SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 35, No. 11, pp. 2683–2690, 2016
Published 2016 SETAC
Printed in the USA

PETROLEUM BIOMARKERS AS TRACERS OF *EXXON VALDEZ* OIL

Mark G. Carls,*† Larry Holland,† Gail V. Irvine,‡ Daniel H. Mann,§ and Mandy Lindeberg†
†Auke Bay Laboratories, National Marine Fisheries Service and National Oceanic and Atmospheric Administration, Juneau, Alaska, USA
‡Alaska Science Center, US Geological Survey, Anchorage, Alaska, USA
§Geography Program, School of Natural Resources, University of Alaska, Fairbanks, Alaska, USA

(Submitted 10 November 2015; Returned for Revision 29 February 2016; Accepted 6 April 2016)

Abstract: Over the past quarter century, petroleum biomarkers have persisted in sequestered *Exxon Valdez* oil in Prince William Sound and the Gulf of Alaska (USA), and hence the oil has remained identifiable. These biomarkers are molecular fossils derived from biochemicals in previously living organisms. Novel pattern matching indicated the presence of Alaska North Slope crude oil (ANSCO) over the entire observation period at most sites (7 of 9) and distinguished this source from several other potential sources. The presence of ANSCO was confirmed with Nordtest forensics, demonstrating the veracity of the new method. The principal advantage of the new method is that it provides sample-specific identification, whereas the Nordtest approach is based on multisample statistics. Biomarkers were conserved relative to other constituents, and thus concentrations (per g oil) in initial beach samples were greater than those in fresh oil because they were lost more slowly than more labile oil constituents such as straight-chain alkanes and aromatic hydrocarbons. However, biomarker concentrations consistently declined thereafter (1989–2014), although loss varied substantially among and within sites. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss. *Environ Toxicol Chem* 2016;35:2683–2690.

Keywords: Biomarker *Exxon Valdez* oil Forensic

INTRODUCTION

Intertidal areas in western Prince William Sound and to the southwest along the Gulf of Alaska (USA) were extensively contaminated with *Exxon Valdez* oil when the tanker grounded in 1989. The source of this oil was Alaska North Slope crude oil (ANSCO), transported from the Prudhoe Bay oil field via the trans-Alaskan pipeline and loaded onto the vessel in Port Valdez. Stranded oil in the coastal zone was persistent and has been studied for more than 2 decades [1–5], yet the focus was on polycyclic aromatic hydrocarbons (PAHs), which are toxic, and study of biomarkers has been delayed until recently [6].

Although the majority of intertidally sequestered oil residues are from the *Exxon Valdez* oil spill, other sources also exist, such as Monterey oil and background hydrocarbons from natural sources [7,8]. Thus there is a need for highly conserved source information to accurately discriminate among oil samples. Petroleum biomarkers are a good choice for this role because they weather very slowly compared with other commonly measured petrogenic hydrocarbons (PAHs and *n*-alkanes); thus they remain recognizable and consistent with the original source for longer periods. Unlike contemporary biomarkers, which are measurable substances from a living organism, petroleum biomarkers are molecular fossils derived from previously living organisms and diagenetically altered during the formation of crude oil [9,10]. The structure of petroleum biomarkers is highly similar or unchanged from their biogenic precursors [10]. Petroleum biomarkers are generally resistant to biodegradation, evaporation, and other weathering processes [9], and thus definitive identification of source oils is possible for long periods. Biomarkers are so persistent that they can be found in sedimentary rock more than 2 billion years old with identifiable biological origins [9]. This persistence allows more definitive identification of sequestered oil than smaller molecules such as previously reported PAHs and *n*-alkanes.

The purpose of the present study was to investigate whether ANSCO can be definitively identified by biomarker content, determine whether the biomarkers were weathering, and distinguish oil sources in time-series samples. Four classes of biomarkers were examined, the isoprenoids (acyclic terpenoids), triterpanes (mostly tricyclic), hopanes (pentacyclic triterpanes), and steranes (tetracyclic terpenoids; Table 1). We apply novel pattern-matching procedures to compare samples with ANSCO to verify its presence in specific samples (or not). Alternative hydrocarbon sources were similarly compared with oil from sample beaches to determine whether these sources were or were not explanatory. The results were confirmed with Nordtest [11] plots. Despite their persistence, biomarker weathering is possible [10], and evidence of weathering in each biomarker class is presented in the present study.

MATERIALS AND METHODS

Sample locations

Sufficient time-series data were available from 9 sites (Figure 1 and Table 2). Time series samples spanned 18 yr to 23 yr at 6 sites. Three additional sites with samples spanning <15 yr and with few data points across time (3–4) were treated with caution but were ultimately accepted as part of the analysis (biomarker composition and change at these sites was consistent with those at the other sites). Study sites were based on shoreline assessment data gathered by Exxon and the Alaska Department

This article includes online-only Supplemental Data.

* Address correspondence to mark.carls@noaa.gov

Published online 11 April 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3454

Table 1. Biomarkers and their abbreviations[a]

Biomarker	Abbreviation		Target ions
Isoprenoids			
Norpristane	norprist		57
2,6,10,14-tetramethylpentadecane (pristane)	prist		57
2,6,10,14-tetramethylhexadecane (phytane)	phyt		57
Triterpanes			
C23 tricyclic terpane	TR23	*	191
C24 tricyclic terpane	TR24	*	191
C25 tricyclic terpane (a)	TR25a	*	191
C25 tricyclic terpane (b)	TR25b	*	191
C24 tetracyclic terpane	TET24	*	191
C26 tricyclic terpane (a)	TR26a	*[b]	191
C26 tricyclic terpane (b)	TR26b		191
C28 tricyclic terpane (a)	TR28a	*	191
C28 tricyclic terpane (b)	TR28b	*	191
C29 tricyclic terpane (a)	TR29a	*	191
C29 tricyclic terpane (b)	TR29b	*	191
Hopanes			
18α(H),21β(H)-22,29,30-trisnorhopane	Ts	*	191
17α(H),21β(H)-22,29,30-trisnorhopane	Tm	*	191
17α(H),18α(H),21β(H)-28,30-bisnorhopane	H28	*	191
17α(H),21β(H)-25-norhopane	NOR25H		191
17α(H),21β(H)-30-norhopane	H29	*	191
18α(H),21β(H)-30-norneohopane	C29Ts	*	191
17α(H),21β(H)-30-norhopane (normoretane)	M29	*	191
18α(H) and 18β(H)-oleanane	OL		191
17α(H),21β(H)-hopane	H30	*	191
17α(H)-30-nor-29-homohopane	NOR30H	*	191
17β(H),21α(H)-hopane (moretane)	M30	*	191
22S-17α(H),21β(H)-30-homohopane	H31S	*	191
22R-17α(H),21β(H)-30-homohopane	H31R	*	191
Gammacerane	GAM	*	191
22S-17α(H),21β(H)-30,31-bishomohopane	H32S	*	191
22R-17α(H),21β(H)-30,31-bishomohopane	H32R	*	191
22S-17α(H),21β(H)-30,31,32-trishomohopane	H33S	*	191
22R-17α(H),21β(H)-30,31,32-trishomohopane	H33R	*	191
22S-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H34S	*	191
22R-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H34R	*	191
22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35S	*	191
22R-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35R	*	191
Steranes			
C_{22} 5α(H),14β(H),17β(H)-sterane	S22	*	217 218
C_{27} 20S-13β(H),17α(H)-diasterane	DIA27S	*	217 218
C_{27} 20R-13β(H),17α(H)-diasterane	DIA27R	*	217 218
C_{27} 20S-5α(H),14α(H),17α(H)-cholestane	C27S	*	217 218
C_{27} 20R-5α(H),14β(H),17β(H)-cholestane	C27bbR	*	217 218
C_{27} 20S-5α(H),14β(H),17β(H)-cholestane	C27bbS	*	217 218
C_{27} 20R-5α(H),14α(H),17α(H)-cholestane	C27R	*	217 218
C_{28} 20S-5α(H),14α(H),17α(H)-ergostane	C28S	*	217 218
C_{28} 20R-5α(H),14β(H),17β(H)-ergostane	C28bbR	*	217 218
C_{28} 20S-5α(H),14β(H),17β(H)-ergostane	C28bbS	*	217 218
C_{28} 20R-5α(H),14α(H),17α(H)-ergostane	C28R	*	217 218
C_{29} 20S-5α(H),14α(H),17α(H)-stigmastane	C29S	*	217 218
C_{29} 20R-5α(H),14β(H),17β(H)-stigmastane	C29bbR	*	217 218
C_{29} 20S-5α(H),14β(H),17β(H)-stigmastane	C29bbS	*	217 218
C_{29} 20R-5α(H),14α(H),17α(H)-stigmastane	C29R	*	217 218

[a]Asterisks mark analytes used for pattern matching; the number of triterpanes, hopanes, and steranes used for modeling were 10, 20, and 15, respectively.
[b]TR26a and TR26b cannot be resolved with current column settings at our laboratory, and thus were combined for modeling.

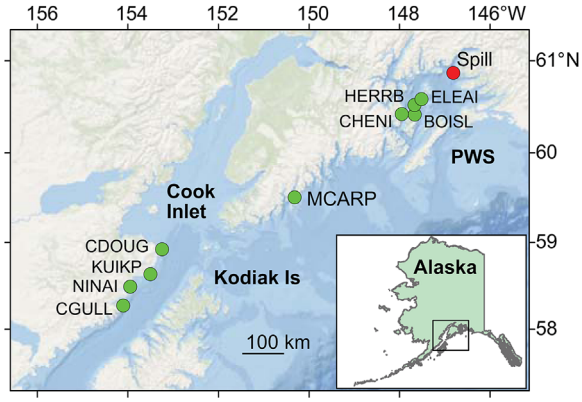

Figure 1. The time-series sample locations included 4 within Prince William Sound (PWS), 4 along the Katmai coast, and one intervening location (McArthur Pass). The red circle marks the location of the *Exxon Valdez* spill. See Table 2 for site abbreviations.

of Environmental Conservation and were limited to sites with persistent oil and repeated observations.

Sediment and oil sample collection methods were previously reported [4,5,7,12–16]. In brief, these samples were generally collected with a spoon or shovel (following various sampling protocols), placed in hydrocarbon-free jars, and frozen pending analysis. Samples were later processed at the Auke Bay Laboratory (Alaska, USA) at various times (depending on collection times and individual study needs) for aliphatic and aromatic hydrocarbons. Study of biomarkers began more recently (~2011–2014 for these samples), and previously archived extracts were used when available. Expected biomarker loss under storage conditions (−20 °C) was negligible, an assumption based on the large size of these molecules, limited potential exchange with atmosphere and water in frozen jars, low temperature (and thus limited potential for microbial decomposition), the inherent resistance of biomarkers to microbial decomposition, and the observation that biomarker patterns often remain unaltered even when oil is undergoing active weathering [10].

Sample processing

Sediment and oil samples were dried with anhydrous sodium sulfate and extracted with dichloromethane. Prior to 2011, samples were exchanged into hexane over steam and separated into aliphatic and aromatic fractions by column chromatography (10 g of 2% deactivated alumina over 20 g of 5% deactivated silica gel). The aliphatic fraction was eluted with 50 mL of pentane, and PAHs were subsequently eluted with 250 mL of 1:1 (v/v) pentane:dichloromethane. From 2011 onward, samples extracted with dichloromethane were reduced in volume over steam to approximately 5 mL. The exact total volume was recorded and an aliquot was archived. The dichloromethane was evaporated from the remaining extract to determine the total mass of extracted oil. This information was used to calculate the application of 7 mg to 10 mg of oil from the archived aliquot to a 6-g silica column. The aliphatic fraction was eluted with 10 mL of pentane, and PAHs were subsequently eluted with 20 mL of 1:1 (v/v) pentane:dichloromethane. All purified extracts were exchanged into 1 mL of hexane over steam and spiked with instrument internal standards prior to instrumental analysis. Reported units were ng PAH g^{-1} oil ($n = 49$) using the amount of oil applied to the silica column as the divisor or ng PAH g^{-1} sediment ($n = 13$).

Table 2. Biomarker pattern match to Alaska North Slope crude oil (ANSCO) by site and date[a]

Site (latitude, longitude)	Abbreviation	SIN	Date	Triterpane model	Hopane model	Sterane model	Combined match	% Combined match
Bay of Isles	BOISL	142343	9/15/1990	1.00	0.90	1.00	1	83
(60.3802, −147.7142)	BOISL	142342	9/15/1990	1.00	0.90	0.93	1	
	BOISL	1203720	7/5/2001	0.50	0.85	0.47	0	
	BOISL	1303346	6/24/2002	1.00	0.85	0.87	1	
	BOISL	1400734	7/12/2003	0.80	0.90	0.87	1	
	BOISL	1502219	6/16/2004	1.00	0.90	1.00	1	
Cape Douglas	CDOUG	302709	7/30/1992	1.00	0.90	0.87	1	100
(58.8818, −153.2950)	CDOUG	1007034	8/8/1999	1.00	0.90	0.93	1	
	CDOUG	20120720	8/3/2012	1.00	1.00	1.00	1	
	CDOUG	20120718	8/3/2012	0.90	0.95	0.93	1	
Cape Gull	CGULL	304902	10/2/1989	0.90	0.90	0.87	1	20
(58.2353, −154.1542)	CGULL	1007040	8/10/1999	0.40	0.55	0.27	0	
	CGULL	1601912	8/21/2005	0.40	0.40	0.07	0	
	CGULL	20120748	8/7/2012	0.50	0.90	0.20	0	
	CGULL	20120750	8/7/2012	0.50	0.90	0.53	0	
Chenega Island	CHENI	7409	7/31/1989	1.00	0.90	0.87	1	33
(60.3872, −148.0025)	CHENI	604711	8/11/1995	0.60	0.90	0.67	0	
	CHENI	1006147	7/13/1999	0.20	0.25	0.40	0	
Eleanor Island	ELEAI	400431	6/19/1993	0.50	0.80	0.53	0	67
(60.5500, −147.5667)	ELEAI	601401	5/13/1995	0.50	0.60	0.67	0	
	ELEAI	1006325	7/14/1999	1.00	0.90	0.60	0	
	ELEAI	1202610	5/19/2001	1.00	0.90	0.93	1	
	ELEAI	1303429	6/26/2002	1.00	0.85	1.00	1	
	ELEAI	1802225	5/21/2007	1.00	0.90	0.93	1	
	ELEAI	20111005	5/23/2011	0.20	0.75	0.27	0	
	ELEAI	20111002	5/23/2011	0.40	0.95	0.47	0	
	ELEAI	20111008	5/23/2011	0.60	0.90	0.60	0	
	ELEAI	20111009	5/23/2011	1.00	0.90	0.93	1	
	ELEAI	20111006	5/23/2011	1.00	0.95	1.00	1	
	ELEAI	20111004	5/23/2011	0.90	0.85	0.93	1	
	ELEAI	20111004	5/23/2011	1.00	0.95	1.00	1	
	ELEAI	20111011	5/23/2011	1.00	0.95	1.00	1	
	ELEAI	20111007	5/23/2011	1.00	0.90	1.00	1	
	ELEAI	20111003	5/23/2011	1.00	0.85	0.93	1	
	ELEAI	20111001	5/23/2011	0.90	0.95	0.93	1	
	ELEAI	20111010	5/23/2011	1.00	0.90	1.00	1	
Herring Bay	HERRB	601826	5/15/1995	0.90	0.90	0.93	1	92
(60.4850, −147.7235)	HERRB	1006314	7/13/1999	0.80	0.85	0.67	1	
	HERRB	1202602	5/10/2001	1.00	0.90	0.93	1	
	HERRB	1301010	5/27/2002	0.50	0.90	0.73	0	
	HERRB	1400725	6/15/2003	1.00	0.90	1.00	1	
	HERRB	1502211	6/13/2004	1.00	0.90	1.00	1	
	HERRB	1603515	7/23/2005	1.00	0.90	1.00	1	
	HERRB	1802213	6/19/2007	1.00	0.90	1.00	1	
	HERRB	20100204	6/23/2010	0.70	0.90	0.80	1	
	HERRB	20100201	6/23/2010	0.80	0.90	0.93	1	
	HERRB	20100203	6/23/2010	0.70	0.90	0.80	1	
	HERRB	20130701	6/27/2013	0.80	0.90	0.67	1	
	HERRB	20140201	2/18/2014	0.80	0.90	1.00	1	
Kiukpalik	KIUKP	302710	7/31/1992	1.00	0.90	0.87	1	100
(58.5968, −153.5531)	KIUKP	1007036	8/9/1999	1.00	0.90	0.93	1	
	KIUKP	20120739	8/4/2012	1.00	0.95	0.93	1	
	KIUKP	20120737	8/4/2012	1.00	0.95	0.93	1	
McArthur Pass	MCARP	1007018	7/28/1999	1.00	0.85	0.93	1	100
(59.4667, −150.3717)	MCARP	1601914	8/22/2005	1.00	0.90	0.93	1	
	MCARP	20120752	8/9/2012	0.90	0.95	0.93	1	
	MCARP	20120751	8/9/2012	1.00	1.00	0.93	1	
Ninagiak Island	NINAI	304903	12/10/1989	1.00	0.90	0.87	1	100
(58.4552, −153.9990)	NINAI	1007038	8/10/1999	1.00	0.90	0.93	1	
	NINAI	1601907	8/19/2005	1.00	0.90	0.93	1	
	NINAI	20120743	8/5/2012	0.90	1.00	1.00	1	
	NINAI	20120742	8/5/2012	1.00	0.95	0.93	1	

[a]Latitude and longitude correspond to the last sample collected in each series. Samples were consistent with ANSCO where scores for all classes were > 0.6 (combined match = 1); overall 77% of the samples matched ANSCO. Site-specific matches are also included. See Supplemental Data, Table S1, for an example of pattern matching.

SIN = sample identification number.

Aliphatic fractions were analyzed for biomarkers by gas chromatography–mass spectroscopy. Many of these extracts were previously analyzed for aliphatics and archived at −20 °C. The data were acquired in the selected ion monitoring mode, and concentrations were determined by the internal standard method with response factors based on 2 representative compounds, 17α (H),21β(H)-hopane (H30) and 5α(H),14α(H),17α(H)-cholestane. The accuracy of the biomarker analyses was approximately ±15% based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was approximately 20%, depending on the biomarker. Biomarker concentrations were not corrected for recovery; surrogate recovery averaged 93% (range 59–125%). Reported biomarkers and their abbreviations are listed in Table 1.

Pattern-matching forensics

Biomarker composition in samples was compared with that in ANSCO (obtained from the T/V *Exxon Valdez* in 1989) to determine whether they matched source oil composition [17]. Concentrations were normalized to total class concentration before comparison. For example, hopane source oil bounds were set from minimum −20% to maximum +20%, expressed in proportional units ($H_i/\sum H_i$), where H_i is the i^{th} hopane concentration, and $\sum H_i$ is the total hopanoid concentration. For each sample, the number of $H_i/\sum H_i$ within corresponding source oil bounds was divided by the total number of hopanes (20) to calculate the fraction of analytes consistent with the source oil. Possible outcomes ranged from 0 to 1, where 1 was a perfect match and 0 was a complete mismatch (Supplemental Data, Figure S1 and Table S1). The probability that an unknown sample was consistent with ANSCO composition was assessed by reference to results of randomly permuting the source oil dataset 10 000 times. The probability of randomly encountering a match >0.55 was <0.0001; thus any score >0.6 was accepted as consistent with ANSCO. Triterpanes and steranes were similarly modeled. Site- and time-specific data were considered matched to the source oil when scores in all 3 classes were >0.6.

Biomarker composition in samples was similarly compared with other potential biomarker sources. These included Monterey oil (spilled as a result of the 1964 Alaska earthquake), coal from the Tyndall Glacier (a possible source of benthic hydrocarbons) [18,19], and Constantine Harbor sediment, which likely collects material transported into Prince William Sound from the Gulf of Alaska [20]. Results for each class (triterpanes, hopanes, and steranes) were independently compared with ANSCO results using Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, because data distributions were not normal (groups were ANSCO, Monterey, coal, and Constantine). Overall site-specific matches that considered the combined results of all 3 classes were also compared among the 4 potential sources.

Nordtest forensics

An alternative forensic method, the Nordtest [21], compares predetermined compound ratios in samples with those in the potential source. The Nordtest uses sample averages (and confidence bounds) to infer whether a sample matched the average source pattern. The 14 ratios used were those recommended by Daling et al. [21] (Table 3).

Each ratio was calculated for each sample at a given site and summarized as a mean. Site means (x-axis in the Nordtest plots) were paired with corresponding means from each of the 4 potential sources (y-axis) in 4 separate tests. The Nordtest method states that if the 95% confidence bounds of all diagnostic ratios overlap the diagonal, then the sample is a positive match to the source oil. Daling et al. [21] also indicate that conclusions can be based on regressions between spill and source samples for the selected suite of measured diagnostic ratios.

Table 3. Nordtest ratio names and formulae as recommended by Daling [21][a]

Ratio name	Ratio formula
%27TS	Ts / (Ts + Tm)
%28ab	H28 / (H28 + H30)
%25nor30ab	NOR25H / (NOR25H + H30)
%29Ts	C29Ts / (C29Ts + H30)
%30O	OL / (OL + H30)
%30G	GAM / (GAM + H30)
%29ab	H29 / (H29 + H30)
%32abS	H32S / (H32S + H32R)
%27dia	(DIA27S + DIA27R) / (DIA27S + DIA27R + C27bbR + C27bbS)
%29aaS	C29S / (C29S + C29R)
%29bb	(C29bbR + C29bbS) / (C29bbR + C29bbS + C29S + C29R)
%27bbSTER	(C27bbR + C27bbS) / (C27bbR + C27bbS + C28bbR + C28bbS + C29bbR + C29bbS)
%28bbSTER	(C28bbR + C28bbS) / (C27bbR + C27bbS + C28bbR + C28bbS + C29bbR + C29bbS)
%29bbSTER	(C29bbR + C29bbS) / (C27bbR + C27bbS + C28bbR + C28bbS + C29bbR + C29bbS)

[a]The compound abbreviations are defined in Table 1.

Weathering

Concentration change over time (per g oil) was examined for each compound with linear regression. Data from all sites were combined for these analyses. Concentrations were log-transformed (natural log). We considered the usefulness of regressions by dividing the observed F value (F_o) by the critical F value (F_c) as suggested by Draper and Smith [22]. Typically a regression is useful if $F_o/F_c \geq 4$, a more restrictive criterion than significance.

RESULTS

Pattern-matching forensics

Biomarker patterns were typically consistent with ANSCO over the entire observation period at contaminated sites (up to 23 yr; Table 2). The combined result of all classes (triterpanes, hopanes, steranes) matched ANSCO in 77% of these samples ($n = 62$; Table 2). Through time at all sites, ANSCO was definitively present except for Chenega Island, where it was not identified in 1995 and 1999, and Cape Gull samples in 1999 and thereafter.

Other potential biomarker sources were not plausible alternatives to ANSCO (Figure 2). When Monterey oil was used as the potential source in the model, class-specific model fits in field samples ($n = 62$) were consistently poorer than with ANSCO as the source ($p_{ANOVA} < 0.05$ for triterpane, hopane, and sterane results; one-way ANOVA on ANSCO, Monterey, coal, and Constantine; Figure 2A and Supplemental Data, Table S2); the combined result for all 3 classes was a 0% fit compared to 77% for ANSCO (Figure 2B). Similarly, when coal and Constantine Harbor were also independently modeled as potential sources, model fits in field samples were consistently poorer than with ANSCO as the source, and neither explained the combined pattern (Figure 2B).

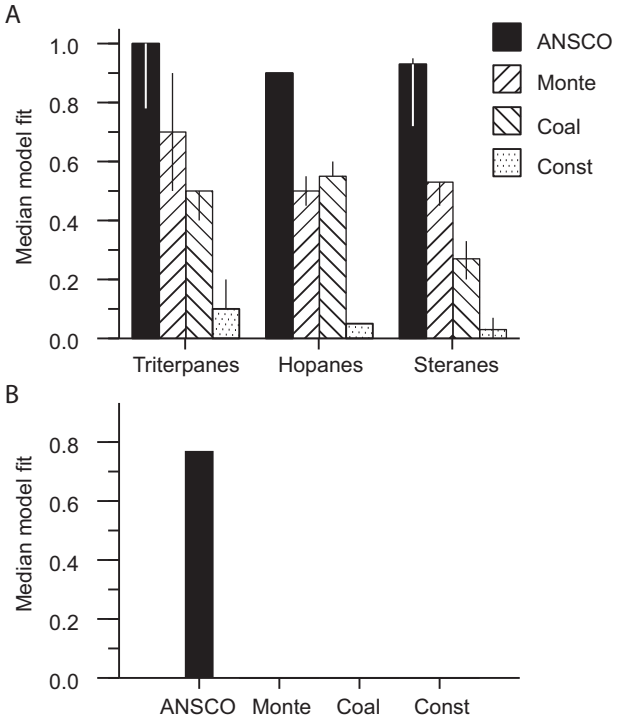

Figure 2. Potential biomarker source comparison. (**A**) Median model results by class (triterpanes, hopanes, and steranes) across samples from all sites. Perfect model fits = 1.0 (100% match), and a complete lack of fit = 0.0. (**B**) Combined results for each potential source (Alaska North Slope crude oil [ANSCO], Monterey oil [Monte], coal, and Constantine Harbor [Const]).

Nordtest forensics

In Nordtest analyses, ANSCO explained the biomarker ratios in the field samples from each site. All regression slopes were near 1, and the 95% confidence interval of these slopes either overlapped 1 or was within 0.01 units of it (Figure 3 and Supplemental Data, Figure S2). Although some error bars did not overlap the diagonal, all were fairly close and regressions were highly significant ($p_{\text{regression}} < 0.001$, 9 sites). Error bars were large at sites with few samples, such as Chenega Island, and at Cape Gull, where weathering was prominent.

Compared with any alternative source, ANSCO matched the field data better (Figure 3). Regression fits were best for ANSCO and worst for Constantine. This was evident by inspecting the *F* values: medians were 701, 65, 19, and 1 for ANSCO, Monterey, coal, and Constantine, respectively ($p < 0.001$, Kruskal–Wallis one-way ANOVA on ranks). Mean Nordtest regression slopes were 0.96, 0.78, 0.96, and 0.36 for ANSCO, Monterey oil, coal, and Constantine, respectively. Although the slope for coal was close to 1, the regression was displaced from $x = y$ and Nordtest ratios were frequently inconsistent with coal as the source (i.e., they did not overlap $x = y$). Nordtest ratios were also frequently inconsistent with $x = y$ for Monterey oil. Constantine ratios were never close to $x = y$. Thus, the Nordtest analysis is consistent with the pattern-matching analysis; ANSCO was frequently detected in samples, and none of the alternative sources were plausible.

Weathering

Initial biomarker concentrations in beached oil were typically greater than in the source oil and declined thereafter (Figure 4 and Supplemental Data, Figure S3). However, concentrations were heterogeneous within and among sites,

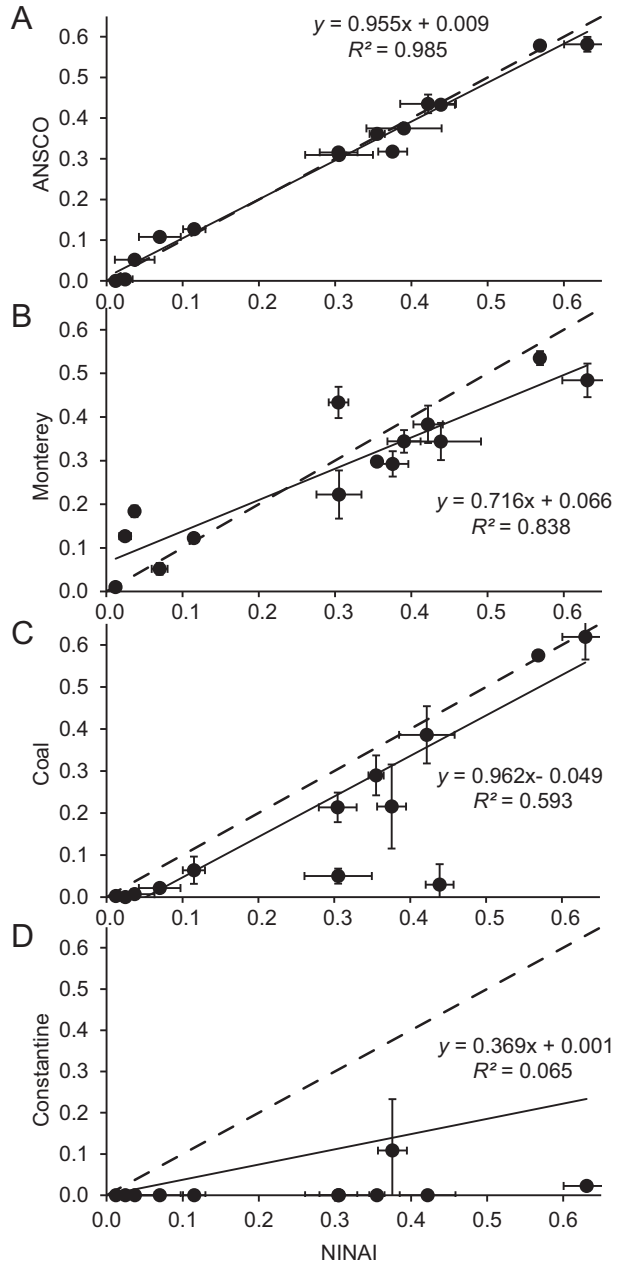

Figure 3. Comparison of potential sources using Nordtest plots. Samples in this example are from Ninagiak Island (NINAI). Potential biomarker sources examined were (**A**) Alaska North Slope crude oil (ANSCO), (**B**) Monterey oil, (**C**) coal, and (**D**) Constantine Harbor (Const). The dashed line is $x = y$. Axes are ratios specified for Nordtest; those from NINAI are on the x-axis and ANSCO, Monterey, Constantine, or coal ratios are on the y-axis. Solid lines are regression fits. Error bars (vertical and horizontal) are 95% confidence bounds.

with some remaining relatively high and others declining. For example, H30 concentrations declined at each site (after the initial increase above that in source oil). Calculated independently for each site, the median H30 slope was −0.06 $\log_e$(concentration) yr^{-1} and ranged from −0.11 ng g oil^{-1} yr^{-1} to 0.01 ng g oil^{-1} yr^{-1} (Figure 5). The combined slope was similar, −0.05 $\log_e$(concentration) yr^{-1}, when all site data were regressed in common; this combined regression was significant ($F_{1,41} = 19.000$, $F_o/F_c = 4.7$, $p < 0.001$). Thus, there was a general decline in H30 content in sequestered oil. Similarly, C27bbS concentration declined with time; the combined slope was negative and significant ($F_{1,41} = 16.280$, $F_o/F_c = 4.0$,

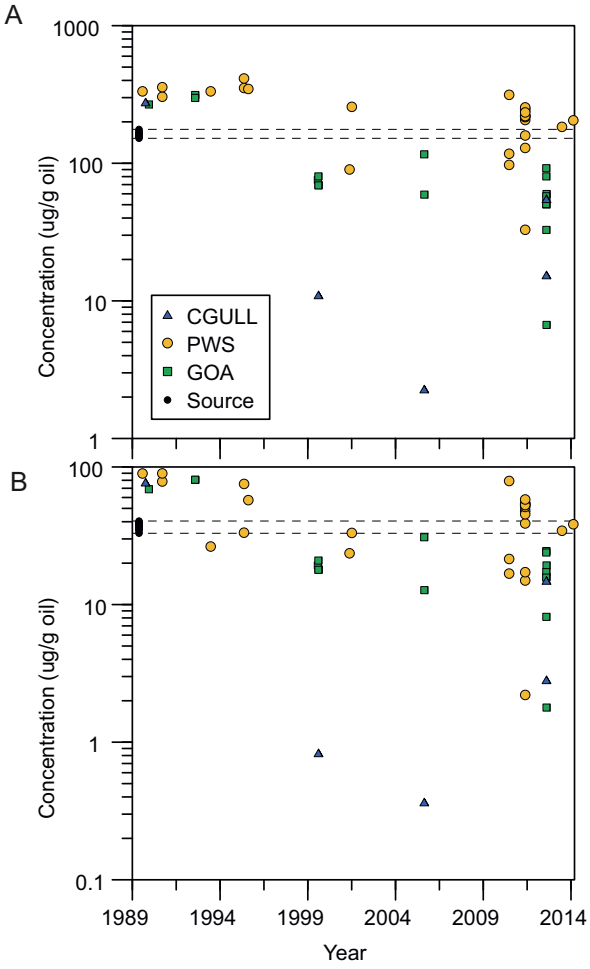

Figure 4. Loss of (**A**) H30, and (**B**) C27bbS over time. Data from all sites were combined to calculate the illustrated linear regressions. Concentrations in *Exxon Valdez* source oil are illustrated with black circles, and the low and high ranges are marked by horizontal dashed lines. Note that scaling is different between the panels. CGull = Cape Gull; PWS = Prince William Sound; GOA = Gulf of Alaska.

$p < 0.001$). Nearly all slopes for all biomarkers were negative (97%; Figure 5). Estimated loss rates were greater for TR28 through TR29b among triterpanes, for NOR25H and OL among hopanes, and from DIA27S through C27bbS among steranes (Figure 5). The same results can be demonstrated with an alternative analysis method (Supplemental Data, Figure S4). Median correlation with time for the isoprenoid, triterpane, hopane, and sterane concentrations (ng/g oil) was −0.747, −0.478, −0.566, and −0.533, respectively (combined site data). Isoprenoid loss was substantially greater than for other biomarkers (Figure 5).

DISCUSSION

Biomarkers can be used to identify hydrocarbon sources, thus allowing discrimination among oil sources where multiple contamination events occurred. For example, in Prince William Sound, Monterey crude oil was spilled 25 yr before the *Exxon Valdez* oil spill, there was limited evidence of site-specific historical contamination within the sound, and coal was a minor source [23]. Biomarkers are also useful for studying the fate, behavior, and weathering of oils in a wide variety of environmental conditions [10].

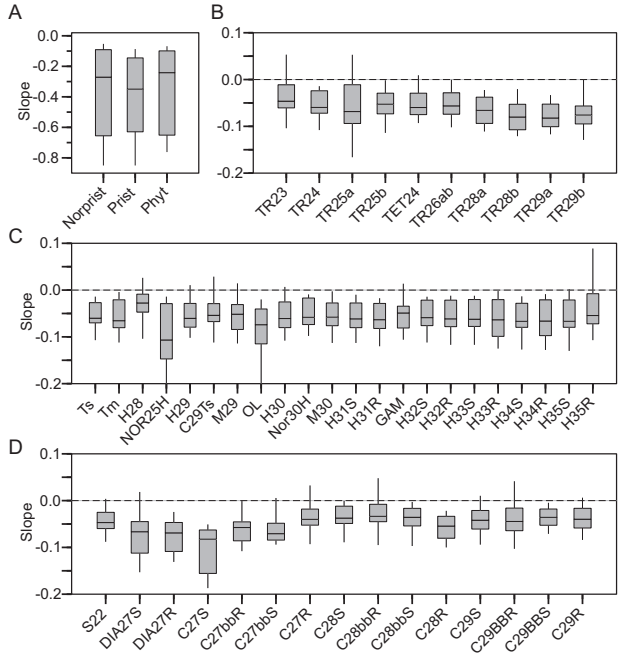

Figure 5. Biomarker loss. (**A–D**) Slopes are $\log_e$(concentration + 0.1)/d. A slope of 0 indicates no change over time. See Table 1 for biomarker abbreviations. Note that y-axis scaling for isoprenoids (**A**) is different from that of the other graphs.

Forensics demonstrated the presence of ANSCO at all sites and generally through time; thus the ensuing changes in biomarker concentration (per g oil) are records of ANSCO weathering. The implications of this weathering are that biomarkers are initially conserved, or more accurately, are lost slowly with respect to more labile oil constituents such as straight-chain alkanes and aromatic hydrocarbons; hence initial biomarker concentrations increased. This increase occurred early and was generally evident in the earliest intertidal samples. However, with continued time, biomarker weathering was also evident, and concentrations often fell below those in the fresh source oil. Weathering was a heterogeneous process with concentrations in some samples remaining relatively high (above initial concentrations) and in other samples falling well below initial concentrations; this scatter was evident within sites as well as among sites (Figure 4). Overall trends were declines in biomarker concentrations from the earliest collections of stranded oil to the present, and this was true whether data from all sites were analyzed collectively or on a site-specific basis.

Biomarker composition at Cape Gull was unusual after 1989 likely because of rapid physical oil loss. Biomarker loss was unusually rapid (Figure 4), and composition was unlike ANSCO in 1999 and thereafter because some triterpanes (TR28a–TR29b) and steranes (DIA27S–C27bbS) were lost. These are the analytes with the highest relative loss rates presented in Figure 5. In addition, the estimated loss rates of these compounds were also high without Cape Gull data. Others have also reported enigmatic conditions at Cape Gull including unusually rapid weathering of PAHs and biomarkers [4–6]. In this analysis, Cape Gull is the most distant site from the spill location, and very little surface and subsurface oil remained by 2012 [6]. The remaining oil was highly biodegraded [6]. The remaining biomarkers are unusual. One hypothesis considered by Irvine et al. [6] is that Cape Gull

biomarkers could represent a secondary contamination event. Given the unusually rapid PAH weathering and oil loss history at this site, we suggest that biomarker weathering may be a more parsimonious explanation for the change in composition, but we have no explanation for the underlying cause.

The pattern-matching method illustrated in the present study definitively discriminated ANSCO from several other potential sources (Supplemental Data, Tables S1 and S2). The pattern-matching method included the normalized value of every reported triterpane, hopane, and sterane analyte, thus making full use of the data (Supplemental Data, Tables S1 and S2). It will not, however, perform well for samples with multiple analytes below detection limits, although it can still provide insight into the source if a few analytes are consistently above detection limits. An alternative method, the Nordtest, which compares specific compound ratios [21], uses sample averages to infer whether they match the source pattern (Figure 3 and Supplemental Data, Figure S2), whereas the new pattern-matching method provides a specific result for each sample and subsequent statistics can follow. Nordtest outcomes become ambiguous when few samples are available or variance is high because confidence limits become large. The pattern-matching approach does not require multiple samples from a site to estimate origins, although several source oil samples are necessary for the model to function (the minimum used in the present study was 5).

Weathering

The biomarker weathering observed in the present study may have been microbial from the point the oil stranded (1989) and was sequestered until present. Rates of evaporation and dissolution were likely negligible for the large complex biomarker molecules under study, although evaporative loss was apparent for smaller compounds ($<C_{16}$) [6]. Microbial removal of *n*-alkanes was apparent at some sites [6]; thus at least some oil constituents were lost by this mechanism. Photooxidation is not likely because the buried oil was shielded from sunlight and resin and asphaltene fractions did not increase [6]. Because microbial degradation and photooxidation are the only 2 natural processes that destroy petroleum hydrocarbons [10] and photooxidation, evaporation, and dissolution were unlikely, microbial degradation may be the most parsimonious explanation for biomarker loss. How microbes manage to remove biomarkers from bulk oil, however, is not obvious; hence there may be other explanations.

The relatively rapid isoprenoid weathering observed in residual oil is consistent with literature reports. Wang and Stout [10] report that they can be severely degraded, although isoprenoids are more recalcitrant than the *n*-alkanes [24]. Our previous experience suggested that isoprenoid loss was too rapid and variable for source modeling, and comparison of ratios (e.g., pristane/phytane) indicated differences between the source oil and samples. Such changes are not surprising because these molecules were lost at different rates.

Weathering rates in other biomarkers varied. Triterpanes TR28a through TR29b tended to weather more rapidly than other triterpanes (Figure 5). Steranes DIA27S through C27bbS weathered more rapidly than other steranes (Figure 5). This suggests a way to estimate the weathering of these compounds in future studies. These results are generally consistent with observations from the *Metula* spill that diasteranes, C27 steranes, and tricyclic terpanes weathered relatively rapidly [10]. Weathering rates were approximately the same among all hopanes in the present study. In contrast, another study noted that H30 and H31 to H34 were degraded relatively rapidly [10,25].

CONCLUSIONS

Biomarkers provide an excellent way of definitively identifying the source of spilled oil over long periods, yet their concentrations change within the oil over time. Differential weathering may cause composition to slowly shift away from that in the source oil, although such shifts did not preclude identification of ANSCO after 25 yr. Biomarkers were clearly retained whereas other oil constituents were lost, explaining their initial concentration increase (per unit oil), yet concentrations declined over time, indicating removal or destruction by some process, possibly microbial. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI:10.1002/etc.3454.

Acknowledgment—We thank M. Zaleski and E. Pihl for processing samples.

Data availability—Data are available at http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files

REFERENCES

1. Spies RB, Rice SD, Wolfe DA, Wright BA. 1996. The effects of the *Exxon Valdez* oil spill on the Alaskan coastal environment. *Am Fish Soc Symp* 18:1–16.
2. Wolfe DA, Hameedi MJ, Galt JA, Watabayashi G, Short J, O'Clair C, Rice S, Michel J, Payne JR, Braddock J, Hanna S, Sale D. 1994. The fate of the oil spilled from the *Exxon Valdez*. *Environ Sci Technol* 28:561A–568A.
3. Babcock MM, Irvine GV, Harris PM, JAC, Rice SD. 1996. Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill. *Am Fish Soc Symp* 18:286–297.
4. Irvine GV, Mann DH, Short JW. 2006. Persistence of 10-year old *Exxon Valdez* oil on Gulf of Alaska beaches: The importance of boulder-armoring. *Mar Pollut Bull* 52:1011–1022.
5. Short JW, Irvine GV, Mann DH, Maselko JM, Pella JJ, Lindeberg MR, Payne JR, Driskell WB, Rice SD. 2007. Slightly weathered *Exxon Valdez* oil persists in Gulf of Alaska beach sediments after 16 years. *Environ Sci Technol* 41:1245–1250.
6. Irvine GV, Mann DH, Carls MG, Holland LG, Reddy CM, Nelson RK, Aeppli C. 2014. Lingering oil on boulder-armored beaches in the Gulf of Alaska 23 years after the *Exxon Valdez* oil spill.
7. Short JW, Lindeberg MR, Harris PM, Maselko JM, Pella JJ, Rice SD. 2004. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the *Exxon Valdez* oil spill. *Environ Sci Technol* 38:19–25.
8. Page DS, Bence AE, Burns WA, Boehm PD, Brown JS, Douglas GS. 2002. A holistic approach to hydrocarbon source allocation in the subtidal sediments of Prince William Sound, Alaska, embayments. *Environ Foren* 3:331–340.
9. Peters KE, Walters CC, Moldowan JM. 2007. *The biomarker guide, Volume 2: Biomarkers and isotopes in petroleum exploration and earth history*. Cambridge University Press, Cambridge, UK.
10. Wang Z, Stout SA. 2007. *Oil spill environmental forensics. Fingerprinting and source identification*. Elsevier, New York, NY, USA.
11. Faksness L-G., Weiss HM, Daling PS. 2002. Revision of the Nordtest methodology for oil spill identification. SINTEF Applied Chemistry, Trondheim, Norway.
12. Carls MG, Babcock MM, Harris PM, Irvine GV, Cusick JA, Rice SD. 2001. Persistence of oiling in mussel beds after the *Exxon Valdez* oil spill. *Mar Environ Res* 51:167–190.
13. O'Clair CE, Short JW, Rice SD. 1996. Contamination of intertidal and subtidal sediments by oil from the *Exxon Valdez* in Prince William Sound. *Am Fish Soc Symp* 18:61–93.

14. Short JW, Maselko JM, Lindeberg MR, Harris PM, Rice SD. 2006. Vertical distribution and probability of encountering intertidal *Exxon Valdez* oil on shorelines of three embayments within Prince William Sound, Alaska. *Environ Sci Technol* 40:3723–3729.
15. Irvine GV. 2000. Persistence of spilled oil on shores and its effects on biota. In Sheppard C, ed, *Seas at the millennium: An environmental evaluation*. Elsevier Science, Oxford, UK, pp 267–281.
16. Irvine GV, Mann DH, Short JW. 1999. Multi-year persistence of oil mousse on high energy beaches distant from the *Exxon Valdez* spill origin. *Mar Pollut Bull* 38:572–584.
17. Carls MG, Larsen ML, Holland LG. 2015. Spilled oils: Static mixtures or dynamic weathering and bioavailability? *Plos One* 10(9):e0134448.
18. Short JW, Kvenvolden KA, Carlson PR, Hostettler FD, Rosenbauer RJ, Wright BA. 1999. Natural hydrocarbon background in benthic sediments of Prince William Sound, Alaska: Oil vs coal. *Environ Sci Technol* 33:34–42.
19. Van Kooten GK, Short JW, Kolak JJ. 2002. Low-maturity Kulthieth Formation coal: A possible source of polycyclic aromatic hydrocarbons in benthic sediment of the northern Gulf of Alaska. *Environ Foren* 3:227–241.
20. Short JW, Heintz RA. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the northwestern Gulf of Alaska based on a PAH weathering model. *Environ Sci Technol* 31:2375–2384.
21. Daling PS, Faksness L-G., Hansen AB, Stout SA. 2002. Improved and standardized methodology for oil spill fingerprinting. *Environ Foren* 3:263–278.
22. Draper NR, Smith H. 1981. *Applied regression analysis*. John Wiley & Sons, New York, NY, USA.
23. Short JW, Kolak JJ, Payne JR, Van Kooten GK. 2007. An evaluation of petrogenic hydrocarbons in northern Gulf of Alaska continental shelf sediments—The role of coastal oil seep inputs. *Org Geochem* 38: 643–670.
24. Payne JR, Driskell WB, FJB, Bailey J, Short JW, Ka'aihue L, Kuckertz TH. 2005. From tankers to tissues—Tracking the degradation and fate of oil discharges in Port Valdez, Alaska, Calgary, Alberta, Canada, pp 32.
25. Frontera-Suau R, Bost FD, McDonald TJ, Morris PJ. 2002. Aerobic biodegradation of hopanes and other biomarkers by crude oil-degrading enrichment cultures. *Environ Sci Technol* 36:4585–4592.