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ORIGINAL PAPER

Preying on commercial fisheries and accumulating paralytic shellfish toxins: a dietary analysis of invasive *Dosidicus gigas* (Cephalopoda Ommastrephidae) stranded in Pacific Canada

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Abstract In fall of 2009, several mass strandings of Humboldt squid (*Dosidicus gigas*) occurred on Vancouver Island (49°7′60N 125°54′0W). Morphological dissections coupled with DNA barcoding of stomach contents revealed *Sardinops sagax* (Pacific sardine) and *Clupea pallasii* (Pacific herring) as their primary prey. Plastic nurdles, fishing line, bull kelp, eelgrass, and a guillemot feather were also discovered. The primary prey, Pacific sardines and Pacific herring, are known to bioaccumulate paralytic shellfish toxins (PSTs); additionally, both PSTs and domoic acid (DA) have been implicated in other mass strandings. Therefore, stomach contents, and other tissues when possible, were tested for PSTs and DA. Testing revealed DA concentrations below regulatory guidance levels for human consumption, yet PSTs were well in excess. Though we cannot conclude that PSTs were the definitive cause of the strandings, our findings are the first report of PSTs in *D. gigas*.

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Introduction

The Humboldt squid (*Dosidicus gigas*) has an historic range extending along the eastern Pacific Ocean from Chile to California (Nigmatullin et al. 2001). Recent invasions of *D. gigas* into northern waters have coincided with ocean warming (Zeidberg and Robison 2007), and since 2003, the range has occasionally extended north to the coast of Alaska (Field et al. 2007) (Fig. 1). The first appearance of *D. gigas* near British Columbia coincided with an El Niño year (Cosgrove 2005).

The native diet of *D. gigas* has been well studied because the species is fished commercially. Main prey items of healthy *D. gigas* from the central East Pacific Ocean include fishes, copepods, amphipods, crustaceans, squids, and octopuses (Ibáñez et al. 2008; Nigmatullin et al. 2001). However, the first records of *D. gigas* near British Columbia, where four healthy specimens were examined, contained only euphausiids in the stomach contents (J. Cosgrove personal communication) and potential shifts in diet associated with the range expansion of this species remain largely unexplored.

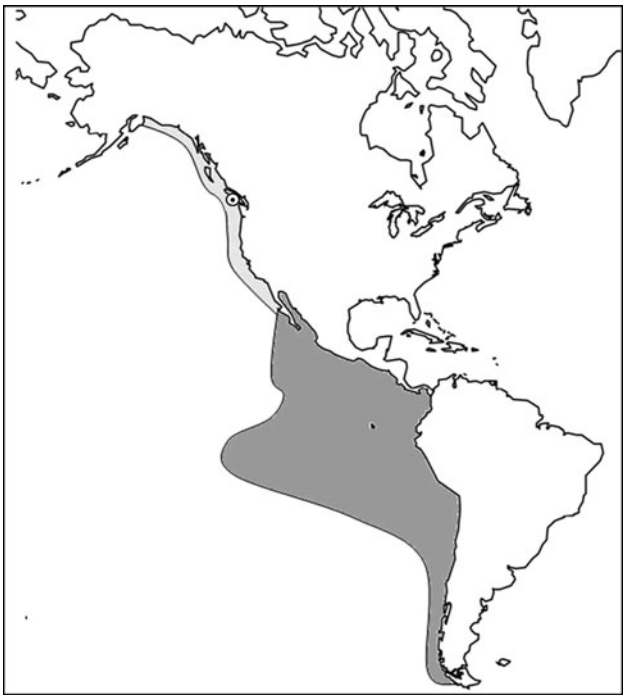

Fig. 1 The native and invasive range of Humboldt squid. (Modified from Gilly 2005). The *dark grey* region is the range until the mid-1980s, while the *lighter grey* region shows the expansion into California. By 2005, they had expanded to Alaska. The location of Tofino, BC, Canada, is indicated by *circle with dot*

Mass strandings are a frequent occurrence for *D. gigas* in the native range; however, the causes have remained unknown (Zeidberg and Robison 2007). Mass strandings provide a unique opportunity for studying the diet of stranded individuals and may be used to try to establish causality. In 2009, an El Niño year (http://www.elnino.noaa.gov/), there was an invasion into Canadian waters, and ten mass stranding events of *D. gigas* occurred on the coast of Vancouver Island between August and October of that year.

In the Pacific Northwest, two common natural toxins in aquatic food webs which have implications for mass strandings are paralytic shellfish toxins (PSTs) and the amnesic shellfish toxin domoic acid (DA). PSTs have been found to bioaccumulate in common prey items of *D. gigas* (Jester et al. 2009), and DA has been implicated in other mass mortality events (e.g. Work et al. 1993; Gulland et al. 2002). The present case study compared Humboldt squid specimens collected from two separate stranding events on the west coast of Vancouver Island. Prey diets were inferred from morphological analysis of stomach contents coupled with DNA barcoding, and assays for the presence of PST and/or DA were also conducted.

Materials and methods

Specimens

Humboldt squid specimens from two strandings on Chesterman Beach, Tofino BC (GPS coordinates: 49.11374, −125.8907608) (DFO 2010), were opportunistically obtained for study (Wilson 2010). The first stranding (S1) occurred between 2 and 4 August 2009, and 20 stomachs were collected on 6 August 2009 by JO. The second stranding (S2) occurred near the end of September 2009, and the contents from 9 stomachs, as well as three whole specimens (mantle length 43–56 cm, 12–20 lbs), were collected on 27 September 2009. Specimens were kept frozen until they were thawed for subsampling. A small sample of squid tissue was taken from each individual squid to verify the species identity of the specimen through DNA barcoding. These samples (usually >3 mm^3) were stored individually in 95% EtOH at −20°C.

Stomach content subsampling

The stomach contents were thawed and then rinsed with 95% EtOH in a sieve (mesh size 1 mm^2), and pieces of bone, tissue, scales, and fins (prey items) that were at least 5 mm^3, as well as parasites, were removed and stored individually in 95% EtOH. Preliminary efforts at exhaustive sampling were taken by extracting the DNA from all the discrete pieces collected from a single stomach ($N = 48$). Subsequently, between 2 and 12 (depending on fullness of the stomach) of the prey items from each stomach were selected for barcoding. We attempted to reveal the maximum amount of prey diversity by selecting the most diverse prey items based on morphological differences (colour and tissue type). Squid sucker rings were identified to species by observing the serrations on the rings under a dissecting microscope. Unusual or non-prey items were rinsed in 95% EtOH, examined, and identified.

DNA barcoding

Each prey item or sample of squid muscle tissue was then subsampled (>3 mm^3 of tissue) into 45 µl of lysis buffer and 5 µl of proteinase K. Subsamples were incubated at 56°C for 18 h prior to DNA extraction using an automated glass fibre technique (Ivanova et al. 2006). The 652-bp barcode region of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene (Hebert et al. 2003) was amplified using four different primer sets (Table 1) following Hebert et al. (2004a). The general thermocycler conditions were an initial hot start at 94°C; followed by 5–6 cycles with an annealing temperature ranging from 45 to 50°C, extension at 72°C, and denaturation at 94°C; then 35 cycles with an

Table 1 Sequences for primer pairs that were used in PCR to obtain DNA barcodes of prey items obtained from the stomach contents of stranded Humboldt squid

Primer pair name	Primer name	Primer sequence (5′–3′)	Thermocycler conditions
Folmer (Folmer et al. 1994)	LCO1490_t1t	**TGTAAAACGACGGCCAGT** GGTCAACAAATCATAAAGATATTGG	Hot start of 94°C for 1 min; 5 cycles of 94°C for 40 s, 45°C for 40 s, 72 for 1 min; 35 cycles of 94°C for 40 s, 51°C for 40 s, 72°C for 1 min; extension at 72°C for 5 min, hold 4°C indefinitely.
	HCO2198_t1t	**CAGGAAACAGCTATGAC** TAAACTTCAGGGTGACCAAAAAATCA	
Crustacean (D. Steinke personal communication)	CrustDF1	GGTCWACAAAYCATAAAGAYATTGG	
	CrustDR1	TAAACYTCAGGRTGACCRAARAAYCA	
Mammal cocktail (Ivanova et al. 2007)	LepF1_t1	**TGTAAAACGACGGCCAGT**ATTCAA CCAATCATAAAGATATTGG	Hot start of 94°C for 1 min; 5 cycles of 94°C for 30 s, 50°C for 40 s, 72°C for 1 min; 35 cycles of 94°C for 30 s, 54°C for 40 s, 72°C for 1 min, extension of 72°C for 10 min, hold 4°C indefinitely.
	VF1_t1	**TGTAAAACGACGGCCAGT**TCTCAA CCAACCACAAAGACATTGG	
	VF1d_t1	**TGTAAAACGACGGCCAGT**TCTCAA CCAACCACAARGAYATYGG	
	VF1i_t1	**TGTAAAACGACGGCCAGT**TCTCAA CCAACCAIAAIGAIATIGG	
	LepR1_t1	**CAGGAAACAGCTATGAC**TAAACTTCT GGATGTCCAAAAAATCA	
	VR1d_t1	**CAGGAAACAGCTATGAC**TAGACTTCT GGGTGGCCRAARAAYCA	
	VR1_t1	**CAGGAAACAGCTATGAC**TAGACTTCT GGGTGGCCAAAGAATCA	
	VR1i_t1	**CAGGAAACAGCTATGAC**TAGACTTCT GGGTGICCIAAIAAICA	
Bird primer (Hebert 2004b)	BirdF1	TTCTCCAACCACAAAGACATTGGCAC	Hot start of 94°C for 1 min; 6 cycles of 94°C for 1 min, 45°C for 1.5 min, 72°C for 1.5 min; 35 cycles of 94°C for 1 min, 55°C for 1.5 min, 72°C for 1.5 min; extension of 72°C for 5 min, hold 4°C indefinitely.
	BirdR1	ACGTGGGAGATAATTCCAAATCCTGG	

The thermocycling protocol for each primer set is also provided

The PCR primers were also used for sequencing except in the case of primers with M13 tails, shown in bold, where only the M13 was used as the sequencing primers

annealing temperature between 51 and 55°C, extension at 72°C, and denaturation at 94°C; and a final extension for 5–10 min at 72°C and then hold indefinitely at 4°C. PCR products were bidirectionally sequenced using BigDye v3.1 on an ABI 3730 DNA Analyzer (Applied Biosystems). Contigs were assembled using Sequencher v. 4.0.5 (Gene Codes). Contigs were uploaded to the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007) public project titled "DNA Barcoding of Squid Stomach Contents" (project code: HBSSC), and GenBank accession numbers are available in Appendix 1 of electronic supplementary material.

Aligned sequences were compared against reference barcodes using the BOLD species ID engine (Ratnasingham and Hebert 2007; in February 2010). Species-level identifications were made based on sequence matches that were at least 99% similar to barcode clusters derived from a single species following Wong and Hanner (2008).

PST testing

The stomach contents from three individuals from S1, as well as the stomach contents, mantle tissue, digestive glands, and gonads from three individuals from S2, were analysed for the presence of PSTs (saxitoxin (STX), decarbamoylsaxitoxin (dcSTX), neosaxitoxin (NEO), gonyautoxins (GTX)1/4 and 2/3, and the N-sulfocarbamoylsaxitoxins C1/2 and GTX5) following AOAC (2005). FDA reference standard STX was obtained from in-house supplies, while all other standards were obtained from the NRC, Institute for Marine Biosciences (Halifax, NS, Canada). All standards were prepared as 1% acetic acid stocks and stored at 4°C (except for the C toxins which were frozen). Due to difficulties arising from the high lipid content found in stomach contents and digestive glands, samples were extracted for PSTs according to Deeds et al. (2008). Post-extraction sample preparations were carried out using

a Caliper RapidTrace® Solid Phase Extraction (SPE) Work Station (Caliper Life Sciences, Hopkinton, MA; currently available through Biotage), programmed to perform sample preparations according to AOAC (2005). An Agilent 1,200 series HPLC system equipped with a reverse-phase C18 column (Supelcosil, 5 mm, 15 × 4.6 mm id) was used to separate and detect PSTs in the prepared extracts. Chromatographic conditions were as described in AOAC (2005). Detection was made using a fluorescence detector with excitation and emission wavelengths at 340 and 395 nm, respectively. ChemStation software (ver. B.02.01) was used to record and process chromatograms. Data were analysed using GraphPad Prism (ver. 5.01) (GraphPad Software, Inc., LaJolla, CA). Samples containing putative PSTs were analysed both with and without oxidation to distinguish toxins from naturally occurring fluorescent compounds that may be present in samples and co-elute with some PSTs (Etheridge et al. 2006).

DA testing

The same tissues analysed for PSTs were also analysed for DA using a commercially available, AOAC-validated (AOAC 2007) test kit (ASP ELISA kit for quantitative determination of domoic acid, Prod. No. A31300401, Biosense Laboratories, Bergen, Norway). All samples were analysed in duplicate at dilutions of 1:200 (minimum recommended by manufacturer) and 1:20,000 (required dilution to detect 20 ppm regulatory guidance level). Data were analysed using GraphPad Prism (ver. 5.01) (GraphPad Software, Inc., LaJolla, CA).

Results

Stomach contents

A total of 235 discrete tissue and bone samples were recovered from 27 squid stomachs, and five stomachs were empty yielding no tissue samples. DNA was extracted from 235 samples, and 205 PCR-amplified fragments were obtained. Of these, 60 yielded poor quality and/or contaminated sequences that were not included in the analysis, leaving 131 unambiguous sequences for further scrutiny. One hundred and seven of these sequences were >500 bp, which could be considered as "complete barcodes" and matched with >99% similarity to unique species profiles in the BOLD Full Length Record Barcode Database (Fig. 2). Twenty sequences yielded no identification in BOLD. The shorter sequences were excluded although reasonable matches could be made in most cases; however, because they represented the same prey species detected by full-length sequences, they are not reported. The barcodes revealed that 19 of the stomachs contained either Pacific herring (*Sardinops sagax*) or Pacific sardines (*Clupea pallasii*). White bate smelt (*Allosmerus elongates*) was found in the stomach of one individual from S1 and two from S2. Other prey species found in single individuals from S1 included Dungeness crab (*Metacarcinus magister*), Pacific sandfish (*Trichondon trichondon*), and Pacific tomcod (*Microgadus proximus*). Prey species unique to S2 were Pacific staghorn sculpin (*Leptocottus armatus*) (one stomach), coho salmon (*Oncorhynchus kitsuch*) (two stomachs), and kelp greenling (*Hexagrammos decagrammus*) (one stomach). A feather was recovered from a single S2 stomach and was identified through barcoding as belonging to the Common Guillemot (*Uria aalge*). Three stomachs from S1 and five stomachs from S2 yielded *D. gigas* sequences from discrete pieces of tissue recovered from the stomach and were believed to be prey items. One stomach contained a sucker ring, which was identified morphologically as Humboldt squid, and one stomach yielded no barcodes.

In addition to those tissues sampled for DNA barcoding, morphological evaluation of stomach contents also revealed the following: fishing line, spruce needles, rocks, and sand. Nurdles (plastic pellets) were present in six out of 17 stomachs from S1 and in two out of 13 stomachs from S2. The maximum amount of nurdles found in one stomach was 11 from an S1 squid, while another stomach had an unusual and significant amount of plant matter [34 g of bull kelp (*Nereocystis luetkana*) and eel grass (*Zostera marina*)].

PST and DA

Of the PSTs tested for, only STX and dcSTX were detected. Stomach contents from S1 contained only STX (Table 2). All three individuals tested from S2 contained predominantly STX, with minor levels of dcSTX. The highest STX equivalent (eq.) levels were found in the stomach contents (range 0.047–0.77 ppm) and digestive gland (range 2.91–4.83 ppm). STX eq. concentrations in the digestive gland were 3.6–6.0 times greater than the regulatory guidance level (in shellfish) of 0.8 ppm (FDA 2001).

Low levels of DA were detected in the stomach contents from one of the three individuals tested from S1 and in all three individuals tested from S2 (Table 2). All levels were <1/100th of the regulatory guidance level in shellfish of 20 ppm (FDA 2001).

Discussion

Morphological sorting coupled with DNA barcoding was successful for identifying prey species from the gut contents of stranded *D. gigas* specimens. However, close to

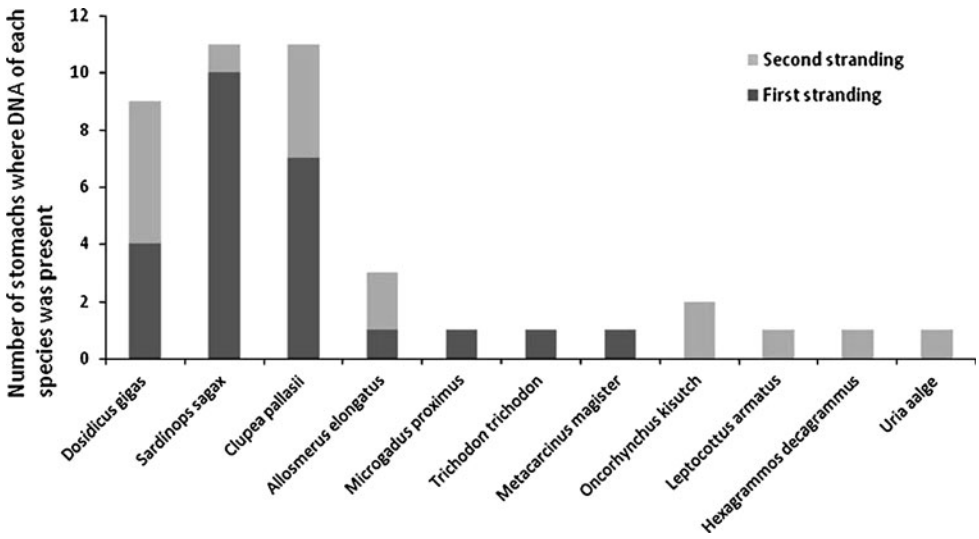

Fig. 2 Prey species consumed by Humboldt squid stranded on Vancouver Island during August and September 2009. Prey species, identified through DNA barcoding, are listed on the *x-axis*, and the *y-axis* indicates the number of stomachs containing sequences belonging to the species

Table 2 Concentrations of PST and DA toxins present in *Dosidicus gigas* from stranding events in British Columbia, Canada

Field ID	Tissue	DA ppm	STX ppm	dcSTX ppm	STX eq* ppm
D. gigas 18	Stomach contents	0	0.77	0	0.77
D. gigas 19	Stomach contents	0.069	0.24	0	0.24
D. gigas 20	Stomach contents	0	0.19	0	0.19
Bella	Mantle	0.029	0.0096	0	0.0096
Bella	Stomach	0.086	0.33	0.033	0.35
Bella	Gonad	0.021	0	0	0
Bella	Digestive gland	0.064	3.75	0.30	3.90
Jacob	Mantle	0.02	0	0.0045	0.0023
Jacob	Stomach contents	0.027	0.047	0	0.047
Jacob	Gonad	0.025	0	0	0
Jacob	Digestive gland	0.029	2.77	0.28	2.91
Ken-Edward	Mantle	0	0.012	0	0.012
Ken-Edward	Stomach	0.085	0.32	0.033	0.34
Ken-Edward	Gonad	0	0.0082	0	0.0082
Ken-Edward	Digestive gland	0.23	4.76	0.134	4.83

nd not detected

* STX eq. = STX + (dcSTX*0.51) (Oshima 1995). DA regulatory guidance level is 20 ppm. STX eq. regulatory guidance level is 0.8 ppm. Field IDs shown in italics refer to squids from the second stranding

one quarter of the sequences recovered were of low quality or contained co-amplified sequences from multiple PCR products, rendering them unusable. These failed sequences were not unexpected due to the nature of stomach content analysis; since stomach contents contain a variety of prey items and universal primers were used. From the successful sequences, numerous prey species that are fished commercially were identified: *Sardinops sagax*, *Clupea pallasii*, *Allosmerus elongates*, *Oncorhynchus kisutch*, *Hexagrammos decagrammus,* and *Microgadus proximus*. Our results are consistent with previous studies on healthy Humboldt squid while in its native range, which found their common prey species to be fishes, other squids, and pelagic crabs (Nigmatullin et al. 2001). Off the coast of California, Field et al. (2007) also found *Sardinops sagax* and *Clupea pallasii* in the stomach contents of Humboldt squid. The presence of Dungeness crabs (*Metacarcinus magister*) is consistent with the finding that Humboldt squid occasionally prey on bottom-dwelling fishes and invertebrates (Field et al. 2007). Cannibalism was suspected when *D. gigas* DNA barcodes were recovered from tissue picked out of eight stomachs, and though contamination from the squid's tissues could not be rule out, cannibalism was later confirmed by the presence of an in tact *D. gigas* sucker ring in one stomach. Cannibalism has been frequently observed in cephalopods (Ibáñez and Keyl 2010), and conspecifics are

known to be a common component of the diet of Humboldt squids (Ehrhardt 1991; Nigmatullin et al. 2001; Markaida and Sosa-Nishizaki 2003; Field et al. 2007; Markaida et al. 2008). Our results show for the first time that prey identifications can be made from partially digested tissue in Humboldt squid stomachs through the use of DNA barcodes.

There were a number of stomach contents that were surprising. The presence of feathers in the stomach of Humboldt squid has previously been reported by Field et al. (2007). Items such as rocks, sand, and plant matter are most likely the result of the stress of the stranding or failed attempts to capture prey (Bolstad and O'Shea 2003). The presence of nurdles most likely resulted from secondary predation due to their small size and negative buoyancy. This finding exemplifies the growing concern over the accumulation and bioaccumulation of plastic in marine ecosystems. Mato et al. (2001) found that nurdles have the ability to accumulate toxic chemicals from the marine environment and nonylphenols accumulated in the tissue of marine animals that consumed them.

Humboldt squid are a potential threat to fisheries in the north-west Pacific Ocean. Several commercially important fish species were found in the stomach contents; however, Pacific herring were one of their primary prey. They could have a significant impact on the Pacific herring fishery in BC as ocean warming trends continue and the occurrence of *D. gigas* becomes more frequent (or resident populations become established). As juveniles, Humboldt squids can grow 5–8% of their mantle length per day, though growth rates decrease to between 0.8 and 1.5% per day in immature adult squid (Nigmatullin et al. 2001). Large quantities of prey are required to maintain this growth. However, the effect that *D. gigas* is currently having on local populations of commercial fishes near BC is difficult to assess because their abundance is unknown.

To our knowledge, this is the first report of PSTs in *D. gigas*. Among cephalopods, PSTs have been found in the arms (Robertson et al. 2004) and digestive gland (Costa et al. 2009) of several species of octopuses. Outside of these studies, only a single squid from the Philippines was reported to have PSTs in its tissue (Llewellyn et al. 2006). The bioaccumulation of PSTs is known in Pacific sardines and Pacific herring, which are the most abundant among the prey species detected in this study (Jester et al. 2009). The level of PSTs in the mantle tissue, the part typically consumed by humans, was below the regulatory guidance levels; however, the high levels in other organs, which predators and scavengers may consume, was well in excess of regulatory guidance levels. *D. gigas* is the largest and one of the most abundant nektonic squid (Nigmatullin et al. 2001); therefore, they are a potential toxin vector for top predators such as tuna, sharks, swordfish, fur seals, and whales. In the case of mass strandings, even terrestrial scavengers, including bears, have been observed to consume the stranded and potentially toxic squid (J. Osborne, personal observation.).

DA was detected in very low levels, well below regulatory guidance levels, in the stomach contents and other tissues. The low amounts of DA found in our study are consistent with these previous results (Gilly 2005; Bargu et al. 2008). There have been numerous studies on the origin and bioaccumulation of DA in marine food webs, with implications for squid and fish as potential vectors to higher trophic positions (Lefebvre et al. 2001, 2005; Bargu et al. 2008). Gilly (2005) hypothesized DA to be the cause of Humboldt squid strandings in Monterey Bay due to its intoxication, which causes neurological defects, yet was unable to detect DA in stranded squid.

Without performing bioassays directly with *D. gigas*, it is difficult to conclusively attribute the 2009 Humboldt squid strandings in Tofino to dietary PSTs. However, there is sufficient evidence to implicate PSTs as a potential contributing cause because PSTs have already been linked to the mass stranding of marine mammals (Landsberg 2002). As well, the squid giant axon (from *Loligo opalescens* and *L. pealei*), on which much of the pioneering work on the mode of action of the saxitoxins was performed, is considered to be extremely sensitive to PSTs (Anderson et al. 2005). Regardless, due to their potentially high biomass, *D. gigas* must now also be considered as a potential vector for PSTs to higher trophic levels in PST endemic areas.

Conclusions

Two mass mortalities in 2009 enabled us to investigate the diet of stranded squid, demonstrating the utility of DNA barcoding for conducting, rapid, accurate, and cost-effective identification of gut contents. Our finding of Pacific sardines and herring as the primary prey in stranded squid stomachs prompted subsequent toxicological work that resulted in the first description of the marine toxin saxitoxin in *D. gigas*. This result suggests there is potential for *D. gigas* to have negative impacts on the top predators that consume them (such as tuna, sharks, seals, and whales) by acting as a vector for PSTs, especially considering their high biomass and extreme consumption of smaller primary consumers of these toxins, such as sardines and herring.

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References

Anderson PAV, Roberts-Misterly J, Greenberg RM (2005) The evolution of voltage-gated sodium channels: were algal toxins involved? Harmful Algae 4:95–107

AOAC (2005) Paralytic shellfish poisoning toxins in shellfish: prechromatographic oxidation and liquid chromatography with fluorescence detection. Official method 2005.06, A. M. In: Official methods of analysis

AOAC (2007) Domoic acid toxins in shellfish: biosense ASP ELISA—a competitive direct enzyme-linked immunosorbent assay. Official method 2006.02, A. M. In: Official methods of analysis

Bargu S, Powell CL, Wang Z, Doucette GJ, Silver MW (2008) Note on the occurrence of *Pseudo-nitzschia australis* and domoic acid in squid from Monterey Bay, CA (USA). Harmful Algae 7:45–51

Bolstad KS, O'Shea S (2003) Gut contents of a giant squid *Architeuthis dux* (Cephalopoda: Oegopsida) from New Zealand waters. N Z J Zool 31:15–21

Cosgrove JA (2005) The first specimens of Humboldt squid in British Columbia. PICES Press 13(2):30–31

Costa PR, Botelho MJ, Rodrigues SM (2009) Accumulation of paralytic shellfish toxins in digestive gland of *Octopus vulgaris* during bloom events including the dinoflagellate *Gymnodinium catenatum*. Mar Pollut Bull 28:1747–1750

Deeds JR, White KD, Etheridge SM, Landsberg JH (2008) Concentrations of saxitoxin and tetrodotoxin in three species of puffers from the Indian River Lagoon, Florida, the location for multiple cases of saxitoxin puffer poisoning from 2002–2004. Trans Am Fish Soc 137:1317–1326

DFO (2010) State of the Pacific Ocean 2009. DFO Can Sci Advis Sec Sci Advis Rep 2010/034

Ehrhardt NM (1991) Potential impact of seasonal migratory jumbo squid (*Dosidicus gigas*) stock on a gulf of California sardine (*Sardinops sagax caerulae*) population. Bull Mar Sci 49:325–332

Etheridge SM, Deeds J, Hall S, White K, Flewelling L, Abbott J, Landsberg J, Conrad S, Bodager D, Jackow G (2006) Detection methods and their limitations: PSP toxins in the southern puffer fish *Sphoeroides nephelus* responsible for human poisoning events in Florida in 2004. Afr J Mar Sci 28:383–387

Field JC, Walker WA, Phillips AJ (2007) Range expansion and trophic interactions of the jumbo squid, *Dosidicus gigas*, in the California Current. Cal Coop Ocean Fish Investig Rep 48:131–146

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Gilly WF (2005) Spreading and stranding of jumbo squid. Ecosystems observations for the Monterey Bay national marine sanctuary 2005:25–26. http://montereybay.noaa.gov/reports/2005/eco/ecoobs2005.pdf. Viewed 1 Mar 2010

Gulland FMD, Haulena M, Fauquier D, Langlois G, Lander ME, Zabka T, Duerr R, Langlois G (2002) Domoic acid toxicity in California sea lions (*Zalophus californianus*): clinical signs, treatment and survival. Vet Rec 150:475–480

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc B 270:313–321

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004a) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci USA 101:14812–14817

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004b) Identification of birds through DNA barcodes. PLoS Biol 2(10):1657–1663

Ibáñez CM, Keyl F (2010) Cannibalism in cephalopods. Rev Fish Biol Fish 20:123–136

Ibáñez CM, Arancibia H, Cubillos LA (2008) Biases in determining the diet of jumbo squid *Dosidicus gigas* (D' Orbigny 1835) (Cephalopoda: Ommastrephidae) off southern-central Chile (34°S–40°S). Mar Res 62:331–338

Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol Ecol Notes 6:998–1002

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548

Jester RJ, Baugh KA, Lefebvre KA (2009) Presence of *Alexandrium catenella* and paralytic shellfish toxins in finfish, shellfish and rock crabs in Monterey Bay, California, USA. Mar Biol 156:493–504

Landsberg JH (2002) The effects of harmful algal blooms on aquatic organisms. Rev Fish Sci 10(2):113–390

Lefebvre KA, Dovel SL, Silver MW (2001) Tissue distribution and neurotoxic effects of domoic acid in a prominent vector species, the northern anchovy *Engraulis mordax*. Mar Biol 138:693–700

Lefebvre KA, Elder NE, Hershberger PK, Trainer VL, Stehr CM, Scholz NL (2005) Dissolved saxitoxin causes transient inhibition of sensorimotor function in larval Pacific herring (*Clupea harengus pallasi*). Mar Biol 147:1393–1402

Llewellyn L, Negri A, Robertson A (2006) Paralytic shellfish toxins in the tropical oceans. Toxin Rev 25:159–196

Markaida U, Sosa-Nishizaki O (2003) Food and feeding habits of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephihdae) from the Gulf of California, Mexico. J Mar Bio Association UK 83:507–522

Markaida U, Gilly WF, Salinas-Zavala CA, Rosas-Luis R, Booth JTA (2008) Food and feeding of jumbo squid *Dosidicus gigas* in the Central Gulf of California during 2005–2007. Cal Coop Ocean Fish Investig Rep 49:90–103

Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T (2001) Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ Sci Technol 35:318–324

Nigmatullin CM, Nesis KN, Arkhipkin AI (2001) A review of the biology of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae). Fish Res 54:9–19

Ratnasingham S, Hebert PDN (2007) BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). Mol Ecol Notes 7:355–364

Robertson A, Stirling D, Robillot C, Llewellyn L, Negri A (2004) First report of saxitoxin in octopi. Toxicon 44:765–771

United States Food Drug Administration (2001) Fish and fisheries products hazards and controls guidance: third edition. Department of Health and Human Services, Center for Food Safety and Applied Nutrition, Washington DC

Wilson JJ (2010) Barcodes help solve riddle of the stranded squid. BARCODE bull 1(4):10. http://ibol.org/news-and-events/newsletter/ Accessed 5 May 2011

Wong EHK, Hanner RH (2008) DNA barcoding detects market substitution in North American seafood. Food Res Int 41(8):828–837

Work TM, Barr B, Beale AM, Fritz L, Quilliam MA, Wright JLC (1993) Epidemiology of domoic acid poisoning in brown pelicans (*Pelicanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. J Zoo Wildl Med 24:54–62

Zeidberg LD, Robison BH (2007) Invasive range expansion by the Humboldt squid, *Dosidicus gigas*, in the eastern North Pacific. Proc Natl Acad Sci USA 104:12948–12950