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Stomach Content Analysis and Concentrations of Chemical Pollutants in the Clymene Dolphin (*Stenella clymene*, Gray 1846) from the Coastal Waters of Ghana

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Abstract

Stomach content analysis constitutes an important component of fisheries management, providing insight into fish feeding patterns and quantitative assessment of food habits. Thus, a study aimed at obtaining dietary information from stomach contents analysis of the most common by-caught dolphin species, *Stenella clymene*, beached from the coastal waters of Ghana, along the Gulf of Guinea was undertaken. The stomachs of 39 by-caught clymene dolphins landed at three fisheries landing beaches along the Ghanaian coast, were analyzed. A further study correlating the chemical contaminant load in tissues of Clymene dolphins to that of their preferred prey was undertaken. The stomach contents were generally composed of digested items. Fish, cephalopods and crustaceans were identified and represented a diversity of 12 species. On taxa level, fish was the most frequent (69%) and numerically the most important prey (46.57) followed by cephalopods (3.05) with crustaceans being present in trace amounts (1.55). However, both cephalopods and fish represented a more balanced share of the diet in biomass (45% and 51%) respectively. Thus, clymene dolphins off the coastal waters of Ghana appear to rely principally on both fish and cephalopods for food. Parasites also dominated the gut contents in relative abundance (48.83%). Prey items accumulated chemical contaminants at relatively the same concentrations (50%) as the blubber, liver and muscle of the clymene dolphins, confirming that food is the main source of exposure to contaminant load for marine mammals. With regards to quantitative analysis of prey species of cetaceans, this study of diet in clymene dolphins is the first recorded in this area.

Keywords: Feeding habits; *S. clymene* dolphins; POPs; Parasites; Marine mammals distribution; Gulf of Guinea

Introduction

The Clymene dolphin, *S. clymene* [1], is one of the poorest known cetaceans, as it was confirmed as a valid species only in 1981 [2]. Recent genetic analysis suggests that, it is most closely related to striped dolphin *Stenella coeruleoalba*, while it appears physically similar to both striped and spinner dolphins *Stenella longirostris* [3,4]. The species has a robust, streamlined body with a moderately short beak and a tall, falcate dorsal fin. The rounded melon is separated from the beak by a distinct crease. These dolphins are recognized by a tricolored pattern on their sides that includes a dark gray cape, moderately gray flanks, and a white or pale gray underside. They also have distinct black lips that appear similar to a "moustache" and a line that extends across the top ridge of their beak. They have 36-52 pairs of small conical teeth in each jaw that are useful for grasping prey [5]. In the western Atlantic, adult body size ranges between 1.70 m and 1.97 m, that is, 1.70 m to 1.90 m in females and 1.76 m to 1.97 m in males and they weigh from 75 kg to 90 kg [6].

Published information on the biology of the Clymene dolphin *S. clymene* were exclusively those from the Western Atlantic Ocean. The species commonly occurs in oceanic waters 250 m to 5,000 m in depth in tropical and warm temperate waters of the Atlantic Ocean [7,8]. They feed on small mesopelagic fish (e.g, myctophids) and cephalopods. Their feeding sometimes occurs at night when their prey vertically migrates towards the surface [9]. The stomach of one stranded specimen contained one pair of small beaks of an unidentified squid and over 800 very small otoliths of fishes of the families Myctophidae, Argentinidae and Bregmacerotidae [9].

The species is poorly known with regards to biology, life history, distribution and migratory habits, hence, research on all aspects of its biology is required. The paucity of information on this species has been attributed to the very low abundance, at least in coastal waters [10]. However, capture records show that *S. clymene* is the most commonly landed cetacean on Ghana's coast, which suggests a high relative abundance in that area [11-14]. In the eastern Atlantic, only a 'West African stock' is recognized, however no population studies have been undertaken [12]. By relative frequency of occurrence in catches, 34.5% in 1998-2000 and 32.1% in 2013-14 as indicator, *S. clymene* is considered the most abundant dolphin in Ghanaian coastal waters [11]. Some Ghanaian fishermen therefore refer to *S. clymene* as the common dolphin. Debrah [14] also identified Clymene dolphins to be the most dominant species caught, followed by *S. attenuata*.

Many dolphin species or populations are recognized internationally as vulnerable or threatened due to their low reproductive potential, which is, giving birth to one calf after a long gestation period of 11-12 months [15,16]. Oil spills and various fishing methods, most notably

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purse-seine fishing for tuna and the use of drift and other gill nets, results in large amounts of dolphins being killed accidentally [17]. In addition, contamination of the oceans, seas, and rivers, is a critical issue of concern, especially with Persistent Organic Pollutants (POPs), heavy metals, plastics, and other industrial and agricultural pollutants. These contaminants do not disintegrate rapidly in the environment hence, dolphins gradually accumulate unusually high levels of these contaminants thus, reducing their populations [18-20]. Clymene dolphins are as well threatened as they are also incidentally captured as by-catch in fishing expeditions using drift gillnets off the coast of West Africa, where they are used as shark bait and for human consumption [21].

The study of stomach contents or feeding habits of fish constitutes an important component of fisheries and it provides the basis for understanding trophic interaction in the aquatic food web. Diets of fishes represent an integration of many important ecological components like habitat use, behavior, condition, energy intake and intra/extra specific interactions. The occurrence, distribution, stomach contents analysis and chemical pollutants have been extensively studied in northern hemisphere cetaceans [22-30]. However, there is insufficient information on population status and trends particularly, with no information available on stomach contents and contaminant loads of marine mammals found in the Gulf of Guinea including paucity of data relating to dolphins beached on the coast of Ghana. In addition, the International Union for Conservation of Nature and Natural Resources (IUCN's) Red List of Threatened Species considers *S. clymene* "Data Deficient" [31].

Thus, this study was undertaken to breach the gap in scientific information on this species, particularly, its feeding ecology and extent of chemical contamination.

Materials and Methods

Sampling

The stomachs of 39 by-caught Clymene dolphins, landed at three fish landing beaches along the Ghanaian coast (Figure 1) were analyzed. Life history data including standard body length (cm), body weight (kg), sex, tooth counts, location and date of by-catch were recorded for each specimen. Fore-, main- and pyloric stomachs were removed from freshly landed dead dolphins, fixed in 40% formalin in polythene bags to arrest post-mortem digestion and, later, examined in the laboratory for their contents, including food matter and endoparasites.

After the dissection of the whole dolphin following standard protocols [20], blubber, muscle, and liver samples were taken, stored in individual aluminium foils and then in polyethylene bags, labelled and transported on ice to the Ecological Laboratory at the University of Ghana where they were kept frozen at −20 °C until analysis was conducted.

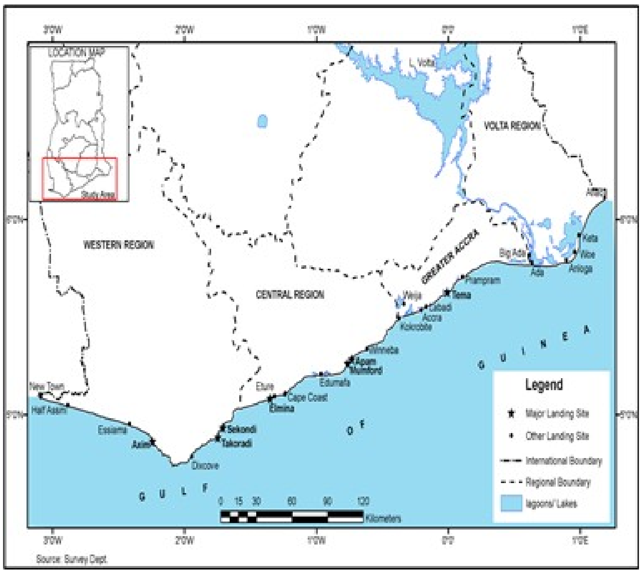

Figure 1: A coastal map of Ghana, showing the location of the study area. This study covers two coastal regions (Central and Western) and consists of two major (Axim, Apam) and one of the other landing sites (Dixcove), all representing areas along the Ghanaian coast where beached dolphins are predominantly found. Sampling was done randomly and fortnightly within a one-year period to obtain the samples.

Sample analysis

Diets were described in terms of prey occurrence, relative abundance, calculated mass and size distribution and followed standard procedures for marine top predators [32-36]. Wet weight of stomach contents was recorded in grams. Each stomach was weighed full, and then emptied into a tray. The empty stomach was weighed again, and the mass of stomach contents obtained by subtraction. The stomach content was washed through a 0.71 μm mesh sieve. All the diagnostic parts were recovered, and identified with the aid of microscopes and hand lenses. Fish bones and otoliths were stored dry, while's cephalopod beaks, crustacean remains as well as any remains with flesh attached were stored in 70% ethanol. Endoparasites were also stored in 70% ethanol. The items found were identified to the lowest taxonomic level possible using published guides [37,38]. Total number of food items was estimated based on paired structures (otoliths, operculum, hyomandibular, dentary and premaxillary) for fishes, upper and lower beaks for cephalopods and eyes/telsons for crustaceans. With the aid of dissecting and light microscopes and parasites identification keys [39], parasites were identified to the lowest taxonomic level possible.

The concentrations of Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in the tissues of the clymene dolphins as well as their prey items (fish, cephalopods) were analyzed following the method described by Kajiwara et al. [40]. Analysis of trace metals was also undertaken following published protocols [41].

Description of stomach contents

The stomach contents of *S. clymene* were described based on three indices. The occurrence of a given prey taxon was the number of stomachs in which this taxon was observed [42]. Relative abundance was given by the number of individuals of the same taxon found throughout the sample set. The calculated biomass was given by the product of the average calculated body mass and the number of individuals of the same taxon in each sample, summed up throughout the sample set. These three indices were expressed by their percentage frequency respectively as, percentage frequency of occurrence (%O), percentage composition by number (%N) and percentage composition by biomass (%M).

Statistical analysis

Concentrations of trace elements in this study were presented on a µg/g dry weight (d.w), since this represents the best bases for comparison of different tissues [43]. Descriptive statistics for mean, standard deviation and range were calculated using SPSS (version 16). Statistical analyses of chemical contaminants data were performed by Analysis of Variance (ANOVA) followed by multiple comparison tests. The significance for statistical analyses was set at α=0.05. To determine the similarity of accumulation of contaminants in the different tissues, Ward's Method of tree clustering, based on analysis of variance, was employed. All analyses of stomach contents were carried out by reference analytical standard methods [44], as a measure for quality control.

Results

Stomach contents

The stomach contents of 39 Clymene dolphins with an average body weight of 61 kg were examined. These comprised 20 males and 19 females. Food remains were recovered from 34 samples (87.2%), with five being empty (12.8%). The total mass of examined food material was 20323.4 g, with a mean mass of 597.7 g ± 152 g (range 0 g to 640 g). In general, food remains were composed of highly digested material (%O=71.8%, %N=12.6 %) and included few identifiable prey remains. Apart from the whole squids which were identified, few cephalopod beaks were found (unidentified, but possibly belonging to the squid, *Sepia* sp.). From direct count, the most important prey family in frequency and numerical terms was the cephalopod, Sepidae (*Sepia sp.)* which accounted for 33.33% and 2.8% respectively of all prey consumed. All other items were teleosts mostly represented by their otoliths and few crustaceans. The most frequent fish prey was the cutlass fishes (Trichiuridae) which occurred in 8 (20.51%) of 34 stomachs examined as compared to the 6 stomachs in which *Decapterus sp* occurred. However, in terms of numbers the mackerel scads, *Decapterus sp.* was of high importance representing 2.6% of prey consumed as indicated in Table 1. Based on variation in morphology, shape and structure, three different otoliths were identified, suggesting the presence of three other fish species. Considering the otoliths present, a minimum of 5 fish species (within 5 families, 5 genera) and two cephalopod species were identified (Table 1). Therefore, on taxa level, fish recorded the highest frequency in terms of occurrence (Figure 2).

Fish, cephalopods, and crustaceans represented a diversity of twelve (12) different species. Average prey diversity was 3.2 ± 2.1 species per sample (N=34 non-empty stomachs).

Stomach Contents	ni	N	%O	%N	%M
Otoliths	7	4	17.95	6.37	0.467
Eyes	15	34	38.46	21.04	1.71
Parasites	20	34	51.28	48.83	3.73
Trichurus lepturus	8	34	20.51	0.79	19.94
Decapterus rhoncus	6	34	15.38	2.63	3.61
Squids (Sepia sp)	13	34	33.33	2.88	45.04
Shrimps	3	34	7.69	1.35	0.21
semi-digested fish (Vertebrae/ skeleton)+ three unidentified fish	28	34	71.79	12.65	25.09
Fish jaws	6	34	15.38	0.46	0.23
Crustacean	3	34	7.69	0.09	0.02
Caudal fins of fish	2	34	5.13	0.06	0.0005
Amphipod	1	34	2.56	0.03	0.01

*Where n is the number of stomachs where the prey i was found and N the total number of stomachs.

Table 1: General composition of items recovered from the stomachs of *S. clymene* landed from the Ghanaian coastal waters.

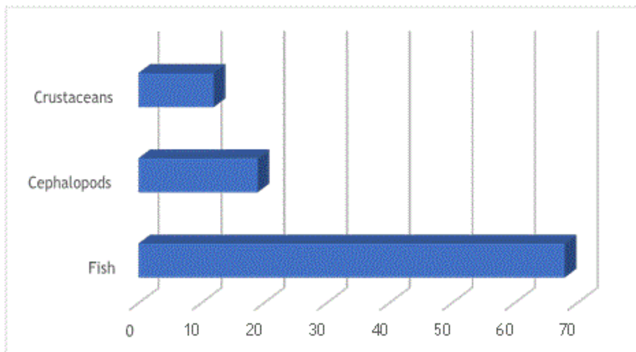

Figure 2: Percent frequency of occurrence of prey items on taxa level found in the stomach of clymene dolphins from Ghanaian coastal waters.

Identification of parasites

Two main types of parasites were identified in the gut of the Clymene dolphins. These were nematodes and acanthocephalans. Nematodes were accurately identified by their smooth, cylindrical, and relatively long body shape whiles the acanthocephalans were differentiated by their characteristic "thorny head", that is, their head region which is armed and encircled with numerous rows of hooks (proboscis). Parasites were identified to family level as Pomphorhynchidae. In terms of abundance, parasites (nematodes), represented equally well and were much comparable to that of fish except that due to their very small body size, it recorded just about 3.73% for its percentage composition by mass (Table 1).

Analysis of chemical contaminants

Analysis of chemical contaminants in tissues indicated a high percentage accumulation of OCPs (gamma-HCH, delta-HCH, Heptaclor, Aldrin Gamma-Chlord, A-endosulfan , p,p'-DDE, Deldrin, Endrin , p,p'-DDT) in the blubber of the dolphin and endoparasitic organisms in the gut (50% for both). Muscle and liver recorded the minimum concentrations of 0.2% respectively (Figure 3). Percentage distribution of 7 PCB congeners analyzed were highest in the blubber (42.86%) followed by both parasites and the liver with a common value of 28.57% (Figure 4). Accumulation of trace metals (As, Cu, Se, Zn, Cd, Hg, Mn, Mg, Cr, Ni, Fe) on the other hand was highest in the blubber (45%), followed by muscle (32%), parasites (20%) and then liver (10%) as shown in Figure 5. Cluster analysis using Ward's method was undertaken to provide an overview of similarities in accumulation patterns in the various tissues of dolphins, as well as the fish and parasites from the stomach content (Figure 6). Based on this analysis, accumulation pattern of OCPs, PCBs and trace metals in the liver and blubber were very similar, likewise that in the stomach contents (fish and parasites).

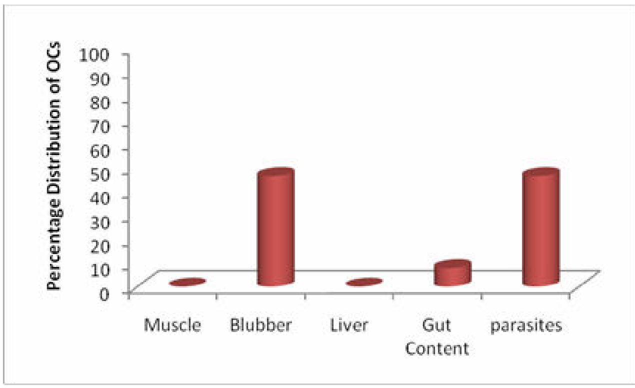

Figure 3: Percentage distribution of OCPS in tissues and stomach content of the clymene dolphin from Ghanaian coastal waters.

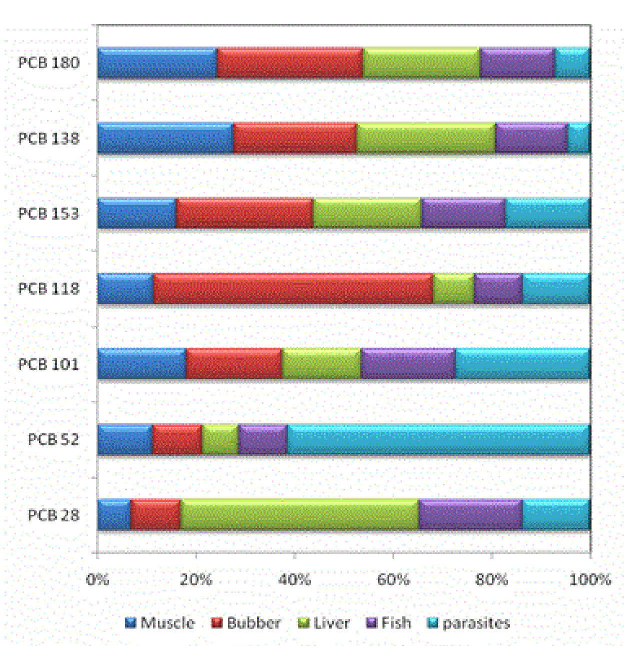

Figure 4: Comparison of the percentage composition of PCB congeners in the various tissues and stomach content of the clymene dolphin from Ghanaian coastal waters.

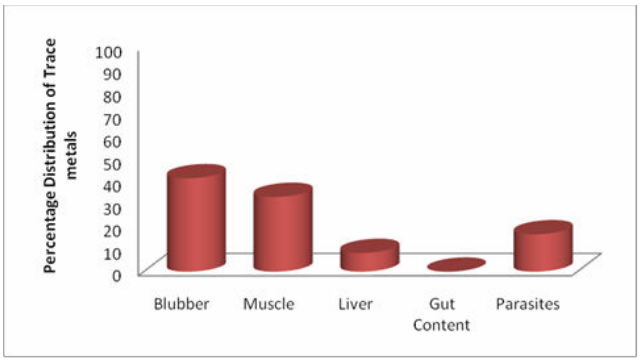

Figure 5: Percentage distribution of trace metals in tissues and stomach content of the clymene dolphin from Ghanaian coastal waters.

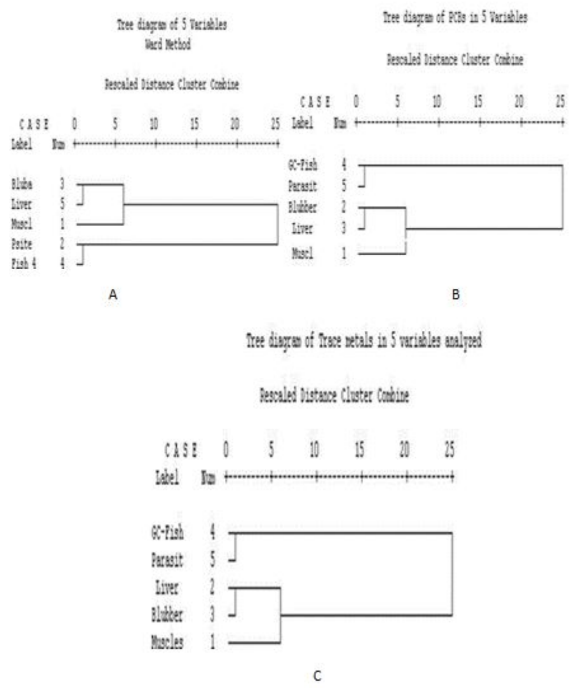

Figure 6: Tree Diagram of (a) OCPs, (b) PCBs and (c) Trace Metals accumulation in the tissues and stomach contents analyzed in the clymene dolphins from Ghanaian coastal waters.

Discussion

This study again confirms *S. clymene* as the most commonly beached dolphin from the coastal waters of Ghana, as all the samples encountered at the landing sites during the one-year sampling period were predominantly of this species. This confirms the preference of this species of dolphins to temperate and tropic waters. From the stomach contents analysis of the Clymene dolphins, fish constituted a major portion of their food, making it the most preferred food item whereas cephalopods constituted an important but minor portion of their diet. It can however be said that both fish and cephalopods represented a more balanced share of the diet in biomass (45.04% and 50.98%) respectively by comparing the number of items that make up the total mass of fish species to the single cephalopod item recorded from each stomach. This is as a result of the generally greater individual body mass of cephalopods relative to fish. Crustaceans accounted for a negligible portion of the diet, by number and by mass. It was observed that the content also included prey of prey, certainly from the fact that many fish species predate on other species. These however constituted the bulk of the digested food. Clymene dolphins off the coastal waters of Ghana thus, appear to rely predominantly on both fish and cephalopods for food. Important prey included typical pelagic groups like the cutlass fishes as well as the bottom-dwelling species, observed from the positioning of the mouth of the unidentified fishes. Studies conducted by Barros et al., [45], in Hong Kong waters indicated that the cutlass fish (*Trichiurus sp*) is consumed by humpback dolphins, finless porpoises and bottlenose dolphins [46], implying they may constitute an important resource for resident and transient cetaceans. This seems to have been confirmed in this study with reference to the frequency at which these species occurred in the diet of Clymene dolphins in Ghanaian coastal waters.

Parasitic infestation of clymene dolphins

In Ghana, Debrah [14] identified some helminth parasites in the fore and main stomachs of dolphins landed on Ghanaian coast, although the numbers recorded were too small for any statistical analysis. Contrary to that research, this study identified, two types of parasites; acanthocephalans, and nematodes, found mainly in fish, and thus may have occurred secondary from ingested prey. This constituted about 48.83 % of the total number of items found in the gut of 20 Clymene dolphins, thus, dominating the gut in relative abundance. Adult parasites especially round worms, live in the stomachs of marine mammals. Large fish tend to be more heavily infested by parasites than small fish of the same species [47]. This is because large fish eat more, and therefore ingest greater numbers of parasites, and also because the larval worms, although inactive, can survive for a long time in fish, and consequently their numbers accumulate as the fish grows older. A similar situation might have occurred in the clymene dolphins and could account for the high percentage of parasites in the guts. Although parasites are a natural occurrence, with small numbers often well tolerated and may not necessarily be contamination, their presence particularly in high amounts as recorded in this study, which can result in excessive interactions, irritations, ulceration or perforations of the stomach hence giving opportunity for secondary infection by bacteria and fungi, can cause significant pathology in the dolphins, likewise in humans through consumption. As there have been cases of human illness [47], caused by the ingestion of some live genus *of nematodes, Phocanema or Anisakis* larvae, in countries where raw or lightly cured fish is commonly eaten.

Chemical contaminants in clymene dolphins

Chemical contaminants (OCPs, PCBs, Trace metals) were found in all tissues of the Clymene dolphin analyzed, as well as in fish, cephalopods and parasites recovered from the stomach. Chemicals enter the marine environment in many ways, typically through fertilizer run offs and use of pesticides in agriculture and home gardening; industrial waste, dumping and airborne emissions, as well as flame retardants used in everyday products. These chemicals are carried around the globe by wind and ocean currents. Regardless the usefulness of these xenobiotics, their increased released into the environment in recent years are causing serious pollution problems.

Marine mammals have an increased likelihood of accumulating these toxins, basically because of high rate of biomagnification due to their elevated trophic level in the marine food chain, their high metabolic rate and long life-span and thus they may serve as a potential indicator of contaminants [18,20,48]. Accumulation of all contaminants analyzed in this study was predominant in the blubber of the dolphin. This is consistent with records of chemical accumulation in marine mammal species [49-51], and justifies the fact that these xenobiotics are lipophilic in nature, thus get deposited in the thick fatty layer of their skin (blubber).

Uptake of most of these chemical pollutants has been reported to be through the lung, skin, diet, placental transfer and milk [52]. However, many studies suggest that diet is the major entry route of elements in marine mammals [18,55]. This is consistent with results from this study, as concentrations of chemical contaminants in the body tissues of the Clymene dolphin were comparable to that recorded from the stomach content. This is particularly so because, dolphins usually consume a wide range of prey species, such as sciaenids available within their geographic ranges [53]. Aside other biological and ecological factors, including geographic area, age, gender, tissue type, metabolic rate and temporal distribution which may influence potential accumulation, feeding strategies also greatly influence the concentrations of chemical contaminants found in dolphins. For instance, particularly high concentrations of trace elements are often encountered in those feeding mainly on cephalopods, which are known to accumulate Cadmium (Cd) in higher levels than fish. Cephalopods are thus, considered to be an important vector of this element to top marine predators [41].

Also, of particular interest in this study, was the high concentration of the organochlorine pesticides in the parasites, relative to the other tissues especially the blubber ($P<0.05$). High concentrations of copper and selenium were also found in the parasite. This supports recent studies which have demonstrated that particular fish parasites can accumulate toxic contaminants (especially metals) from aquatic environment to concentrations that surpass those in the best-established accumulation indicators like the sessile mussel [56-58]. This in a way provides evidence that parasites could be important indicators of contaminant load in marine mammals and might be valuable as environmental indicators, principally for assessing water quality.

Conclusion

The results presented in this study provide evidence of the presence and abundance *of S. clymene* in Ghanaian coastal waters. Fish and cephalopods constitute the most preferred prey items for the Clymene dolphins with high endoparasitic infestation. Analysis confirmed the presence of chemical contaminants in the tissues of the Clymene dolphins as well as in their prey items, at comparable concentrations, suggesting diet as possible route of entry. Based on this study, further investigations on the parasitology of the Clymene dolphins are recommended.

Acknowledgments

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Competing Interests

The authors have declared that no competing interests exist.

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