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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

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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).

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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,