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Exposure assessment of the biotoxin domoic acid in California sea lions: application of a bioenergetic model

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ABSTRACT: The biotoxin domoic acid (DA), produced by diatoms of the genus *Pseudo-nitzschia*, has caused massive California sea lion *Zalophus californianus* mortalities and live strandings along the California coast. Since quantifying the field DA dose that causes toxic effects in sea lions is logistically difficult, a bioenergetic model that uses age/sex-specific energy requirements was developed to estimate DA doses, assuming ingestion of 2 important vector species: anchovies *Engraulis mordax* and sardines *Sardinops sagax*. In this model, uncertainty and variability were incorporated by assigning sampling distributions to each model variable. Variables included: (1) vector energy density and assimilation efficiency of gross fish energy; (2) sea lion weight and energy requirements adjusted for energy expenditures associated with foraging, growth and reproduction; and (3) DA concentration in the vector species. Model outputs were analyzed relative to thresholds that cause adverse effects in other mammal species (1 and 2.71 mg DA kg^{-1} body weight). Based on DA concentrations measured in fish during a previous *Pseudo-nitzschia* bloom, consumption of anchovies versus sardines as 20% of the sea lions' daily intake would result in a 4-fold increase in risk of non-lethal toxic effects. Across age classes, the median DA dose in pups (7 to 12 mo old) was twice that estimated for juveniles and was between 2 and 4 times greater than for adults. In DA dose estimates most of the variability resulted from the uncertainty associated with the energy density of the vector species and DA concentration in sardines rather than from the uncertainty associated with sea lions. Finally, we highlight the most relevant areas of research needed for determining a definitive risk of sea lion exposure to DA.

KEY WORDS: Domoic acid · Exposure assessment · California sea lion · Probabilistic model

INTRODUCTION

Massive marine mammal mortalities have been linked to the occurrence of harmful algal blooms dominated by marine diatoms of the genus *Pseudo-nitzschia* (Gulland 2000, Scholin et al. 2000). *Pseudo-nitzschia* produces and releases the phycotoxin domoic acid (DA), a tricarboxylate amino acid analog of the excitatory neurotransmitter L-glutamate that binds with high affinity to kainate and amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) subtypes of the glutamate receptors. DA activation of kainite/AMPA receptors results in the release of glutamate and other excitatory amino acids, followed by the secondary activation of Ca^+-conducting N-methyl-D-aspartate (NMDA) subtype of glutamate receptors and L-type

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voltage-dependent calcium channels, as well as reversal of the Na^+/Ca^+ exchanger (Berman & Murray 1997). As a result, a cascade of Ca^+-dependent events is initiated, causing neuronal damage and neurodegeneration, particularly in glutaminergic tracks of the hippocampus. In humans, DA is responsible for the excitotoxic syndrome known as amnesic shellfish poisoning (ASP), so-named for its associated short-term memory loss due to hippocampal damage (Perl et al. 1990).

The onset of frequent *Pseudo-nitzschia* blooms coincides with a regime shift in the north Pacific Ocean that occurred in the mid-1990s (Chavez et al. 2003), favoring cooler water, stronger upwelling with increased nutrient input expected to support greater phytoplankton productivity, and a larger northern anchovy *Engraulis mordax* population in the eastern Pacific. The predicted 25 yr tenure of this cycle suggests that such events may become routine on the California coast, with unknown consequences to susceptible animal populations. Toxic blooms of *Pseudo-nitzschia* have occurred almost annually on the California coast since 1998, with each event associated with extensive deaths and live strandings of California sea lions *Zalophus californianus* and reproductive failure in surviving females (Gulland 2000, Scholin et al. 2000, Brodie et al. 2006). The Marine Mammal Center (TMMC) reported the stranding of 70 and 184 sea lions in 1998 and 2000, respectively, with typical symptoms of DA intoxication (seizures, ataxia and scratching behavior, brain and heart lesions; Gulland 2000). Some of these individuals showed quantifiable amounts of DA in body fluids (i.e. urine, serum, and feces). Anchovies and krill (euphausiids) are 2 of the identified trophic vectors of DA from primary producers to higher trophic levels such as squid, salmon, marine birds, and marine mammals (Fritz et al. 1992, Lefebvre et al. 1999, 2002a, Scholin et al. 2000). In the case of sea lions, the presence of *Pseudo-nitzschia australis* frustules and anchovy remains in sea lion feces and the simultaneous detection of DA in *P. australis* cells and anchovy body fluids, along with the temporal and spatial association of toxic *Pseudo-nitzschia* blooms with these mass mortalities provide strong evidence for DA trophic transfer from anchovies (Gulland 2000, Scholin et al. 2000).

Despite a decrease in the number of pup births during the 1983 and 1992 El Niño events (36 and 26%, respectively) (DeLong et al. 1991), the California sea lion population increased steadily from 145 000 individuals in 1983 to ~170 000 in 1994 (Barlow et al. 1995). Recent estimates indicate that the size of the sea lion population ranges between 237 000 and 244 000 individuals (US EPA 2005), with an estimated 5.2% annual increase in pup birth rates (Barlow et al. 1995, Lowry & Maravilla-Chavez 2005). However, the long-term effects on the sea lion population resulting from annual acute exposures to DA remain largely unknown.

The purpose of the present study was to develop the exposure component of an ecological risk assessment specific for California sea lions exposed to DA. In order to assess the effects of DA on the California sea lion population, it is essential to first estimate the DA dose that would result from the ingestion of potential DA vectors. Although the diet of sea lions is diverse and many prey species may serve as DA vectors, this research focuses primarily on Pacific sardines *Sardinops sagax* and northern anchovies *Engraulis mordax* as the primary vectors. These 2 species, in which measured DA has been higher than that of other prey (Lefebvre et al. 2002b), occupy the lowest trophic level among all prey species, thus linking directly the DA-producing diatoms and sea lions. To estimate the levels of DA in sea lions, we utilized a bioenergetic model coupled with measured concentrations of DA in these 2 vectors. Models of this kind are not only cost effective, but also are useful in that they help to conceptualize complex systems (Winship et al. 2002). This particular bioenergetic model estimated food consumption based on 2 important components: growth and metabolism (Kleiber 1975). Using this approach, we developed age/sex-specific estimates of DA dose in sea lions preying on anchovies and sardines. We employed a probabilistic bioenergetic model, and incorporated model and parameter uncertainties by means of Monte Carlo analysis.

MATERIALS AND METHODS

Domoic acid receptor species. California sea lions *Zalophus californianus* are found along the Pacific coast of North America from British Columbia, Canada, to southern Mexico (Odell 1981). Their average life span is 20 yr, with sexual maturity being reached between the 3rd and 5th yr (Riedman 1990) and female and male adulthood being reached at the ages of 5 and 8, respectively (TMMC unpubl. data). Information on length and weight for 680 female and 620 male California sea lions extracted from the TMMC database were used to generate sex-specific length–weight curves of the form: $\mathrm{Ln}(W) = b_0 + b_1 \times \mathrm{Ln}(L)$, where W and L represent the sea lion's weight and length, respectively. These regressions (with their associated standard deviations) plus age–length relationships from Greig et al. (2005) were incorporated into a probabilistic model (see below) to generate age/sex-specific weights. Weights drawn from a normal distribution for each age/sex class were then used to estimate daily energy requirements. Age stages were defined according to TMMC guidelines as follows: pup, <1 yr old; juvenile female, 1 to <5 yr old; juvenile male, 1 to <8 yr old; adult female, 5 to 20 yr old, and adult male, 8 to 20 yr old. Female pregnancy and lactation costs were assumed to start at the age of 5, while males were assumed to be reproductively active at the age of 8 (Reidman 1990, TMMC unpubl. data).

Domoic acid vector species. California sea lions are opportunistic feeders, switching prey species depending on prey distribution and abundance (Antonelis et al. 1984). The diet of sea lions is comprised of >20 different prey items (anchovies, sardines, mackerel, rockfish, hake, squid, among others; Lowry et al. 1990), many of which may serve as domoic acid (DA) vectors. However, our research focused primarily on anchovies and sardines, planktivorous fish that, relative to other prey species, have been found to contain the highest amounts of DA in viscera (Lefebvre et al. 2002b). The contribution of these 2 prey items to the diet of California sea lions is highly variable depending on season and environmental variables (i.e. sea surface temperature; Costa et al. 1991, DeLong et al. 1991, Chavez et al. 2003). To determine the sensitivity of DA estimates to assumptions of prey proportion for a sea lion's daily diet, we simulated daily dietary contributions of anchovies and sardines between 10 and 100% in 10% increments. Mean concentration of DA in these species during a peak hypothetical algal bloom episode were assumed to be similar to those found by Lefebvre et al. (2002b) during September of 2000 in Monterey Bay (136.53 ± 35.67 µg DA g^{-1} anchovy and 45.89 ± 13.00 µg DA g^{-1} sardine).

Description of the exposure model. Comprehensive bioenergetic models estimate food consumption based on growth, reproduction, metabolism, and digestion (see Winship et al. 2002). For the purpose of our study we opted to use a simpler model based primarily on metabolism. Kleiber (1975) established that across terrestrial and marine mammal species the basal metabolic rate (BMR, also referred to as standard metabolic rate; Worthy 2001) or the energy required to sustain life (i.e. metabolic activity of tissues and cells, as well as the circulation, respiration, and gastrointestinal processes) is a function of body mass. Kleibers' BMR (kcal d^{-1}) for a resting adult mammal at its thermally neutral zone is described as: $\text{BMR} = 70 \times M^{0.75}$, where M is body mass (kg). Modifications to this equation have been suggested to compensate for food habits (McNab 1986) and intestinal length (Williams et al. 2001). Although we recognize the value of the above-mentioned models, we selected the model proposed by Kleiber (Kleiber 1975) and incorporated additional energy expenditures associated with various physiological processes (Costa et al. 1991, Worthy 2001, Winship et al. 2002). Additional expenditures included costs resulting from foraging trips at sea (Costa et al. 1991), growth (i.e. pup and juvenile growth), and reproduction (i.e. pregnancy and lactation; Worthy 2001, Winship et al. 2002) (Table 1).

To estimate an acute (i.e. single day) DA dose in sea lions, the daily food intake (DFI, kg) of a given prey species was calculated as: $\text{DFI} = P_i \times (\text{BMR}/\text{ED}_i) \times F_{\text{ae-}i}$ where P_i represents the proportion of the diet comprised by prey item i (i.e. either anchovy or sardine), ED the energy density of prey item i (kcal g^{-1}), and $F_{\text{ae-}i}$ the prey item i assimilation efficiency of gross fish energy (%). The daily DA dose (mg kg^{-1}) in sea lions was calculated as: $\text{DA dose} = (\text{DFI} \times \text{DA}_i)/W$, where DA_i represents the amount of DA in prey item i (µg DA

Table 1. *Zalophus californianus*. Input parameters associated with California sea lions. F: female; M: male; W: weight (kg); L: standard length (cm); a: age (yr); BMR: basal metabolic rate

Input parameters	Equation	Source
Age vs. length relationship	F: $L = 166.4139 \times (1 - e^{-0.3523 \times [a - (-1.5988)]})$ M: $L = 278.6571 \times (1 - e^{-0.0882 \times [a - (-4.6688)]})$	Greig et al. (2005)
Length vs. weight relationship	$\text{Ln}(W) = b_0 + b_1 \times \text{Ln}(L)$ F: $b_0 = -10.92 \pm 0.19$; $b_1 = 2.99 \pm 0.04$; $r^2 = 0.90$ M: $b_0 = -9.78 \pm 0.19$; $b_1 = 2.74 \pm 0.04$; $r^2 = 0.89$	Present paper
Additional cost for pup growth (7 to 12 mo) (kcal d^{-1})	3.5 (down to 2.5) × BMR—linear decrease with increasing age	Winship et al. (2002)
Additional cost for juvenile growth (F: 1 to <5 yr; M: 1 to <8 yr) (kcal d^{-1})	1.75 (down to 1) × BMR—linear decrease with increasing age	Modified from Winship et al. (2002)
Additional cost for pregnancy (kcal d^{-1})	1.2 × BMR (min.–max.: 1.1–1.7 × BMR)	Worthy (2001), Winship et al. (2002)
Additional cost for lactation (kcal d^{-1})	1.75 × BMR (min.–max.: 1.6–1.95 × BMR)	Worthy (2001), Winship et al. (2002)
Additional cost for at-sea activities (kcal d^{-1})	4.8 × BMR	Costa et al. (1991)

g^{-1} fish), and W the sea lion's body weight (kg body wt) (Table 2). Estimated DA doses in sea lions are presented herein as mg kg^{-1} body wt d^{-1}.

In order to assess critical levels of DA doses, we compared the estimated DA doses in sea lions to oral threshold doses from surrogate species at which we would expect to see toxic effects. Specifically, we used a non-lethal threshold of 1 mg kg^{-1} body wt, which is the oral dose shown to cause vomiting in monkeys (Truelove et al. 1997), and a lethal threshold of 2.71 mg kg^{-1} body wt (modified from Perl et al. 1990). The latter threshold was estimated, via logistic regression (Logit link; Statistica), as the mid-point between the highest DA doses that caused acute gastrointestinal symptoms in humans (i.e. subacute cases) and the DA doses that caused toxic effects requiring hospitalization or resulting in admission to the intensive care unit (i.e. severe cases; Perl et al. 1990). These human cases showed signs of DA intoxication hours after exposure. For the computation of this threshold, we assumed an average body weight of 70 kg. Modeled doses at or above 1 mg kg^{-1} body wt were considered to cause non-lethal effects in sea lions, while doses at or above 2.71 mg kg^{-1} body wt were considered to cause lethal effects.

Probabilistic approach. A probability approach, as advocated by the US Environmental Protection Agency (US EPA 1992), was employed for the exposure component of this ecological risk assessment. Specifically, Monte Carlo simulation was used to incorporate uncertainty by assigning probability distributions, based on the information available in the literature, to each of the input parameters used to estimate DA daily doses (Table 2). Input parameter distributions were assigned as follows: normal for variables that are symmetric around the mean; uniform for variables bounded by a lower and upper point estimates; triangular for variables with an estimate of central tendency bounded by a lower and upper limits; log-normal for right-skewed variables with a lower limit of zero and no upper bound; point estimate for variables for which data are limited or unavailable. DA doses, applying the equation described above, were generated by drawing 1000 values randomly from each of the assigned distributions per input variable.

Simulation description. With this model we are not trying to address the temporal and spatial variability associated with either sea lions or the vector species. Therefore, for simulation purposes, we assumed a worst case scenario of overlapping distribution of sea lions and fish containing the DA. Also, simulations were conducted under the assumption that sea lions are exposed to DA through ingestion of fish containing the biotoxin during foraging trips. Thus, we first used the bioenergetic model to evaluate the sea lion's at-sea energy expenditures for all age/sex classes. Further, we estimated the amount of each vector species, on a sea lion body weight base, necessary to maintain their estimated at-sea requirements.

Table 2. *Zalophus californianus*. Input parameters used in the probabilistic exposure assessment of domoic acid (DA) toxicity to California sea lions. Mean ± 1 SD

Input parameter	Specific parameter	Distribution	Value	Source
Vector species				
Anchovy	Percent diet (%)	Uniform	10–100	
	Energy density (kcal g^{-1})	Uniform	0.73–2.808	Mollet et al. (2002), Benoit-Bird (2004)
	Assimilation efficiency (%)[a]	Point estimate	91.6	
Sardine	Percent diet (%)	Uniform	10–100	Worthy (2001)
	Energy density (kcal g^{-1})	Uniform	0.97–2.3	Mollet et al. (2002), Benoit-Bird (2004)
	Assimilation efficiency (%)[a]	Point estimate	91.6[b]	
California sea lion	Weight (kg)	Normal	See Table 1	See Table 1
Additional costs				
	Growth (kcal d^{-1})	Point estimate	See Table 1	Winship et al. (2002)
	Pregnancy (kcal d^{-1})	Triangular	See Table 1	Worthy (2001), Winship et al. (2002)
	Lactation (kcal d^{-1})	Triangular	See Table 1	Worthy (2001), Winship et al. (2002)
Domoic acid				
	Concentration in anchovies (µg DA g^{-1} fish)	Log-normal	136.53 ± 35.67	Lefebvre et al. (2002b)
	Concentration in sardines (µg DA g^{-1} fish)	Log-normal	45.89 ± 13.00	Lefebvre et al. (2002b)

[a] Assimilation efficiency of gross fish energy; [b] Assimilation efficiency of sardines was assumed to be equal to that of anchovies

Simulation scenarios (1000 iterations per case) were evaluated using the exposure model described above. A first scenario assessed the influence of the proportion of either anchovy or sardine (i.e. 10 to 100% of the daily diet in 10% increments) on the DA dose in sea lions. Simulations were performed using a single age/sex class, specifically 10 yr old, non-lactating, non-pregnant adult females. Since we assumed that sea lions are exposed to DA through ingestion of fish containing DA during foraging trips, simulations were performed correcting for energy requirements of at-sea expenditures (see Table 1). In a second scenario, we estimated the DA dose across all age/sex classes, assuming that exposure to DA results solely from direct ingestion of the vector species. In the case of pups, since we did not account for maternal transfer of DA through lactation, only older pups (7 to 12 mo old) were considered in our simulations. Melin (1995) stated that sea lion pups approaching weaning (i.e. 7 to 12 mo old) ingest solid food, which suggests that direct exposure to DA through vector species is a likely event. We assumed a realistic proportion (10%) of the vectors in the California sea lion daily diet, correcting for at-sea energy requirement.

Uncertainty analysis. An uncertainty analysis was performed to determine the contribution of the individual input variables to the overall model uncertainty. Input variables with assigned probability distributions (fish energy density and dose, sea lion weight) were set to a nominal value (i.e. mean), while continuing to draw the remaining variables from their respective distributions. Comparisons via output variance were made with the original model (i.e. uncertainty in all variables).

The potential influence of distribution choice was of some concern, particularly for dose in vector species, for which very limited data were available. A log-normal distribution was implemented in the simulation because this type of distribution is often used to represent environmental concentrations that actually represent the product of a number of individual components (Brattin et al. 1996). In addition, the log-normal distribution is bounded at zero, which is necessary to represent values that cannot be negative. Nonetheless, to explore the influence of this distribution choice, we repeated the simulations using a normal distribution rather than a log-normal distribution for DA concentration in vector species.

Comparison of model results with measured DA levels. Modeled doses were compared to estimate original DA doses from stranded animals. We employed time-series measurements of DA concentration in urine samples collected from 3 adult female sea lions to estimate their doses. Urine collection started on the day of stranding and continued until DA fell below detection limit (i.e. 3 consecutive days). These 3 stranded females were found within the same area and during the bloom event from which the DA concentrations in sardines and anchovies used in our model were determined (Lefebvre et al. 2002b). The total original oral dose of DA in these females corresponds to a portion of the oral dose that was assimilated through the digestive tract and eliminated as urine via the kidneys, plus the dose that was eliminated directly through feces. Since the urine collected on the last day was below the detection limit for all 3 sea lions, the cumulative DA from prior days should represent the total proportion eliminated via the kidneys from the original oral dose. The only known experimental study that has quantified the proportion of DA eliminated as urine, estimated a rate of 4 to 7% for Cynomolgus monkeys (Truelove et al. 1997). We used the total DA from the available urine samples to estimate a lower bound on the original oral dose by assuming a minimum daily urine estimate via interspecies scaling ($60 \times W^{0.75}$; Edwards 1975) and a conservative DA assimilation efficiency of 4%.

RESULTS

Sea lion energy requirements

Executions of the bioenergetic model (1000 per age/sex class) performed to evaluate the energy requirements for foraging individuals of *Zalophus californianus* indicated rapid changes in requirements for female (min. to max.: 6206 to 7148 kcal d^{-1}) and male (min. to max.: 8131 to 8887 kcal d^{-1}) pups (7 to 12 mo old only), and female (min. to max.: 6263 to 11 033 kcal d^{-1}) and male (min. to max.: 7456 to 11 491 kcal d^{-1}) juveniles in response to their energy demands for growth. The estimated foraging energy requirements of sexually mature females showed higher requirements for lactating and pregnant females (min. to max.: 14 032 to 18 897 kcal d^{-1} and 9123 to 17 244 kcal d^{-1}, respectively) than for non-lactating, non-pregnant females (min. to max.: 8006 to 10 739 kcal d^{-1}) of the same age (Table 3). In contrast, the estimated energy requirement of foraging males (min. to max.: 9920 to 18 491 kcal d^{-1}) increased with age in response to continuous weight gains. On a per weight basis, foraging pups, followed by juveniles and lactating females, have the highest metabolic requirements. For instance, female and male pups feeding on anchovies or sardines would require 4 times more food than adults of the same sex.

Table 3. *Zalophus californianus*. Min. and max. daily at-sea metabolic requirements (Costa et al. 1991) for California sea lion age/sex classes (kcal kg^{-1} body wt d^{-1}) using Kleiber's allometric scaling (Kleiber 1975), and anchovy or sardine food requirements (g fish kg^{-1} body wt d^{-1}) relative to sea lion body weight. Each simulation per age/sex class was executed 1000 times

Sea lion age class	Age (yr)	Weight (kg)	At-sea energy requirement (kcal kg^{-1} body wt d^{-1})	Food requirements Anchovy (g fish kg^{-1} body wt d^{-1})	 Sardine (g fish kg^{-1} body wt d^{-1})
Pup female	0.58–1	12–17	413– 528	233–298	253–323
Pup male	0.58–1	18–22	404–455	228–257	247–279
Juvenile female	1–5	17–58	190–376	107–212	116–230
Juvenile male	1–8	21–95	121–353	68–199	74–216
Adult female	5–20	57–81	133–140	75–79	81–86
Pregnant female	5–20	57–80	161– 215	91–121	98–131
Lactating female	5–20	56–80	236–251	133–142	144–153
Adult male	8–20	90–208	89–110	50–62	54–67

Simulation 1: effects of the vector species on DA dose

Simulations (1000 per case) were performed to evaluate the influence of the proportion of anchovies or sardines (i.e. 10 to 100%) in a sea lion's daily diet on the DA dose. Adult females of the same age (10 yr old) and physiological condition (non-lactating, non-pregnant) foraging at sea were used for these simulations. Due to the limited experimental data on the DA dose that would cause toxic effects in California sea lions, we assumed that effects would be seen at concentrations at or above the 2 adopted oral exposure thresholds: 1 mg DA kg^{-1} body wt (i.e. non-lethal effects; Truelove et al. 1997) and 2.71 mg DA kg^{-1} body wt (i.e. lethal effects; modified from Perl et al. 1990).

The exposure model indicates that ingestion of both vector species would result in females with DA concentrations above the thresholds. For instance, assuming anchovies as the main DA vector, the exposure model estimated a median DA dose ranging from 0.94 to 9.35 mg DA kg^{-1} body wt d^{-1}, resulting from ingestion of a daily diet comprised of 10 to 100% anchovies (Fig. 1A). Relative to the 1 mg DA kg^{-1} body wt threshold, a 10% anchovy diet would result in a 0.45 risk of a non-lethal toxic effect, increasing rapidly to 0.95 with a 20% diet. Relative to the 2.71 mg DA kg^{-1} body wt threshold, a 10% anchovy diet would result in a 0.01 risk of lethal effects increasing sigmoidally to 0.99 at 70% anchovy ingestion. In contrast, simulations with sardines as the primary DA vector showed an estimated median DA dose, ranging from 0.34 to 3.42 mg DA kg^{-1} body wt d^{-1} with ingestion of 10 to 100% sardines, respectively (Fig. 1B). Risks from the sardine diet were much lower, with a 50 and 100% sardine diet resulting in 0.9 and 0.7 risks of non-lethal and lethal effects, respectively. These simulations suggest that

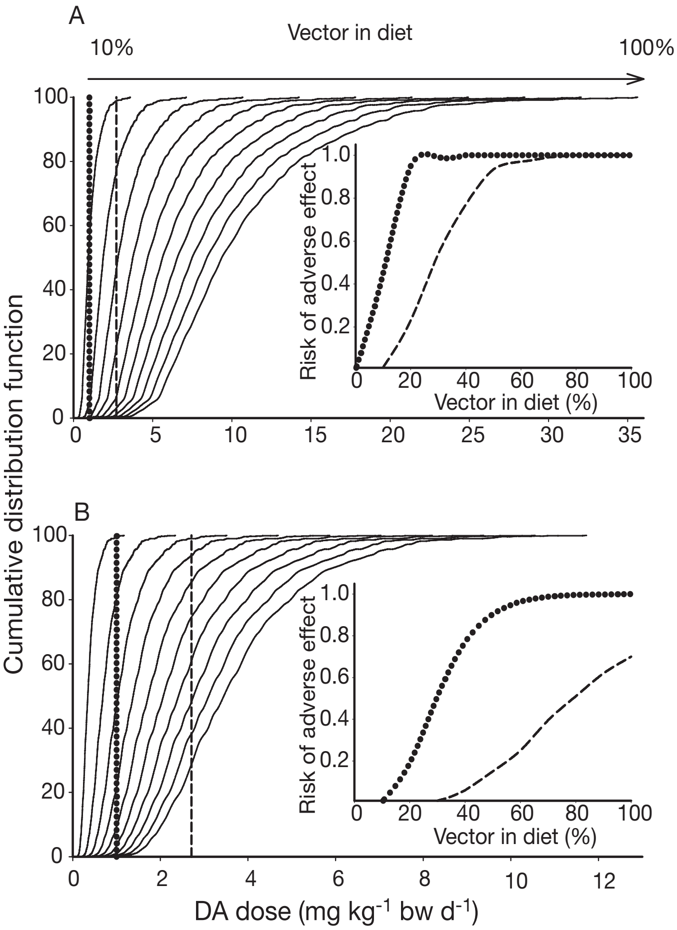

Fig. 1. *Zalophus californianus*. Estimated domoic acid (DA) dose (mg kg^{-1} body wt d^{-1}) in a 10 yr adult female California sea lion, with a daily diet comprised of 10 to 100% (A) anchovies or (B) sardines in 10% increments. Inset: results were compared to non-lethal (1 mg DA kg^{-1} body wt, dotted line) and lethal (2.71 mg DA kg^{-1} body wt, dashed line) oral thresholds. Risk of adverse effects represents the number of simulations with estimated doses at or above thresholds

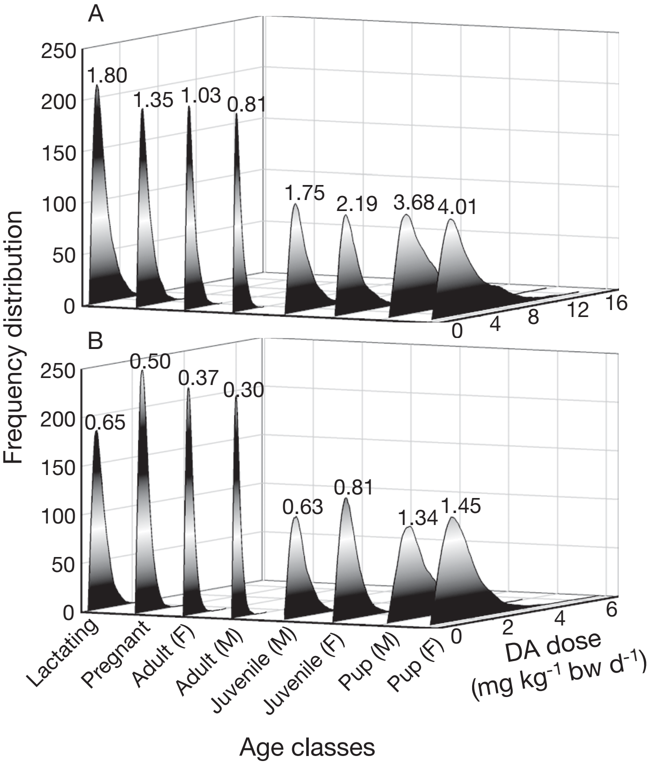

Fig. 2. *Zalophus californianus*. Frequency distributions of estimated domoic acid (DA) doses (mg kg^{-1} body wt d^{-1}) for all California sea lion age/sex classes (F: female; M: male). Pups included in the simulations were limited to those between 7 and 12 mo old. Numbers above peaks: median DA dose. Simulations performed assuming a daily diet with 10% (A) anchovies or (B) sardines

anchovies are a much more potent vector of DA for sea lions as compared to sardines. For example, compared to the non-lethal threshold, a 20% anchovy diet would result in a 4-fold increase in risk as compared to a 20% sardine diet.

Simulation 2: DA dose across age/sex classes

A final set of simulations was performed to investigate variability in estimated doses of DA across age/sex classes assuming at-sea metabolic requirements. Since DA exposure via lactational transfer was not accounted for in the model, only DA doses in pups ages 7 to 12 mo were estimated. Frequency distribution of DA doses, assuming an environmentally realistic proportion of the vectors in the California sea lion daily diet (10% each), showed that the dose would be expected to vary greatly across age/sex classes. Regardless of the vector, the median DA dose in sea lion pups is nearly double that estimated for juveniles and between 2 and 4 times greater than that for adult sea lions (Fig. 2A,B). These simulations also show that within age classes, there is greater dose variability in pups and juveniles compared to adults, and that the median DA dose in females is slightly higher than that in males.

Model outputs relative to the thresholds showed that a 10% anchovy diet would pose a much greater risk of non-lethal and lethal effects across all age classes than a 10% sardine diet (Fig. 3). For example, anchovies would result in 0.75 to 0.80 lethal risk to pups, while sardines would result in <0.06 risk. Also, a 10% sardine diet would only pose a relatively small risk of non-lethal effects to adult sea lions (<0.16).

Uncertainty analysis

Uncertainty analysis was performed using a 10 yr old, non-lactating, non-pregnant female, with at-sea energy requirements. Model uncertainty analysis of DA doses resulting from ingestion of either anchovies or sardines was performed by setting individual variables (i.e. fish's energy density and DA concentration, and sea lion weight) to a nominal value one at a time. The model uncertainty in DA dose estimation when uncertainty was present in all variables (original model) resulted in variances of 0.282 and 0.029 in simulations with anchovies and sardines, respectively. Simulations with anchovies as the sole DA vector indicated that by holding the vector's energy density constant, the model variance was reduced by 78% compared to the original model, while a relatively smaller

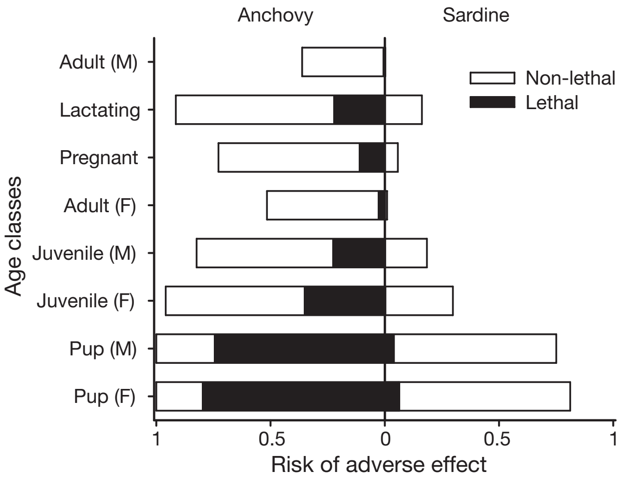

Fig. 3. *Zalophus californianus*. Comparison of domoic acid (DA) adverse effects between a 10% anchovy and a 10% sardine daily diet across all California sea lion age/sex classes (F: female; M: male). Pups included in the simulations were limited to those between 7 and 12 mo old. 'Risk of adverse effect' represents the proportion of simulations with estimated doses at or above the non-lethal (1 mg DA kg^{-1} body wt) and lethal (2.71 mg DA kg^{-1} body wt) oral thresholds

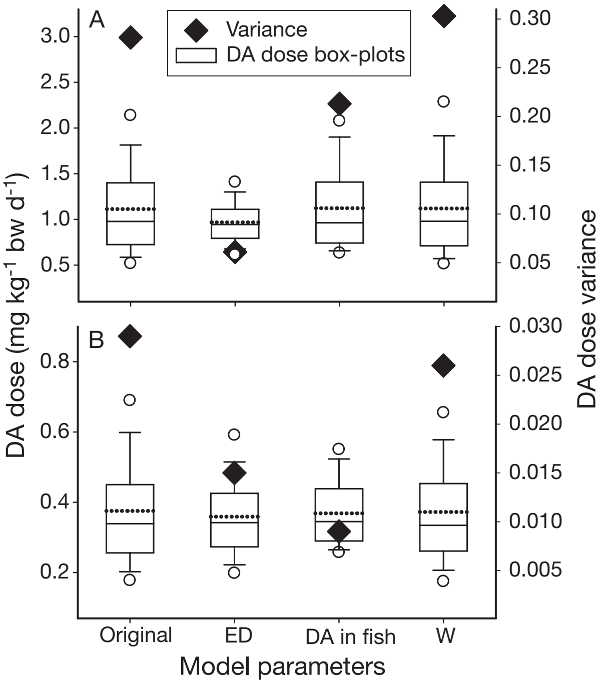

Fig. 4. *Zalophus californianus*. Uncertainty analysis of domoic acid (DA) dose (mg kg^{-1} body wt d^{-1}) using a 10 yr old, non-lactating, non-pregnant female, with at-sea energy requirements and a 10% (A) anchovy or (B) sardine diet. The original model indicates uncertainty present in all input variables. Variables include ED: fish energy density; DA in fish: DA concentration in the vector species; W: sea lion's body weight. Box-plots include median (solid line in box), mean (dotted line in box) and 10th, 25th, 75th, 90th percentiles (other horizontal lines)

variability (24%) resulted from the DA concentration in fish (Fig. 4A). In contrast, in simulations with sardines, DA concentration in fish was the most important source of uncertainty, resulting in a 70% decrease in the overall model variance when held constant. In these simulations a smaller variability (48%) in model estimates was associated with the energy density of the sardines (Fig. 4B). For both vector scenarios the sea lion weight contributes little to the overall model uncertainty. Overall median values within vector species were similar across uncertainty simulations.

An additional simulation (data not shown) of the DA doses using either the minimum or maximum anchovy energy density values (see Table 2) yielded doses 117% above and 26% below, respectively, those estimated with the original model (i.e. energy density uncertainty included). In comparison, minimum or maximum sardine energy density values yielded DA doses 59% above and 43% below, respectively, those of the original model.

The above simulations (each variable being drawn from assigned distributions, none held at nominal value) were repeated with DA concentration in vectors modeled as a normal distribution rather than log-normal distribution. Resulting estimates of DA dose in sea lions did not change significantly (results not shown).

Comparison of model results with measured DA levels

In the 3 stranded adult females (weights = 73, 76, and 71 kg), the cumulative DA concentrations in urine collected over 3 consecutive days from the day of stranding were 913, 473, and 847 ng DA ml^{-1} urine, respectively. Assuming a minimum daily urine output of 1.56, 1.52, and 1.48 l, respectively, estimated via interspecies scaling ($60 \times W^{0.75}$; Edwards 1975) and a conservative DA assimilation efficiency of 4% (Truelove et al. 1997), we estimated minimum DA doses for these 3 sea lions to be 0.48, 0.24, and 0.44 mg DA kg^{-1} body wt d^{-1}. For all 3 individuals, the concentration on the last day was below the detection limit, indicating that the entire DA dose had been effectively eliminated. However, we do not know the time lapse between exposure and stranding, or whether or not urine was released prior to the first sampling for DA analysis; therefore, our estimated doses must be considered a lower bound. Nonetheless, these doses fall within the 95th percentiles of the daily modeled DA doses for adult sea lions ingesting a 10% sardine diet (95th percentiles = 0.17 to 0.74 mg kg^{-1} body wt d^{-1}), and close to the lower end of the estimated dose for a 10% anchovy diet (95th percentiles = 0.52 to 2.48 mg kg^{-1} body wt d^{-1}).

DISCUSSION

Numerous studies have shown the devastating effects of harmful algal blooms on marine wildlife, particularly on mammals (for review, see Van Dolah 2005). Although the exposure of biotoxins to marine mammals may occur through several routes, exposure to DA occurs through the food web, via ingestion of planktivorous fish. In the case of California sea lions *Zalophus californianus*, anchovies and sardines are the most important sources of DA intoxication (Lefebvre et al. 2002b). The probabilistic approach presented here indicated that the magnitude of the effects would depend on the prey item of choice (anchovy vs. sardine) and the proportion of the vector in the diet. This model also showed anchovies to be a greater vector of concern than sardines, where, for example, same age classes ingesting a daily diet comprised of 10% anchovies would have DA doses equivalent to a 30%

sardine diet. Based on these model results, we speculate that the magnitude of sea lion mortalities/strandings during the 1998 *Pseudo-nitzschia* toxic bloom would have been greatly reduced, and perhaps the sea lions would have been impacted to a lesser degree, had they been feeding primarily on sardines instead of anchovies (Gulland 2000). Interestingly, these 2 vectors are not only similar in their biology, position in the marine food web, and geographic distribution, but also in their dominance and abundance in the California Current System (Ahlstrom 1966). Their abundance, however, fluctuates in response to large-scale and multidecadal (i.e. 40 to 60 yr) ocean temperature fluctuations (Chavez et al. 2003). Specifically, warmer sea surface temperatures favor the proliferation of sardines, while a colder regime favors anchovies (Chavez et al. 2003). Thus, greater DA effects on sea lions may be observed as the system shifts from a sardine to an anchovy regime. Under an ideal scenario sea lions could reduce their risk of exposure to DA by diversifying their diet and reducing the intake of planktivorous fish, and by ingesting prey species with higher energy density or prey with similar energy density, but occupying a higher trophic level (i.e. salmon, Pacific herring). Since these are opportunistic feeders, the likelihood of exposure is entirely dependent on the prey species (and their associated trophic level) present during foraging trips.

Several physiological and behavioral traits of sea lions may contribute to a high likelihood of sea lion exposure to this extremely water-soluble biotoxin. Water intake in California sea lions comes primarily from their food (Ridgway & Harrison 1981), which they ingest whole. This, added to a high fish assimilation efficiency (>90%), may favor an efficient transfer of the dissolved DA from the vector's body fluids into the sea lion's blood stream. Although Truelove et al. (1997) reported a 4 to 7% DA assimilation efficiency in monkeys, the efficient digestive physiology of sea lions suggests a higher assimilation efficiency. Our model highlights the need for quantifying the assimilation of DA specific for sea lions, perhaps through the use of suitable surrogate markers for experimentation with wildlife. If sea lions do indeed assimilate DA more efficiently, then the oral toxic threshold for sea lions would be lower than the thresholds for monkeys (1 mg kg^{-1} body wt; Truelove et al. 1997) and humans (2.71 mg kg^{-1} body wt; modified from Perl et al. 1990), and the risk values presented here would be underestimated.

Our model specifically focused on acute exposures rather than chronic exposures due to the demonstrated rapid elimination of the DA. Experimental studies have determined serum half-lives of approximately 20 min for rats (Suzuki & Hierlihy 1993, Truelove & Iverson 1994) and approximately 2 h for Cynomolgus monkeys (Truelove & Iverson 1994). Although the rate of clearance of DA in sea lions is not known, it can be estimated using interspecies scaling (Edwards 1975). Interspecies scaling assumes that allometric equations can be used to predict pharmacokinetic parameters on the basis of body weight. The general form of the allometric equation is: $y = aW^b$, where y is the pharmacokinetic parameter of interest, W represents body weight, and a and b are fitted coefficients. Using pharmacokinetic parameters measured in rats and Cynomolgus monkeys (Truelove & Iverson 1994) to fit allometric equations, we estimated the volume of distribution (V_d = 130.8 ml kg^{-1}) and clearance (Cl = 0.17 ml min^{-1} kg^{-1}) for a 70 kg sea lion. Based on these estimates, the expected half-life of DA for sea lions would be 8.9 h; thus, the focus of our exposure model on a single acute exposure (based on daily energy requirement) appears appropriate. Although the estimates for pharmacokinetic parameters based on interspecies scaling using only 2 alternate species is tenuous, the results are consistent with DA levels measured from urine of stranded animals. Assuming a first-order elimination process described by: $C_t = C_0\,\text{e}^{-kt}$, where C_t is the DA concentration at time t, C_0 is the initial concentration, and k is the elimination rate constant (Cl/V_d), the complete DA dose would be cleared after about 48 h. This is consistent with measured values from the 3 stranded animals that maintained measurable DA levels in their urine 1 d following admittance to the rehabilitation facility (at least 24 h post-exposure), but were below detection limit within 2 d.

In this model, differences in DA estimated doses across age classes are a direct consequence of the increased energy demands associated with growth. In particular, DA dose estimates in sea lion pups (7 to 12 mo old) were nearly double that of juveniles and between 3 and 6 times greater than that of adult sea lions. An additional exposure to DA in pups approaching weaning would likely occur via lactation, while being the primary source of DA for young pups (<7 mo old). Although we did not account for maternal transfer of DA to pups via lactation, we suspect that this is an important mechanism for DA transfer particularly in newborn pups (not included here) whose energy demands are supplied entirely through milk. Studies in rats (Maucher & Ramsdell 2005) found measurable DA in milk of lactating females and showed that pups exposed to DA-spiked milk had measurable amounts of DA in plasma, indicating that this biotoxin is transferable from exposed mothers to lactating pups. DA transfer through lactation in sea lion pups could be evaluated as additional information becomes available.

The uncertainty analysis of the exposure assessment model highlighted the importance of the vector's energy density, especially of that of anchovies, on DA dose estimation. For the sardine simulations, variance in DA concentration in the prey contributed most significantly to uncertainty in the exposure model. This is contrary to the simulations for anchovies, in which prey DA concentration was much higher and thus more likely to produce a dose above the toxic threshold, even though DA concentrations in the 2 prey species exhibited a similar degree of relative variation (sardine CV = 28 %, anchovy CV = 26 %). This analysis underscores the need for accurate estimation of metabolic parameters, specifically energy density, for vector species in the size classes preferred by sea lions. A recent study on the foraging ecology of California sea lions suggested that differences in prey selection exist between male and female sea lions, with less prey selectivity by adult males (Weise 2006). Prey selection of specific anchovy and sardine size classes may further indicate selection of specific energy densities, which, in turn, can lead to higher or lower DA doses. The metabolic parameters specifying increased energy requirements for growth, lactation/pregnancy, and at-sea foraging were not included in the uncertainty analysis because there was insufficient information to accurately characterize distributions or ranges for these parameters. As these parameters are multipliers for the baseline metabolic rate, it is intuitively obvious that uncertainty in these parameters would contribute significantly to variability in model results.

While our results indicate that uncertainty in metabolic parameters greatly influences variability in the current exposure model, this uncertainty in predictions of dose at the individual level is likely to be minimal when uncertainty in exposure of the overall population is considered. The current exposure model incorporates a distribution for DA concentration in prey species, but all individuals are assumed to be exposed equally, i.e. we do not model bloom patchiness or spatial variation in prey or sea lion foraging. The uncertainty in sea lion and prey movements and bloom patchiness, once incorporated into an overall population model, is likely to be the dominant source of variation in determining exposure. Future efforts should therefore concentrate on quantifying DA and spatial variation in vector species during bloom periods, particularly from foraging areas frequented by sea lions, as this important piece of information will improve sea lion DA dose estimates.

In this as in any probabilistic model, the level of uncertainty increases with the number of variables used in DA dose estimation. Common to all variables in our model is the limited availability of experimental data. A handful of metabolic measurements in California sea lions (Boyd et al. 1995, Hurley & Costa 2001) have shown elevated rates compared to those estimated by Kleiber's allometric equation. Hurley & Costa (2001) measured the metabolic rate of resting adult sea lions showing that Kleiber's allometric scaling underestimates this rate by a factor of 1.9 to 3. Similarly, Boyd et al. (1995) used heart rate and doubly labeled water (DLM) as measures of field metabolic rate, and stated that this might be between 2 and 4 times that estimated using Kleiber's scaling. This later observation is in agreement with model adjustments for the at-sea foraging activities (Costa et al. 1991) presented here. Although we used a conservative approach for estimating energy requirements as a basis for food ingestion and DA dose estimation, additional experimental data on this important model parameter, specifically on age/sex-specific energy requirements of foraging sea lions, would likely improve model estimates.

While the purpose of this model was primarily to estimate individualized age/sex-specific DA doses, it is clear that further modeling efforts should include a behavioral as well as a population component. For example, algal blooms containing high levels of DA along the coast of California often occur prior to the breeding season (May through August) of sea lions. Brodie et al. (2006) indicated that reproductive failure (i.e. abortion, premature parturition of pups, and death of pregnant sea lions) in 209 female sea lions exposed late in their pregnancy resulted from intoxication with DA. In the 1998 sea lion mortality event in the Monterey Bay area, the majority of the 70 stranded animals (77 %) were adult females, of which 50 % were pregnant (Gulland 2000). Similarly, of the 184 sea lions affected in the 2000 DA event, 80 % were adult females (Gulland et al. 2002). The apparent susceptibility of females is most likely indicative of a particular population structure during the breeding season, as well as of life stage specific behavioral differences. For instance, Aurioles-Gamboa & Zavala-Gonzalez (1994) and Francis & Heath (1991) studied the breeding population of California sea lions and found that the number of adult males across several breeding grounds was about one-fifth that of adult females. Other studies have also indicated that during the breeding season females and juveniles spend a larger amount of time foraging at sea than males (Odell 1981, Heath et al. 1991, Worthy 2001), most of which fast during this season.

The validation of the current model is rather limited due to the infeasibility of conducting experimental trials with sea lions and DA (i.e. dose–response data). However, given information limitations, time-series measurements of DA concentration in urine samples collected from the 3 stranded female sea lions yielded estimated doses within the range or below those predicted by the model. Additional DA time-series analysis from serum and urine collected from animals showing DA signs of intoxication soon after stranding would allow a more thorough model validation. As a result, this model is proposed to highlight areas in which research is needed to conduct a definitive risk analysis. Areas in which additional experimental data are required include the sea lions' bioenergetic measurements, diet analysis, DA assimilation efficiency, and a better estimate of the toxic DA threshold dose in these marine mammals.

The model presented here comprises the first attempt at a more comprehensive tiered ecological risk assessment for California sea lions exposed to DA. Thus, as data become available, the model will be further optimized to better characterize the risk of sea lion exposure to this biotoxin. Future modeling efforts will also incorporate information on sea lion life stage specific behavior and population dynamics to properly quantify environmentally realistic effects.

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