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Current practices in aquatic animal supplementation

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Abstract

Aquatic animals have been managed under human care for centuries. Limitations in the variety of foods available to feed to these animals, as well as the use of frozen fish products in current dietary protocols, makes supplementation of some nutrients necessary. Limited research has been done on species-specific requirements for vitamins or minerals and there are few standardised recommendations for these species other than for thiamin and vitamin E, for which recommendations are based on deficiencies created under controlled situations in pinnipeds. In recent years, advances have been made in the way fish are caught, processed, stored, thawed and fed to animals. Additionally, many facilities analyse their feeder fish items for caloric content and base their diets on calories consumed instead of strictly on an as-fed weight basis. However, vitamin supplementation practices have often not been modified to reflect these changes. Finally, more recent nutritional concerns have arisen; many facilities have experienced cases of iron storage disease in both pinniped and cetacean species and vitamin C supplementation may contribute to this pathology by enhancing the absorption of dietary iron. An aquatic animal nutritional survey was distributed to zoos and aquariums worldwide focusing on marine mammals, penguins and sharks. Results were returned from 70 facilities. The range of supplements being fed to aquatic animals is significant and much of the variation is due to the manner in which supplements are being dosed. Although over-supplementation of water-soluble vitamins may be tolerated by these species, over-supplementation of some fat-soluble vitamins is potentially harmful. These data can inform and be used to improve feeding practices for aquatic animals.

Introduction

Since the mid 1800s, aquatic animals have been displayed in public zoos and aquariums for both entertainment and education. Aquatic animals in the wild are opportunistic feeders and consume a wide variety of prey items. In zoos and aquariums, however, their diets are limited and typically restricted to frozen, thawed, commercially harvested feeder fish. These food items are purchased in bulk due to seasonal catch, and to assure routine availability, often resulting in prolonged freezer storage times (Crissey 1998). The most common species, chosen because they are readily available at a relatively low cost, are herring (*Clupea harengus*), capelin (*Mallotus villosus*), mackerel (*Scomber scombrus*) and squid (*Illex* or *Loligo*) (Bernard and Allen 2002). Other seafood items are chosen by institutions based on nutrient content, quality and animal preference. The nutrient profiles of these foods often vary, depending upon factors including species, season, age and sex of the catch. Offering a variety of feeder fish types is essential to avoiding dependence on any one particular commodity, as availability is not guaranteed from year to year. It has been recommended that aquatic animal diets be composed of more than one fish variety, including high and low-fat options, to ensure a balanced diet (Geraci 1978). The diets of fish-eating animals often include vitamin or mineral supplements to make up for nutrient losses during storage and thawing (Crissey 1998).

To better understand current feeding and supplementation practices for aquatic animals, a survey was conducted. The vitamins and minerals focused on in this survey included vitamins A, B1 (thiamin), C, E and iron. Each of these have been discussed in the aquatic animal literature previously with respect to requirements, deficiencies, toxicities, or disease. Thiamin is of concern due to degradation post-mortem by thiaminase enzymes, found in many of the foods consumed by fish-eating animals (Geraci 1974; Fitzsimons et al. 2005). Research in harp

Table 1. Institutions which responded to the aquatic animal supplementation survey, which solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided.

Adventure Aquarium – NJ, USA	Grupo Via Delphi – Xcaret, Mexico	Prospect Park Zoo – NY, USA
Alaska SeaLife Center – AK, USA	Gulf World Marine Park – FL, USA	Riverbanks Zoo and Garden – SC, USA
Atlantis, the Palm – Dubai, UAE	Henry Vilas Zoo – WI, USA	Roger Williams Park Zoo – RI, USA
Audubon Zoo – LA, USA	Houston Zoo – TX, USA	Roosevelt Park Zoo – ND, USA
Barcelona Zoo – Barcelona, Spain	Indianapolis Zoo – IN, USA	Saint Louis Zoo – MO, USA
Biodome de Montreal – Montreal, Canada	Jenkinson's Aquarium – NJ, USA	San Diego Zoo – CA, USA
BioPark Zoo – NM, USA	John Ball Zoo – MI, USA	Sea Life Park Hawaii – HI, USA
Blank Park Zoo – IA, USA	Long Island Aquarium – NY, USA	Sea World San Antonio – TX, USA
Bronx Zoo – NY, USA	Los Angeles Zoo – CA, USA	Sea World San Diego – CA, USA
Brookfield Zoo – IL, USA	Marineland of Canada – Ontario, Canada	Seattle Aquarium – WA, USA
Buffalo Zoo – NY, USA	Melbourne Zoo – Melbourne, Australia	SeaWorld Orlando – FL, USA
Cabo Dolphins – Cabo San Lucas, Mexico	Memphis Zoo – TN, USA	Sedgwick County Zoo – KS, USA
Caldwell Zoo – TX, USA	Milwaukee County Zoo – WI, USA	Seneca Park Zoo – NY, USA
Children's Zoo at Celebration Square – MI, USA	Minnesota Zoo – MN, USA	Shedd Aquarium – IL, USA
Cincinnati Zoo – OH, USA	Monterey Bay Aquarium – CA, USA	Smithsonian National Zoo – DC, USA
Columbus Zoo and Aquarium – OH, USA	Mystic Aquarium – CT, USA	Tampa's Lowry Park Zoo – FL, USA
Como Park Zoo – MN, USA	National Aquarium – MD, USA	The Marine Mammal Center – CA, USA
Detroit Zoo – MI, USA	New England Aquarium – MA, USA	The Mirage – NV, USA
Disney's Animal Programs – FL, USA	New York Aquarium – NY, USA	The National Aviary – PA, USA
Dolfinarium Harderwijk – the Netherlands	Oklahoma City Zoo – OK, USA	Theater of the Sea – FL, USA
Dolphins Plus – FL, USA	Oregon Zoo – OR, USA	Utah's Hogle Zoo – UT, USA
Fort Wayne Children's Zoo – IN, USA	Pacific Marine Center – CA, USA	Vancouver Aquarium – BC, Canada
Georgia Aquarium – GA, USA	Point Defiance Zoo & Aquarium – WA, USA	Zoo de Granby – Quebec, Canada
Great Plains Zoo – SD, USA		

seals resulted in a recommendation that 25 to 33 mg thiamin/kg food should be added to marine mammal diets (Geraci 1972). Also in harp seals, examination of vitamin E deficiency, resulting in electrolyte imbalances, irregular moults and low plasma vitamin E concentrations, led to a recommendation of the addition of 100 IU vitamin E/kg of fish for marine mammals (Engelhardt and Geraci 1978).

In contrast to concerns about deficiency, vitamin A concentrations are generally very high in whole fish (Dierenfeld et al. 1991; Bernard and Allen 2002) and in some of the available aquatic animal supplements. High vitamin A may present a risk of toxicity, but also can impair vitamin E absorption; a study in northern fur seals showed decreased serum vitamin E concentrations when animals were supplemented with high concentrations of vitamin A (Mazzaro et al. 1995). Finally, vitamin C has been recommended for dolphins (200–250 mg/day) (Barck Moore 1980), and scurvy has been reported in dolphins (Miller and Ridgway 1963). However, reports of iron storage disease in dolphins (Johnson et al. 2009; Phillips et al. 2011), fur seals (Mazzaro et al. 2004), and sea lions (Garcia et al. 2000) have led some institutions to remove vitamin C supplementation from these animals as vitamin C can increase iron absorption (Teucher et al. 2004).

The history of how and why marine mammal supplements were formulated is limited to personal communication but specific supplements were manufactured to avoid the labour of multiple supplements associated with feeding fish to aquatic animals. The formulations were based on published literature, other carnivore diets and established fish nutrient composition. Vitamin A levels were typically high in these supplements due to depressed circulating vitamin A concentrations that had been determined at the time in certain marine mammal species (L. Cornell and J. McBain, pers. comm.). However, it is now recognised that vitamin A is tightly regulated in the blood (Raila et al. 2000; Simms and Ross 2000), and thus blood values did not accurately reflect a low vitamin A status. Additionally, changes in commercial fishing practices including handling, storage and the time required for freezing has significantly improved over time. These, combined with advancements in the way fish are stored, thawed and handled at individual facilities has resulted in changes to previously established industry-standardised methodologies. Finally, more supplementation options (e.g. paste, tablets, multiple types of nutrient profiles etc.) are now available to institutions. This survey was performed to determine how current practices at individual facilities might benefit from updated feed supplement recommendations. Calculations of estimated nutrient intake were made based on current fish nutrient analyses in addition to data from previously published literature.

Methods

A nutrition survey (see supplementary material) was created and disseminated to zoos and aquariums via the Penguin and Marine Mammal Taxonomic Advisory Groups, the International Association for Aquatic Animal Medicine newsletter and the

Table 2. Food items (percentage of the diet) provided to aquatic animals based on survey results. The survey was completed by 70 institutions, and solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided.

Food	Pinnipeds	Cetaceans	Freshwater otters	Sea otters	Penguins	Elasmobranchs
Herring	0–100%	8–84%	0–50%	0–12%	0–100%	0–70%
Capelin	0–85%	0–80%	0–90%	0–38%	0–100%	0–50%
Squid	0–73%	0–15%	0–20%	0–50%	0–10%	0–75%
Mackerel	0–33%	0–30%	0–50%		0–10%	0–80%
Smelt	0–20%	0–30%	0–95%		0–60%	0–14%
Silversides					0–100%	
Invertebrates				8–70%		
Total no. foods	1–10	2–6	1–9	2–6	1–9	2–11
Mean no. foods	3.9	4	3.2	3.7	3.4	4.7
Most frequent no. foods	4	4	3	2	3	4

AZA's Nutrition Advisory Group listserve. Pinnipeds, cetaceans, otters, penguins and sharks were the animals of interest. The survey solicited information on the type of facility (public display, research, stranding), the types of dietary items fed (based on common names of species) with percentages and identification of vitamin and mineral supplements (including form used) and dosing methods for each group of animals. The survey did not ask about the number of animals held at each institution, so the data presented represent the percentage of institutions holding animals as opposed to the percentage of animals being maintained on a diet/supplement. The surveys were completed by qualified personnel (husbandry, nutrition and/or veterinary staff) at each facility and information was returned via email or fax. Institutions responding (n=70) were primarily from North America, although institutions from UAE, Australia, Spain and the Netherlands also participated (Table 1). The survey data represents 60 institutions holding marine mammals, 41 institutions holding penguins and 24 institutions holding sharks.

In addition to the survey results, samples of four of the major feeder fish reported in the survey, Atlantic mackerel (*Scomber scombrus*), capelin (*Mallotus villosus*), Atlantic herring (*Clupea* harengus) and European squid (*Loligo vulgaris*) were freshly caught (i.e. less than 30 days), individually quick frozen (IQF; BionicBait Pompano Beach, FL 33064) and analysed for selected nutrient content in a commercial laboratory. Specific nutrients analysed included vitamins A and E, ascorbic acid, thiamin and iodine (NP Analytical Labs, St Louis MO), as well as dry matter, protein, fat, calcium, phosphorus, sodium, potassium, magnesium, iron, zinc, manganese and copper (Dairy One, 730 Warren Road, Ithaca NY). To quantify the stability of specific nutrients under prolonged freezer storage, capelin and mackerel samples were submitted for analysis at 0, 6 and 12 months post-catch. All samples were maintained at -20°C for the duration of the testing period and packaged and submitted for analysis using identical protocols.

Results

The survey demonstrated that herring, capelin, squid and mackerel were fed at the highest percentages to all animal groups (Table 2). The variety of food choices offered at individual facilities varied from one to 11. In addition to the four main dietary items, 35 other food items including other fish species, invertebrates and complete diets were reported to be used in smaller proportions to add variety to diets. Fish species included anchovy (*Engraulis* family spp.), bluefish (*Pomatomus saltatrix*), bonito (Scombridae *f*amily spp.), butterfish (*Peprilus triacanthus*), croaker (*Micropogonias undulatus*), goatfish (*Mullidae* family spp.), menhaden (*Brevoortia tyrannus*), milkfish (*Chanos chanos*), pilchards (*Clupeidae* family spp.), pollock (*Pollachius* spp.), pompano (*Trachinotus* spp.), redbait (*Emmelichthys nitidus),* sand eels (Ammodytidae family spp.), trout and salmon (Salmonidae family spp.), sardines (Clupeidae family spp.), saury (*Cololabis*

Table 3. Method of supplementation to aquatic animals based on survey results. The survey was completed by 70 institutions, and solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided.

Supplement dosing regime	Pinnipeds and cetaceans	Otters	Penguins	Elasmo-branchs
By diet	66%	22%	12%	54%
By body weight	25%	0%	2%	0%
Specified no. tablets/day	0%	33%	86%	21%
Thiamin and Vit E only	13%	8%	7%	0%
Additional supplements	23%	8%	26%	14%
No supplements	0%	36%	0%	7%

Table 4. Type of supplement fed to aquatic animals, based on survey results. The survey was completed by 70 institutions, and solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided.

Supplement content	Pinnipeds and cetaceans	Otters	Penguins	Elasmo-branchs
No or low Vit A	55%	36%	40%	0%
High Vit A	39%	19%	58%	75%
With Vit C	63%	42%	79%	75%
Without Vit C	32%	14%	19%	0%
With iron	0%	8%	19%	36%
Without iron	95%	47%	79%	39%

Table 5. Nutrient composition of capelin (*Mallotus villosus*), from various time-points post-harvest, and from published literature. Data represent nutrients on dry matter basis, and are means +/- SEM for n>3 samples from 2011 harvest. Means within rows with different superscripts are significantly different (p<0.05). BLD = below limit of detection.

	1 month frozen	8 months frozen	13 months frozen	Bernard and Allen 2002	Dierenfeld et al. 1991	Lawson et al. 1998
Dry matter (%)	21.3±0.3	20.3±0.3	21.4±0.4	14.6–22.8	25	31.8±10.1
Crude protein (%)	74.3±0.8a	71.4±1.0ab	68.7±0.5b	56.0–76.5		50.6±15.6
Crude fat (%)	17.9±0.8	19.7±0.7	18.3±1.0	7.0–23.3		43.1±24.8
Ash (%)	12.1±0.4a	12.0±0.1ab	10.7±0.3b	8.6–12.8		6.6±1.4
Ca (%)	1.7±0.1	1.6±0.1	1.5±0.1	1.2–2.2		
P (%)	1.8±0.1	1.9±0.1	1.9±0.1	1.3–2.3		
Mg (%)	0.20±0.00	0.18±0.00	0.18±0.01	0.08–0.23		
K (%)	1.4±0.1b	1.6±0.1ab	1.7±0.1a	0.78–2.0		
Na (%)	1.5±0.1a	1.3±0.1a	1.0±0.1b	0.39–1.7		
Fe (ppm)	71±7a	55±1ab	51±1b	36–140		
Zn (ppm)	66±2	63±3	62±2	41–87		
Cu (ppm)	2.0±0.0	1.0±0.0	3.0±0.0	3.0–10		
Mn (ppm)	2.0±0.0	2.0±0.0	2.0±0.0	2.0–5.0		
Iodine (ppm)	BLD		BLD			
Thiamin (ppm)	BLD		BLD			
Vit A (IU/kg)	9,702±1,754a		4,263±406b	8,904–189,829	13,212±1,608	
Vit C (ppm)	BLD		BLD			
Vit E (IU/kg)	BLD		BLD	23–360	68±9	

Table 6. Nutrient composition of Atlantic mackerel (*Scomber scombrus*), from various time-points post-harvest, and from published literature. Data represent nutrients on dry matter basis, and are means +/- SEM for n>3 samples from 2011 harvest. Means within rows with different superscripts are significantly different (p<0.05). BLD = below limit of detection.

	1 month frozen	8 months frozen	13 months frozen	Bernard and Allen 2002	Dierenfeld et al. 1991
Dry matter (%)	31.3±1.6	31.3±1.0	32.4±1.7	31.2–36.9	25
Crude protein (%)	59.0±4.1	61.8±2.5	55.1±3.4	41.8–50.1	
Crude fat (%)	32.1±4.0	33.0±2.6	31.5±3.9	42.3–48.4	
Ash (%)	8.6±0.5[a]	8.4±0.3[a]	6.5±0.4[b]	6.2–6.4	
Ca (%)	1.3±0.1[b]	1.6±0.1[a]	1.1±0.1[b]	0.73–1.2	
P (%)	1.8±0.1	1.8±0.1	1.8±0.1	1.1–1.2	
Mg (%)	0.13±0.00	0.13±0.00	0.11±0.01	0.10–0.14	
K (%)	0.94±0.04	1.1±0.1	0.97±0.08	0.77–1.0	
Na (%)	0.48±0.06	0.57±0.01	0.40±0.02	0.38–0.48	
Fe (ppm)	70±4	80±1	64±5	63–100	
Zn (ppm)	44±3	52±3	45±4	39–46	
Cu (ppm)	2.0±0.0	2.0±0.0	4.0±0.0	5.0–10	
Mn (ppm)	2.0±0.3	2.0±0.3	1.0±0.3	2.0–3.0	
Iodine (ppm)	BLD	BLD	BLD		
Thiamin (ppm)	1.3±1.3	BLD	BLD		
Vit A (IU/kg)	107,843±10,091[a]	101,293±12,503[a]	53,681±9,094[b]	59,108	56,532±4,228
Vit C (ppm)	BLD	BLD	16±0.1		
Vit E (IU/kg)	BLD	BLD	11±5.5	33	54±8.4

Table 7. Nutrient composition of Atlantic herring (*Clupea harengus*) at 1-month post-capture, and from published literature. Data represent nutrients on dry matter basis for n=1 sample from 2011 harvest. BLD = below limit of detection.

	1 month frozen	Bernard and Allen 2002	Dierenfeld et al. 1991	Lawson et al. 1998
Dry matter (%)	25.0	20.6-28.6	25.0	35.0±3.4
Crude protein (%)	70.8	44.2-70.2		57.4±3.7
Crude fat (%)	20.6	16.4-38.3		39.1±11.1
Ash (%)	13	7.3-11.9		2.3±0.2
Ca (%)	2.2	1.6-1.9		
P (%)	1.7	1.0-2.1		
Mg (%)	0.20	0.10-0.24		
K (%)	1.3	0.94-1.9		
Na (%)	0.76	0.35-0.85		
Fe (ppm)	71	64-132		
Zn (ppm)	68	44-80		
Cu (ppm)	1	4-6		
Mn (ppm)	4	3-7		
Iodine (ppm)	9.0			
Thiamin (ppm)	BLD			
Vit A (IU/kg)	33,704	10,704-31,864	13,492±536	
Vit C (ppm)	BLD			
Vit E (IU/kg)	BLD			

Table 8. Nutrient composition of squid (*Loligo vulgaris*) at 1-month post-capture, and from published literature. Data represent nutrients on dry matter basis for n=1 sample from 2011 harvest. BLD = below limit of detection.

	1 month frozen	Bernard and Allen 2002; *Ilex and Loligo sp.*	Lawson, et al. 1998; *Illex coindetii*	Lawson, et al. 1998; *Gonatus fabrici*	Pennino et al. 1991
Dry matter (%)	17.2	15.4-18.8	24.9±1.5	26.5±6.0	19.4 ± 1.0
Crude protein (%)	79.5	65.4-77.3	68.3±2.6	51.7±10.2	85.6 ± 4.4
Crude fat (%)	9.1	8.3-11.4	26.5±6.2	41.1±14.3	10.8 ± 1.0
Ash (%)	11.7	4.8-6.4	5.6±0.6	7.2±2.1	10.6
Ca (%)	0.17	0.11-0.17			
P (%)	0.83	1.1-1.2			
Mg (%)	0.33	0.21-0.23			
K (%)	1.1	0.66-1.2			
Na (%)	2.0	0.84-0.90			
Fe (ppm)	24	12-29			
Zn (ppm)	74	63-87			
Cu (ppm)	291	106-245			
Mn (ppm)	3	0-2			
Iodine (ppm)	BLD				
Thiamin (ppm)	7.2				
Vit A (IU/kg)	6,813				22,333 ± 7,000
Vit C (ppm)	BLD				
Vit E (IU/kg)	179				331 ± 69

Table 9. Example of nutrient intake versus vitamin recommendations when supplementation is dosed based on food intake. Example based on equivalent energy consumption across feeder fish types.

	High energy fish e.g. mackerel	Moderate energy fish e.g. herring	Low energy fish e.g. capelin
Amount of food (kg/day, as fed)	13	21	31
Vit E from suppl. (IU/d)[1]	944	1525	2251
Vit E from diet (IU/d)[2]	0-220	0-377	0-613
Total Vit E intake (IU/d)	944-1164	1525-1902	2251-2864
Recommended Vit E (IU/d) (Engelhardt and Geraci 1978)	1300	2100	3100
Thiamin from suppl. (mg/d)[1]	1230	1987	2933
Thiamin from diet (mg/d)[2]	0	2.4	0
Total thiamin intake (mg/d)	1230	1987-1989	2933
Recommended thiamin (mg/d) (Geraci 1972)	429	693	1023
Vit A from suppl (IU/d)[1]	45760	73920	109120
Vit A from diet (IU/d)[2]	219,125-440,215	60,332-189,969	29,073-1,294,634
Total Vitamin A intake (IU/d)	264,886-485,975	134,252-263,889	138,193-1,403,754

[1]Supplement calculated to provide 73 IU vitamin E, 95 mg thiamin, and 3520 IU vitamin A per kg fish as fed based on common tablet supplementation guidelines. [2]Vitamin levels from diet determined from values from this project and the literature (Table 5-8).

saira), skate (Rajidae family spp.), sprat (*Sprattus* spp.), tommy rough (*Arripis georgianus*), and whiting (*Gadus chalcogrammus*). Invertebrates fed included razor clams (*Siliqua patula*), surf clams (*Spisula solidissima*), crab (species not noted), krill (Euphausiidae family spp.), mussels (Mytilidae family spp.), scallops (Pectinidae family spp.), and shrimp (species not noted). Other diet items included carnivore, feline, canine, horsemeat and gel-based diets (specific product information not provided). Stranding facilities generally offered less variety than zoos and aquariums. Only sea otters (*Enhydra lutris*) were reported to receive invertebrates as a significant portion of their diet.

The type of vitamin/mineral supplementation reported in the survey varied by animal type (Table 3). In general, tableted/pill products were used for supplementation, although paste-type products (e.g. Thiamin E paste) were also used. Pinnipeds and cetaceans were mainly supplemented based on the amount of diet consumed (number of tablets/kg diet consumed), while a smaller percentage of institutions based their supplement dosing strategy on body weight of the animal (number of tablets/kg body weight). Some institutions holding penguins, otters and sharks reported other methods of dosing their animals, including a specific number of tablets per day or days per week.

The nutrition provided by supplementation to animals also varied by species (Table 4). All facilities maintaining pinnipeds, cetaceans and penguins reported adding a supplement to diets, while some facilities housing otters and sharks reported no addition of a vitamin or mineral supplementation via the diet. For all groups of animals, 8–26 % of facilities fed vitamin E and thiamin (B1) supplements in addition to a multi-vitamin. A small percentage of pinniped, otter and cetacean facilities reported feeding only vitamin E and thiamin (B1) to their animals.

To better understand the total nutrient intake level of aquatic animals, the primary feeder fish were assessed for selected nutrient composition over 1 year of storage (3 time periods), and data were also compared to previous reports for capelin (Table 5) and Atlantic mackerel (Table 6). Analysis of Atlantic herring (Table 7) and squid (Table 8) was conducted at one month post-capture and compared to previous reports. Fish analysed contained low or no iodine, vitamin C or vitamin E, low to moderate iron levels, and moderate to high levels of vitamin A. Squid had relatively high copper levels and detectable thiamin. For fish analysed over time, in general, ash content was reduced over time, as was vitamin A content.

Discussion

It is apparent that much variation continues to exist in methods of supplementation and types of supplements provided to aquatic animals. The high degree of variability in vitamin A and E supplementation reported here for pinnipeds is virtually unchanged from that suggested over 20 years ago (Mazzaro et al. 1995). Toxicities from over-exposure to fat-soluble metabolites such as vitamin A are rare; however, there is a significant quantity of literature indicating the potential for toxic accumulation of this metabolite (NRC 1987; Russell 2000). In many species, clinical pathology results from chronic consumption of 100 to 1000 times the actual nutritional requirement and hypervitaminosis A has been reported in animals at as low as 10 times the level of species-specific requirement (NRC 1987). When vitamin requirements are not clearly defined, as is commonly the case with aquatic animals, over-exposure becomes more likely.

Based on multiple examinations of fish nutrient composition in captive scenarios (e.g. Dierenfeld et al. 1991; Bernard and Allen 2002), the preponderance of dietary vitamin A comes from endogenous concentrations found in the feeder fish themselves. Consequently, the current best practice of feeding only whole non-eviscerated forage fish to captive animals may justify the discontinuation or reduction of supplementation with high levels of vitamin A, as has been previously suggested (Dierenfeld et al. 1991). From the standpoint of economics, additional supplementation may be costly, and at worst, ill-advised from the perspective of preventative animal health care. For smaller animals, for which chopped, filleted or gutted feeder fish may constitute the basis of the animals' ration, additional supplementation with vitamin A may be necessary, as the primary location for vitamin A storage is in the liver, and deficiencies of vitamin A continue to be reported (St Leger et al. 2011). Other nutrients examined, including vitamins E and C, are not typically considered as potentially toxic, even at elevated levels of intake. However, the endogenous concentrations of E and C contained within the feeder fish analysed in our research and by others appears quite low, and thus, continued supplementation is warranted. Of note is that supplementation with vitamin C (ascorbic acid) has been questioned due its propensity for stimulating iron absorption (Teucher et al. 2004) and the concern over iron storage disorders in marine mammals. However, further research is required to further quantify supplementation recommendations.

Finally, the practice of supplementation based on the amount of fish fed leads to dramatically different levels of nutrient intake (Table 9). For example, supplementation of high-fat fish (which contain higher calories and thus are fed at a lower total quantity) results in much lower supplementation of vitamin E as compared to supplementation of low-fat fish (which contain lower caloric density and thus are fed at a greater total quantity). For the majority of species studied, vitamin E requirements parallel the intake of polyunsaturated fatty acids (e.g. Harris and Embree 1963; NRC 1993, 1994, 2006). It is likely that the same relationship exists in aquatic animals, and thus supplementation of vitamin E should be higher when fish containing high fat and high polyunsaturated fatty acids are fed, as has been previously stated (Dierenfeld et al. 1991). Lacking further detail on vitamin E content of diet ingredients specifically, supplementation based on body weight of the animal may provide a more standardised intake of nutrients, when body weight measurements are feasible.

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