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Anaerobic bacteria from the digestive tract of North Atlantic fin whales (*Balaenoptera physalus*)

(Baleen whale; forestomach microbiology; colon microbiology, methanogen; sulfate-reducer; *Vibrio* spp; enteric bacteria)

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1. SUMMARY

Samples were collected from the forestomach and colon of North Atlantic fin whales (*Balaenoptera physalus*) landed at the commercial whaling station at Hvalfjördur, Iceland during three whaling seasons. Techniques were used to enrich for and enumerate anaerobic bacteria, methanogens, and sulfate reducers. Anaerobic bacteria ranged from 10^8 to 10^{10} per ml of digesta in the colon, and from 10^5 to 10^9 per ml of digesta in the forestomach. Methanogens and sulfate-reducing bacteria were found in the majority of forestomach and colon samples, with sulfate-reducing bacteria usually occurring at higher concentrations. Enteric bacteria, *Vibrio,* and *Listonella* spp. were found in the colon. Volatile fatty acids were detected in significant concentrations in the forestomach of many of the whales. These results support previous findings which suggest that a microbial fermentation occurs in the forestomach of baleen whales.

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2. INTRODUCTION

The mammalian order Cetacea which includes the whales, porpoises and dolphins is divided into two living suborders. The suborder Mysticeti contains the baleen whales, and the suborder Odontoceti includes the toothed whales. Baleen whales feed largely on krill. The krill are trapped by the baleen plates and then pass through a multichambered stomach typical of all cetaceans.

The purpose of this study was to examine the microbiology of the forestomach and colon contents of healthy baleen whales. Since most of the previous microbiological studies of cetaceans have been done with captive or stranded odontocetes, the normal microflora of mysticetes in the wild is poorly understood. For this study North Atlantic fin whales were examined since these animals were available through a commercial whaling operation in Iceland.

One additional aspect of this investigation was to evaluate whether a microbial fermentation occurs in the forestomach of fin whales. Though some believe the forestomach of whales serves simply as a storage chamber [1–3], recent results suggest that it may be functionally analogous to

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the forestomach of land ruminants in which a microbial fermentation occurs [4]. Evidence for this hypothesis was derived from the discovery of significant amounts of acetic, propionic and butyric as well as other volatile fatty acids in forestomach samples of gray (*Escherichtius robustus*) and bowhead (*Balaena mysticetus*) whales.

3. MATERIALS AND METHODS

3.1. Fin whale harvesting

Fin whales were taken by catcher boats owned by the Icelandic whaling company, Hvalur H.F., and towed back to the shore station located at Hvalfjördur, where the animals were flensed. Catcher boats traveled 80–160 km off the west coast of Iceland during the summer whaling season and usually harpooned two whales each trip. To maintain the quality of the whale meat an incision is made in the abdominal cavity at sea to permit sea water (9–10°C) to cool the internal organs. All catcher boats adhered to a strict time limit for the interval between time when the whale was taken and when it was delivered to the station. Scientists were permitted to remove samples shortly after the whale arrived at the station. Fin whales were sampled during the 1983, 1984, and 1985 Icelandic whaling seasons.

3.2. Sampling protocol

At the shore-based station, incisions were made through the outer wall of the forestomach and colon and approx. 300 ml of contents was placed in sterile beakers. The samples were taken to the nearby Icelandic Whale Research Center. Using a 10-ml disposable syringe, modified into a 'microcorer' by removing the tapered end, 7 ml of contents was removed from the beakers. A sterile spatula was used to stuff the forestomach contents into the syringe. The 7-ml sample was mixed with 28 ml of pre-reduced diluent and blended in a sterile 37-ml Waring blender for 30 s. Sterile disposable 1-ml syringes and pre-reduced diluent were used to dilute the blended samples. Various media were inoculated to permit the enrichment and enumeration of different kinds of anaerobic bacteria. In addition, during the 1983 season, digestive tract contents were examined for enteric bacteria and vibrios.

3.3. Examination of colon contents for members of the Enterobacteriaceae

For isolation of bacterial species belonging to the family Enterobacteriaceae, contents from the colon were inoculated onto MacConkey Agar (Difco) and incubated at 37°C. From one whale, the contents from the small intestine were also examined. Representative colonies were picked and restreaked onto MacConkey Agar. The oxidase test [5] was performed on all of the MacConkey Agar isolates to eliminate oxidase-positive organisms. This was followed by a screening procedure [5]. Selected isolates were identified using the API 20E system (Analytab Products, Plainview, NY, USA).

3.4. Examination of forestomach, small intestine, and colon contents for mesophilic members of the Vibrionaceae

For the Vibrionaceae, samples of colon contents were swabbed onto TCBS Agar (thiosulfate–citrate–bile–sucrose agar) (Difco) and incubated at 37°C. From one fin whale, samples were also taken from the forestomach and second stomach. After purification of representative colonies on TCBS Agar, a limited taxonomic screening was performed using the following media and tests: 0/129 (BDH Chemicals Ltd.) sensitivity at 10 μg and 150 μg concentrations per sensitivity disk; oxidase test; catalase test; growth on CLED (cystine-lactose-electrolyte deficient) medium (with Andrade indicator; Oxoid) containing 0% and 1% NaCl; lysine decarboxylase, arginine dihydrolase, and ornithine decarboxylase using Moeller Decarboxylase Base (Difco); acid production from L-arabinose, lactose, maltose, D-mannitol, sucrose, and glucose; indole production; Voges-Proskauer test; and production of urease using urea agar base (Difco). Media were supplemented with 1% NaCl to permit growth of the halophilic vibrios. Media and procedures for the vibrio identification

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are outlined by Furniss et al. [6] and West and Colwell [7].

3.5. Enumeration of viable heterotrophic anaerobes, methanogens, and sulfate-reducers

Media and diluent to permit enumeration of the stringent anaerobes were prepared in Seattle and transported to Iceland. Except where noted below media were sterilised in glass tubes containing butyl rubber stoppers. Media were prepared using techniques outlined by Hungate [8] and the Virginia Polytechnic Institute Anaerobe Manual [9]. Gases used for preparation of the anaerobic media were deoxygenated using a heated copper column. In Iceland, N_2 used for gassing and rinsing syringes was deoxygenated by bubbling the gas through a titanium citrate solution [10]. Before inoculation with the diluted samples, media were reduced with the addition of a reducing agent, (cysteine-HCL–Na_2S, sodium dithionite). Sterile disposable 1-ml hypodermic needles and syringes were used to dilute the samples and inoculate the media. All media for the stringent anaerobes contained a half-strength artificial seawater base (ASW1/2), which included: 12.0 g NaCl, 3.5 g $MgSO_4 \cdot 7H_2O$, 2.6 g $MgCl \cdot 6H_2O$, 0.55 g $CaCl_2$, and 0.35 g KCl in 1000 ml of distilled H_2O. All concentrations for media components listed below are per 1000 ml ASW1/2. All inoculated media were incubated at 37°C. Minor changes were made in media composition between sampling seasons and are described below.

3.5.1. Anaerobic heterotrophic bacteria

Medium 10 [11] was modified for the enumeration of anaerobic heterotrophic bacteria found in the digestive tract of fin whales and called Cetacean medium (CM). During the 1983 and 1984 seasons CM contained 0.5 g glucose, 0.5 g yeast extract, 2.0 g tryptone, 1.0 ml hemin solution, 0.5 g NH_4Cl, 0.2 g K_2HPO_4, 10.0 ml vitamin mix [12], 1.0 ml resazurin solution (0.1% w/v), 20.0 g agar. The pH was adjusted with $NaHCO_3$ to 6.8. This medium was prepared in screw-cap tubes covered with Hungate butyl rubber septa [13] and the headspace was filled with N_2 in 1983 and with 10% H_2/5% CO_2/85% N_2 mix in 1984 and 1985. In 1985 the quantity of tryptone in the formula was increased to 10.0 g. The medium was heated before use to melt the agar and held at 50°C before the addition of the diluted sample. After addition of the sample the medium was rapidly cooled in an ice-water bath and solidified on the side of the tube. Media were reduced before inoculation with the addition of Na_2S–cysteine-HCl solution. Bacterial colonies were counted with the aid of a dissecting microscope after 3 weeks of incubation.

3.5.2. Methanogenic bacteria enrichments

Techniques used for the enrichment of methanogen bacteria were modifications of those presented by Balch et al. [14]. Methanogenic bacteria were enriched in the following broth medium: 0.5 g NH_4Cl, 0.2 g K_2HPO_4, 1.0 ml trace element solution [15], 10.0 mM Mops (4-morpholinepropanesulfonic acid), 2.5 g sodium acetate, 2.5 g sodium formate, 2.0 g yeast extract (Difco), 2.0 g tryptone (Difco), 1.0 ml of a 0.1% (v/v) resazurin solution. For the preliminary study in 1984, the medium was pressurized to 2 atmospheres with H_2 gas. In 1985, a 10% CO_2/90% H_2 mixture was used and the Mops buffer was replaced with 10.0 mM Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid). Before use the medium was reduced with a Na_2S–cysteine-HCl solution and sodium bicarbonate was used to adjust the pH to 7.0. Enrichments were considered positive if methane was detected in the headspace using a gas chromatographic technique. A 0.2-ml headspace sample was ingested into a Poropak R (80-100 mesh) stainless steel column (6 ft by 0.125 in). The column temperature was set at 30°C, and the carrier gas flow rate was set at 20 ml of He per min. A Carle Analytical Gas Chromatograph (Model 111) with a thermal conductivity detector was used. Enrichments were examined periodically for a period of 1 month.

3.5.3. Sulfate-reducing bacteria

Sulfate-reducing bacteria were enriched using the following medium: 0.5 g NH_4Cl, 0.2 g K_2HPO_4, 1.0 ml trace element solution without iron [15], 10.0 mM Mops, 2.5 g sodium acetate, 5.0 ml sodium lactate (50% solution), 1.0 g yeast

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extract (Difco), 1.0 ml resazurin solution with N_2. Sodium dithionite and a $FeSO_4 \cdot 7H_2O$ solution were added before inoculation. Enrichments were scored positive when the tube turned a deep black color.

3.6. pH and determination of volatile fatty acids

The pH of the stomach and colon contents was determined using a pH meter. Using a gas liquid chromatograph, the volatile fatty acid (VFA) composition and concentration of the forestomach contents were determined on samples that had been either fixed with formalin or frozen [4].

4. RESULTS

Samples for microbiological and chemical evaluation were removed primarily from the forestomach and colon of fin whales landed at the Icelandic whaling station. In addition, a dissection of several fin whales was performed. The forestomach was found to be approximately equal in size to the main stomach. The morphology of the upper digestive tract of fin whales was examined and is schematically represented in Fig. 1. From linear measurements taken of full forestomachs

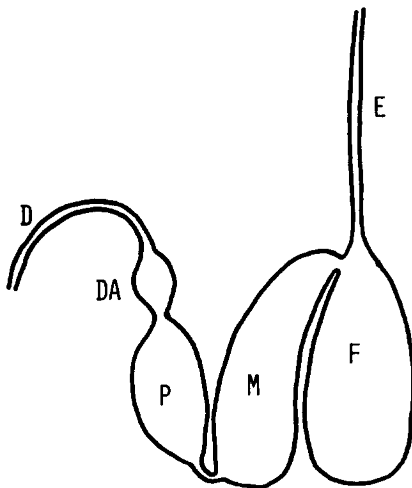

Fig. 1. Upper digestive tract morphology of fin whale, showing esophagus (E), forestomach (F), main stomach (M), pyloric stomach (P), duodenal ampulla (DA), and duodenum (D).

from mature fin whales, we estimated the volume to be approximately 250–300 l. Fin whales off the coast of Iceland were feeding primarily on krill (*Megacytiphanes norvegica*) and rarely upon fish (J. Sigurjónsson, personal communication). During the 1983 season, a higher proportion of fin whale stomachs contained fish that was tentatively identified as blue whiting, *Micromesistius patassa* (J. Sigurjónsson, personal communication).

The counts of viable anaerobic bacteria in the forestomach samples ranged from 10^5–10^9 per ml and from 10^8–10^{10} per ml in the colon (Table 1). Forestomach samples also generally appeared to be slightly acidic. Volatile fatty acids were detected in all samples and ranged in concentration from < 1-247 mM. The forestomach samples were not uniform in appearance and several samples appeared to be diluted with seawater which may enter the forestomach during death of the whales or during transit of the whales back to the flensing station. Many whales had stomachs completely filled with seawater and void of krill. The forestomachs of these whales were not sampled. Although the VFA concentration varied between samples, the total straight-chain (normal) VFAs were found in higher concentrations than the total branched-chain (iso) VFAs (Table 2). Acetic acid was found in highest concentration in all of the samples. The other normal-chain VFAs found were propionic, butyric, and valeric acids; the iso-VFAs included isobutyric, isovaleric, and isocaproic acids.

Forestomach and colon samples were also examined for sulfate-reducers and methanogens. Table 3 presents data for the colon samples. In 1984, 3 of the 5 forestomach and 5 of the 7 colon samples were positive for at least one of these groups. In 1985 attempts were made to quantitate the levels of these bacteria in the forestomach and colon. Sulfate-reducers and methanogens were examined in the 10^{-1}, 10^{-3}, and 10^{-5} dilutions of samples. Slight modifications of both media were made to improve the recovery of sulfate-reducers and methanogens. In 1985, only sulfate-reducers were found in the 10^{-1} dilution of forestomach samples from 2 whales. However, both methanogens and sulfate-reducers were found in all colon samples. Sulfate-reducers were found in the 10^{-5}

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Table 1

Characteristics of fin whales that were sampled; counts of anaerobic bacteria, pH and total volatile fatty acid (VFA) concentration in forestomach samples; counts of anaerobic bacteria and pH of colon samples

Fin whales were assigned a number and measured in units of feet at the whaling station. The body weights of these whales could not be determined. In a separate study (C.H. Lockyer and T.D. Waters, personal communication) conducted by Sea Mammal Research Unit (Cambridge, U.K.) the body weight of a 61-ft male fin whale (No. 42, landed in 1984) was 36900 kg and a 52-ft male fin whale (No. 107, landed in 1985) was 25300 kg. N.D. = Not determined

Characteristics of whales				Forestomach samples			Colon samples	
Whale No.	Sex	Length (ft)	Hours after death sampled	Viable anaerobic bacteria per ml	pH	Total VFA (mM)	Viable anaerobic bacteria per ml	pH
1983 Season								
108	M	48	20	2×10^8	6.7	9	approx. 10^9	6.5
111	M	62	22	N.D.	6.9	50	N.D.	N.D.
112	M	54	22	N.D.	7.5	1	N.D.	N.D.
117	M	61	23	2×10^5	6.9	1	N.D.	N.D.
118	F	58	19	N.D.	N.D.	N.D.	2.1×10^8	7.4
119	M	58	22	N.D.	N.D.	N.D.	1.0×10^9	6.4
120	F	60	21	6×10^6	6.5	2	5.5×10^8	7.1
121	F	68	22	N.D.	N.D.	N.D.	6.3×10^8	6.3
122	M	54	18	2.1×10^6	6.2	2	4.8×10^9	6.2
123	M	62	21	N.D.	N.D.	N.D.	3.2×10^9	6.5
124	F	73	20	2.6×10^8	6.6	23	1.8×10^9	6.8
1984 Season								
106	F	60	20	N.D.	N.D.	N.D.	6.9×10^9	6.2
107	M	57	21	1.5×10^7	7.4	<1	2.8×10^9	6.4
112	F	60	23	4.8×10^7	6.8	1	1.5×10^{10}	6.7
113	F	47	25	N.D.	N.D.	3	N.D.	N.D.
114	F	61	23	N.D.	N.D.	N.D.	4.0×10^9	6.6
115	M	61	27	N.D.	6.2	5	N.D.	N.D.
116	F	60	19	3×10^8	6.6	21	N.D.	N.D.
119	M	53	22	N.D.	N.D.	N.D.	8.2×10^9	6.6
121	F	60	22	1.6×10^7	N.D.	<1	9.0×10^9	6.2
140	F	63	16	1.7×10^9	6.9	7	2.7×10^9	6.4
1985 Season								
89	M	60	25	N.D.	N.D.	N.D.	6.8×10^8	6.7
92	F	59	26	N.D.	N.D.	N.D.	4.2×10^9	6.5
101	F	52	25	approx. 10^7	6.8	247	N.D.	N.D.
103	F	56	19	N.D.	N.D.	N.D.	4.4×10^9	6.3
105	M	56	18	1.3×10^9	6.8	98	5.4×10^9	6.6
109	F	65	24	N.D.	N.D.	N.D.	2.6×10^8	6.4

dilution in all colon samples. The dilution at which methanogens were detected in the colon varied among whales: 2 of 5 whales contained methanogens at the 10^{-5} dilution, 2 more at 10^{-3}, and all whales at the 10^{-1} dilution. Since the 10^{-5} dilution was the highest dilution tested, methanogens and sulfate-reducers may occur at higher concentrations in the colon of some fin whales.

65 oxidase-negative isolates from MacConkey Agar were examined in the Enterobacteriacea screen. The majority of the cultures were urease-positive (50/65). Nearly half of the isolates showed reactions typical of *Proteus vulgaris* [5]. The following species were found: *P. vulgaris, Enterobacter cloacae, Escherichia coli, Morganella morganii, Citrobacter freundii, Serratia* spp. and *Hafnia alvei.* The quality of the identifications by the API 20E computer-based system was considered to be excellent or very good for the majority of identified isolates, except for some *E. cloacae* isolates.

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Table 2

Mole percentage of volatile fatty acids in forestomach samples of North Atlantic fin whales that contained more than 1 mM of total volatile fatty acids

Average mol% VFA are calculated for the fin whales, and previous results of analyses of gray and bowhead whales forestomach contents are shown for comparison.

Whale No.	Normal VFAs				Iso-VFAs		
	Acetic	Propionic	Butyric	Valeric	Isobutyric	Isovaleric	Isocaproic
1983 Season							
108	62	6	11	– [a]	5	7	4
111	70	15	10	–	1	3	–
112	79	7	6	–	–	8	–
117	77	5	5	–	5	8	–
120	76	7	7	–	4	6	–
122	66	10	8	–	7	9	–
124	65	13	12	–	2	6	1
1984 Season							
113	68	10	18	–	–	4	–
115	70	8	6	–	3	10	2
116	53	8	20	1	6	7	3
140	67	8	21	–	< 1	4	–
1985 Season							
101	51	7	19	2	6	8	6
105	64	9	19	–	< 1	6	–
Average percentages							
Fin whales	67.2	8.9	12.6	< 1	3.0	6.6	< 1
Gray whales [b]	61	13	16	< 1	3	5	1
Bowhead whales [b]	61	10	15	1	4	5	1

[a] Not detected.

[b] Data from Herwig et al. [4].

Table 3

Enrichments for sulfate-reducers and methanogens in colon samples from fin whales

1985 enrichments were examined at 3 different dilutions.

Whale No.	Methanogens			Sulfate-reducers		
1984 Season						
106	–			–		
107	+			–		
112	+			+		
114	+			–		
119	+			+		
121	+			–		
140	–			–		
1985 Season						
Dilution	10^{-1}	10^{-3}	10^{-5}	10^{-1}	10^{-3}	10^{-5}
89	+	+	+	+	+	+
92	+	+	+	+	+	+
103	+	+	–	+	+	+
105	+	–	–	+	+	+
109	+	+	–	+	+	+

Table 4

Results of biochemical and physical tests to tentatively identify TCBS agar isolates as *V. parahaemolyticus*, *V. alginolyticus*, and *L. damsela*

Test	*V. parahaemolyticus*	*V. alginolyticus*	*L. damsela*
Growth on TCBS Agar, 37°C	+	+	+
Require NaCl for growth	+	+	+
Oxidase test	+	+	+
Argine dihydrolase	–	–	+
Lysine decarboxylase	+	+	D [a]
Ornithine decarboxylase	+	D	–
0/129 sensitivity	+, 150 [b]	+, 150 [b]	+, 10 [b]
Acid production:			
L-arabinose	D	+	+
Lactose	–	–	–
Sucrose	–	+	–
Indole	–	D	–
Voges-Proskauer	–	+	+
Urease	D	–	+

[a] D = result may differ between isolates. [b] μg

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Table 5

Presence of Enterobacteriaceae and Vibrionaceae isolates in the digestive tract of North Atlantic fin whales sampled during the 1983 season

Whale No.	Region	Enteric species							*Vibrio* species		
		C. freundii	*E. cloaceae*	*E. coli*	*H. alvei*	*M. morganii*	*P. vulgaris*	*Serratia* sp.	*V. alginolyticus*	*V. parahaemolyticus*	*L. damsela*
118	Colon	+				+			+	+	
119	Colon								+	+	
120	Colon				+					+	+
122	Colon			+						+	
123	Small intestines		+				+				+
	Colon	+	+			+		+			
124 [a]	First stomach									+	
	Second stomach									+	
	Colon									+	

[a] No examination for Enterobacteriaceae species was performed for fin whale 124.

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Table 6

Enterics and vibrios found in the gastrointestinal tract of cetaceans

Bacterial species	Cetacean species	Common name	Reference
Enterics			
Edwardsiella tarda	*Phocoena phocoena*	Harbor porpoises	[22]
	– [a]	Porpoises [b]	[23]
Escherichia coli	*Globicephala melaena*	Long-finned pilot whale	[24]
	–	Porpoises [b]	[23]
Klebisiella / Enterobacter aerogenes	–	'wild porpoise'	[25]
(*Aerobacter aerogenes*) [c]	–	Porpoises [b]	[23]
Morganella morganii	*G. melaena*	Long-finned pilot whale	[24]
(*Proteus morganii*) [c]	–	Dolphins	[26]
	–	Porpoises [b]	[23]
Proteus sp.	*Tursiops truncatus*	Bottlenose dolphin	[27]
Proteus mirabilis	*G. melaena*	Long-finned pilot whale	[24]
	–	Porpoises [b]	[23]
Proteus rettgeri	–	Porpoises [b]	[23]
Yersinia enterocolitica	–	Dolphins	[26]
Vibrios			
Vibrio alginolyticus	*G. melaena*	Long-finned pilot whale	[24]
	T. truncatus	Bottlenose dolphin	[27]

[a] –, not reported. Some authors referred to the examined animals only by common name or combined results of different species.
[b] Porpoises included *Delphinus delphis* (common dolphin) and *Lagenorhynchus obliquidens* (Pacific whiteside dolphin).
[c] Species name listed in original paper but changed for table because of more recent reclassification.

In these cases, the identification was considered to be acceptable. Ten isolates could not be identified using the API 20E system.

In 1983, the digestive contents from 6 whales were examined for mesophilic species of the Vibrionaceae. 78 isolates obtained from the TCBS agar were tentatively identified as belonging to *Vibrio parahaemolyticus, Vibrio alginolyticus,* and *Listonella damsela.* Table 4 lists the results of biochemical or physiological tests to identify the TCBS agar isolates as *V. parahaemolyticus, V. alginolyticus,* or *L. damsela.* The results of key taxonomic tests and systematic evaluations of vibrios by other investigators [17–19] was considered. A large percentage of the vibrios were found to be urease-positive, including many of the *V. parahaemolyticus* isolates and all of the *L. damsela* isolates.

Table 5 summarizes the results of the examination of the digestive tract samples for Enterobacteriaceae and Vibrionaceae isolates.

5. DISCUSSION

A major problem in studying the microbiology of baleen whales is obtaining samples. Several attempts were made to sample the forestomach and colon of fin whales immediately after harvest by catcher boats at sea, but this task proved to be exceedingly difficult and dangerous. Thus, it was necessary to rely on the return of catcher boats to the shore-based station at Hvalfjördur. Despite this long period, i.e., 16–25 h between death and sampling (Table 1), it should be noted that the forestomach and colon contents were cooled by the ambient seawater (9–10°C) during the trip to the station.

The presence of volatile fatty acids, the relative proportion of the individual volatile fatty acids, and the significant levels of viable anaerobic bacteria in the forestomach is suggestive of a microbial fermentation. These results are consistent with results from our previous study [4] in

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which we examined the levels of bacteria stained with acridine orange and the VFAs that were present in fixed forestomach samples from bowhead and gray whales. The levels of bacteria and VFAs varied between individual whales. Some of these differences may be attributed to the time of last feeding, partial dilution of the contents with seawater, differences in the microbial flora between whales, and the heterogenicity within a large forestomach.

In this study, we also wanted to determine whether methanogens and sulfate-reducers served metabolically as terminal acceptors for electrons generated during a microbial fermentation. In the forestomach samples, methanogens were detected in only one sample, and the sulfate-reducers were found in 4 of the 7. Finding the sulfate-reducers and methanogens in several of the forestomach samples is consistent with, but not a requirement for, a microbial fermentation. Certainly, a forestomach fermentation completely analogous to that found in terrestrial plant-consuming ruminants in which methanogens occur is highly unlikely, because of the differences in composition of food eaten by baleen whales and terrestrial herbivores. Protein and lipid are the principal components of krill, while carbohydrate occurs in very low concentrations [20]. A portion of the krill protein appears to be hydrolyzed and fermented as suggested by the presence of the branched-chain VFAs in the chromatographic analysis. What about the lipids? Krill lipid is dominated by phospholipids and triacylglycerols [20]. Many of the fatty acids are unsaturated. Perhaps, as in land ruminants, both microbial lipolysis and hydrogenation of unsaturated fatty acids is occurring. Oxidation of fatty acids is also possible, but would probably be unimportant because of the slow growth of bacteria that are known to perform this activity [21].

The colon contents of fin whales contained 10^8–10^{10} viable anaerobic bacteria per ml. The release of this number of bacteria into pelagic waters could have a significant impact on the food available for zooplankton and microflagellates that consume bacteria. Perhaps 10^{13}–10^{15} bacteria (300 l of fecal material $\times$ (10^8–10^{10} cells) ml^{-1}) could be released by a single fin whale in a day into a marine environment containining a 'background' of approx. 10^5 bacteria per ml.

In preliminary examinations of whale colon samples, growth of enteric bacteria was not detected on 10^{-6} dilutions on MacConkey Agar. Only low dilutions ranging from 1/5 to 10^{-2} consistently yielded growth on MacConkey Agar. Additionally, for some whales, bacteria were enumerated aerobically using a medium similar to Cetacean Medium, but lacking $NaHCO_3$. Aerobes and facultative anaerobes enumerated by this procedure ranged between 10^4 and 10^5 per ml for fin whales 122 and 123, considerably less than the 10^9 concentration determined for the obligate anaerobes. Therefore, we conclude that the aerobic and facultative anaerobic bacteria (including the enterics) comprise an extremely low percentage of the lower gastrointestinal microflora of fin whales. Further work is needed to characterize and identify the predominant anaerobic bacteria.

Many of the same species of enterics and vibrios found in North Atlantic fin whales have been reported in other cetaceans (Table 6). Johnson and Fung [23] found many of the same enteric bacteria in the anus of 24 captured porpoises, while Buck [24] recovered only 2 enteric species, *E. coli* and *P. mirabilis,* from the anus of a juvenile pilot whale before the animal was introduced to captivity. Many of the enteric bacteria isolated from the fin whale are considered opportunistic pathogens that play an occasional role in intestinal or extraintestinal disease, primarily as secondary invaders rather than primary pathogens.

V. parahaemolyticus, V. alginolyticus, and the closely related *L. damsela* were found to be part of the normal gastrointestinal microflora of fin whales. Since only a limited number of phenotypic characteristics was examined for each vibrio isolate, and genetic analyses (e.g. DNA homologies) were not performed, the identification of the vibrios in the study is considered tentative. Nevertheless, the results suggest that marine mammals may serve as a reservoir for mesophilic vibrios. *L. damsela* is reported to be pathogenic for 3 fish species, the blacksmith (*Chromis punctipinnis*), the garibaldi (*Hypsypops rubicunda*) and the spiny dogfish (*Squalus acanthias*), and was isolated from a brown shark (*Carcharhinus plumbeus*) [17,18]. *V.*

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parahaemolyticus and *V. alginolyticus* have been studied for years and are known to be potential pathogens for humans [16]. In some estuaries, these microorganisms are associated with planktonic crustaceans during the warmer periods of the year and survive in the sediments during the winter. In the colder marine waters of the temperate and polar regions of the world, the warm, nutrient-rich environment found in the gastrointestinal tract of marine mammals may be among the few habitats where some mesophilic marine vibrios survive and multiply.

The presence of urease-positive microorganisms, such as *P. vulgaris,* may be noteworthy. *P. vulgaris* may cause primary and secondary infections in other mammals. *Proteus* spp. are often found in urinary tract infections. A contributing factor to the pathogenicity of *Proteus* in the urinary tract is the activity of urease in producing ammonia with a resultant rise in pH. *Morganella* may also serve a pathogenic role in urinary tract infections. Many of the *V. parahaemolyticus* isolates and all of the *L. damsela* isolates were also urease-positive. The role of urease-positive organisms in the intestine is not clear, though these bacteria may assist in the hydrolysis of urea or more likely, their major role may be the oxidative deamination of amino acids [28].

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