. Despite these estimated incidents of exposure exceeding the ARfD, there have been no documented cases of amnesic shellfish poisoning reported in Washington State, potentially due to unrecognized and unreported symptoms, or due to the order of magnitude safety margin incorporated in the ARfD calculation. This safety margin could be increasingly tested in the future as toxic algal blooms are predicted to

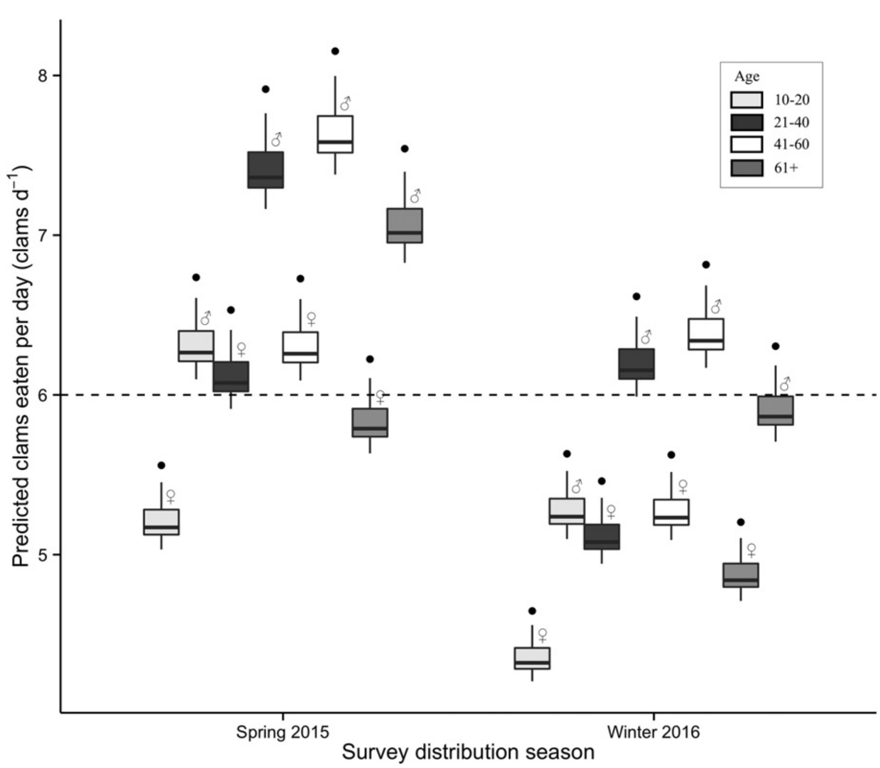

Fig. 4. Predicted consumption rates (clams·d^{-1}), when eating clams, summarizing each month across age group, gender, and season of survey distribution. Colors represent four age groups of 10–20 years (light gray), 21–40 years (dark gray), 41–60 years (white), and 61+ years (medium gray), and patterns represent gender (female = solid, male = hatched). The dashed line represents the clam consumption rate (6 clams·d^{-1}) used as the basis of current regulatory limits. Boxplots represent, first quartile (lower horizontal line), median (middle horizontal line), and third quartile (higher horizontal line). Vertical lines represent data points between the first or third quartile and 1.5 times the interquartile range.

Table 3
Summary of model parameter values at which predicted human DA exposure equals the acute reference dose (ARfD: 0.075 mg·kg^{-1}) for each gender, age group, and survey population.

Age (year)	Gender	Weight (kg)	#Clams eaten per day		Clam DA values (mg·kg^{-1}) at which human exposure = AFrD, calculated using mean (±SE) consumption rates	
			Winter mean (SE)	Spring mean (SE)	Winter population	Spring population
10–20	Female	59.1	4.4 (1.2)	5.2 (1.1)	22.5 (17.7–30.9)	18.8 (15.5–23.8)
	Male	65.8	5.3 (1.2)	6.3 (1.1)	20.7 (16.8–26.8)	17.3 (14.7–21.0)
21–40	Female	74.7	5.1 (1.3)	6.1 (1.2)	24.2 (19.5–32.0)	20.2 (17.0–25.0)
	Male	88.3	6.2 (1.3)	7.4 (1.2)	23.6 (19.6–29.7)	19.7 (17.0–23.5)
41–60	Female	76.1	5.3 (1.3)	6.3 (1.2)	23.9 (19.4–31.3)	20.0 (16.9–24.5)
	Male	92.9	6.4 (1.3)	7.7 (1.2)	24.1 (20.1–30.1)	20.2 (17.5–23.8)
61+	Female	73.9	4.9 (1.3)	5.8 (1.2)	25.1 (20.0–33.8)	21.0 (17.5–26.3)
	Male	89	5.9 (1.3)	7.1 (1.2)	18.6 (16.0–22.1)	20.9 (17.9–25.1)

increase in frequency under future ocean warming conditions (Lewitus et al., 2012; Moore et al., 2008).

The order of magnitude safety factor built into the ARfD is intended to protect consumer groups most sensitive to domoic acid toxicity including young children, pregnant women, elderly populations, and those with renal dysfunction, largely based on evidence from non-human animal models. Postnatal mice in critical stages of brain development have shown increased sensitivity to DA toxicity, resulting in permanent behavioral and histochemical consequences (Doucette et al., 2004). Prenatal DA exposure has led to memory loss, learning deficits, hippocampal degradation, long-lasting neurological and behavioral effects in rats, and reproductive failure in California sea lions (Dakshinamurti et al., 1993; Doucette et al., 2000; Goldstein et al., 2009; Levin et al., 2005; Maucher and Ramsdell, 2007). Elderly consumers had higher susceptibility to DA toxicity in the 1987 Canadian DA poisoning event summarized by Perl et al. (1990) and aged rats have shown higher susceptibility to DA toxicity, potentially due to impaired renal function, increased blood-brain barrier permeability, or loss of intrinsic neuroprotective mechanisms during brain aging (Hesp et al., 2007; Hesp et al., 2004; Kerr et al., 2002). Renal filtration is the primary route for DA elimination and impaired renal function can increase DA toxicity in mice and rats (Funk et al., 2014; Suzuki and Hierlihy, 1993).

While the human health effects of chronic, low level DA exposure are still being determined, this study demonstrates that some recreational clam harvesters consume clams at rates that meet and exceed existing chronic exposure thresholds (Grattan et al., 2016). Extrapolating our survey results to the 397,000 recreational diggers in the 2014–2015 WA season, approximately 11,910 razor clam harvesters were chronically exposed to low levels of DA as defined as 15 clams per month for 12 months (Ayres, 2015b). Of additional concern, a subset of the chronically exposed population would also be at risk to elevated exposure above the ARfD, due to consumption rates higher than the average used to base existing regulations (6 clams·day^{-1}) during periods

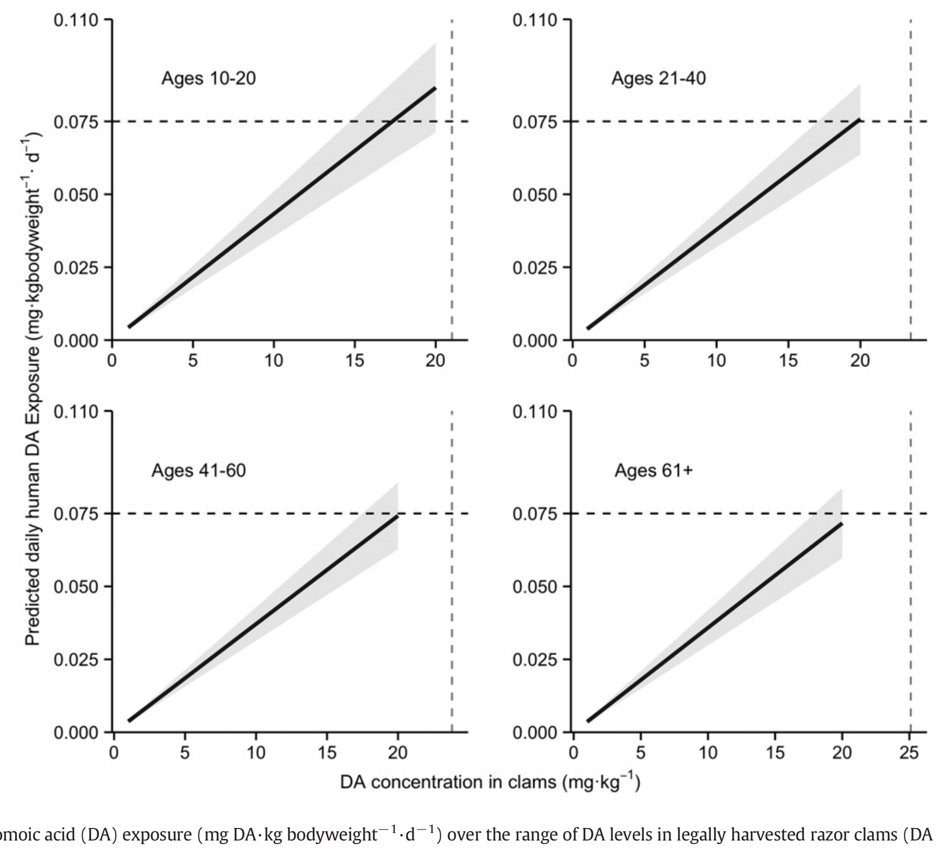

Fig. 5. Predicted daily oral domoic acid (DA) exposure (mg DA·kg bodyweight^{-1}·d^{-1}) over the range of DA levels in legally harvested razor clams (DA = 1–20 mg·kg·d^{-1}) for male consumers from the Spring 2015 survey population across all age groups: (a) 10–20 years, (b) 21–40 years, (c) 41–60 years, and (d) 61+ years. The shaded areas represent ± one standard error. The dashed, horizontal line is the acute reference dose (ARfD: 0.075 mg DA·kg bodyweight^{-1}·d^{-1}) for human DA exposure used by US management agencies. The dashed, vertical line denotes the DA value in clams at which that consumer group exceeds the ARfD.

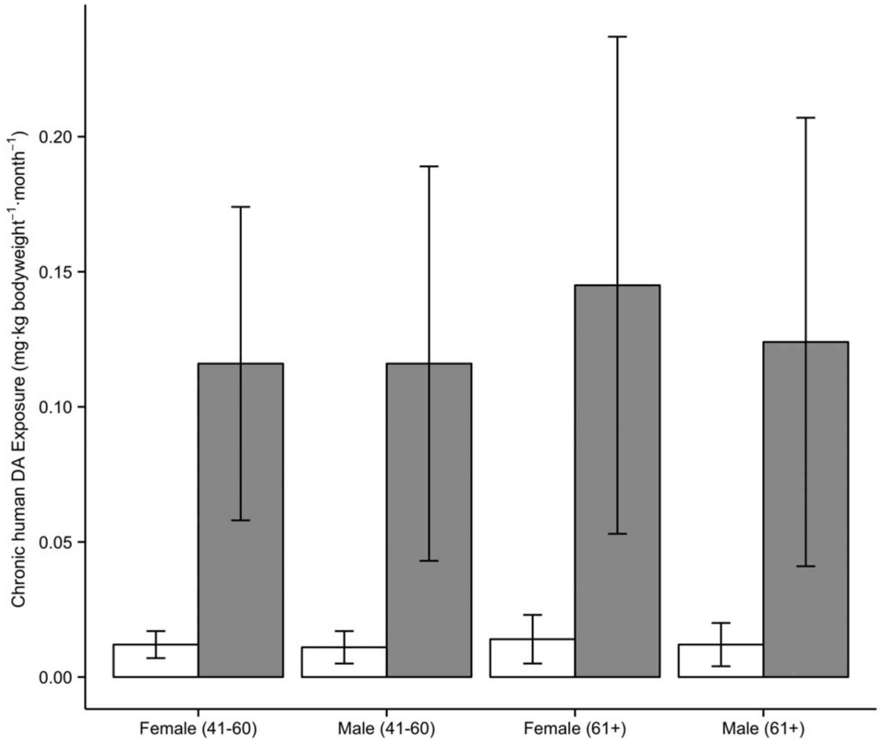

Fig. 6. Average (±SD) monthly DA exposure (mg DA·kg bodyweight^{-1}·d^{-1}) for chronic consumers (eating 15 clams per month for 12 months) from May 2014–April May 2015 (prior to the DA bloom: white) and after the fishery reopened from January–Dec, 2016 (after the DA bloom: black).

of elevated DA levels in clams. Limited studies have shown the potential for human health impacts from chronic, low level DA exposure. Repetitive, low dose experiments simulating chronic exposure have shown increased toxin susceptibility and impaired mitochondrial function in zebrafish after 20 weeks and 18 weeks respectively (Hiolski et al., 2014; Lefebvre et al., 2012). Truelove et al. (1996) and Truelove et al. (1997) found no signs of toxicity after repeated, low dose DA exposure in cynomolgus monkeys (30 day exposure) and rats (64 day exposure), although these studies focused on traditional clinical indicators of toxicity such as gross histologic lesions and blood chemistry. In the first human chronic exposure study, Grattan et al. (2016) assessed cognitive function and clam consumption rates of subsistence razor clam harvesters in Washington State over four years and determined memory impairment was associated with consumption of 15 or more razor clams per month over twelve months. However, the authors question the clinical significance of this finding as the memory impairment was within the normal range of memory measures. We could not directly compare our estimated DA exposure levels of chronically exposed consumers to Grattan et al. (2016), as they did not publish the DA exposure levels in their human study.

Obtaining accurate seafood consumption rates that reflect the diversity of consumers is essential to protecting the population from exposure to related toxins (Andjelkovic et al., 2012; Burger and Gochfeld, 2011; O'Neill, 2000; The Suquamish Tribe, 2000; US Environmental Protection Agency, 1988; WA Department of Ecology, 2013). Exposure to unsafe levels of toxins due to elevated consumption rates has been documented among other recreational fisher communities (Burger, 2013; Burger et al., 1999; Mayfield et al., 2007; Picot et al., 2013) and the US Environmental Protection Agency has recognized the need for seafood consumption rates that represent broader demographics (US Environmental Protection Agency, 2014). Unrepresentative data can lead to a lack of confidence in fisheries management, resulting in harvesters consuming seafood at rates that will result in higher than recommended toxin exposure levels (Roberts et al., 2016). Conversely, harvesters may reduce their seafood consumption due to unwarranted safety concerns, with potential adverse effects on personal health (Beehler et al., 2002; Burger, 2013; Burger and Gochfeld, 2009). Our study provided preliminary DA exposure estimates of age and gender subgroups within the recreational population, however the survey design did not obtain a representative sample of all demographics. Our survey population did not include equal representation from minorities, Native Americans, and younger demographics (<40 years of age). We were also dependent on harvesters returning the survey to us, biasing the results towards those who have the interest and ability to participate in the study. Now that the presence of acute and chronic exposure has been identified in this population, a more structured follow-up survey should be conducted to obtain more accurate exposure estimates across the diversity of the population.

5. Conclusions

Current management guidelines for human DA exposure via razor clams are based on consumption rates and bodyweights that do not reflect the diverse recreational harvesting community. The inclusion of demographically and temporally specific data in DA exposure calculations resulted in the identification of recreational razor clam harvester groups with estimated acute DA exposures higher than the current ARfD. A group of harvesters was also identified that was chronically exposed to low levels of DA over multiple months, reinforcing the importance of investigating the effects of repetitive, low level DA exposure. While this study provides valuable, first estimates of the average DA exposures of recreational razor clam harvesters, biases in the survey participants suggest the need for a structured follow up study to inform policy recommendations. The extent of these two types of exposures across the diversity of clam harvesters can be determined by a more intensive, structured study that ensures adequate representation of all consumer groups. It will become increasingly important to understand and communicate the limits of environmental safety regulations as ocean conditions change and toxic algal blooms become more frequent (Wells, 2015).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2017.01.006.

Acknowledgements

This study was supported by an NIH/NSF Ocean's and Human Health Program (R01ES021930/OCE1314088) to DJM/KAL and the NIH supplement (RES021930A) to BF. The clam consumption surveys were distributed by the Washington Department of Fish and Wildlife staff, including: John Deibert, Clayton Parson, John Beck, Bruce Kauffman, Travis Haring, Zack Forster, Robert Morgan, Jamie Fuller, Kyron Dierick, Carol Henry. Daniel Huppert and Tom Leschine contributed valuable comments to the consumption survey design.

References

Andjelkovic, M., Vandevijvere, S., Van Klaveren, J., Van Oyen, H., Van Loco, J., 2012. Exposure to domoic acid through shellfish consumption in Belgium. Environ. Int. 49, 115–119.

Ayres, D., 2015a. US congressional briefing on 2015 west coast HAB event. November 6, 2015. https://www.researchgate.net/profile/Dan_Ayres/publications (accessed May 13, 2016).

Ayres, D., 2015b. Washington Razor Clam Management: Setting the 2015–2016 Season. WA Department of Fish and Wildlife:p. 69 (Available at http://wdfwwagov/fishing/shellfish/razorclams/2015_razor_clam_season_settingpdf).

Beehler, G.P., Weiner, J.M., McCann, S.E., Vena, J.E., Sandberg, D.E., 2002. Identification of sport fish consumption patterns in families of recreational anglers through factor analysis. Environ. Res. 89, 19–28.

Bejarano, A.C., VanDola, F.M., Gulland, F.M., Rowles, T.K., Schwacke, L.H., 2008. Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a review. Hum. Ecol. Risk. Assess. 14, 544–567.

Burger, J., 2013. Role of self-caught fish in total fish consumption rates for recreational fishermen: average consumption for some species exceeds allowable intake. J. Risk Res. 16, 1057–1075.

Burger, J., Gochfeld, M., 2009. Perceptions of the risks and benefits of fish consumption: individual choices to reduce risk and increase health benefits. Environ. Res. 109, 343–349.

Burger, J., Gochfeld, M., 2011. Conceptual environmental justice model for evaluating chemical pathways of exposure in low-income, minority, Native American, and other unique exposure populations. Am. J. Public Health 101, S64–S73.

Burger, J., Stephens, W.L., Boring, C.S., Kuklinski, M., Gibbons, J.W., Gochfeld, M., 1999. Factors in exposure assessment: ethnic and socioeconomic differences in fishing and consumption of fish caught along the Savannah River. Risk Anal. 19, 427–438.

Cook, P.F., Reichmuth, C., Rouse, A.A., Libby, L.A., Dennison, S.E., Carmichael, O.T., Kruse-Elliott, K.T., Bloom, J., Singh, B., Fravel, V.A., Barbosa, L., Stuppino, J.J., Van Bonn, W.G., Gulland, F.M.D., Ranganath, C., 2015. Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for strandings. Science 350, 1545–1547.

Dakshinamurti, K., Sharma, S.K., Sundaram, M., Watanabe, T., 1993. Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. J. Neurosci. 13, 4486–4495.

Donatuto, J., Harper, B., 2008. Issues in evaluating fish consumption rates for Native American tribes. Risk Anal. 28.

Doucette, T.A., Strain, S.M., Allen, G.V., Ryan, C.L., Tasker, R.A.R., 2000. Comparative behavioural toxicity of domoic acid and kainic acid in neonatal rats. Neurotoxicol. Teratol. 22, 863–869.

Doucette, T.A., Bernard, P.B., Husum, H., Perry, M.A., Ryan, C.L., Tasker, R.A., 2004. Low doses of domoic acid during postnatal development produce permanent changes in rat behaviour and hippocampal morphology. Neurotox. Res. 6, 555–563.

Dyson, K., Huppert, D.D., 2010. Regional economic impacts of razor clam beach closures due to harmful algal blooms (HABs) on the Pacific coast of Washington. Harmful Algae 9, 264–271.

Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – domoic acidEFSA J. 1181, 1–61.

Fryar, C., Gu, Q., Ogden, C., 2012. Anthropometric reference data for children and adults: United States, 2007–2010. National Center for Health Statistics Vital Health Stat, p. 11.

Funk, J.A., Janech, M.G., Dillon, J.C., Bissler, J.J., Siroky, B.J., Bell, P.D., 2014. Characterization of renal toxicity in mice administered the marine biotoxin domoic acid. J. Am. Soc. Nephrol. 25, 1187–1197.

Gill, D.A., Bastlund, J.F., Watson, W.P., Ryan, C.L., Reynolds, D.S., Tasker, R.A., 2010. Neonatal exposure to low-dose domoic acid lowers seizure threshold in adult rats. Neuroscience 169, 1789–1799.

Gill, D.A., Perry, M.A., McGuire, E.P., Perez-Gomez, A., Tasker, R.A., 2012. Low-dose neonatal domoic acid causes persistent changes in behavioural and molecular indicators of stress response in rats. Behav. Brain Res. 230, 409–417.

Gochfeld, M., Burger, J., 2011. Disproportionate exposures in environmental justice and other populations: the importance of outliers. Am. J. Public Health 101 (Suppl. 1), S53–S63.

Goldstein, T., Mazet, J.A.K., Zabka, T.S., Langlois, G., Colegrove, K.M., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P.A., Barakos, J., Williams, D.C., Dennison, S., Haulena, M., Gulland, F.M.D., 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. Proc. R. Soc. B Biol. Sci. 275, 267–276.

Goldstein, T., Zabka, T.S., DeLong, R.L., Wheeler, E.A., Ylitalo, G., Bargu, S., Silver, M., Leighfield, T., Van Dolah, F., Langlois, G., Sidor, I., Dunn, J.L., Gulland, F.M.D., 2009. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. J Wildl Dis 45, 91–108.

Grattan, L.M., Boushey, C., Tracy, K., Trainer, V., Roberts, S.M., Schluterman, N., Morris Jr., J.G., 2016. The association between razor clam consumption and memory in the CoASTAL cohort. Harmful Algae 57, 20–25.

Hesp, B.R., Wrightson, T., Mullaney, I., Kerr, D.S., 2004. Kainate receptor agonists and antagonists mediate tolerance to kainic acid and reduce high-affinity GTPase activity in young, but not aged, rat hippocampus. J. Neurochem. 90, 70–79.

Hesp, B.R., Clarkson, A.N., Sawant, P.M., Kerr, D.S., 2007. Domoic acid preconditioning and seizure induction in young and aged rats. Epilepsy Res. 76, 103–112.

Hiolski, E.M., Kendrick, P.S., Frame, E.R., Myers, M.S., Bammler, T.K., Beyer, R.P., Farin, F.M., Wilkerson, H.W., Smith, D.R., Marcinek, D.J., Lefebvre, K.A., 2014. Chronic low-level domoic acid exposure alters gene transcription and impairs mitochondrial function in the CNS. Aquat. Toxicol. 155, 151–159.

Kerr, D.S., Razak, A., Crawford, N., 2002. Age-related changes in tolerance to the marine algal excitotoxin domoic acid. Neuropharmacology 43, 357–366.

Lefebvre, K.A., Robertson, A., 2010. Domoic acid and human exposure risks: a review. Toxicon 56, 218–230.

Lefebvre, K.A., Frame, E.R., Gulland, F., Hansen, J.D., Kendrick, P.S., Beyer, R.P., Bammler, T.K., Farin, F.M., Hiolski, E.M., Smith, D.R., Marcinek, D.J., 2012. A novel antibody-based biomarker for chronic algal toxin exposure and sub-acute neurotoxicity. PLoS One 7.

Levin, E.D., Pizarro, K., Pang, W.G., Harrison, J., Ramsdell, J.S., 2005. Persisting behavioral consequences of prenatal domoic acid exposure in rats. Neurotoxicol. Teratol. 27, 719–725.

Lewitus, A.J., Horner, R.A., Caron, D.A., Garcia-Mendoza, E., Hickey, B.M., Hunter, M., Huppert, D.D., Kudela, R.M., Langlois, G.W., Largier, J.L., Lessard, E.J., RaLonde, R., Rensel, J.E.J., Strutton, P.G., Trainer, V.L., Tweddle, J.F., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. Harmful Algae 19, 133–159.

Marien, K., 1996. Establishing tolerable dungeness crab (*Cancer magister*) and razor clam (*Siliqua patula*) domoic acid contaminant levels. Environ. Health Perspect. 104, 1230–1236.

Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J., Tyre, A.J., Possingham, H.P., 2005. Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecol. Lett. 8, 1235–1246.

Maucher, J.M., Ramsdell, J.S., 2007. Maternal-fetal transfer of domoic acid in rats at two gestational time points. Environ. Health Perspect. 115, 1743–1746.

Mayfield, D.B., Robinson, S., Simmonds, J., 2007. Survey of fish consumption patterns of King County (Washington) recreational anglers. J. Expo. Sci. Environ. Epidemiol. 17, 604–612.

McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D., Gulland, F.M.D., Thomson, R.E., Cochlan, W.P., Trainer, V.L., 2016. An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. Geophys. Res. Lett. 43.

Moore, S.K., Trainer, V.L., Mantua, N.J., Parker, M.S., Laws, E.A., Backer, L.C., Fleming, L.E., 2008. Impacts of climate variability and future climate change on harmful algal blooms and human health. Environ. Health 7, 12.

Muha, N., Ramsdell, J.S., 2011. Domoic acid induced seizures progress to a chronic state of epilepsy in rats. Toxicon 57, 168–171.

O'Neill, C.A., 2000. Variable justice: environmental standards, contaminated fish, and "acceptable" risk to native peoples. Stanford Environ. Law J. 19, 46–51.

Perl, T.M., Bedard, L., Kosatsky, T., Hockin, J.C., Todd, E.C.D., Remis, R.S., 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. N. Engl. J. Med. 322, 1775–1780.

Picot, C., Limon, G., Durand, G., Parent-Massin, D., Roudot, A.C., 2013. Probabilistic dietary exposure to phycotoxins in a recreational shellfish harvester subpopulation (France). J. Expo. Sci. Environ. Epidemiol. 23, 435–441.

Quilliam, M.A., Xie, M., Hardstaff, W.R., 1991. A rapid extraction and clean-up procedure for the determination of domoic acid in tissue samples. Technical Report 64. National Research Council of Canada, Institute for Marine Biosciences, Halifax, NS.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (ISBN 3-900051-07-0, URL http://www.R-project.org/).

Roberts, S.M., Grattan, L.M., Toben, A.C., Ausherman, C., Trainer, V.L., Tracy, K., Morris, J.G., 2016. Perception of risk for domoic acid related health problems: a cross-cultural study. Harmful Algae 57, 39–44.

Schwarz, M., Jandová, K., Struk, I., Marešová, D., Pokorný, J., Riljak, V., 2014. Low dose domoic acid influences spontaneous behavior in adult rats. Physiol. Res. 63, 369–376.

Suzuki, C.A., Hierlihy, S.L., 1993. Renal clearance of domoic acid in the rat. Food Chem. Toxicol. 31, 701–706.

The Suquamish Tribe, 2000. Fish Consumption Survey of the Suquamish Tribe of the Port Madison Indian Reservations, Puget Sound Region. The Suquamish Tribe, 15838 Sandy Hook Road, Post Office Box 498, Suquamish, WA 98392.

Toth, J.F., Brown, R.B., 1997. Racial and gender meanings of why people participate in recreational fishing. Leis. Sci. 19, 129–146.

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., Trick, C.G., 2012. Pseudo-nitzschia physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14, 271–300.

Truelove, J., Mueller, R., Pulido, O., Iverson, F., 1996. Subchronic toxicity study of domoic acid in the rat. Food Chem. Toxicol. 34, 525–529.

Truelove, J., Mueller, R., Pulido, O., Martin, L., Fernie, S., Iverson, F., 1997. 30-Day oral toxicity study of domoic acid in cynomolgus monkeys: lack of overt toxicity at doses approaching the acute toxic dose. Nat. Toxins 5, 111–114.

US Environmental Protection Agency, 1988. Guidance for conducting fish and wildlife consumption surveys. Report No EPA-823-B-98-007. US Environmental Protection Agency, Washington, DC.

US Environmental Protection Agency, 2014. Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003–2010) EPA-820-R-14-002. US Environmental Protection Agency, Washington DC.
WA Department of Ecology, 2013. Fish consumption rates: a review of data and information about fish consumption in Washington. Publication no 12-09-058. Available at. wwwecywagov/biblio/1209058html.
Weirich, C.A., Miller, T.R., 2014. Freshwater harmful algal blooms: toxins and children's health. Curr. Probl. Pediatr. Adolesc. Health Care 44, 2–24.
Wekell, J.C., Gauglitz, E.J., Barnett, H.J., Hatfield, C.L., Eklund, M., 1994. The occurrence of domoic acid in razor clams (*Siliqua patula*), dungenss crab (*Cancer magister*), and anchovies (*Engrauls mordax*). J. Shellfish Res. 13, 587–593.
Wekell, J.C., Trainer, V.L., Ayres, D., Simons, D., 2002. A study of spatial variability of domoic acid in razor clams: recommendations for resource management on the Washington coast. Harmful Algae 1, 35–43.
Wekell, J.C., Hurst, J., Lefebvre, K.A., 2004. The origin of the regulatory limits for PSP and ASP toxins in shellfish. J. Shellfish Res. 23, 927–930.
Wells, M.L., 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. Harmful Algae 49.
Zeileis, A., 2008. Regression models for count data in R. J. Stat. Softw. 27.
Zuur, A., 2009. Mixed Effects Models and Extensions in Ecology With R (Statistics for Biology and Health) ed^eds. Springer, New York, NY.