# Chapter X. Review of PAH and alkane retention in sediment oiled by the *Exxon Valdez*

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**Abstract.** Polynuclear aromatic hydrocarbons (PAHs) and alkanes have remained in sediment oiled by the *Exxon Valdez* spill for 25 years. Because PAHs are toxins, this is cause for concern. However, relatively little oiled sediment remains and recent passive sampler data and the exposure history of mussels, harlequin ducks, and sea otters suggest these oil deposits have essentially become decoupled from macrofauna outside the sediment. Hydrocarbon retention in oil was proportional to molecular mass. The greater loss of small PAHs was likely a function of thermodynamic transfer of highly insoluble molecules from oil to water and air. Data from a second Alaskan spill, the *Selendang Ayu*, was used to independently confirm this relationship. Much of this PAH loss from Alaska North Slope crude oil occurred before the first samples were collected (October 1989, several months after the spill), yet exposure records indicate that diminishing PAH concentrations were biologically available for extended periods of time.

## Introduction

The supertanker *Exxon Valdez* grounded on Bligh Reef in northeastern Prince William Sound (PWS), Alaska, on March 24, 1989. The resultant 42 million liter oil spill was the largest in US history and was ultimately distributed over approximately 28,500 $km^2$ (Gundlach et al. 1990). The slick moved southwesterly across PWS, surrounding the Naked Island group (Naked Island, Peak Island, and Storey Island), the Knight Island archipelago, and southwest islands before entering the Gulf of Alaska (GOA). The oil became more viscous with time and distance as it emulsified (Short et al. 2007). It did not reach the northern and eastern mainland in PWS, nor Hinchinbrook Island and those to the east, and did not reach some areas on the western mainland (Gundlach et al. 1990). About 40% of the spilled oil was beached in PWS (Wolfe et al. 1994) and there was visible oiling on 1160 km of shoreline (Gundlach et al. 1990). Spill impacts on mammals, birds, fish, and intertidal communities were immediate, often devastating, and affected sensitive species for many years (Peterson 2001). Efforts to clean beaches were intensive in 1989 to 1990 but only about 10% of the oil was removed (Mearns 1996).

The purpose of this paper is to examine polynuclear aromatic hydrocarbon (PAH) content in oil sequestered intertidally over a 25 year period and by molecular mass because these molecules are toxic. If PAHs remain in oil, then toxic potential remains. In theory, molecular mass should influence PAH loss through thermodynamic mechanisms. Differential PAH loss from oil is summarized as weathering (Short and Heintz 1997). Hence, by understanding the underlying weathering processes and mechanisms, reasons for why the oil preferentially retained some PAHs is comprehensible. The second purpose was to similarly examine *n*-alkane retention in oil. To independently verify ANSCO results, the same processes were examined in a second oil spill that occurred in the Aleutian Islands (Carls et al. 2015). Retention of biomarkers (triterpanes, hopanes, and steranes) in sequestered ANSCO oil is the topic of another paper (Carls et al. 2016) and is not included here. Our third goal was to place the results into context by discussing the amount of remaining ANSCO in beaches and review the history of biological exposure to it.

The study area was beaches along the northern GOA and in western PWS (Fig. 1). This paper extends previous reports of oil retention in this area (Irvine et al. 1999; Short et al. 2004; Irvine et al. 2006; Short et al. 2006; Short

et al. 2007; Irvine et al. 2014). Several of those papers are focused on the persistence and amount of sequestered oil. This work is instead focused only on the chemistry of the remaining oil and does not estimate the total amount of oil remaining on beaches. Previous studies identified conditions at Cape Gull, located within the GOA, as unusual (Irvine et al. 2006; Short et al. 2007), hence we analyzed it independently instead of including it with statistics describing the remainder of GOA sites.

## Methods

*Data sources and collection*

Hydrocarbon data were obtained from the publically available State/Federal *Exxon Valdez* Trustee Council hydrocarbon database (Short et al. 1996; Carls and Masuda 2014). All samples from PWS and the GOA that were processed as oil (resulting in concentrations expressed per gram oil) from sites with three or more sample years were included in the analysis. There were 143 samples from 13 sites collected between 1989 and 2014 and 39 additional samples collected in 2015. Sample sites were Bay of Isles (BOISL), Cape Douglas (CDOUG), Cape Gull (CGULL), Chenega Island (CHENI), Eleanor Island (ELEAI), Green Island (GREEI), Herring Bay (HERRB), Kashvik Bay (KASHB), Kiukpalik Bay (KIUKP), Knight Island (KNIGI), Latouche Island (LATOI), McArthur Pass (MCARP), Morning Cove (MORNC), Ninagiak Island (NINAI), Sleepy Bay (SLEEB), and Smith Island (SMITI). To confirm the *Exxon Valdez* results, 101 oil samples collected between 2004 and 2008 from a second spill, the *Selendang Ayu* (*S. Ayu*), were also analyzed. The latter spill occurred in the Aleutian Islands, thus both the oil type and locations were independent.

Sediment and oil sample collection methods were previously reported (O'Clair et al. 1996; Brodersen et al. 1999; Irvine et al. 1999; Murphy et al. 1999; Irvine 2000; Carls et al. 2001; Short et al. 2004; Irvine et al. 2006; Short et al. 2006; Short et al. 2007; Carls et al. 2015). 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 at various times (depending on collection times and individual study needs) for aliphatic and aromatic hydrocarbons. The most recent analyses occurred in late 2015.

*Hydrocarbon extraction and measurement*

Oil samples were obtained by extracting sediment samples. About 50 g of homogenized sediment was placed in a 250 ml Teflon extraction vessel and dried with about 40 ml anhydrous sodium sulfate. The oil was extracted with 50 ml dichloromethane (DCM) with shaking and 30 minutes in an ultrasonic bath. Extracts were poured through combusted, DCM-rinsed 17 cm glass fiber filters into 250 ml round bottom flasks. The remainder was re-extracted with 40 ml DCM and 20 minutes sonication and added to the first aliquot through the filter. The extraction vessel was rinsed twice with 25 ml DCM and filtered into the flask. Extract volumes were reduced by boiling at 80°C and quantitatively transferred to 50 ml conical tipped centrifuge tubes. Aliquots were removed for gravimetric analysis and gas chromatography. Gravimetric measures were completed by allowing the DCM to evaporate from a tared aluminum weigh pan. Pans were then placed in a vacuum oven, evacuated to 50 mm Hg and allowed to remain 45 to 60 minutes without heat. The oven was subsequently vented and pans remained at atmospheric pressure for about 15 minutes before weighing in grams to the $5^{th}$ decimal. The injection target oil mass for PAH and alkane analysis was 3 to 6 mg; appropriate volumes were removed from remaining aliquots, and spiked with 500 µL deuterated surrogate spiking solution. The solvent was exchanged to

remove DCM; volumes were reduced under a stream of nitrogen that just agitated the surface of the extract. When the volume reached 0.5 ml (the volume of the surrogate spike in hexane), several drops of hexane were added and the volume was reduced again.

Aromatic fractions were analyzed for PAHs by gas chromatography – mass spectroscopy. Data were acquired in selected ion monitoring mode and concentrations were determined by the internal standard method (Short et al. 1996). Measured PAHs were naphthalenes (N0 to N4), biphenyl (BPH), acenaphthylene (ACN), acenaphthene (ACE), fluorenes (F0 to F4), dibenzothiophenes (D0 to D4) phenanthrenes (P0 to P4), anthracene (ANT), fluoranthene (FLU), pyrene (PYR), fluoranthene/pyrenes (FP1 to FP4) benzo(a)anthracene (BAA), chrysenes (C0 to C4), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(e)pyrene (BEP), benzo(a)pyrene (BAP), perylene (PER), indeno(1,2,3-cd)pyrene (ICP), dibenzo(a,h)anthracene (DBA), and benzo(ghi)perylene (BZP). Several of these were not routinely measured until 2004 (F4, D4, FP2, FP3, and FP4). Concentrations below method detection limits were set to zero. A PAH source model was used to estimate origins of observed PAHs (Carls 2006; Carls et al. 2015).

Aliphatic fractions were analyzed for *n*-alkanes using gas chromatography and flame ionization detection (Short et al. 1996). Analyte concentrations were determined by the internal standard method. Measured normal alkanes ranged from *n*−C9 through *n*−C36 plus pristane and phytane. Several of these were not routinely measured until 2004 (*n*−C9, *n*−C31, *n*−C33, *n*−C35, and *n*−C36). Concentrations below method detection limits were set to zero.

*Source oil samples*

Twenty-one source oil sample measurements were used to determine analyte concentrations in fresh *Exxon Valdez* oil (Alaska North Slope crude oil, ANSCO) and eight source measurements were used for *S. Ayu* oil (SAO). An additional six source measurements were prepared and analyzed in 2015 as quality controls. Analytical measurements were completed as previously described. Retention was estimated by dividing observed analyte concentrations (ng/g oil) by the mean analyte concentration in fresh oil (ng/g oil). Estimates were expressed as percentages. Analytes included PAHs and *n*-alkanes; these were analyzed separately.

*Problem samples*

PAH retention in samples collected in 2011 at ELEAI was unusually high; these 12 samples were not included in the PAH analysis. Retention patterns were similar to all other data, but shifted by an unknown systematic error. The 2011 ELEAI alkane data were also removed from analysis; *n*-C31, *n*-C33, and *n*-C35 were underestimated in this data set.

*Statistics*

To understand why molecules were lost or retained by oil, geometric mean retention was regressed against molecular mass to determine if it was influenced by size. The PAH regression models were determined by testing a large number of models for best fit – but a sigmoidal model was usually the best descriptor ($R = D + (V_{max} \bullet w^n) / (w^n + Km^n)$, where R = retention, w = molecular weight and D, $V_{max}$, n, and Km were parameters fit by the model; XLFit plugin for Excel). Means were compared with single-factor analysis of variance (ANOVA). Non-parametric ANOVA was used (Kruskal-Wallis ANOVA on ranks) when normality or equal variance tests failed

(Shapiro-Wilk and Brown-Forsythe tests, respectively). Statistics were completed with SigmaPlot and Minitab software.

Principal component analysis (PCA) was used to compare PAH retention patterns among samples (Minitab). Additional regressions were completed to interpret PCA results (XLfit).

The usefulness of regressions was estimated using the methods of Draper and Smith (Draper and Smith 1981). In general, if the observed F value ($F_o$) divided by the critical F value ($F_c$) is > 4, then the regression is useful.

## Results

*PAH in ANSCO*

The ANSCO samples were consistently petrogenic at all sites (SI 1). PAH source model estimates tended to be least where weathering was most advanced because information is lost as analytes are depleted.

Oil weathering tended to be greater in PWS than in the GOA, as evidenced by larger proportions of chrysenes in PWS (Fig. 1). Weathering was greatest at CGULL, a site previously identified as unusual in the GOA (Irvine et al. 2006; Short et al. 2007). Weathering in all other GOA samples was generally less than in PWS ($P_{ANOVA} < 0.001$). Weathering described by chrysene proportions was related to the modeled weathering estimates of (Short and Heintz 1997) ($r^2 = 0.828$, $P_{sigmoidal\ regression} < 0.001$; SI 2).

Estimated by area medians, PAH analyte retention was generally least at CGULL, intermediate in PWS, and greatest in the GOA (Table 1). This pattern was evident in 29 of 38 individual analytes. Analytes that were not consistently measured were not included in this comparison (F4, D4, FP2 – FP4) because results can be biased based on when they were (or were not) measured.

PAH retention in ANSCO was directly proportional to molecular mass (Fig. 2a). Scatter was large, thus correlation was moderate: $r = 0.449$ ($r^2 = 0.202$). However, the regression was highly significant ($P < 0.001$; $n = 7306$; $F_o/F_c = 1361$).

Retention of PAH in GOA, PWS, and CGULL oil converged at the same location in PCA space (Fig. 3). The convergence area represents high PAH loss and all CGULL samples collected after 1989 were in this region. The primary change across component 1 (PC 1), which explained 56% of the variance, was increasing retention; correlation (r) between PC 1 and median retention was 0.969 (logistic regression). The general divergence of PWS and GOA samples in component 2 (PC 2) was caused by better low- and mid-range PAH retention in the GOA. Retention of HMW PAH was similar between regions.

Retention ranged from relatively small to relatively large across PC 1 for PWS and the GOA; consistent time trends were generally not evident (Fig. 3). However, PWS and GOA samples were more similar in 1989; by 2010 they generally diverged across PC 2.

*Alkanes in ANSCO*

Alkanes in ANSCO weathered by preferentially losing smaller compounds. The proportion of heavy alkanes (of total alkanes) was greater in beaches (median = 73% at CGULL, 51% in PWS, and 19% in the GOA) than in fresh oil (13%; $0.001 < P_{ANOVA} \leq 0.031$).

Alkane retention was generally least at CGULL, intermediate in PWS, and greatest in the GOA for *n*−C13 through *n*−C34, including pristane and phytane (Table 3). Retention was near zero for *n*−C10 to *n*−C12. Alkanes with incomplete measurements (*n*−C9, *n*−C31, *n*−C33, *n*−C35, and *n*−C36) were not included in the analysis.

Alkane retention in the remaining ANSCO was directly proportional to molecular mass (Fig. 4a). Scatter was large, thus correlation was only moderate (0.412). Nonetheless, the regression was highly significant ($P < 0.001$; $n = 4466$; $F_o/F_c = 238$).

*PAH in SAO*

The SAO samples were petrogenic (the mean PAH source model result was 0.991) and weathering increased with time. Chrysene proportions were significantly greater 3 years after the spill (2008) than in the first year of the spill ($P_{ANOVA} = 0.009$), indicating loss of smaller PAHs, hence increasing relative chrysene quantities. Weathering in SAO was variable in intertidal sediment, ranging from fresh to weathered 3 years after the spill. Weathering described by chrysene proportions was related to the modeled weathering estimates of (Short and Heintz 1997) ($r^2 = 0.870$, $P_{sigmoidal\ regression} < 0.001$; SI 2).

PAH retention in SAO was directly proportional to molecular mass (Fig. 2b). Correlation was strong (0.770) and the regression was highly significant ($P < 0.001$; $n = 4343$; $F_o/F_c = 9221$). Scatter was smaller than in the ANSCO data set. Large PAHs were typically not concentrated beyond 100%. Geometric mean retention was 103% for C4 and 108% for BEP; all others were < 100%. Mean PAH retention in ANSCO, which was observed over a longer period of time, was smaller than in SAO (Fig. 2).

*Alkanes in SAO*

Alkanes in SAO weathered by preferentially losing smaller compounds. The proportion of heavy alkanes (of total alkanes) was greater 3 years after the spill (median = 53%) than in fresh oil (3%) and in beaches the first year of the spill (10%; $P_{ANOVA} < 0.001$).

Alkane retention in remaining SAO was directly proportional to molecular mass with no indications of an asymptote (Fig. 4b). Correlation was strong (0.770) and the regression was highly significant ($P < 0.001$; $n = 2828$; $F_o/F_c = 1.6 \times 10^6$). The larger alkanes became concentrated; geometric mean retention was > 100% for *n*-C29 and above and ranged up to 16 times the original concentration for *n*-C36.

## Discussion

Alaska North Slope crude oil remains in some GOA and PWS beaches after 25 years and has retained characteristic PAH and alkane constituents. Enough PAHs were retained that the oil remained clearly identifiable as petrogenic by PAH source modeling (Carls et al. 2015). Larger alkanes were also retained.

Biomarkers, which are large molecules, remain in this oil, hence ANSCO remains definitively identifiable at every measured site (CGULL, ELEAI, HERRB, KIUKP, LATOI, MCARP, and NINAI) except BOISL (Carls et al. 2016). Hopanes and steranes matched ANSCO at BOISL and we expect additional samples would yield the ANSCO signature at BOISL.

Weathering of ANSCO was generally more rapid in PWS than in the GOA with the exception of one site, CGULL, where it has been unusually rapid. Molecule retention (and loss) is likely controlled by thermodynamics and relates to molecular mass. Evaporation and dissolution, the two most likely loss compartments (NRC 2003; Wang and Stout 2007), are controlled by first order loss rate kinetics with rate constants determined by the enthalpy of vaporization through the Arrhenius equation (Short and Heintz 1997). Thus, small molecules, whether PAH or alkane, preferentially left the oil, and larger molecules were retained. Retention of PAHs that can dissolve in meaningful quantities (through about C2 chrysenes) means that oil toxicity can be activated by physical disturbance, allowing greater interaction with surrounding water and access to organisms.

Counterbalancing this problem, only about 8% of the sediment area remains oiled in beaches that were originally moderately to heavily oiled and the volume remaining is about 0.14 to 0.28% (Short et al. 2004; Short et al. 2006; Short et al. 2007). Furthermore, recent passive sampler data and the exposure history of mussels, harlequin ducks, and sea otters suggest these oil deposits have essentially become decoupled from macrofauna outside the sediment (Carls et al. 2001; Esler et al. 2002; Page et al. 2005; Ballachey et al. 2014; Esler et al. 2014). Total PAH concentrations in mussels declined with time after the spill as the oil became depleted, was physically removed, or became isolated by formation of weathered asphalt encasements around oil deposits. Significant mussel exposure ceased after about 12 years (Page et al. 2005).

Some animals, notably harlequin ducks and sea otters were also physically exposed to oil through foraging activity or other behavior and were exposed to PAHs for a longer period of time. This disturbance-based, diminishing exposure process persisted for about 22 years and has now also dropped to insignificant levels (Ballachey et al. 2014; Esler et al. 2014). Thus the remaining oil deposits have essentially become decoupled from macrofauna outside the sediment and should remain decoupled except when they are disturbed by occasional, unusually high energy events.

Retention of PAHs and alkanes as a function of molecular mass in these long term oil deposits clearly indicates the presence of a fundamental underlying thermodynamic process (Figs. 2 and 4). The data are relatively noisy, but by examining many samples, clear trends emerge. This same process was evident in an unrelated, geographically distinct spill with a different crude oil (SAO), confirming the ANSCO results. Furthermore, this retention pattern consistently emerged when ANSCO data were subdivided into 5-year increments (SI 3). The subdivided data suggest relatively greater retention in recent years but this is likely the consequence progressively narrowing sample collection to the most problematic beaches.

A second artifact in the ANSCO data, an apparent decline in retention among higher molecular mass PAHs (SI 3) is explained by occasional failure to detect these compounds during analysis. For example, among chrysenes, the probability that C4 chrysenes were not detected was higher than for C0 chrysenes, thus reducing mean retention estimates. This problem can be corrected by including only data above method detection limits (SI 4).

The corrected values make more sense because chrysene content consistently increases with weathering (Bence and Burns 1995; Bao et al. 2014; Carls et al. 2015). The apparent uncorrected HMW loss pattern in ANSCO is consistent with photo-oxidation (Garrett et al. 1998) but that explanation is unlikely. The oil was buried, hence shielded from radiation and the loss pattern for smaller molecular mass PAHs was inconsistent with photo-oxidation. Thus the most parsimonious explanation for the drop off is the occasional failure to detect some relatively low concentration HMW compounds and the subsequent influence of representing below method detection limit data as zeros instead of removing them from the data set.

Estimated PAH retention (e.g., Fig. 2) can be treated as a rate and applied to PAH composition to simulate weathering. This is accomplished by starting with the PAH composition of fresh ANSCO, with each analyte expressed as a percentage of TPAH, and sequentially subtracting a fraction of the estimated retention. The simulated weathering pattern sequence (Fig. 5) is highly consistent with real weathering patterns (Short and Heintz 1997; Wang and Stout 2007); the lightest compounds are lost most quickly both among and within homologous groups. Further support that the simulation yields the real weathering pattern is that two different source models identify the simulated patterns as oil (Short and Heintz 1997; Carls 2006; Carls et al. 2015) with the caveat that the source models progressively lose the ability to identify oil as increasing numbers of analyte concentrations reach zero. Previous work has identified the underlying weathering driver as thermodynamics (Short and Heintz 1997; Carls et al. 2015). This study supports that observation; thermodynamics is the rate controlling step, and it consistently yields specific retention patterns related to molecular mass.

Scatter plots for the differential loss process in naturally weathered oil (Fig. 2) gives the impression that thermodynamic control is a highly variable, sloppy process. It is not. The reason for the high scatter is that the weathering clock runs at different rates in different places; it is dependent on circumstance (volume, exposure, burial, capping, etc.) not the exact tick of time. The evidence for thermodynamic precision are the composition patterns in recovered oil, which are always somewhere on the weathering curve, and never in some strange configuration inconsistent with the mass-dependent weathering process. Thus, the reason for the scatter is that there are many different retention curves as a result of many different weathering clocks - yet central tendency always converges on the same pattern (SI 4).

The differential rate of PAH and alkane loss between PWS and the GOA suggests there were physical differences between these locations. The change in weathering patterns evidently occurred within the beaches because retention patterns were more similar between regions in early years than later (Fig. 3). Weathering differences may have been a consequence of weathering as the oil was transported from PWS to the GOA by water; increased contact time and mixing energy created oil-water emulsions and increased viscosity (Short et al. 2007). Viscous ANSCO emulsions penetrated the more porous GOA beaches and hence became protected from disturbance by the beach structure (Irvine et al. 1999; Short et al. 2007; Irvine et al. 2014). In contrast, less viscous oil stranded in the generally finer-grained beaches in PWS (Short et al. 2007). Thus weathering rates are more rapid in PWS than in the GOA despite greater wave energy in the latter and despite similarities at the time of deposition.

PAHs that were lost from the oil entered both air and water. Evaporative processes are typically the most rapid (10 to 50% by weight), followed by dissolution (1 to 3%), and microbial degradation (slower than dissolution)

(Wang and Stout 2007). Our data cannot distinguish which compartments were responsible for the observed loss, but both air and water are possible because the oil was collected from intertidal sediment and was potentially in contact with them. Oil in beaches is often detectable by olfaction when deposits are disturbed, thus there is evidence of evaporation. PAHs were also detected in nearby organisms such as mussels (Carls et al. 2001; Page et al. 2005), evidence of dissolution. Passive samplers deployed in PWS, the GOA, and Unalaska (SAO) also provided evidence of dissolution (Carls et al. 2004; Short et al. 2008; Springman et al. 2008; Irvine et al. 2014; Carls et al. 2015).

## Conclusions

Higher molecular weight alkanes and PAHs from two different spills were retained in oil intertidally sequestered in beaches. Retention was consistent with thermodynamics; the probability that smaller molecules are lost to surrounding air or water is relatively large compared to larger molecules, thus composition undergoes characteristic weathering shifts. Retention of PAHs means that remaining oil retains toxicity. However, biological studies have demonstrated that exposure to PAHs from ANSCO has diminished below significance for all species examined as part of the Natural Resources Damage Assessment process. In addition, the stability of remaining oil volumes (Short et al. 2007) indicates little exchange with the surrounding environment.

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**Table 1.** Median PAH retention (%) in ANSCO by area.

*Retrospective PAH and alkane Dec 2015 v2.xlsm, sheet "mean by"*

| PAH | CGULL | PWS | GOA |
|---|---|---|---|
| N0 | 0.000 | 0.013 | 0.000 |
| N1 | 0.000 | 0.019 | 0.078 |
| N2 | 0.000 | 0.107 | 1.240 |
| N3 | 0.010 | 0.881 | 5.681 |
| N4 | 0.062 | 1.739 | 7.210 |
| BPH | 0.000 | 0.000 | 0.098 |
| ACN | 0.000 | 0.000 | 0.000 |
| ACE | 0.000 | 0.189 | 0.000 |
| F0 | 0.000 | 0.248 | 2.391 |
| F1 | 0.000 | 1.224 | 6.634 |
| F2 | 0.141 | 2.450 | 10.642 |
| F3 | 0.956 | 3.655 | 9.940 |
| D0 | 0.000 | 0.621 | 4.771 |
| D1 | 0.055 | 1.444 | 9.215 |
| D2 | 1.062 | 2.864 | 11.445 |
| D3 | 2.347 | 3.601 | 16.857 |
| P0 | 0.000 | 0.450 | 4.737 |
| P1 | 0.023 | 1.104 | 10.303 |
| P2 | 0.537 | 2.859 | 14.285 |
| P3 | 1.797 | 4.878 | 15.281 |
| P4 | 4.559 | 6.191 | 20.400 |
| ANT | 0.000 | 6.861 | 29.772 |
| FLU | 0.000 | 7.195 | 13.003 |
| PYR | 1.639 | 5.907 | 13.267 |
| FP1 | 3.593 | 6.391 | 13.009 |
| BAA | 2.103 | 2.408 | 6.377 |
| C0 | 4.467 | 4.831 | 15.358 |
| C1 | 2.777 | 5.207 | 11.256 |
| C2 | 2.437 | 4.789 | 7.485 |
| C3 | 3.043 | 5.643 | 5.505 |
| C4 | 0.000 | 1.381 | 1.193 |
| BBF | 7.999 | 11.172 | 20.357 |
| BKF | 0.000 | 0.000 | 0.000 |
| BEP | 6.308 | 9.235 | 15.685 |
| BAP | 18.719 | 6.386 | 19.504 |
| PER | 11.276 | 17.358 | 2.446 |
| DBA | 0.000 | 1.555 | 0.000 |
| BZP | 5.267 | 23.863 | 11.538 |

**Table 3.** Mean alkane retention in ANSCO by area.
*Retrospective PAH and alkane Jan 2016.xlsm, sheet "more ALK loss"*

| Alkane | CGULL | PWS | GOA |
|---|---|---|---|
| *n*-C10 | 0.00 | 0.00 | 0.00 |
| *n*-C11 | 0.00 | 0.00 | 0.00 |
| *n*-C12 | 0.00 | 0.00 | 0.16 |
| *n*-C13 | 0.00 | 0.00 | 1.51 |
| *n*-C14 | 0.00 | 0.00 | 2.04 |
| *n*-C15 | 0.00 | 0.04 | 4.14 |
| *n*-C16 | 0.00 | 0.03 | 5.43 |
| *n*-C17 | 0.01 | 0.04 | 7.91 |
| PRISTANE | 2.61 | 1.25 | 22.70 |
| *n*-C18 | 0.04 | 0.00 | 7.51 |
| PHYTANE | 1.96 | 0.80 | 19.16 |
| *n*-C19 | 0.03 | 0.00 | 7.83 |
| *n*-C20 | 0.31 | 0.23 | 9.18 |
| *n*-C21 | 0.08 | 0.19 | 8.92 |
| *n*-C22 | 0.22 | 0.23 | 12.57 |
| *n*-C23 | 0.18 | 0.29 | 14.11 |
| *n*-C24 | 0.00 | 0.07 | 15.79 |
| *n*-C25 | 0.68 | 0.03 | 19.00 |
| *n*-C26 | 0.34 | 0.46 | 21.52 |
| *n*-C27 | 0.30 | 0.72 | 17.84 |
| *n*-C28 | 0.90 | 0.95 | 17.38 |
| *n*-C29 | 1.64 | 3.89 | 18.62 |
| *n*-C30 | 1.00 | 0.84 | 16.88 |
| *n*-C32 | 5.46 | 4.01 | 26.11 |
| *n*-C34 | 4.70 | 1.58 | 24.05 |

**Fig. 1.** Mean proportion chrysenes (of total PAHs) at each sample site in Prince William Sound and the Gulf of Alaska. Site numbers are 1) BOISL, 2) CDOUG, 3) CGULL, 4) CHENI, 5) ELEAI, 6) HERRB, 7) KASHB, 8) KIUKP, 9) LATOI, 10) MCARP, 11) MORNC, 12) NINAI, and 13) SLEEB.
\\nmfs.local\AKC-ABL\RECA\Chemistry\Hydrocarbons\Projects\Retrospective PAHs\maps\weathering.mxd.

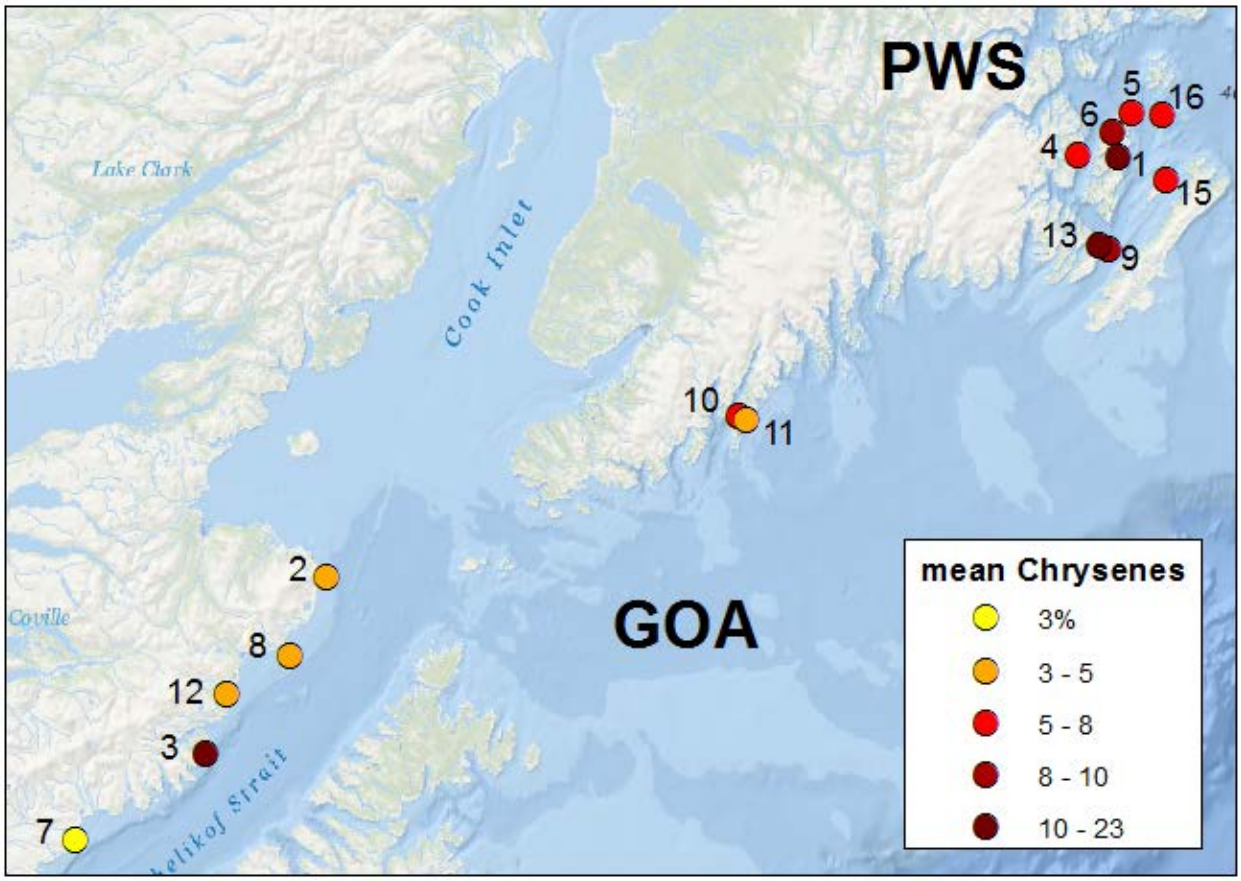

**Fig. 2.** Polynuclear aromatic hydrocarbon (PAH) retention as a function of molecular mass. The red circles are geometric means overlain on scatter plots of all data (gray). Regressions were based on all data. Top panel: PAH retention in Alaska North Slope crude oil from the Gulf of Alaska and Prince William Sound (1989 to 2015). Bottom panel: PAH retention in *Selendang Ayu* oil (Aleutian Islands). Retrospective PAH and Alkane Dec 2015 v2.xlsm, sheet "PAH loss (3)" Selendang PAH and Alkane Dec 2015.xlsm, sheet "PAH loss (3)" and PAH retention.eps, .jpg.

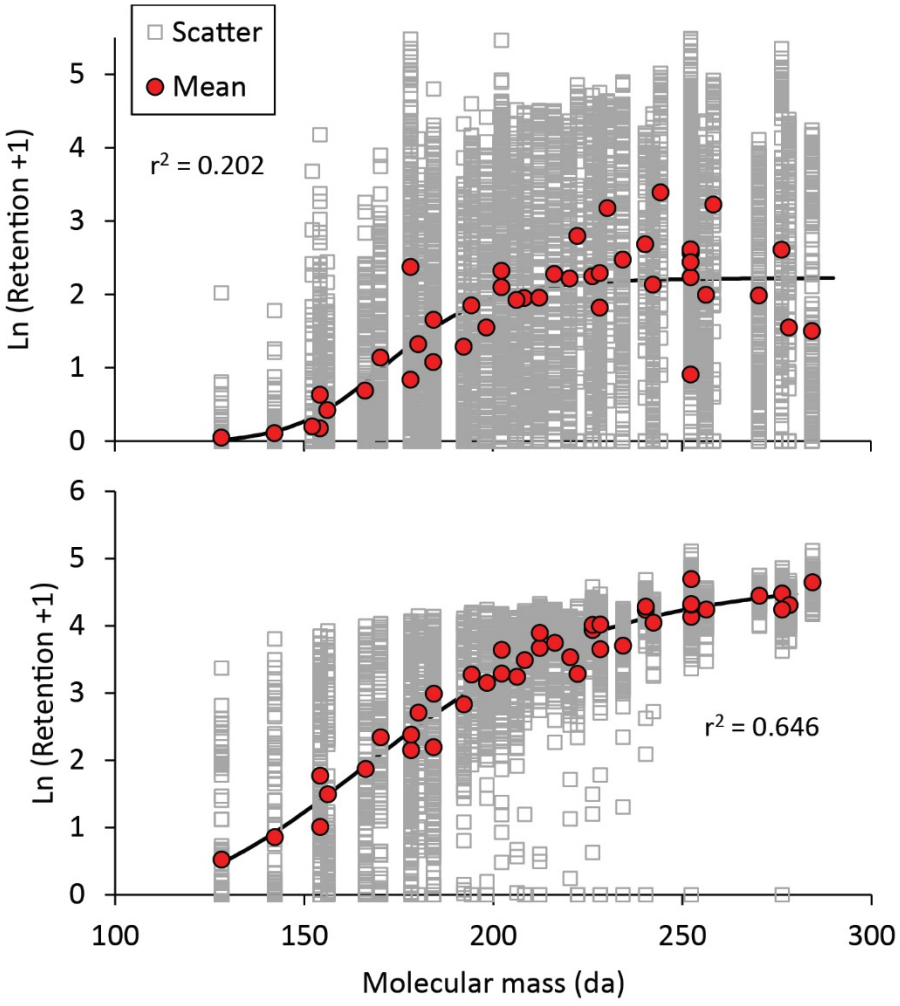


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**Fig. 3.** Principal component analysis of PAH retention in ANSCO. Axes are components 1 (PC 1) and 2 (PC 2). Component 1 explained 56% of the variance and PC 2 explained 13%.

Retrospective PAH and Alkane Jan 2016.xlsm, sheet "PCA"

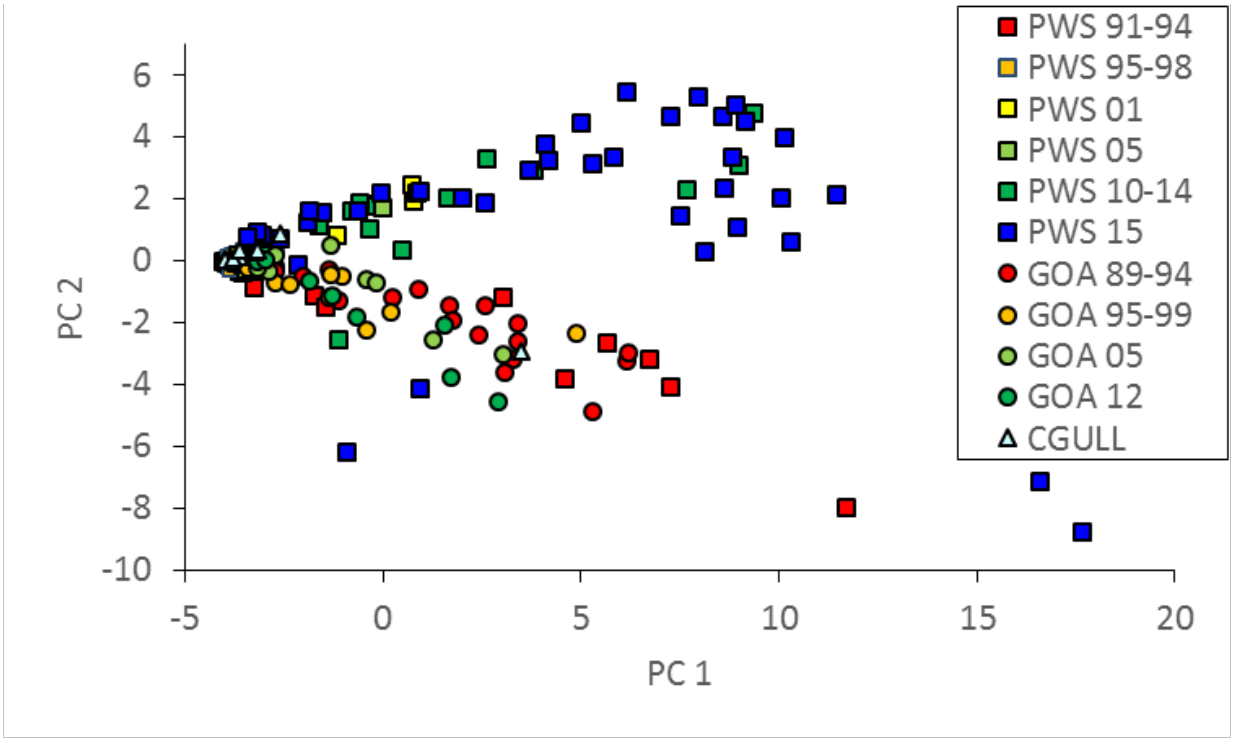


**Fig. 4.** Alkane retention was directly proportional to molecular mass. The red circles are geometric means overlain on scatter plots of all data (gray). Regressions were based on all data. Top panel: retention in Alaska North Slope crude oil samples collected from 13 sites in Prince William Sound and the Gulf of Alaska (1989 to 2015). Bottom panel: alkane retention in *Selendang Ayu* oil, 2004 to 2008. Retrospective PAH and Alkane Dec 2015 v2.xlsm, sheet "More ALK loss." Selendang PAH and Alkane Dec 2015.xlsm, sheet "ALK loss." Alkane retention.eps, .jpg.

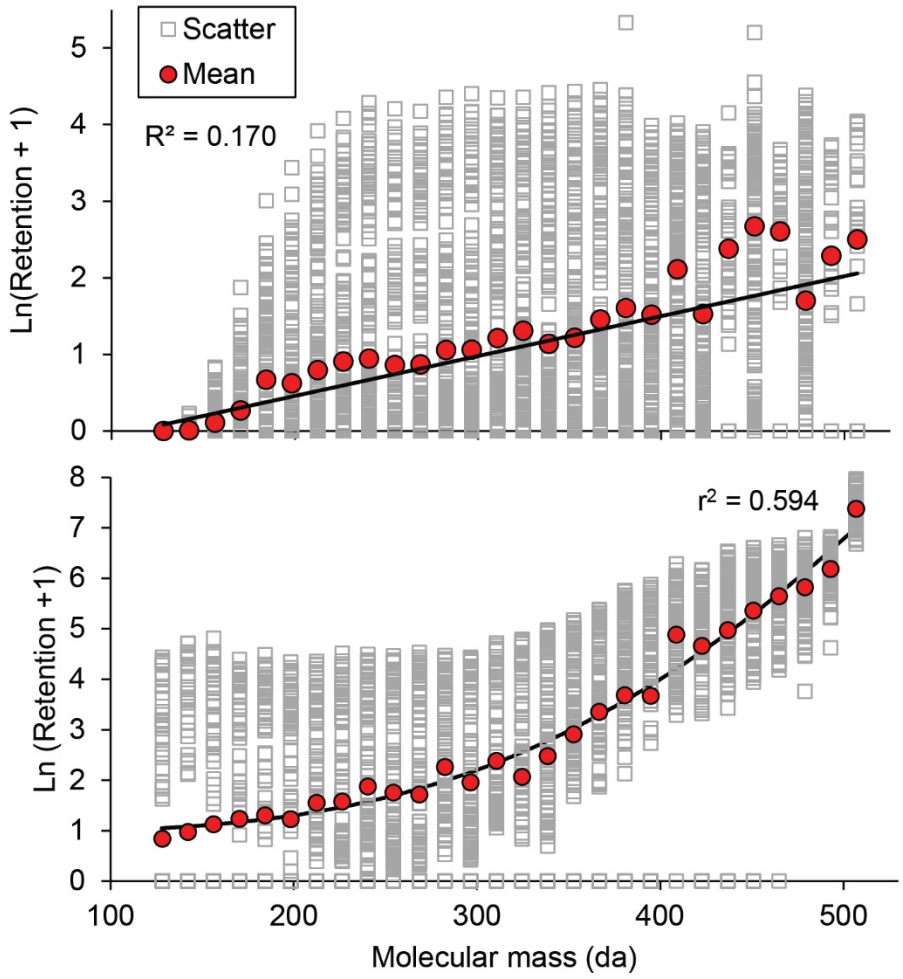
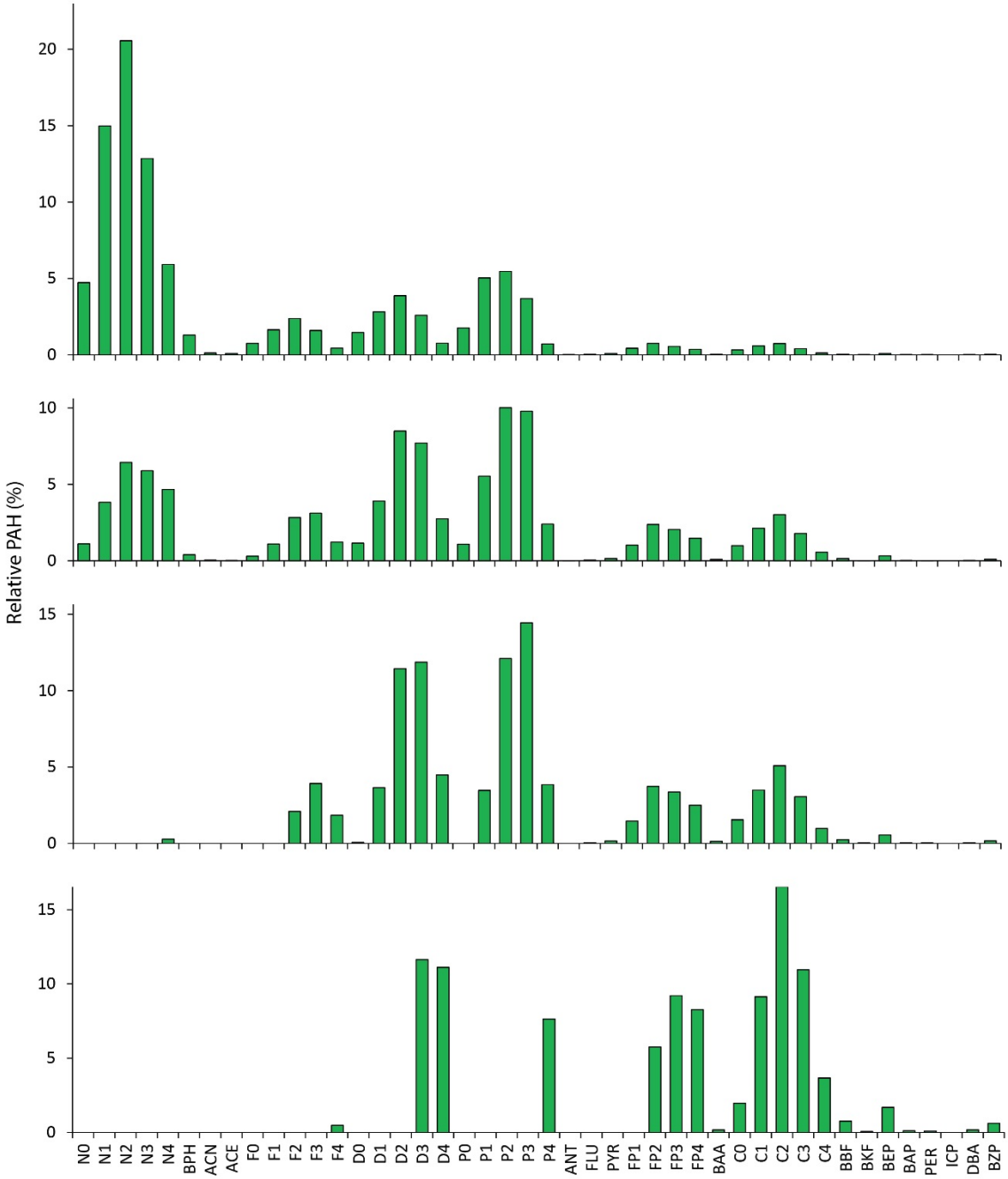
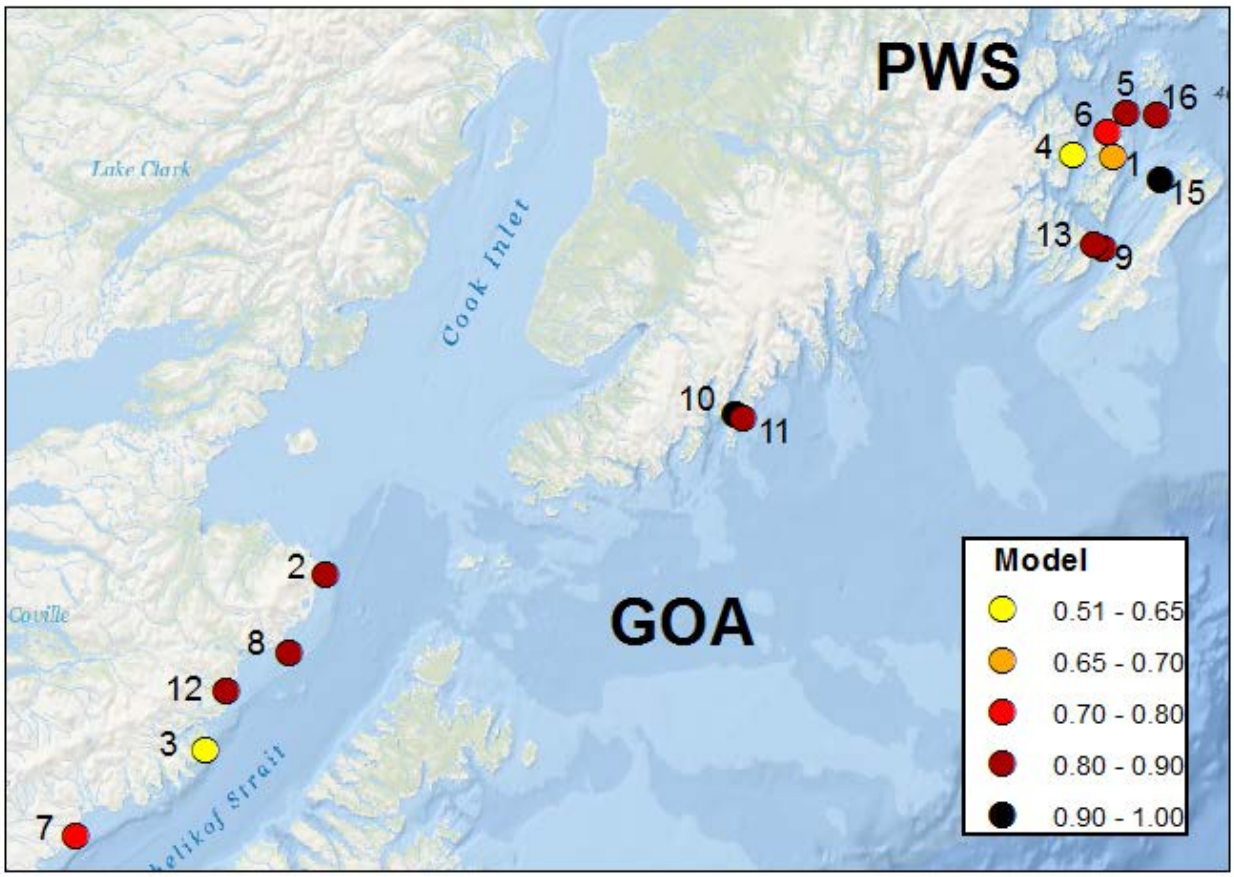


**Fig. 5.** Simulated weathering based on PAH retention, from unweathered (top panel) to considerably weathered (bottom panel).

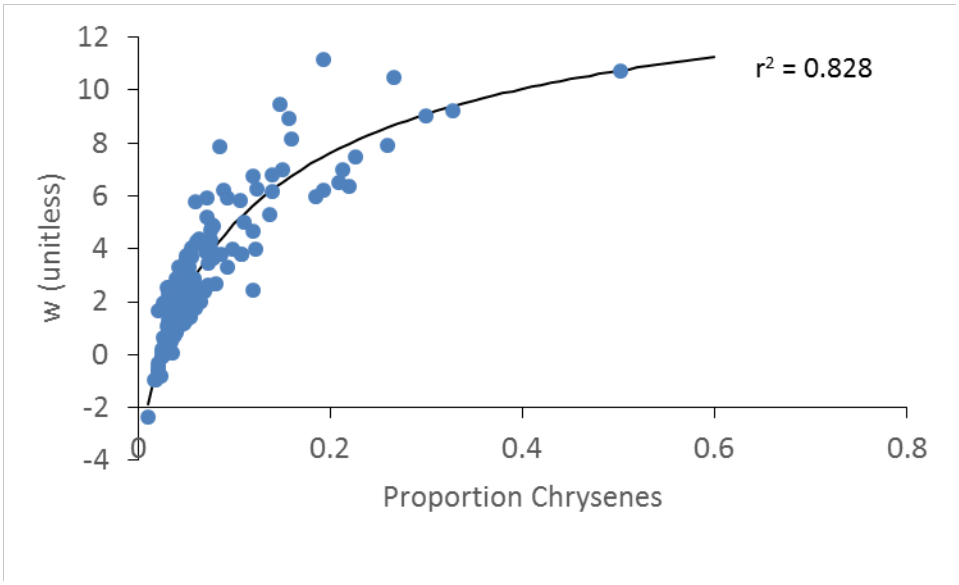


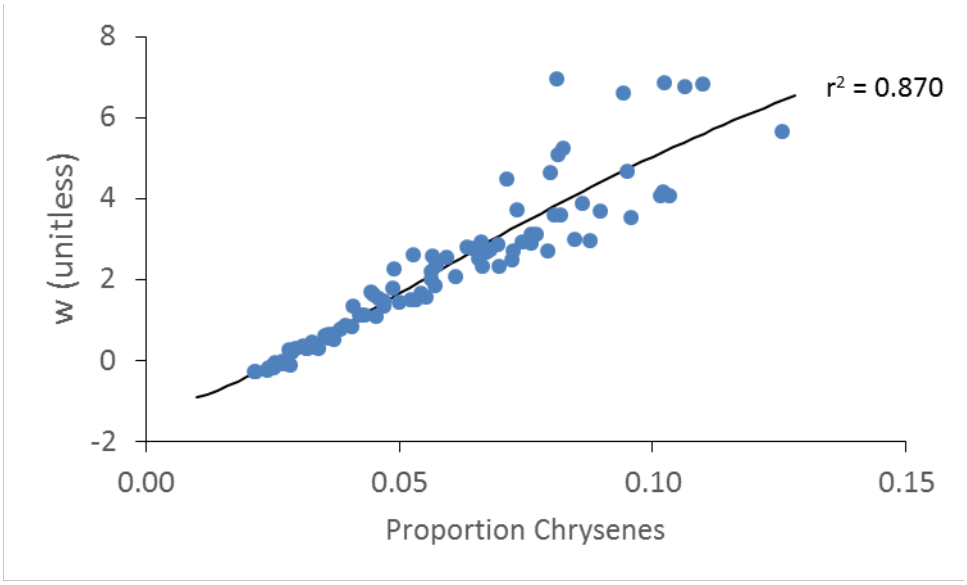
**SI 1.** Source identification: mean PAH source model results at each sample site in Prince William Sound and the Gulf of Alaska. Site numbers are 1) BOISL, 2) CDOUG, 3) CGULL, 4) CHENI, 5) ELEAI, 6) HERRB, 7) KASHB, 8) KIUKP, 9) LATOI, 10) MCARP, 11) MORNC, 12) NINAI, and 13) SLEEB.

PAH source 2.mxd, .png, layer "PAH source model result"

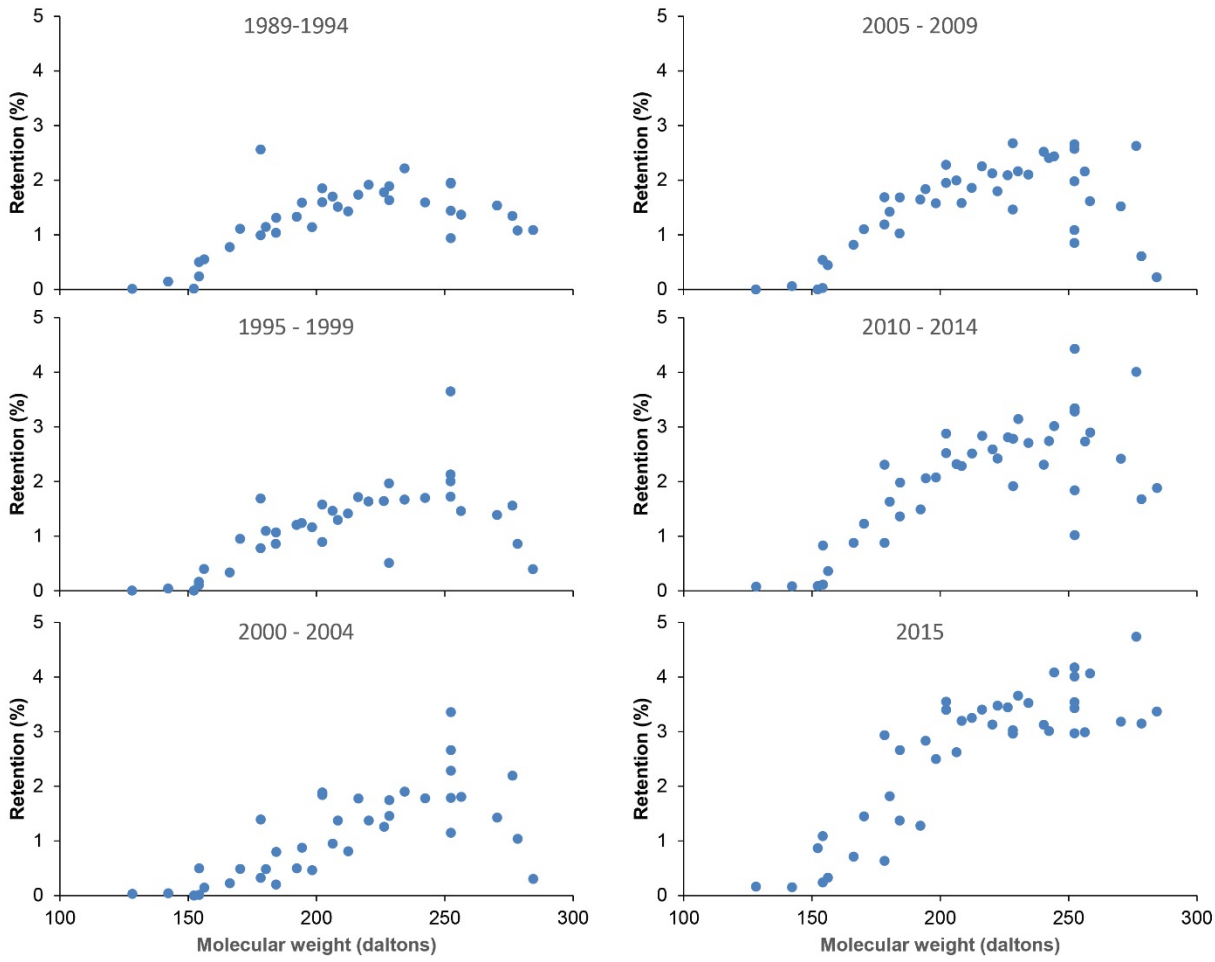


**SI 2.** Comparison of weathering estimates. Chyrsenes proportions are illustrated on the x-axis and unitless weathering factor w, estimated with the Short and Heintz (1997) model are illustrated on the y-axis. The top panel is Alaska North Slope crude oil and the bottom panel is *Selendang Ayu* oil.
Retrospective PAH and Alkane Oct 2015 v4.xlsm, sheet "weathering"

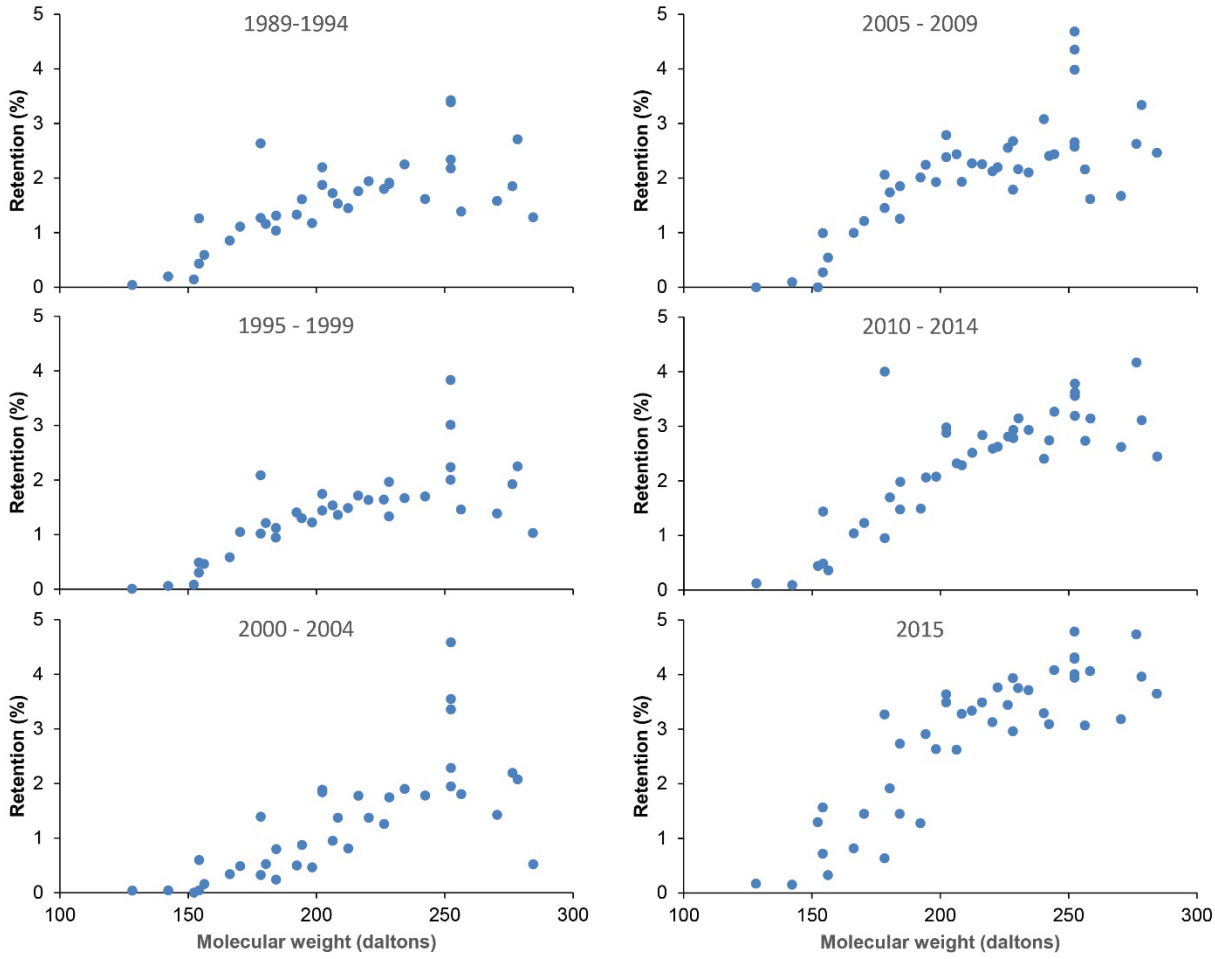


SI 3. PAH retention in Alaska North Slope crude oil as a function of molecular mass in 5-year increments.



SI 4. PAH retention in Alaska North Slope crude oil as a function of molecular mass in 5-year increments restricted to data above method detection limits.



## Biomarkers as tracers of *Exxon Valdez* oil

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## Abstract

Over the past quarter century, biomarkers persisted in sequestered *Exxon Valdez* oil in Prince William Sound and the Gulf of Alaska, hence the oil remained identifiable. 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 multi-sample statistics. Biomarkers were conserved relative to other constituents, 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 to 2014), though loss varied substantially among and within sites. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss. Loss rates of steranes tended to be least.

Keywords: biomarker, Exxon Valdez oil, forensic

## INTRODUCTION

Intertidal areas in western Prince William Sound and to the southwest along the Gulf of Alaska 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 two decades [1-5], yet the focus was on polycyclic aromatic hydrocarbons (PAHs), which are toxic, and study of biomarkers was delayed until recently [6].

Although the majority of intertidally sequestered oil residues are from the *Exxon Valdez* oil spill, other sources also exist [7, 8]. Thus there is a need for highly conserved source information to accurately discriminate among oil samples. Biomarkers are a good choice for this role, as they weather very slowly and contain considerable source-specific information. The purpose of this study was to investigate if ANSCO can be definitively identified by biomarker content, determine if the biomarkers were weathering, and distinguish oil sources in time-series samples.

Four classes of biomarkers were examined in this project, the isoprenoids (acyclic terpenoids), triterpanes (mostly tricyclic), hopanes (pentacyclic triterpanes), and steranes (tetracyclic terpenoids; Table 1). All originated in living organisms and reached their present-day form as a result of geochemical processes leading to the formation of oil [9]. Biomarkers are generally resistant to biodegradation, evaporation, and other weathering processes [9] thus definitive identification of source oils is possible for long periods of time. 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. In this study we apply novel pattern-matching procedures to compare samples with to ANSCO to verify its presence in specific samples (or not). Alternative hydrocarbon sources were similarly compared to oil from sample beaches to determine if these sources were or were not explanatory. Results were confirmed with Nordtest [10] plots. Despite their persistence, biomarker weathering is possible [11] and evidence of weathering in each biomarker class is presented in this paper.

## METHODS

### Sample locations

Sufficient time series data were available from 9 sites (Fig. 1; Table 2). Time series samples spanned 18 to 23 years at six sites. Three additional sites with samples spanning < 15 years and with few data points across time (3 to 4) were treated with caution but were ultimately accepted as part of the analysis. Study sites were based on shoreline assessment data gathered by Exxon and the Alaska Department 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 at various times (depending on collection times and individual study needs) for aliphatic and aromatic hydrocarbons. Study of biomarkers began more recently (approximately 2011 to 2014 for these samples) and previously archived extracts were used when available. Expected biomarker loss under storage conditions (−20°C) was negligible.

## Sample processing

Sediment and oil samples were dried with anhydrous sodium sulfate and extracted with dichloromethane (DCM). 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:DCM. From 2011 onward, samples extracted with DCM were reduced in volume over steam to approximately 5 ml. The exact total volume was recorded and an aliquot was archived. The DCM was evaporated from the remaining extract to determine the total mass of extracted oil. This information was used to calculate the application of 7 to 10 mg of oil from the archived aliquot to a 6 g silica column. The aliphatic fraction was eluted with 10 ml pentane and PAHs were subsequently eluted with 20 mL of 1:1 (v/v) pentane:DCM. 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 silica column as the divisor or ng PAH $g^{-1}$ sediment (n = 13).

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 SIM mode, and concentrations were determined by the internal standard method with response factors (RF) based on two representative compounds, 17α (H),21β(H)-hopane (H30) and 5α(H),14α(H),17α(H)-cholestane. The accuracy of the biomarker analyses was about ± 15% based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was about 20%, depending on the biomarker. Biomarker concentrations were not corrected for recovery; surrogate recovery averaged 93% (range 59 to 125%). Reported biomarkers and their abbreviations are listed in Table 1.

## Pattern-matching forensics

Biomarker composition in samples was compared to that in ANSCO (obtained from the T/V *Exxon Valdez* in 1989) to determine if 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 ($H_i$ / ∑ $H_i$), where $H_i$ is the $i^{th}$ hopane concentration and ∑$H_i$ is the total hopanoid concentration. For each sample, the number of $H_i$ / ∑ $H_i$ 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 (SI 1). The probability that an unknown sample was consistent with ANSCO composition was assessed by reference to results of randomly permuting the source oil data set 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 three classes were > 0.6.

Biomarker composition in samples was similarly compared to 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 PWS from the Gulf of Alaska [20]. Results for each class (triterpanes, hopanes, and steranes) were independently compared to ANSCO results with Kruskal-Wallis one-way ANOVA on ranks, as data distributions were not normal (groups were ANSCO, Monterey, coal, and Constantine). Overall site-specific matches that considered the combined results of all three classes were also compared among the four potential sources.

### Nordtest forensics

An alternative forensic method, the Nordtest [21], compares pre-determined compound ratios in samples with those in the potential source. The Nordtest uses sample averages (and confidence bounds) to infer if a sample matched the average source pattern. The 14 ratios used were those recommended by Daling et al. (2002): %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)], and %29bbSTER [(C29bbR + C29bbS) / (C27bbR + C27bbS + C28bbR + C28bbS + C29bbR + C29bbS)]. The ratio names are those given by (Daling et al. 2002); the compound names are as defined in Table 1.

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 four potential sources (y-axis) in four 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. (2002) also indicate that conclusions can be based on regressions between spill and source samples for the selected suite of measured diagnostic ratios.

### 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 years; Table 2). The combined result of all classes (triterpanes, hopanes, steranes) matched ANSCO in 77% of these samples (n = 62; Table 2). ANSCO was definitively present through time at all sites except Chenega Island, where it was not identified in 1995 and 1999, and Cape Gull samples subsequent to 1989.

Other potential biomarker sources were not plausible alternatives to ANSCO (Fig. 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; Fig. 2a); the combined result for all three classes was a 0% fit compared to 77% for ANSCO (Fig. 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 (Fig. 2b).

## Nordtest forensics

ANSCO explained the biomarker ratios in the field samples from each site in Nordtest analyses. 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 (Fig. 3 and SI 3). Although some error bars did not overlap the diagonal, all were fairly close and regressions were highly significant ($P_{regression} < 0.001$, 9 sites). Error bars were large at sites with few samples, such as Chenega Island, and at Cape Gull where weathering was prominent.

ANSCO matched the field data better than any alternative source (Fig. 3). Regression fits were best for ANSCO and worst for Constantine. This was evident by inspecting 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 (Fig. 4 and SI 4). However, concentrations were heterogeneous within and among sites, 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 −20.1 ng g oil$^{-1}$ day$^{-1}$ and ranged from −26.5 to −9.1 ng g oil$^{-1}$ day$^{-1}$. The

combined slope was similar, −20.8 ng g oil$^{-1}$ day$^{-1}$, 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; Fig. 4a). Thus, there was a general decline in H30 content in sequestered oil. Similarly, C27bbS concentration declined with time (Fig. 4b); the combined slope was negative and significant ($F_{1,41}$ = 16.280, $F_o/F_c$ = 4.0, P < 0.001). The declines illustrated by these two compounds can be generalized for all biomarkers. 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 (Fig. 5).

## DISCUSSION

Forensics demonstrated the presence of ANSCO at all sites and generally through time, thus the ensuing changes in biomarker concentration (per gram 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 (Fig. 4). Overall trends were declines in biomarker concentrations from the earliest collections of stranded oil to 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 for unknown reasons. Biomarker loss was unusually rapid (Fig. 4) and composition was unlike ANSCO after 1989. 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]. Remaining oil was highly biodegraded [6]. The remaining biomarkers are unusual. One hypothesis considered by [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 have no explanation for the underlying cause.

The pattern-matching method illustrated in this paper definitively discriminated ANSCO from several other potential sources (SI 1 and 2). The pattern-matching method included the normalized value of every reported triterpane, hopane, and sterane analyte, thus making full use of the data (SI 1 and 2). 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 if they matched the source pattern (Fig. 3 and SI 3), 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 this paper was 5).

### Weathering

The biomarker weathering observed in this 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 two natural processes that destroy petroleum hydrocarbons [11] 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 [11] report that they can be severely degraded, although isoprenoids are more recalcitrant than the n-alkanes [23]. Previous experience suggested 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 and weathered more rapidly than other triterpanes (Fig. 5). Steranes DIA27S through C27bbS weathered more rapidly than other steranes (Fig. 5). These results are generally consistent with observations from the Metula spill that diasteranes, C27 steranes, and tricyclic terpanes weathered relatively rapidly [11]. Weathering rates were about the same among all hopanes in our study. In contrast, another study observed H30 and H31 to H34 were degraded relatively rapidly [11, 24].

## CONCLUSIONS

Biomarkers provide an excellent way of definitively identifying the source of spilled oil over long periods of time, 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 years. Biomarkers were clearly retained while 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. The largest steranes tended to be the most persistent constituents.

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**Table 1.** Biomarkers and their abbreviations. Asterisks mark analytes used for pattern matching; the number of triterpanes, hopanes, and steranes used for modeling were 10, 20, and 15, respectively.

| Abbreviation | | Biomarker: Isoprenoids | Target Ions |
|---|---|---|---|
| norprist | | norpristane | 57 |
| prist | | 2,6,10,14-tetramethylpentadecane (pristane) | 57 |
| phyt | | 2,6,10,14-tetramethylhexadecane (phytane) | 57 |

| Abbreviation | | Biomarker: Triterpanes | Target Ions |
|---|---|---|---|
| TR23 | * | C23 tricyclic terpane | 191 |
| TR24 | * | C24 tricyclic terpane | 191 |
| TR25a | * | C25 tricyclic terpane (a) | 191 |
| TR25b | * | C25 tricyclic terpane (b) | 191 |
| TET24 | * | C24 tetracyclic terpane | 191 |
| TR26a | *[a] | C26 tricyclic terpane (a) | 191 |
| TR26b | | C26 tricyclic terpane (b) | 191 |
| TR28a | * | C28 tricyclic terpane (a) | 191 |
| TR28b | * | C28 tricyclic terpane (b) | 191 |
| TR29a | * | C29 tricyclic terpane (a) | 191 |
| TR29b | * | C29 tricyclic terpane (b) | 191 |

| Abbreviation | | Biomarker: hopanes | Target Ions |
|---|---|---|---|
| Ts | * | 18α(H),21β(H)-22,29,30-trisnorhopane | 191 |
| Tm | * | 17α(H),21β(H)-22,29,30-trisnorhopane | 191 |
| H28 | * | 17α(H),18α(H),21β(H)-28,30-bisnorhopane | 191 |
| NOR25H | | 17α(H),21β(H)-25-norhopane | 191 |
| H29 | * | 17α(H),21β(H)-30-norhopane | 191 |
| C29Ts | * | 18α(H),21β(H)-30-norneohopane | 191 |
| M29 | * | 17α(H),21β(H)-30-norhopane (normoretane) | 191 |
| OL | | 18α(H) and 18β(H)-oleanane | 191 |
| H30 | * | 17α(H),21β(H)-hopane | 191 |
| NOR30H | * | 17α(H)-30-nor-29-homohopane | 191 |
| M30 | * | 17β(H),21α(H)-hopane (moretane) | 191 |
| H31S | * | 22S-17α(H),21β(H)-30-homohopane | 191 |
| H31R | * | 22R-17α(H),21β(H)-30-homohopane | 191 |

| | | | |
|---|---|---|---|
| GAM | * | Gammacerane | 191 |
| H32S | * | 22S-17α(H),21β(H)-30,31-bishomohopane | 191 |
| H32R | * | 22R-17α(H),21β(H)-30,31-bishomohopane | 191 |
| H33S | * | 22S-17α(H),21β(H)-30,31,32-trishomohopane | 191 |
| H33R | * | 22R-17α(H),21β(H)-30,31,32-trishomohopane | 191 |
| H34S | * | 22S-17α(H),21β(H)-30,31,32,33-tetrakishomohopane | 191 |
| H34R | * | 22R-17α(H),21β(H)-30,31,32,33-tetrakishomohopane | 191 |
| H35S | * | 22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane | 191 |
| H35R | * | 22R-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane | 191 |

| **Abbreviation** | | **Biomarker: steranes** | **Target Ions** |
|---|---|---|---|
| S22 | * | $C_{22}$ 5α(H),14β(H),17β(H)-sterane | 217,218 |
| DIA27S | * | $C_{27}$ 20S-13β(H),17α(H)-diasterane | 217,218 |
| DIA27R | * | $C_{27}$ 20R-13β(H),17α(H)-diasterane | 217,218 |
| C27S | * | $C_{27}$ 20S-5α(H),14α(H),17α(H)-cholestane | 217,218 |
| C27bbR | * | $C_{27}$ 20R-5α(H),14β(H),17β(H)-cholestane | 217,218 |
| C27bbS | * | $C_{27}$ 20S-5α(H),14β(H),17β(H)-cholestane | 217,218 |
| C27R | * | $C_{27}$ 20R-5α(H),14α(H),17α(H)-cholestane | 217,218 |
| C28S | * | $C_{28}$ 20S-5α(H),14α(H),17α(H)-ergostane | 217,218 |
| C28bbR | * | $C_{28}$ 20R-5α(H),14β(H),17β(H)-ergostane | 217,218 |
| C28bbS | * | $C_{28}$ 20S-5α(H),14β(H),17β(H)-ergostane | 217,218 |
| C28R | * | $C_{28}$ 20R-5α(H),14α(H),17α(H)-ergostane | 217,218 |
| C29S | * | $C_{29}$ 20S-5α(H),14α(H),17α(H)-stigmastane | 217,218 |
| C29bbR | * | $C_{29}$ 20R-5α(H),14β(H),17β(H)-stigmastane | 217,218 |
| C29bbS | * | $C_{29}$ 20S-5α(H),14β(H),17β(H)-stigmastane | 217,218 |
| C29R | * | $C_{29}$ 20R-5α(H),14α(H),17α(H)-stigmastane | 217,218 |

[a]TR26a and TR26b cannot be resolved with current column settings at our laboratory, thus were combined for modeling.

**Table 2.** Biomarker pattern match to Alaska North Slope crude oil (ANSCO) by site and date. 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 SI 1 for an example of pattern matching.

| Site (latitude, longitude) | Abbrev-iation | SIN | Date | triterp model | Hopane model | Sterane model | Combined match | % combined match |
|---|---|---|---|---|---|---|---|---|
| Bay of Isles (60.3802, | BOISL | 142343 | 9/15/1990 | 1.00 | 0.90 | 1.00 | 1 | 83 |
| −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 (58.8818, | CDOUG | 302709 | 7/30/1992 | 1.00 | 0.90 | 0.87 | 1 | 100 |
| −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 (58.2353, | CGULL | 304902 | 10/2/1989 | 0.90 | 0.90 | 0.87 | 1 | 20 |
| −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 (60.3872, | CHENI | 7409 | 7/31/1989 | 1.00 | 0.90 | 0.87 | 1 | 33 |
| −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 (60.5500, | ELEAI | 400431 | 6/19/1993 | 0.50 | 0.80 | 0.53 | 0 | 67 |
| −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 | |

**Fig. 1**. Time-series sample locations included four within Prince William Sound (PWS), four 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.

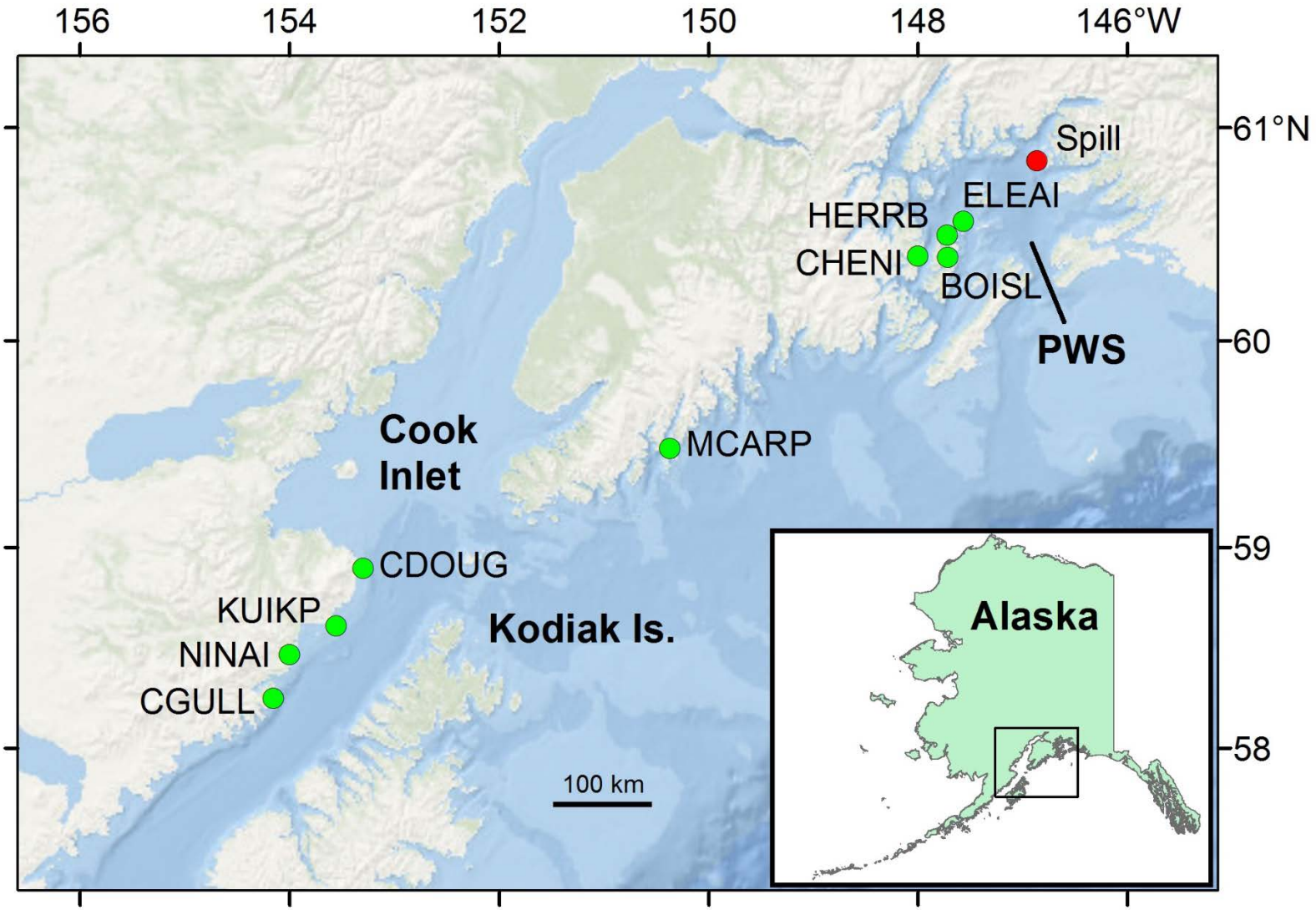


**Fig. 2.** Potential biomarker source comparison. Upper panel: median model results by class (triterpanes, hopanes, and steranes) across samples from all sites. Perfect model fits = 1.0 (100% match), a complete lack of fit = 0.0. Bottom panel: combined results for each potential source.

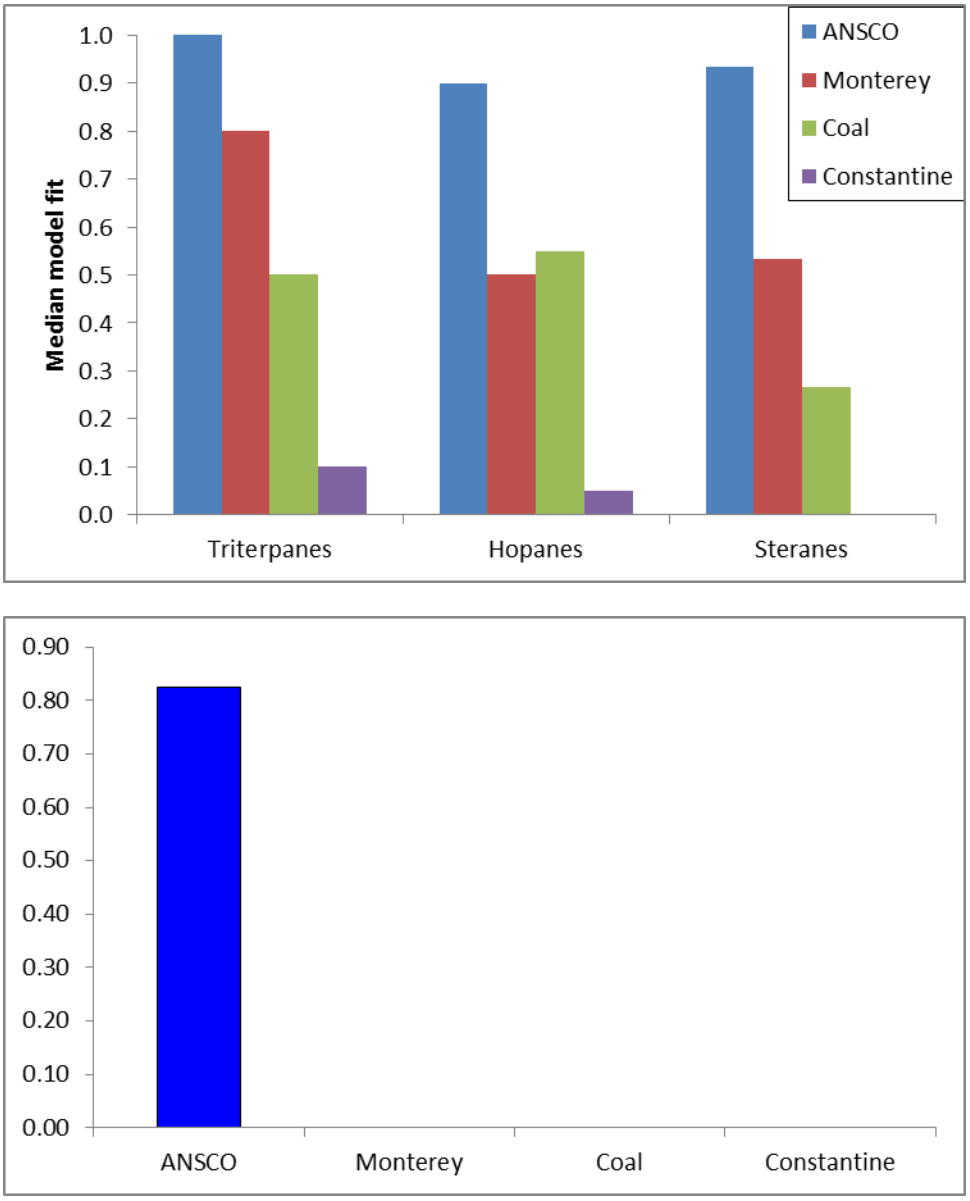


**Fig. 3.** Comparison of potential sources using Nordtest plots. Samples in this example are from NINAI (Ninagiak Island). Potential biomarker sources examined were ANSCO, Monterey oil, Constantine Harbor, and coal (counter clockwise from top left). The dotted line is x = y. Axes are ratios specified 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.

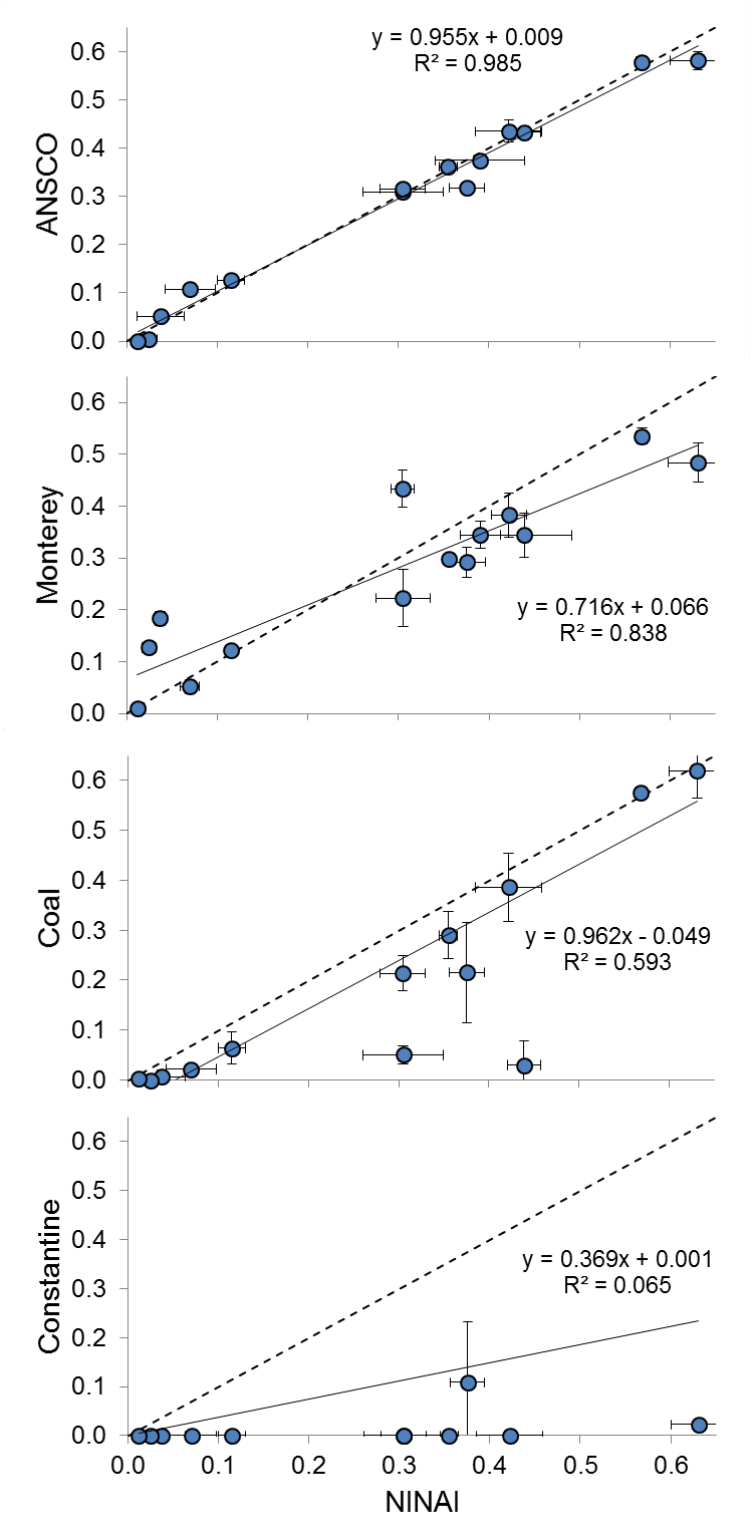
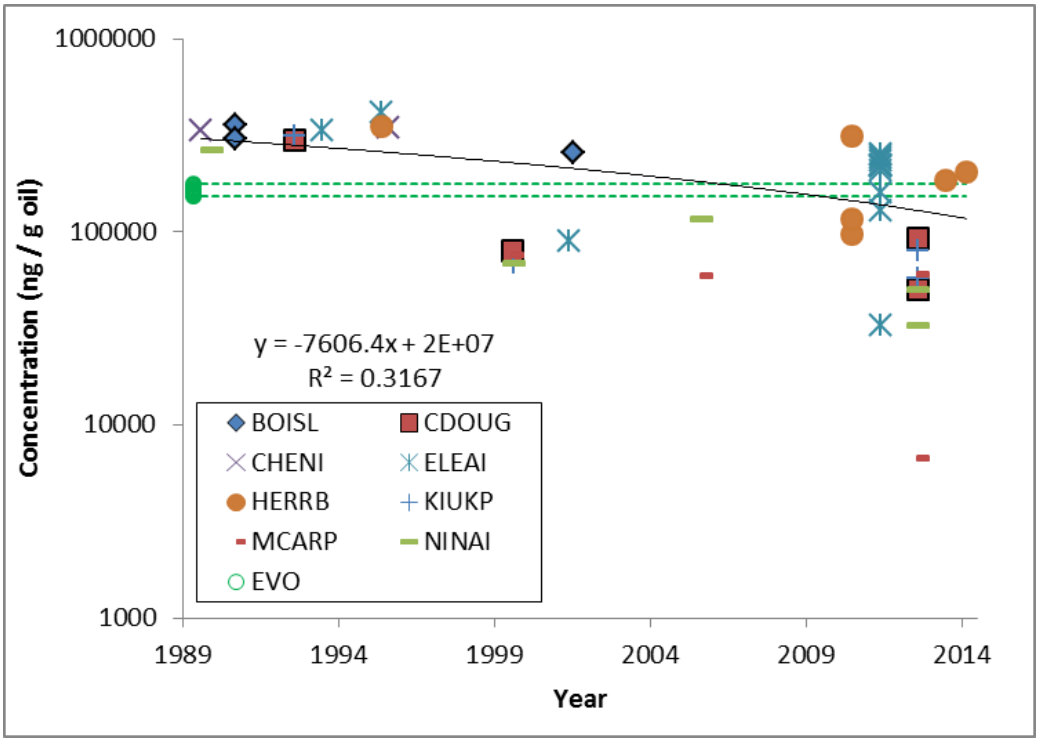


**Fig. 4.** Loss of H30 (panel A) and C27bbS (panel B) over time. Data from all sites were combined to calculate the illustrated linear regressions. Concentrations in source oil (EVO) are illustrated with black circles and the low and high ranges are marked by horizontal dashed lines.



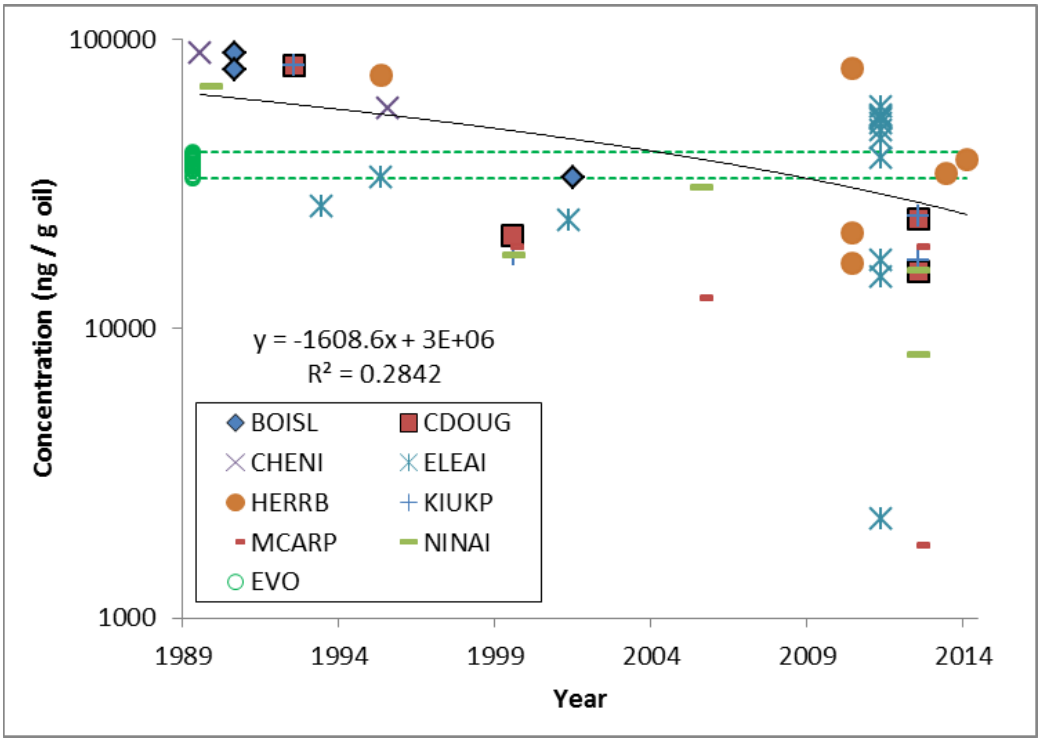
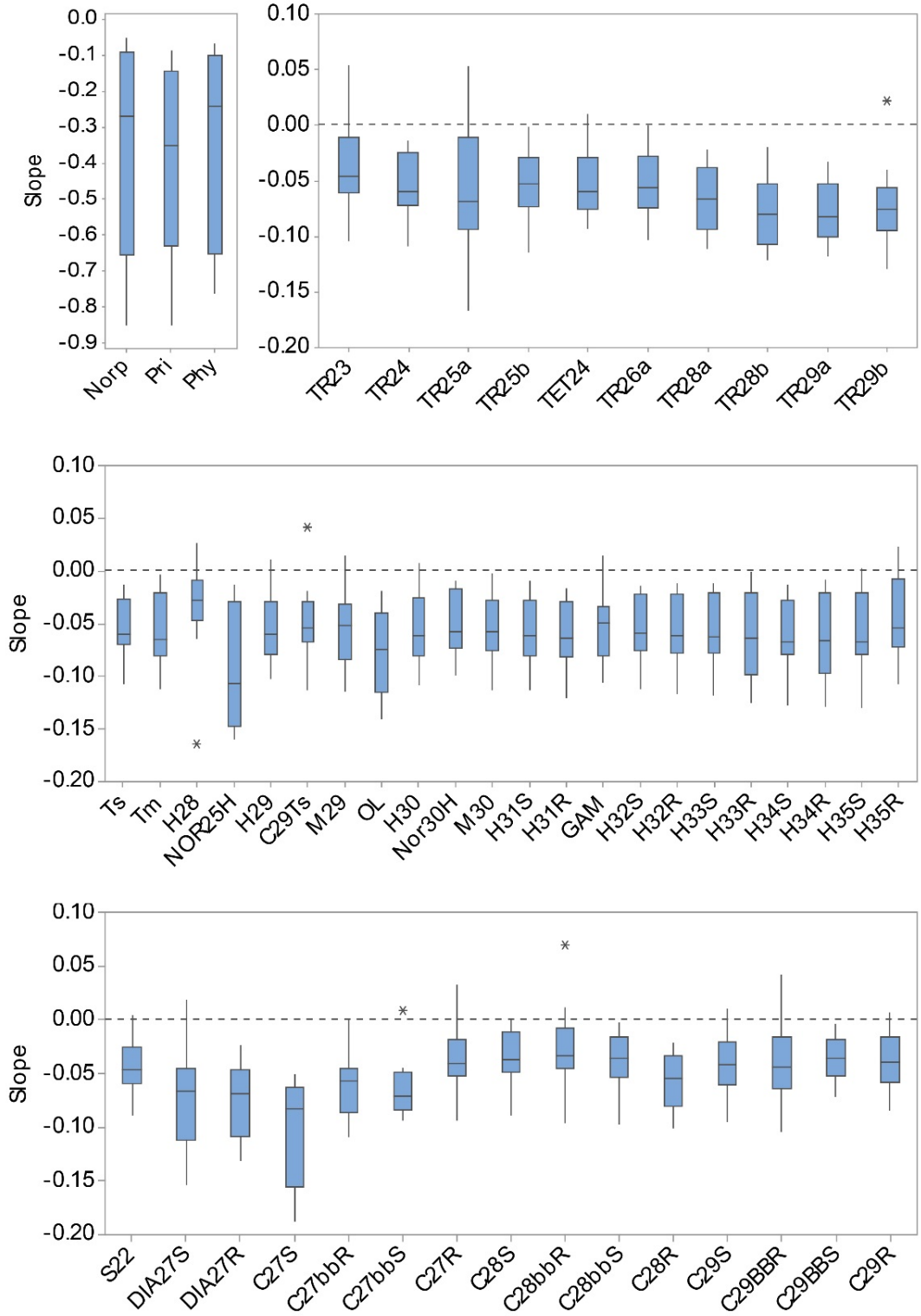



**Fig. 5.** Biomarker loss. Slopes are $\log_e$(concentration+0.1) / day. A slope of 0 indicates no change over time. Note that y-axis scaling for isoprenoids is different than for all other graphs.



## SUPPLEMENTAL DATA

**SI 1. Biomarker pattern matching.** Composition of biomarkers in field samples was compared to ANSCO triterpane, hopane, and sterane composition. Vertical bars (black) indicate the range ± 20% expected in source oil (between the horizontal tics only) as determined from 20 ANSCO source oil samples. Modeled analytes are indicated in Table 1 of the paper. The example below (panel A) is a Bay of Isles sample collected in 1990. Nearly all analytes match the ANSCO pattern except H28, GAM, and C28S, thus the model scores were 1.00, 0.90, and 0.93 for triterpanes, hopanes, and steranes, respectively. The mean score was 0.97, and all scores were > 0.6, thus the sample was consistent with ANSCO (code = 1). In contrast, a Monterey oil sample collected from Green Island in 2001 does not match ANSCO (panel B).

A



B

The table below provides pattern-matching outcomes. Many of the field samples from PWS and the Gulf of Alaska included in this study match ANSCO (these are labeled BOISL, CDOUG, CHENI, … NINAI; combined = 1). In contrast, none of the alternative sources match ANSCO (combined = 0), including Monterey oil, coal, Constantine Harbor sediment, Selendang Ayu oil (spilled in the Aleutian Islands), and Deepwater Horizon oil from the Gulf of Mexico.

| | | | Model results | | | | |
|---|---|---|---|---|---|---|---|
| | | | **Triterpane** | **Hopane** | **Sterane** | **Mean** | **combined** |
| Monterey oil | 1203707 | 7/2/2001 | 0.500 | 0.650 | 0.467 | 0.539 | 0 |
| Monterey oil | 1203703 | 6/29/2001 | 0.700 | 0.600 | 0.467 | 0.589 | 0 |
| Monterey oil | 1203608 | 6/22/2001 | 0.600 | 0.550 | 0.267 | 0.472 | 0 |
| Monterey oil | 1203736 | 7/10/2001 | 0.700 | 0.550 | 0.400 | 0.550 | 0 |
| Monterey oil | 1203541 | 6/19/2001 | 1.000 | 0.650 | 0.467 | 0.706 | 0 |
| Monterey oil | 1203536 | 6/17/2001 | 0.800 | 0.550 | 0.400 | 0.583 | 0 |
| Tyndall Glacier | 1503203 | 7/5/2004 | 0.200 | 0.350 | 0.133 | 0.228 | 0 |
| Tyndall Glacier | 1503209 | 7/5/2004 | 0.200 | 0.350 | 0.200 | 0.250 | 0 |
| Tyndall Glacier | 1503218 | 7/8/2004 | 0.200 | 0.250 | 0.200 | 0.217 | 0 |
| Tyndall Glacier | 1503213 | 7/5/2004 | 0.100 | 0.250 | 0.133 | 0.161 | 0 |
| Tyndall Glacier | 1503211 | 7/5/2004 | 0.000 | 0.350 | 0.133 | 0.161 | 0 |
| Tyndall Glacier | 1503212 | 7/5/2004 | 0.100 | 0.200 | 0.133 | 0.144 | 0 |
| Constantine | 1700144 | 3/4/2006 | 0.000 | 0.050 | 0.000 | 0.017 | 0 |
| Constantine | 1801132 | 7/12/2007 | 0.000 | 0.000 | 0.000 | 0.000 | 0 |
| Constantine | 1700145 | 3/4/2006 | 0.000 | 0.050 | 0.000 | 0.017 | 0 |
| Constantine | 1601735 | 7/19/2005 | 0.100 | 0.050 | 0.000 | 0.050 | 0 |
| Constantine | 1700143 | 3/4/2006 | 0.100 | 0.000 | 0.067 | 0.056 | 0 |
| Selendang Ayu | 1902444 | 12/31/2004 | 0.400 | 0.900 | 0.600 | 0.633 | 0 |
| Selendang Ayu | 1902445 | 1/5/2005 | 0.400 | 0.900 | 0.600 | 0.633 | 0 |
| Selendang Ayu | 1902445 | 1/5/2005 | 0.500 | 0.900 | 0.733 | 0.711 | 0 |
| Selendang Ayu | 1902446 | 12/19/2004 | 0.500 | 0.950 | 0.867 | 0.772 | 0 |
| Selendang Ayu | 1902446 | 12/19/2004 | 0.400 | 0.950 | 0.600 | 0.650 | 0 |
| Selendang Ayu | 1902452 | 1/5/2005 | 0.300 | 0.100 | 0.400 | 0.267 | 0 |
| Deepwater | 20100603 | 5/20/2010 | 0.500 | 0.500 | 0.667 | 0.556 | 0 |
| Deepwater | 20100603 | 5/20/2010 | 0.800 | 0.300 | 0.533 | 0.544 | 0 |
| Deepwater | 20100605 | 5/21/2010 | 1.000 | 0.500 | 0.600 | 0.700 | 0 |
| Deepwater | 20100605 | 5/21/2010 | 0.900 | 0.550 | 0.733 | 0.728 | 0 |
| Deepwater | 20100605 | 5/21/2010 | 0.900 | 0.600 | 0.733 | 0.744 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BOISL | 142343 | 9/15/1990 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| BOISL | 142342 | 9/15/1990 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| BOISL | 1203720 | 7/5/2001 | 0.500 | 0.850 | 0.467 | 0.606 | 0 |
| BOISL | 1303346 | 6/24/2002 | 1.000 | 0.850 | 0.867 | 0.906 | 1 |
| BOISL | 1400734 | 7/12/2003 | 0.800 | 0.900 | 0.867 | 0.856 | 1 |
| BOISL | 1502219 | 6/16/2004 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| CDOUG | 302709 | 7/30/1992 | 1.000 | 0.900 | 0.867 | 0.922 | 1 |
| CDOUG | 1007034 | 8/8/1999 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| CDOUG | 20120720 | 8/3/2012 | 1.000 | 1.000 | 1.000 | 1.000 | 1 |
| CDOUG | 20120718 | 8/3/2012 | 0.900 | 0.950 | 0.933 | 0.928 | 1 |
| CGULL | 304902 | 10/2/1989 | 0.900 | 0.900 | 0.867 | 0.889 | 1 |
| CGULL | 1007040 | 8/10/1999 | 0.400 | 0.550 | 0.267 | 0.406 | 0 |
| CGULL | 1601912 | 8/21/2005 | 0.400 | 0.400 | 0.067 | 0.289 | 0 |
| CGULL | 20120748 | 8/7/2012 | 0.500 | 0.900 | 0.200 | 0.533 | 0 |
| CGULL | 20120750 | 8/7/2012 | 0.500 | 0.900 | 0.533 | 0.644 | 0 |
| CHENI | 7409 | 7/31/1989 | 1.000 | 0.900 | 0.867 | 0.922 | 1 |
| CHENI | 604711 | 8/11/1995 | 0.600 | 0.900 | 0.667 | 0.722 | 0 |
| CHENI | 1006147 | 7/13/1999 | 0.200 | 0.250 | 0.400 | 0.283 | 0 |
| ELEAI | 400431 | 6/19/1993 | 0.500 | 0.800 | 0.533 | 0.611 | 0 |
| ELEAI | 601401 | 5/13/1995 | 0.500 | 0.600 | 0.667 | 0.589 | 0 |
| ELEAI | 1006325 | 7/14/1999 | 1.000 | 0.900 | 0.600 | 0.833 | 0 |
| ELEAI | 1202610 | 5/19/2001 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| ELEAI | 1303429 | 6/26/2002 | 1.000 | 0.850 | 1.000 | 0.950 | 1 |
| ELEAI | 1802225 | 5/21/2007 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| ELEAI | 20111005 | 5/23/2011 | 0.200 | 0.750 | 0.267 | 0.406 | 0 |
| ELEAI | 20111002 | 5/23/2011 | 0.400 | 0.950 | 0.467 | 0.606 | 0 |
| ELEAI | 20111008 | 5/23/2011 | 0.600 | 0.900 | 0.600 | 0.700 | 0 |
| ELEAI | 20111009 | 5/23/2011 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| ELEAI | 20111006 | 5/23/2011 | 1.000 | 0.950 | 1.000 | 0.983 | 1 |
| ELEAI | 20111004 | 5/23/2011 | 0.900 | 0.850 | 0.933 | 0.894 | 1 |
| ELEAI | 20111004 | 5/23/2011 | 1.000 | 0.950 | 1.000 | 0.983 | 1 |
| ELEAI | 20111011 | 5/23/2011 | 1.000 | 0.950 | 1.000 | 0.983 | 1 |
| ELEAI | 20111007 | 5/23/2011 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| ELEAI | 20111003 | 5/23/2011 | 1.000 | 0.850 | 0.933 | 0.928 | 1 |
| ELEAI | 20111001 | 5/23/2011 | 0.900 | 0.950 | 0.933 | 0.928 | 1 |
| ELEAI | 20111010 | 5/23/2011 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| HERRB | 601826 | 5/15/1995 | 0.900 | 0.900 | 0.933 | 0.911 | 1 |
| HERRB | 1006314 | 7/13/1999 | 0.800 | 0.850 | 0.667 | 0.772 | 1 |
| HERRB | 1202602 | 5/10/2001 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| HERRB | 1301010 | 5/27/2002 | 0.500 | 0.900 | 0.733 | 0.711 | 0 |
| HERRB | 1400725 | 6/15/2003 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HERRB | 1502211 | 6/13/2004 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| HERRB | 1603515 | 7/23/2005 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| HERRB | 1802213 | 6/19/2007 | 1.000 | 0.900 | 1.000 | 0.967 | 1 |
| HERRB | 20100204 | 6/23/2010 | 0.700 | 0.900 | 0.800 | 0.800 | 1 |
| HERRB | 20100201 | 6/23/2010 | 0.800 | 0.900 | 0.933 | 0.878 | 1 |
| HERRB | 20100203 | 6/23/2010 | 0.700 | 0.900 | 0.800 | 0.800 | 1 |
| HERRB | 20130701 | 6/27/2013 | 0.800 | 0.900 | 0.667 | 0.789 | 1 |
| HERRB | 20140201 | 2/18/2014 | 0.800 | 0.900 | 1.000 | 0.900 | 1 |
| KIUKP | 302710 | 7/31/1992 | 1.000 | 0.900 | 0.867 | 0.922 | 1 |
| KIUKP | 1007036 | 8/9/1999 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| KIUKP | 20120739 | 8/4/2012 | 1.000 | 0.950 | 0.933 | 0.961 | 1 |
| KIUKP | 20120737 | 8/4/2012 | 1.000 | 0.950 | 0.933 | 0.961 | 1 |
| MCARP | 1007018 | 7/28/1999 | 1.000 | 0.850 | 0.933 | 0.928 | 1 |
| MCARP | 1601914 | 8/22/2005 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| MCARP | 20120752 | 8/9/2012 | 0.900 | 0.950 | 0.933 | 0.928 | 1 |
| MCARP | 20120751 | 8/9/2012 | 1.000 | 1.000 | 0.933 | 0.978 | 1 |
| NINAI | 304903 | 12/10/1989 | 1.000 | 0.900 | 0.867 | 0.922 | 1 |
| NINAI | 1007038 | 8/10/1999 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |
| NINAI | 1601907 | 8/19/2005 | 1.000 | 0.900 | 0.933 | 0.944 | 1 |

**SI 2.** Comparison of biomarker composition matching to four alternative sources[1], Alaska North Slope crude oil (ANSCO), Monterey oil, coal, and Constantine Harbor sediment. In contrast to the analysis in SI 1, each of these oils was set as the potential source and each field sample was tested against that source. Overall summary statistics (mean, minimum, … se) precede tabled model results for each field sample for tricyclic triterpanes (T), hopanes (H), and steranes (S). Exact matches are 1.0; 0.0 is a complete mismatch. Combined results (C) are 1 when all matches are > 0.6, else 0; 77% of these matched ANSCO, and none matched Monterey oil, coal, or Constantine. See Table 2 for site abbreviations. Date is sample collection date, SIN is sample identification number.

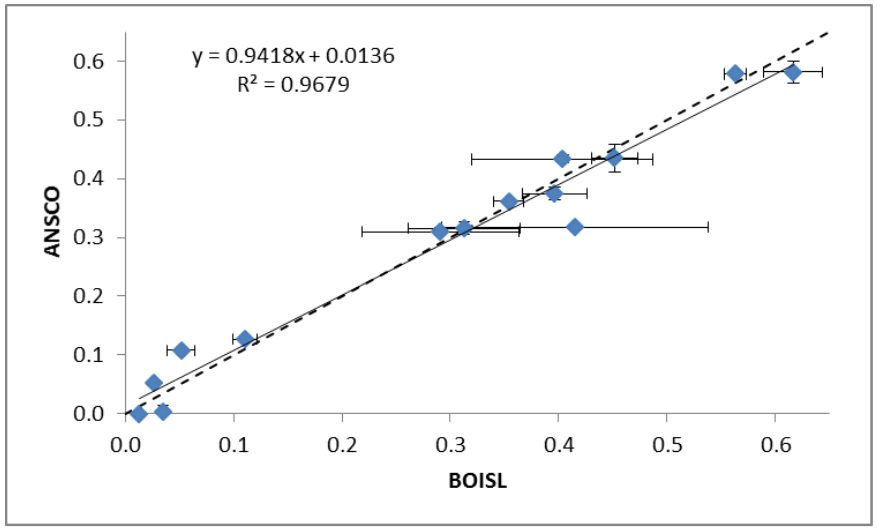
| | | | **ANSCO as source** | | | | **Monterey as source** | | | | **Coal as source** | | | | **Constantine as source** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mean | 0.85 | 0.87 | 0.82 | | 0.68 | 0.48 | 0.50 | | 0.46 | 0.56 | 0.28 | | 0.15 | 0.05 | 0.04 | |
| | | min | 0.20 | 0.25 | 0.07 | | 0.10 | 0.15 | 0.13 | | 0.30 | 0.40 | 0.20 | | 0.10 | 0.00 | 0.00 | |
| | | max | 1.00 | 1.00 | 1.00 | | 1.00 | 0.60 | 0.73 | | 0.60 | 0.80 | 0.73 | | 0.60 | 0.20 | 0.33 | |
| | | s | 0.23 | 0.12 | 0.23 | | 0.24 | 0.07 | 0.11 | | 0.06 | 0.05 | 0.10 | | 0.09 | 0.02 | 0.05 | |
| | | n | 62 | 62 | 62 | 48 | 62 | 62 | 62 | 0 | 62 | 62 | 62 | 0 | 62 | 62 | 62 | 0 |
| | | se | 0.03 | 0.02 | 0.03 | | 0.03 | 0.01 | 0.01 | | 0.01 | 0.01 | 0.01 | | 0.01 | 0.00 | 0.01 | |
| | | percent | | | | 77% | | | | 0% | | | | 0% | | | | 0% |
| **Site** | **Date** | **SIN** | **T** | **H** | **S** | **C** | **T** | **H** | **S** | **C** | **T** | **H** | **S** | **C** | **T** | **H** | **S** | **C** |
| BOISL | 9/15/1990 | 142343 | 1.00 | 0.90 | 1.00 | 1 | 0.80 | 0.45 | 0.47 | 0 | 0.50 | 0.60 | 0.33 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| BOISL | 9/15/1990 | 142342 | 1.00 | 0.90 | 0.93 | 1 | 1.00 | 0.50 | 0.47 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| BOISL | 7/5/2001 | 1203720 | 0.50 | 0.85 | 0.47 | 0 | 0.40 | 0.40 | 0.53 | 0 | 0.50 | 0.60 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| BOISL | 6/24/2002 | 1303346 | 1.00 | 0.85 | 0.87 | 1 | 0.80 | 0.45 | 0.40 | 0 | 0.50 | 0.55 | 0.27 | 0 | 0.10 | 0.00 | 0.00 | 0 |
| BOISL | 7/12/2003 | 1400734 | 0.80 | 0.90 | 0.87 | 1 | 0.50 | 0.55 | 0.67 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| BOISL | 6/16/2004 | 1502219 | 1.00 | 0.90 | 1.00 | 1 | 1.00 | 0.45 | 0.40 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| CDOUG | 7/30/1992 | 302709 | 1.00 | 0.90 | 0.87 | 1 | 1.00 | 0.50 | 0.47 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| CDOUG | 8/8/1999 | 1007034 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.40 | 0.60 | 0.27 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| CDOUG | 8/3/2012 | 20120720 | 1.00 | 1.00 | 1.00 | 1 | 0.70 | 0.55 | 0.33 | 0 | 0.50 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| CDOUG | 8/3/2012 | 20120718 | 0.90 | 0.95 | 0.93 | 1 | 0.70 | 0.55 | 0.53 | 0 | 0.50 | 0.50 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |

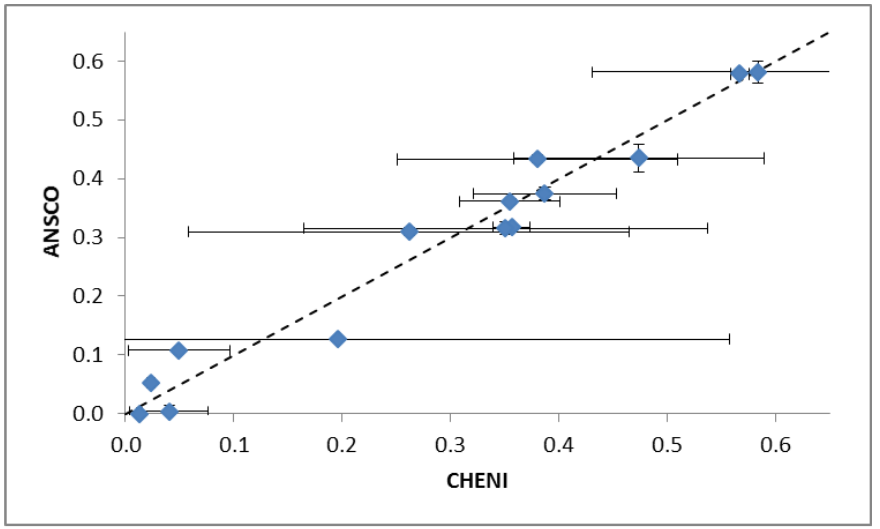
[1] Retrospective biomarker data and graphics Jan 2015.xlsm, sheet “source model comparisons” near column CD

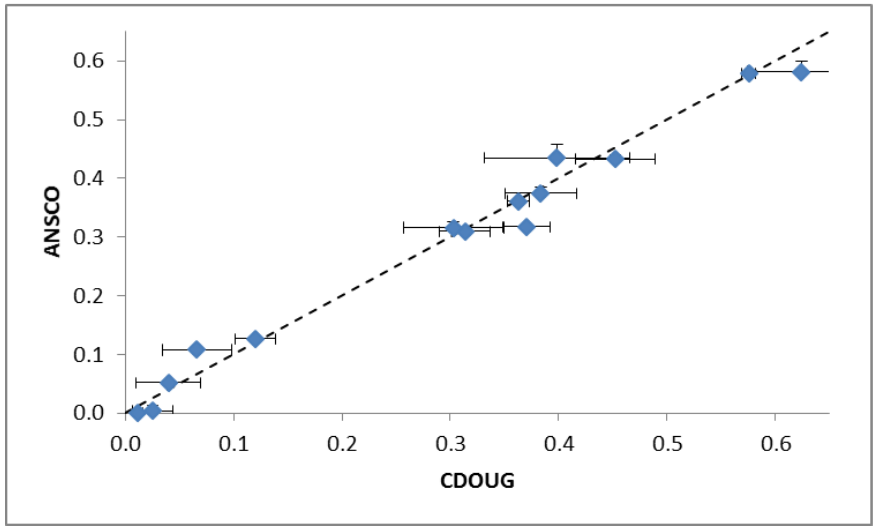
| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CGULL | 10/2/1089 | 304902 | 0.90 | 0.90 | 0.87 | 1 | 1.00 | 0.45 | 0.47 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| CGULL | 8/10/1999 | 1007040 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.50 | 0.33 | 0 | 0.60 | 0.70 | 0.47 | 0 | 0.20 | 0.10 | 0.07 | 0 |
| CGULL | 8/21/2005 | 1601912 | 0.40 | 0.40 | 0.07 | 0 | 0.20 | 0.15 | 0.13 | 0 | 0.60 | 0.40 | 0.73 | 0 | 0.50 | 0.20 | 0.33 | 0 |
| CGULL | 8/7/2012 | 20120748 | 0.50 | 0.90 | 0.20 | 0 | 0.30 | 0.45 | 0.60 | 0 | 0.40 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| CGULL | 8/7/2012 | 20120750 | 0.50 | 0.90 | 0.53 | 0 | 0.30 | 0.55 | 0.47 | 0 | 0.50 | 0.55 | 0.47 | 0 | 0.20 | 0.05 | 0.13 | 0 |
| CHENI | 7/31/1989 | 7409 | 1.00 | 0.90 | 0.87 | 1 | 0.90 | 0.45 | 0.47 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| CHENI | 8/11/1995 | 604711 | 0.60 | 0.90 | 0.67 | 0 | 0.50 | 0.50 | 0.60 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| CHENI | 7/13/1999 | 1006147 | 0.20 | 0.25 | 0.40 | 0 | 0.20 | 0.50 | 0.53 | 0 | 0.40 | 0.60 | 0.40 | 0 | 0.30 | 0.05 | 0.07 | 0 |
| ELEAI | 6/19/1993 | 400431 | 0.50 | 0.80 | 0.53 | 0 | 0.30 | 0.55 | 0.60 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/13/1995 | 601401 | 0.50 | 0.60 | 0.67 | 0 | 0.40 | 0.45 | 0.60 | 0 | 0.40 | 0.80 | 0.33 | 0 | 0.10 | 0.10 | 0.00 | 0 |
| ELEAI | 7/14/1999 | 1006325 | 1.00 | 0.90 | 0.60 | 0 | 0.60 | 0.50 | 0.20 | 0 | 0.50 | 0.60 | 0.40 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/19/2001 | 1202610 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.40 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 6/26/2002 | 1303429 | 1.00 | 0.85 | 1.00 | 1 | 0.80 | 0.45 | 0.53 | 0 | 0.40 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/21/2007 | 1802225 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.40 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/23/2011 | 20111005 | 0.20 | 0.75 | 0.27 | 0 | 0.30 | 0.45 | 0.27 | 0 | 0.60 | 0.60 | 0.53 | 0 | 0.60 | 0.05 | 0.13 | 0 |
| ELEAI | 5/23/2011 | 20111002 | 0.40 | 0.95 | 0.47 | 0 | 0.30 | 0.60 | 0.67 | 0 | 0.50 | 0.55 | 0.33 | 0 | 0.20 | 0.10 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111008 | 0.60 | 0.90 | 0.60 | 0 | 0.30 | 0.50 | 0.67 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.20 | 0.10 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111009 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.47 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/23/2011 | 20111006 | 1.00 | 0.95 | 1.00 | 1 | 0.90 | 0.40 | 0.53 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111004 | 0.90 | 0.85 | 0.93 | 1 | 0.80 | 0.55 | 0.47 | 0 | 0.40 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111004 | 1.00 | 0.95 | 1.00 | 1 | 0.80 | 0.45 | 0.53 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/23/2011 | 20111011 | 1.00 | 0.95 | 1.00 | 1 | 0.80 | 0.50 | 0.53 | 0 | 0.50 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| ELEAI | 5/23/2011 | 20111007 | 1.00 | 0.90 | 1.00 | 1 | 0.90 | 0.50 | 0.60 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111003 | 1.00 | 0.85 | 0.93 | 1 | 0.60 | 0.50 | 0.53 | 0 | 0.50 | 0.65 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111001 | 0.90 | 0.95 | 0.93 | 1 | 0.60 | 0.45 | 0.53 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| ELEAI | 5/23/2011 | 20111010 | 1.00 | 0.90 | 1.00 | 1 | 0.70 | 0.55 | 0.33 | 0 | 0.50 | 0.55 | 0.27 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| HERRB | 5/15/1995 | 601826 | 0.90 | 0.90 | 0.93 | 1 | 0.60 | 0.55 | 0.60 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| HERRB | 7/13/1999 | 1006314 | 0.80 | 0.85 | 0.67 | 1 | 0.70 | 0.55 | 0.53 | 0 | 0.30 | 0.60 | 0.33 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| HERRB | 5/10/2001 | 1202602 | 1.00 | 0.90 | 0.93 | 1 | 0.70 | 0.40 | 0.47 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |

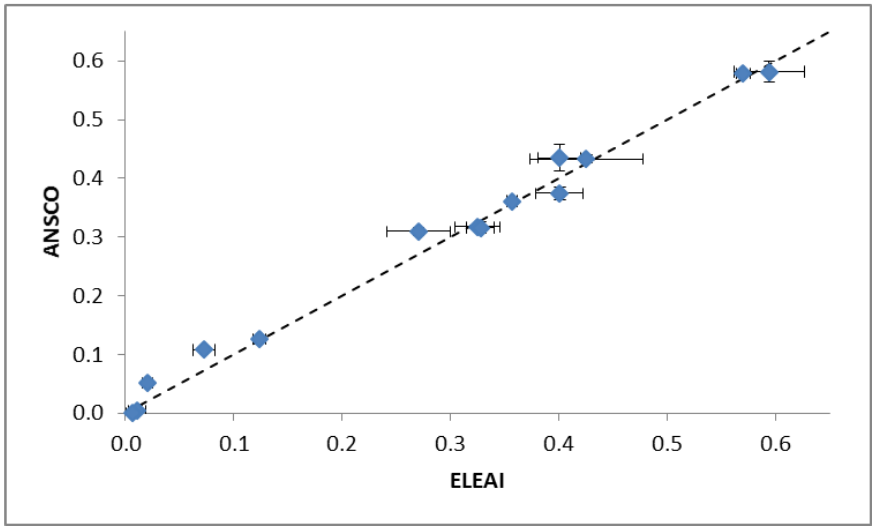
| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HERRB | 5/27/2002 | 1301010 | 0.50 | 0.90 | 0.73 | 0 | 0.40 | 0.50 | 0.73 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| HERRB | 6/15/2003 | 1400725 | 1.00 | 0.90 | 1.00 | 1 | 0.80 | 0.50 | 0.40 | 0 | 0.50 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| HERRB | 6/13/2004 | 1502211 | 1.00 | 0.90 | 1.00 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| HERRB | 7/23/2005 | 1603515 | 1.00 | 0.90 | 1.00 | 1 | 0.80 | 0.50 | 0.53 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| HERRB | 6/19/2007 | 1802213 | 1.00 | 0.90 | 1.00 | 1 | 0.80 | 0.55 | 0.47 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| HERRB | 6/23/2010 | 20100204 | 0.70 | 0.90 | 0.80 | 1 | 0.50 | 0.50 | 0.73 | 0 | 0.50 | 0.60 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| HERRB | 6/23/2010 | 20100201 | 0.80 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.47 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| HERRB | 6/23/2010 | 20100203 | 0.70 | 0.90 | 0.80 | 1 | 0.50 | 0.45 | 0.67 | 0 | 0.50 | 0.60 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| HERRB | 6/27/2013 | 20130701 | 0.80 | 0.90 | 0.67 | 1 | 0.50 | 0.55 | 0.67 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| HERRB | 2/18/2014 | 20140201 | 0.80 | 0.90 | 1.00 | 1 | 0.50 | 0.50 | 0.60 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| KIUKP | 7/31/1992 | 302710 | 1.00 | 0.90 | 0.87 | 1 | 1.00 | 0.45 | 0.47 | 0 | 0.40 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.00 | 0 |
| KIUKP | 8/9/1999 | 1007036 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.50 | 0.60 | 0.20 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| KIUKP | 8/4/2012 | 20120739 | 1.00 | 0.95 | 0.93 | 1 | 0.60 | 0.55 | 0.40 | 0 | 0.50 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| KIUKP | 8/4/2012 | 20120737 | 1.00 | 0.95 | 0.93 | 1 | 0.70 | 0.45 | 0.40 | 0 | 0.40 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| MCARP | 7/28/1999 | 1007018 | 1.00 | 0.85 | 0.93 | 1 | 0.90 | 0.40 | 0.47 | 0 | 0.50 | 0.55 | 0.27 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| MCARP | 8/22/2005 | 1601914 | 1.00 | 0.90 | 0.93 | 1 | 0.70 | 0.55 | 0.40 | 0 | 0.50 | 0.55 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| MCARP | 8/9/2012 | 20120752 | 0.90 | 0.95 | 0.93 | 1 | 0.80 | 0.45 | 0.40 | 0 | 0.40 | 0.50 | 0.33 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| MCARP | 8/9/2012 | 20120751 | 1.00 | 1.00 | 0.93 | 1 | 0.60 | 0.55 | 0.40 | 0 | 0.50 | 0.50 | 0.20 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| NINAI | 12/10/1989 | 304903 | 1.00 | 0.90 | 0.87 | 1 | 0.90 | 0.55 | 0.40 | 0 | 0.50 | 0.55 | 0.33 | 0 | 0.20 | 0.05 | 0.07 | 0 |
| NINAI | 8/10/1999 | 1007038 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.45 | 0.53 | 0 | 0.40 | 0.60 | 0.20 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| NINAI | 8/19/2005 | 1601907 | 1.00 | 0.90 | 0.93 | 1 | 0.90 | 0.40 | 0.53 | 0 | 0.40 | 0.55 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |
| NINAI | 8/5/2012 | 20120743 | 0.90 | 1.00 | 1.00 | 1 | 0.60 | 0.55 | 0.47 | 0 | 0.50 | 0.50 | 0.40 | 0 | 0.10 | 0.05 | 0.00 | 0 |
| NINAI | 8/5/2012 | 20120742 | 1.00 | 0.95 | 0.93 | 1 | 0.80 | 0.55 | 0.47 | 0 | 0.40 | 0.50 | 0.27 | 0 | 0.10 | 0.05 | 0.07 | 0 |

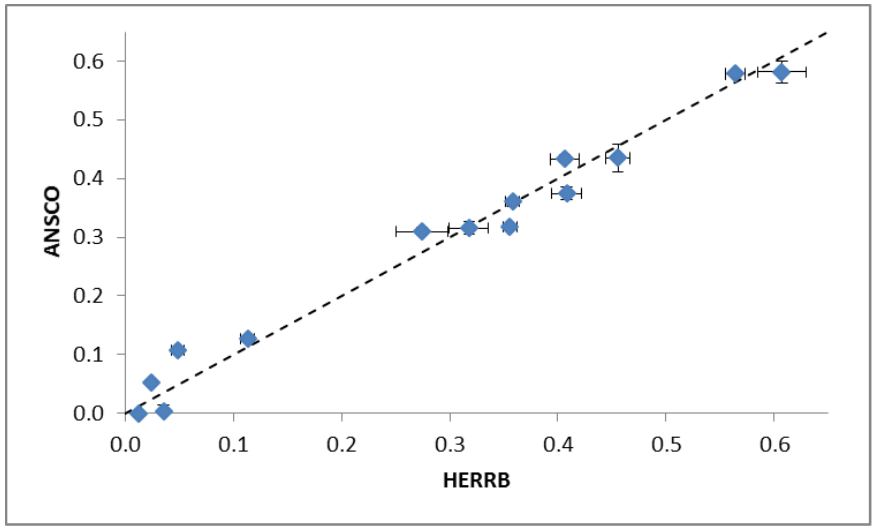
**SI 3.** Nordtest plots for each site versus ANSCO as the potential source. The dotted line is x = y. Solid lines are regression fits. Error bars (vertical and horizontal) are 95% confidence bounds.

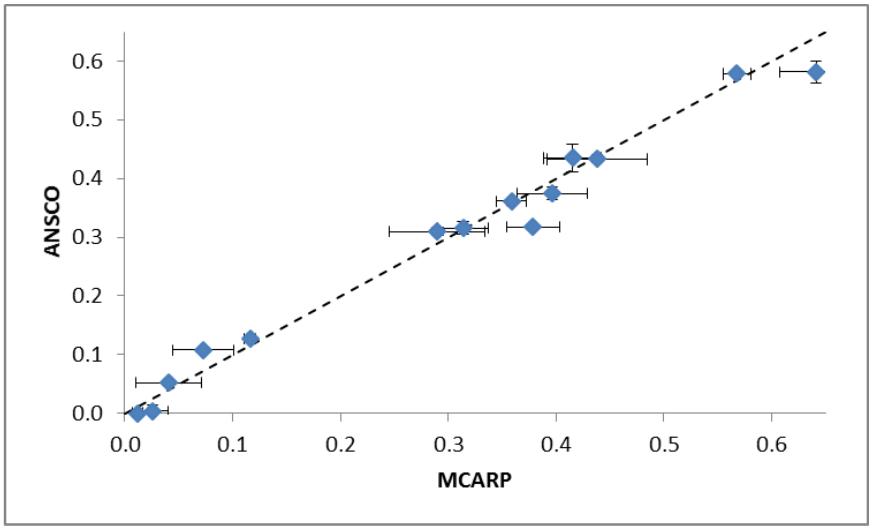


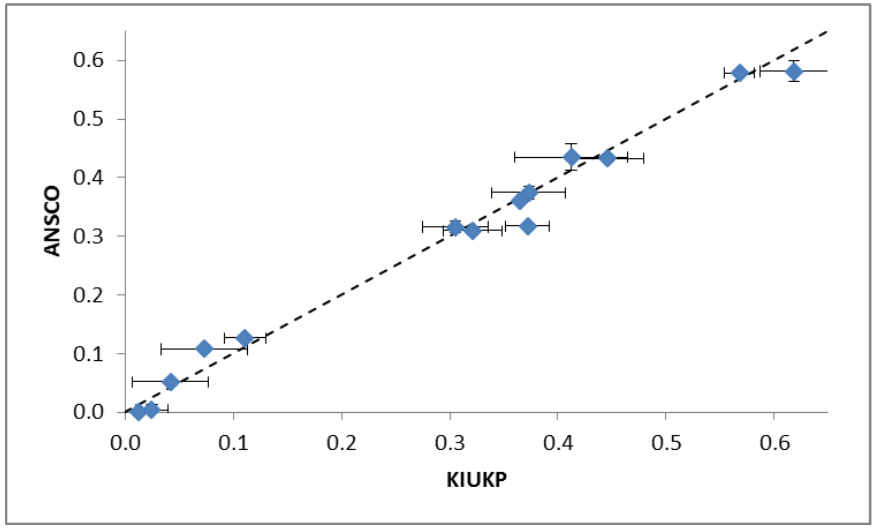



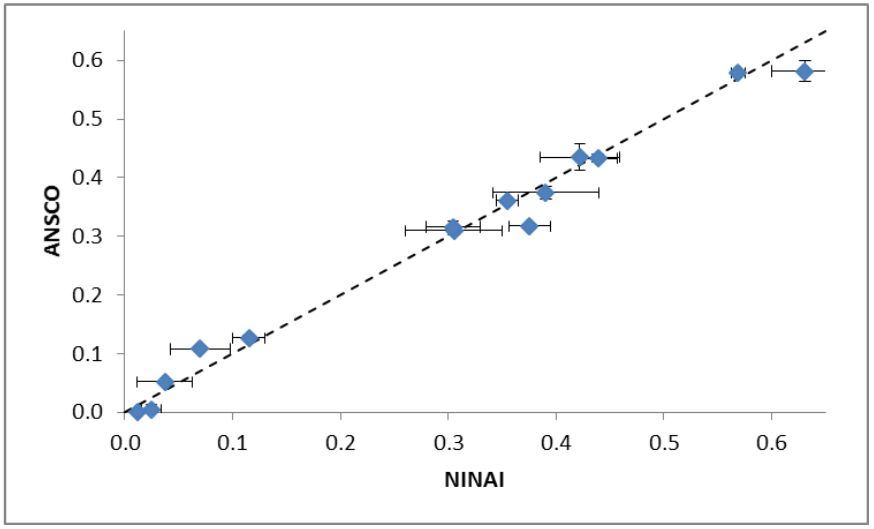



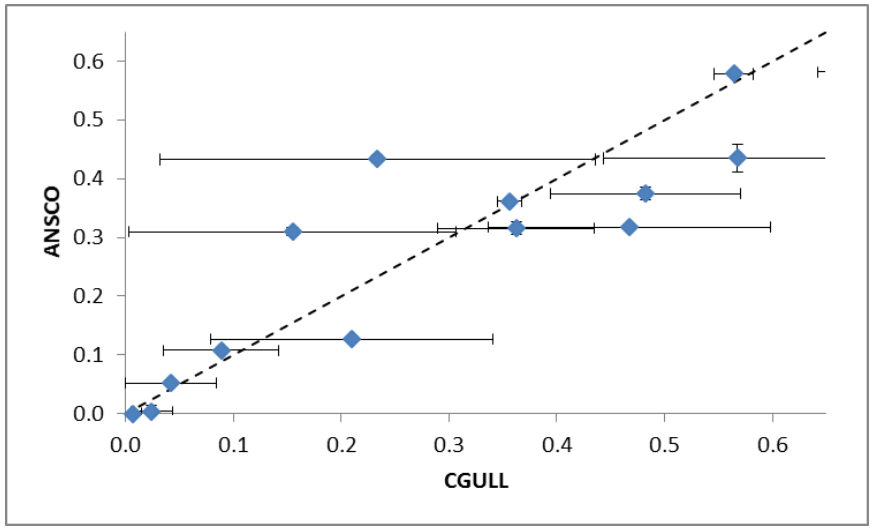




ANSCO
HERRB
0.0
0.1
0.2
0.3
0.4
0.5
0.6

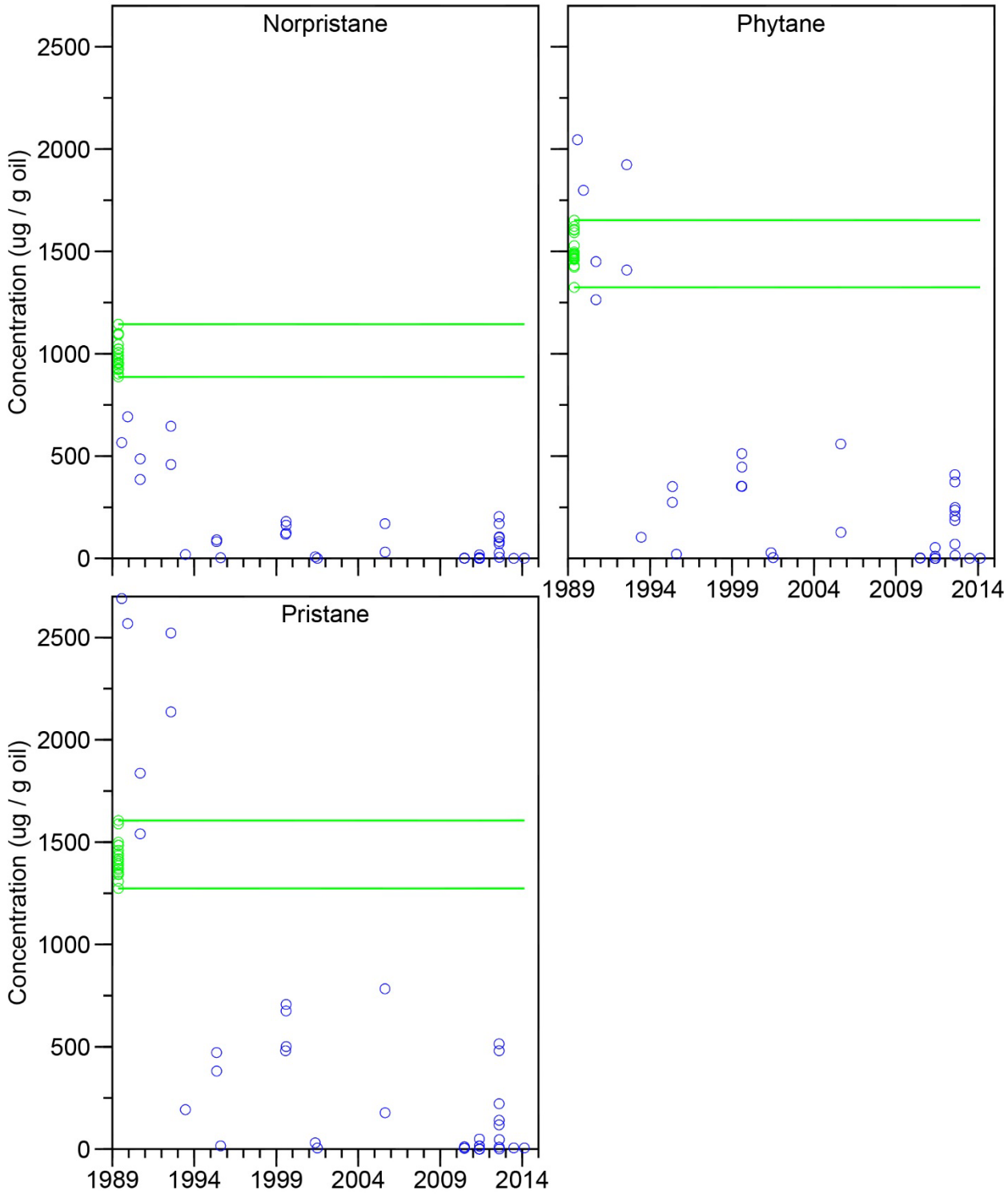

ANSCO
MCARP
0.0
0.1
0.2
0.3
0.4
0.5
0.6

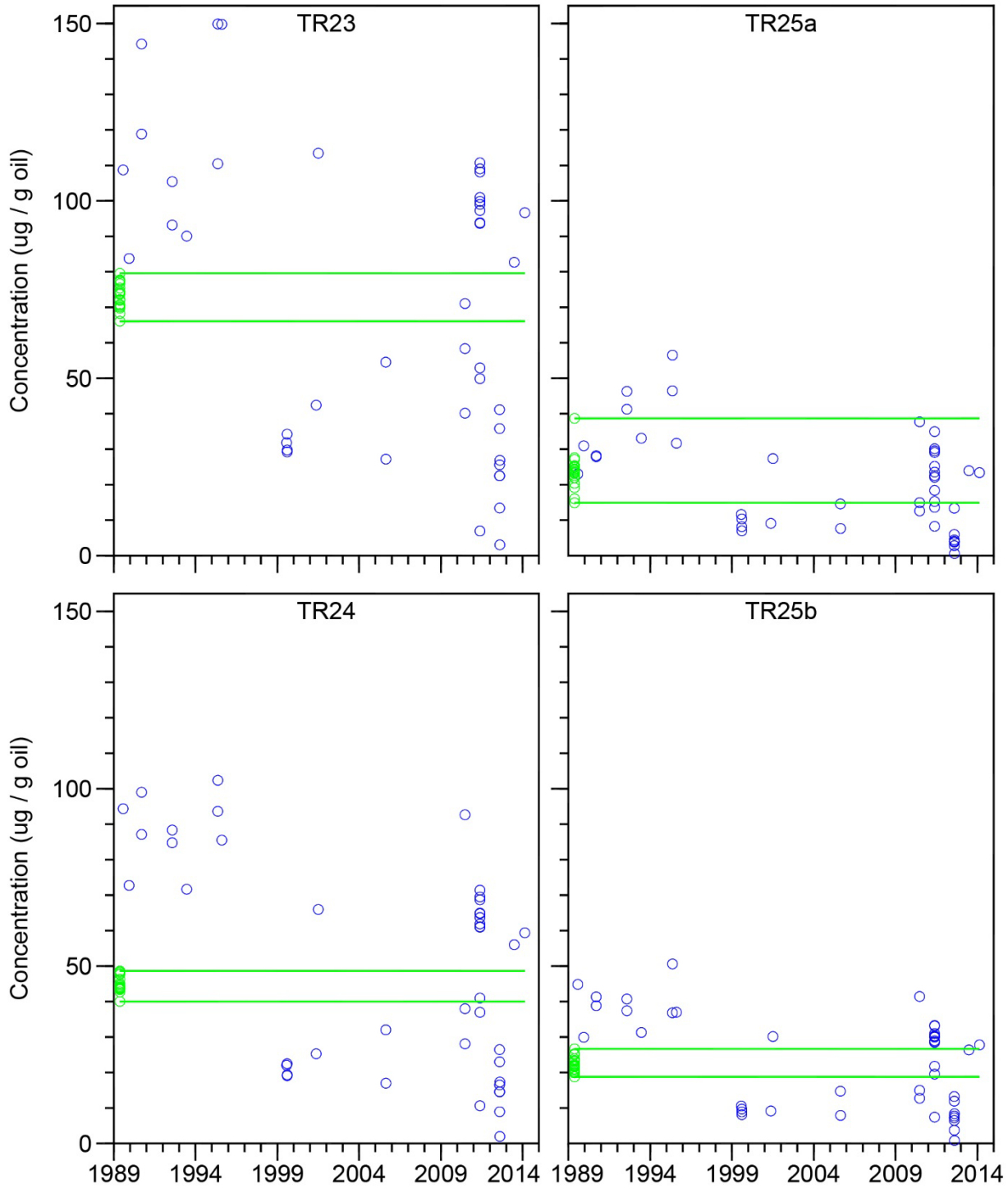

ANSCO
KIUKP
0.0
0.1
0.2
0.3
0.4
0.5
0.6

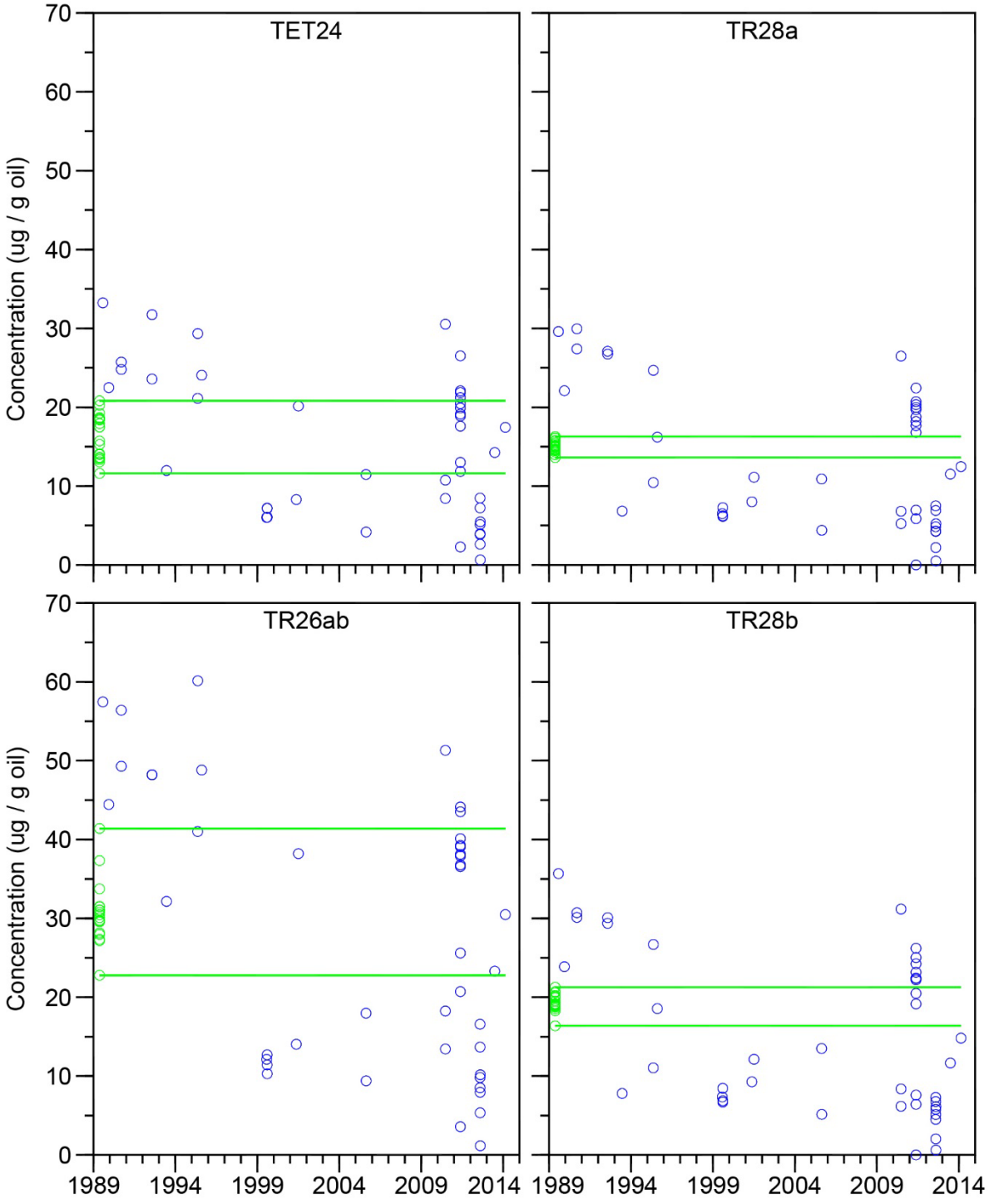

ANSCO
NINAI
0.0
0.1
0.2
0.3
0.4
0.5
0.6

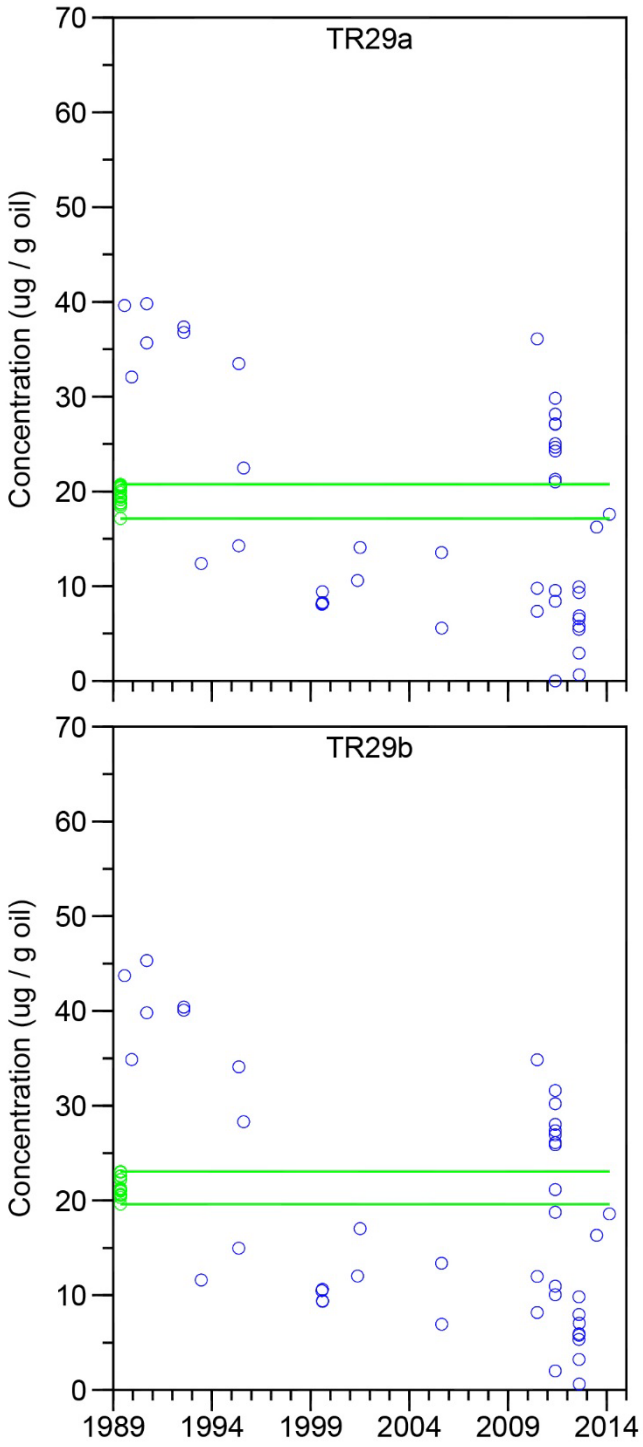

ANSCO
CGULL
0.0
0.1
0.2
0.3
0.4
0.5
0.6

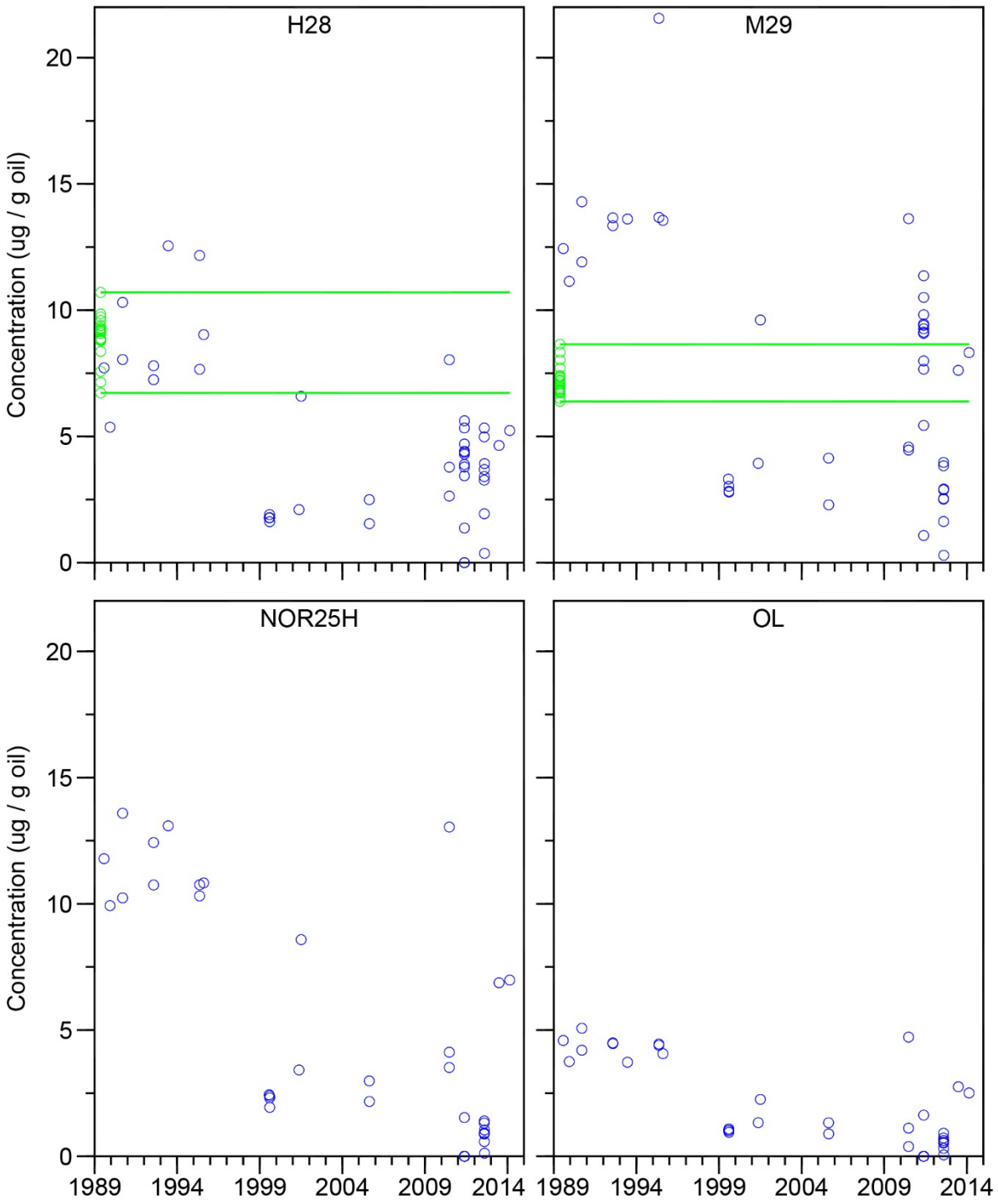
**SI 4**. Biomarker concentration change over time. Blue symbols are data from all field sites. Source oil (EVO) is illustrated in green with the low and high range marked by horizontal lines.

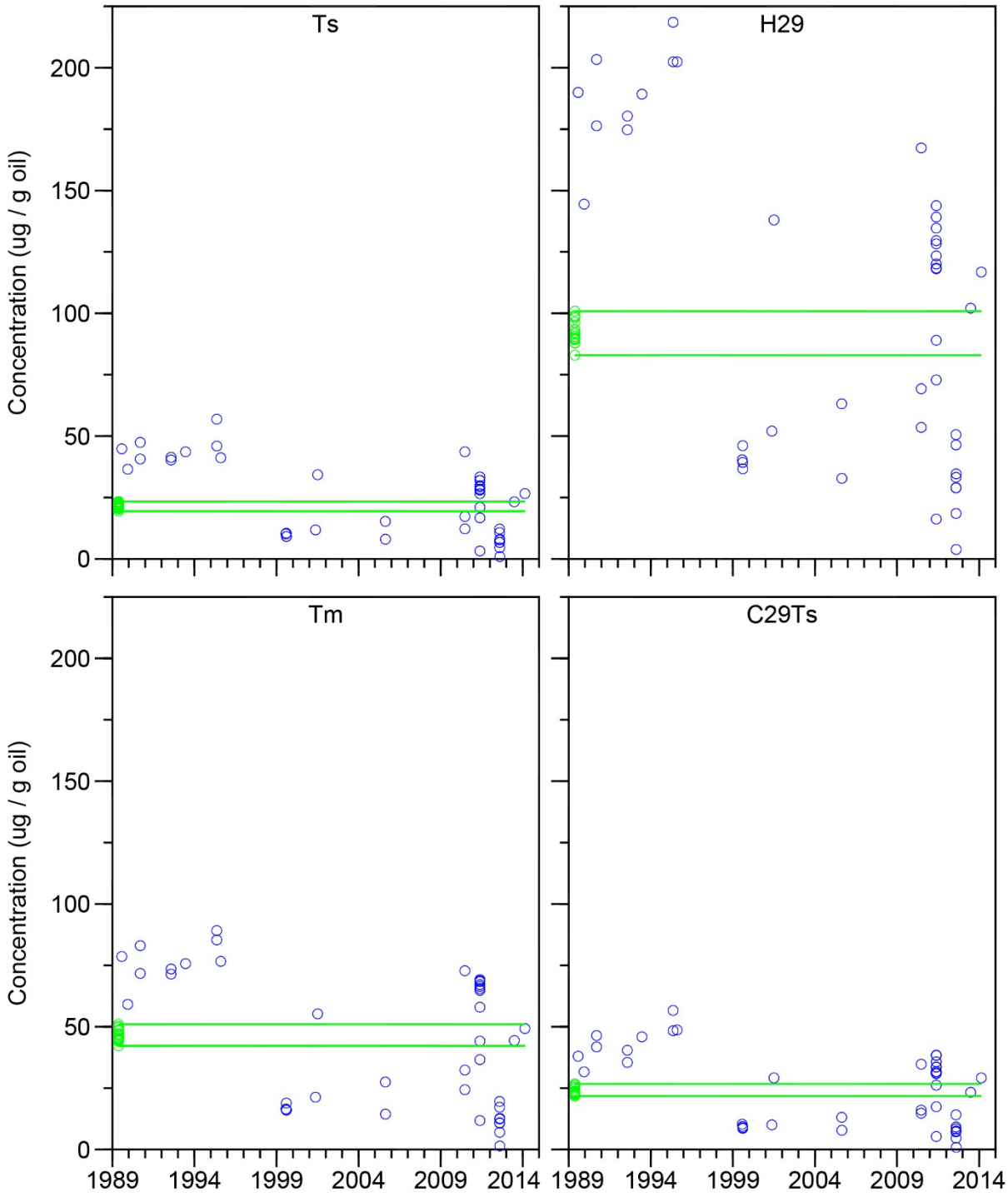


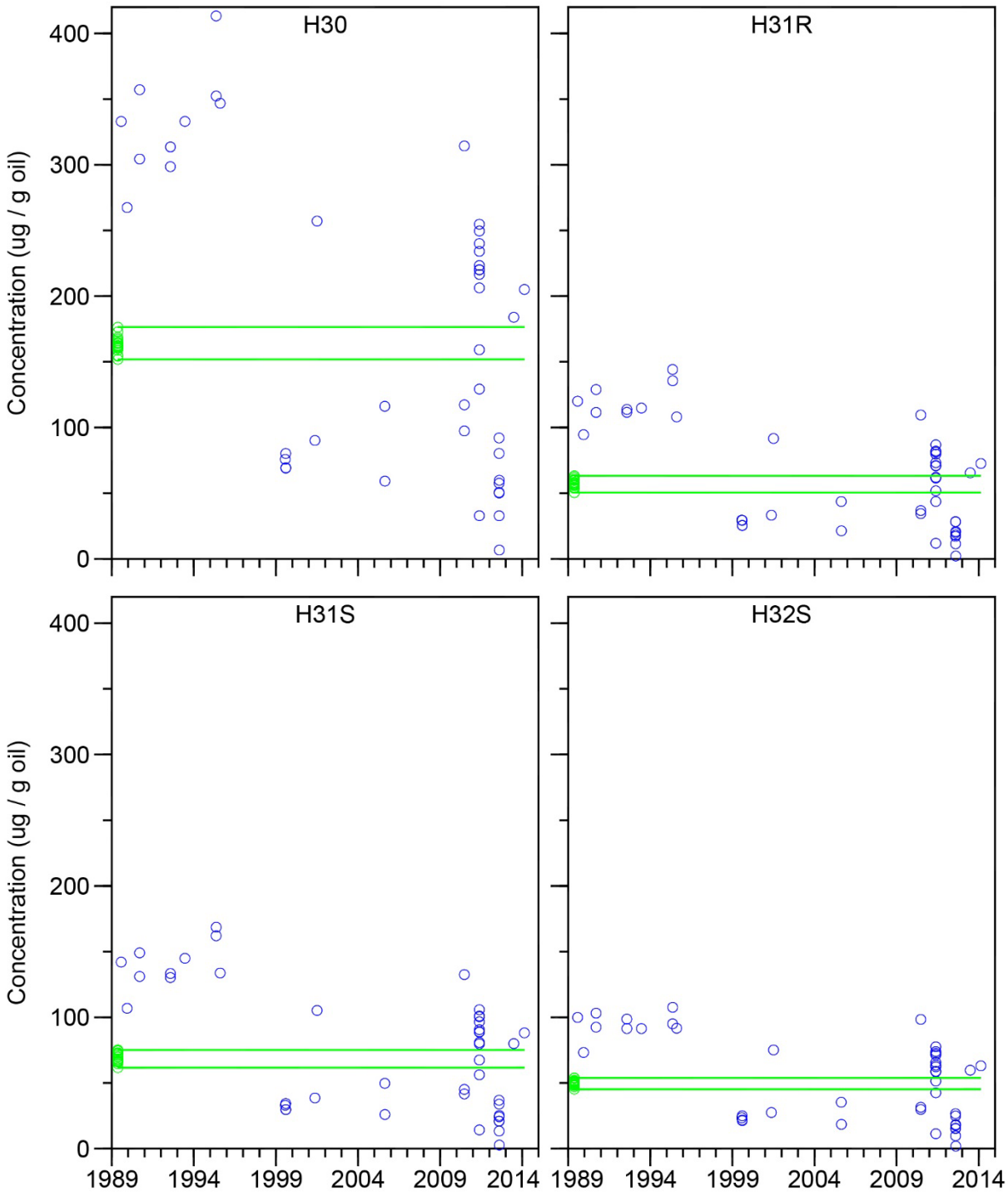

TR23
TR25a
TR24
TR25b
Concentration (ug / g oil)
150
100
50
0
1989
1994
1999
2004
2009
2014

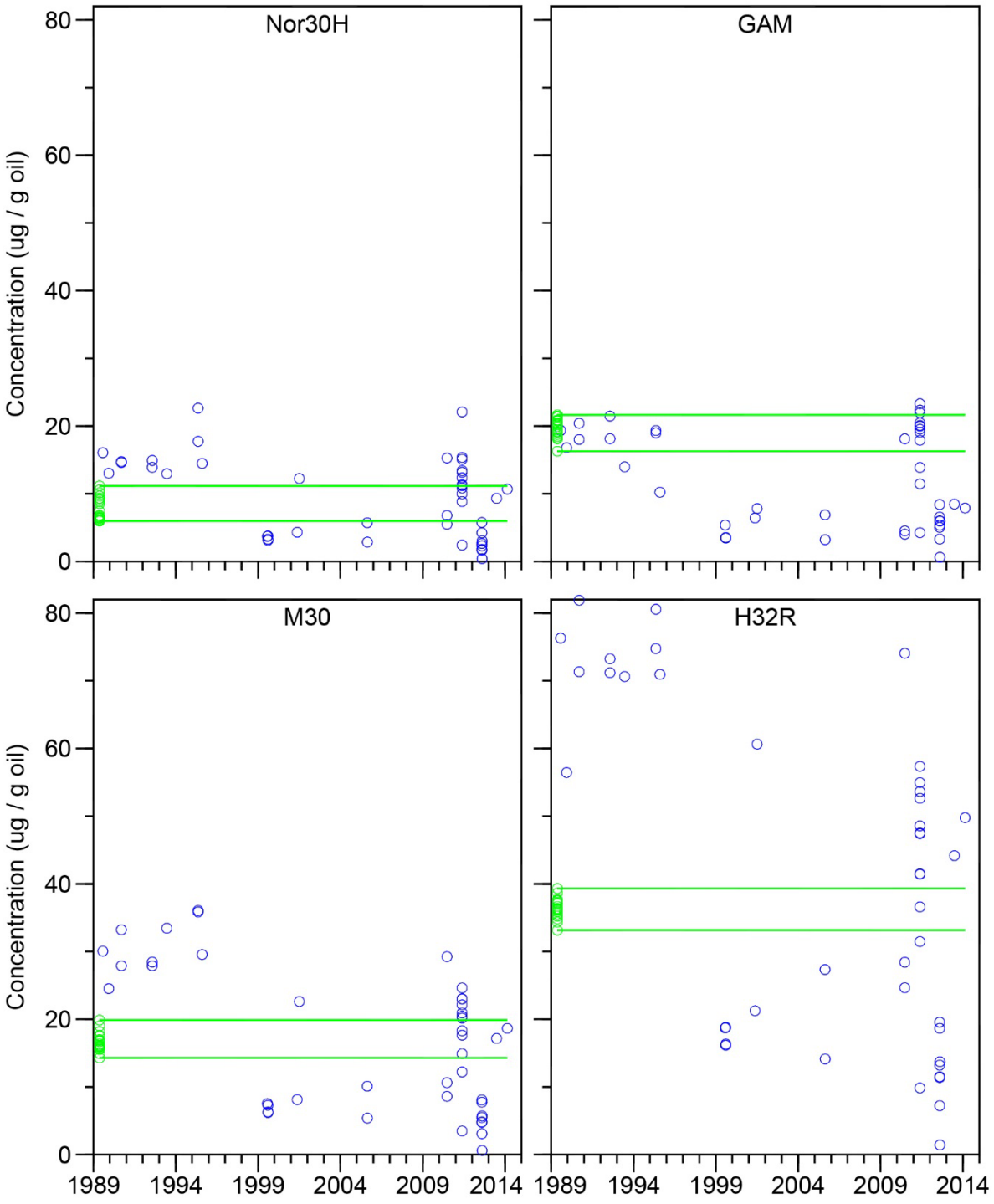

TET24
TR28a
TR26ab
TR28b
Concentration (ug / g oil)
Concentration (ug / g oil)
0
10
20
30
40
50
60
70
1989
1994
1999
2004
2009
2014

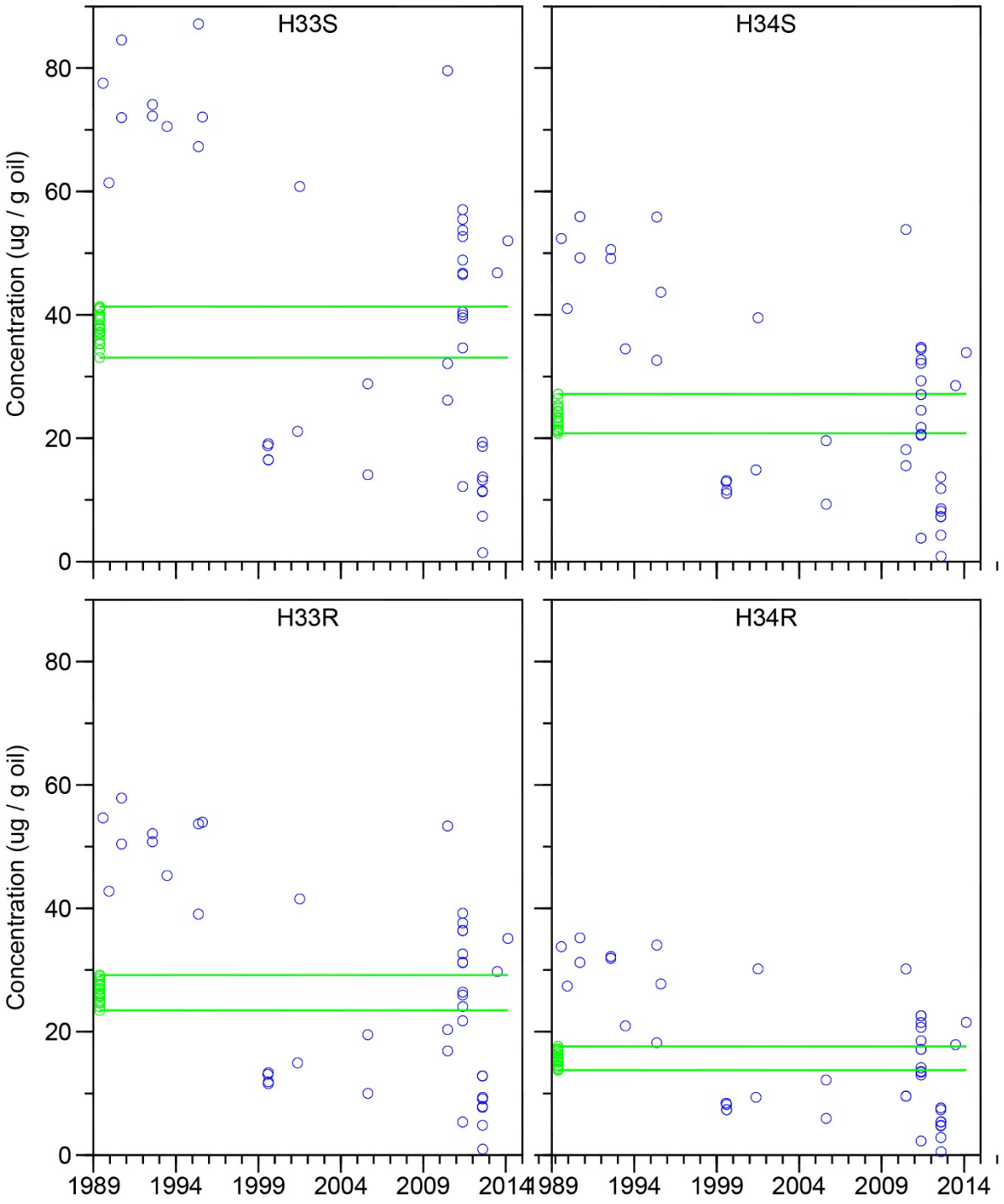

TR29a
Concentration (ug / g oil)
70
60
50
40
30
20
10
0
TR29b
Concentration (ug / g oil)
70
60
50
40
30
20
10
0
1989
1994
1999
2004
2009
2014

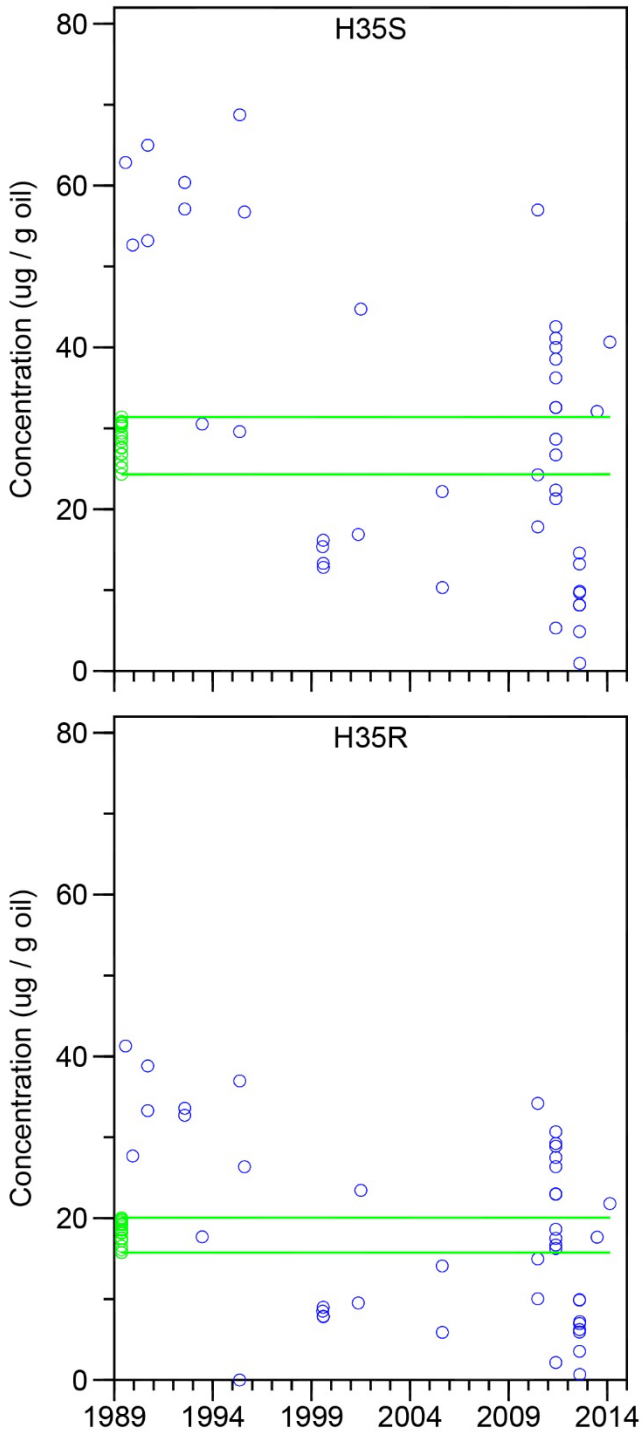

H28
M29
NOR25H
OL
Concentration (ug / g oil)
Concentration (ug / g oil)
0
5
10
15
20
1989
1994
1999
2004
2009
2014

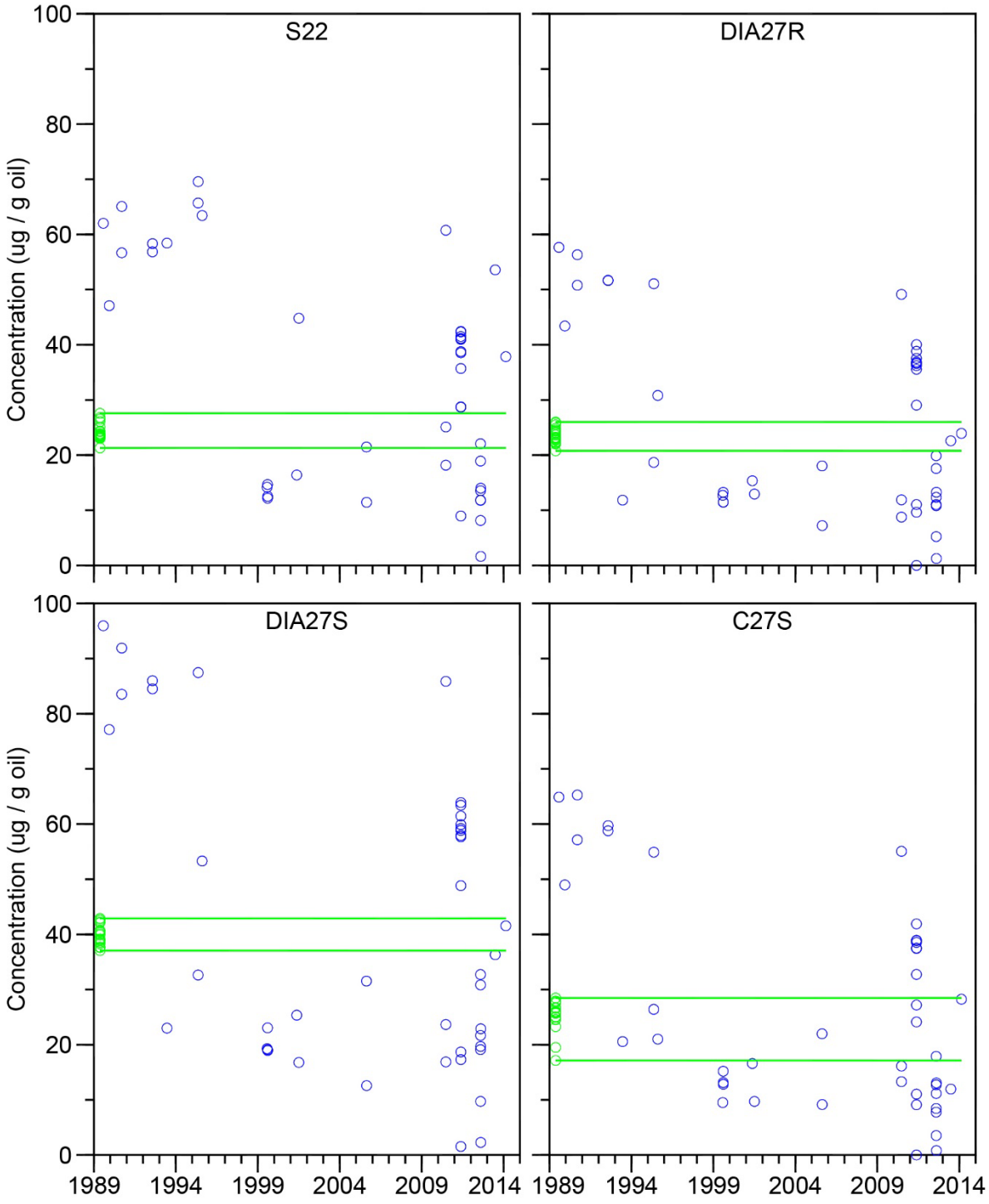

Ts
H29
Tm
C29Ts
Concentration (ug / g oil)
Concentration (ug / g oil)
0
50
100
150
200
1989
1994
1999
2004
2009
2014

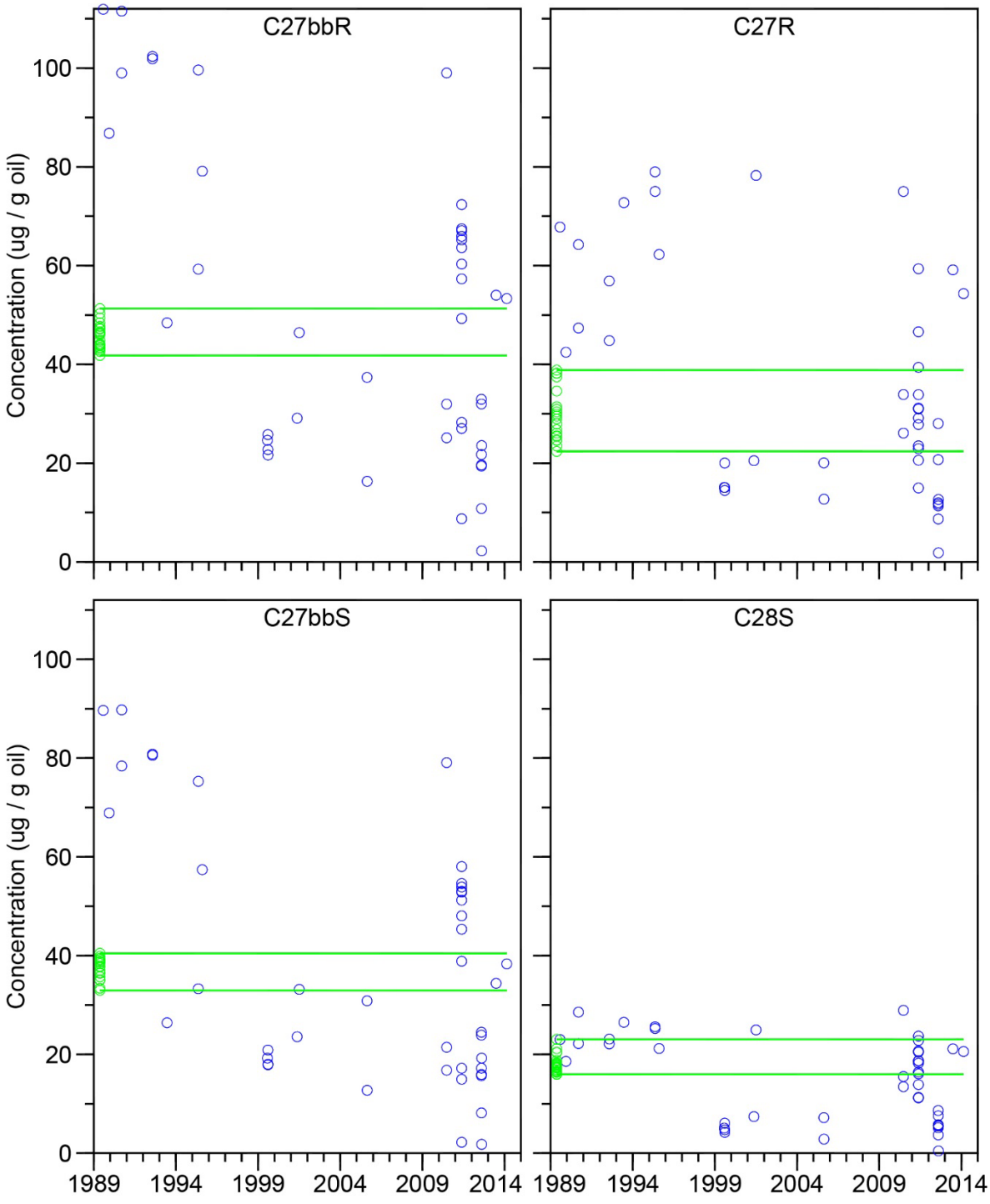

H30
H31R
H31S
H32S
Concentration (ug / g oil)
Concentration (ug / g oil)
0
100
200
300
400
1989
1994
1999
2004
2009
2014

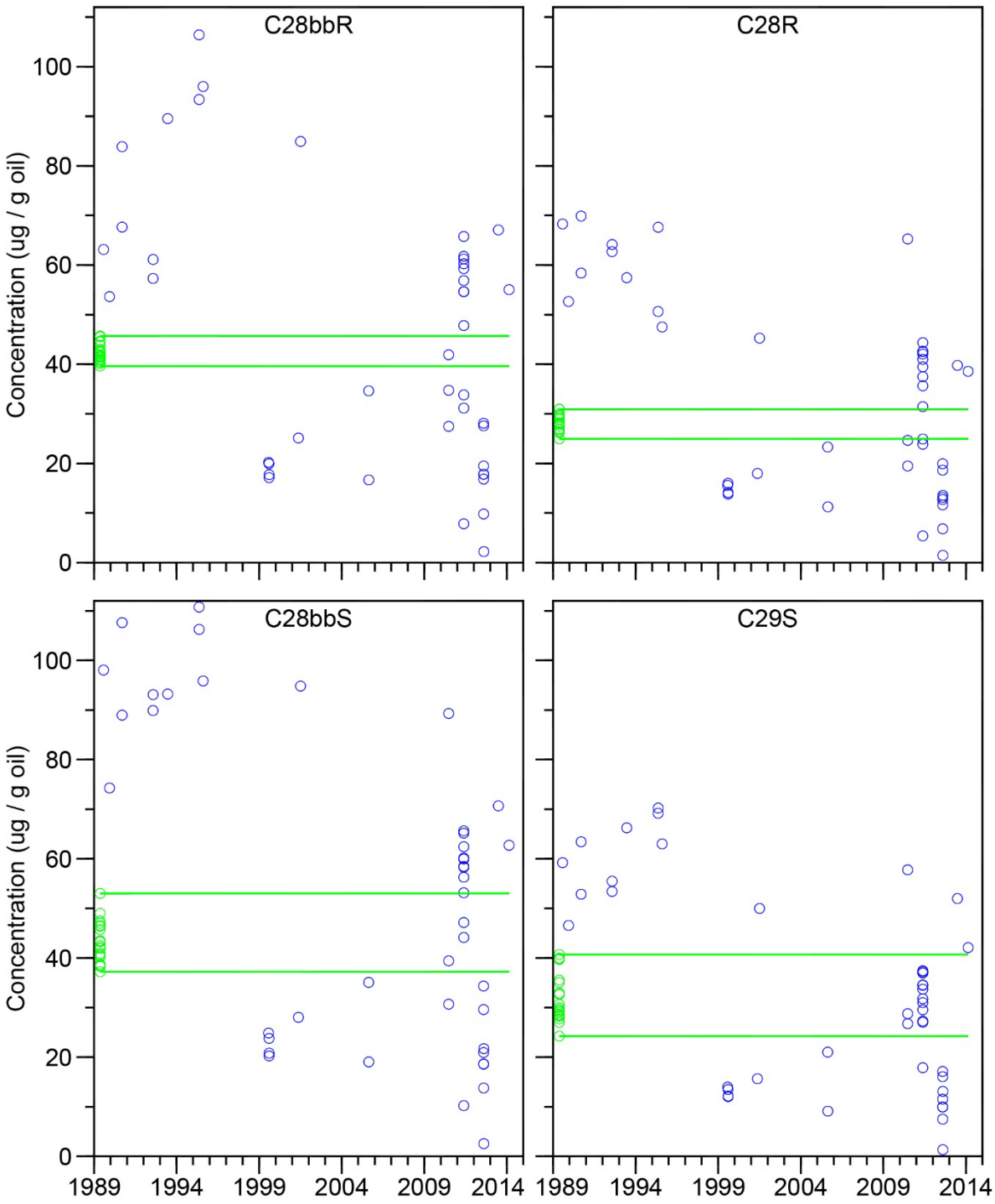

Nor30H
GAM
M30
H32R
Concentration (ug / g oil)
Concentration (ug / g oil)
0
20
40
60
80
1989
1994
1999
2004
2009
2014

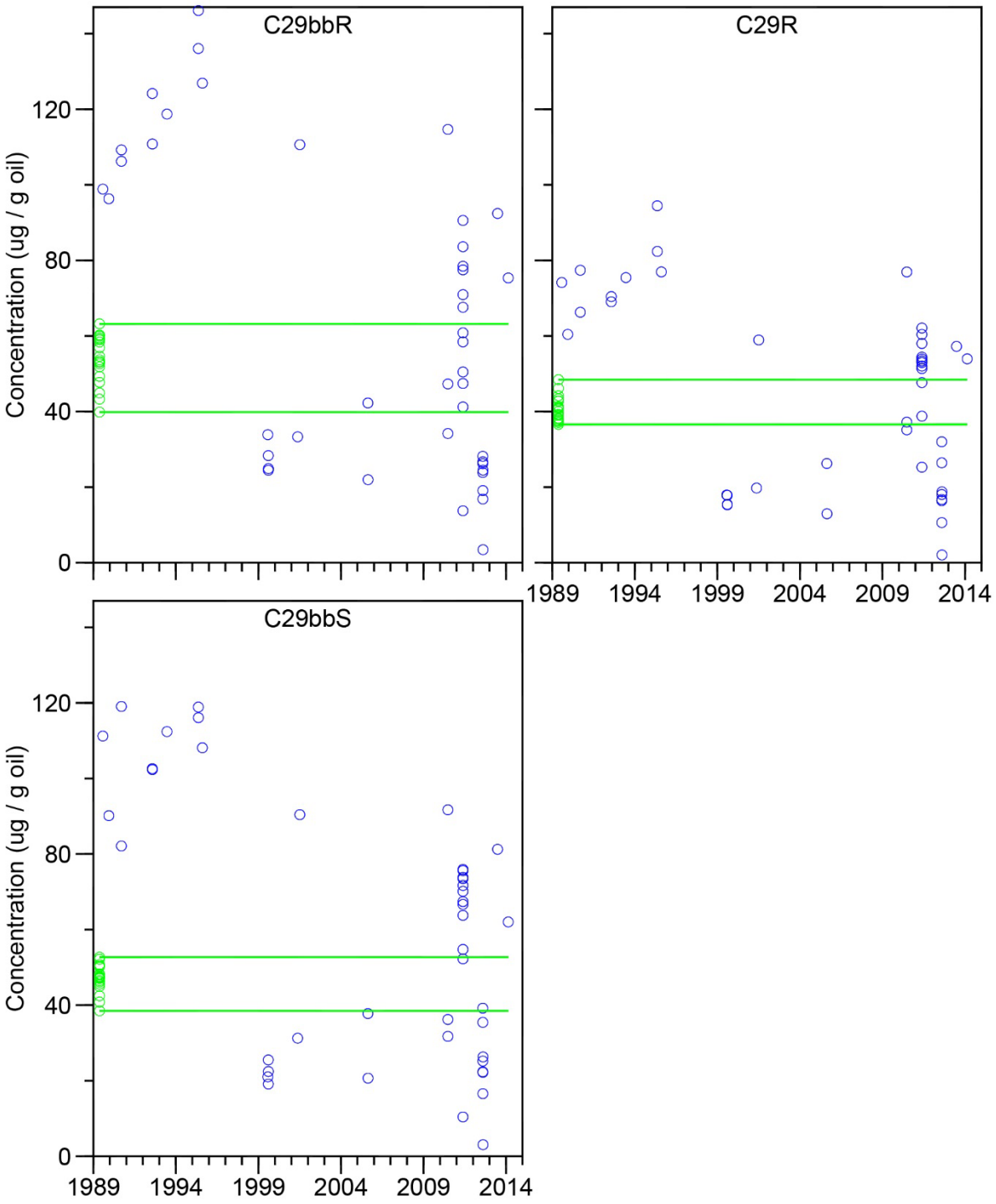

H33S
H34S
H33R
H34R
Concentration (ug / g oil)
0
20
40
60
80
1989
1994
1999
2004
2009
2014


H35S
Concentration (ug / g oil)
0
20
40
60
80
H35R
Concentration (ug / g oil)
0
20
40
60
80
1989
1994
1999
2004
2009
2014


S22
DIA27R
DIA27S
C27S
Concentration (ug / g oil)
Concentration (ug / g oil)
0
20
40
60
80
100
1989
1994
1999
2004
2009
2014


C27bbR
C27R
C27bbS
C28S
Concentration (ug / g oil)
Concentration (ug / g oil)
0
20
40
60
80
100
1989
1994
1999
2004
2009
2014


C28bbR
C28R
C28bbS
C29S
Concentration (ug / g oil)
0
20
40
60
80
100
1989
1994
1999
2004
2009
2014


C29bbR
C29R
C29bbS
Concentration (ug / g oil)
0
40
80
120
1989
1994
1999
2004
2009
2014

# Chapter Z. Biomarkers in *Exxon Valdez* oil from Prince William Sound, 2015

Mark G. Carls, Larry Holland, Corey Fugate, and Mandy Lindeberg

**Abstract**

Oil spilled by the *Exxon Valdez* has remained in some western PWS beaches for > 25 years and it remains definitively identifiable with biomarker forensic modeling. Nonetheless, biomarkers, particularly some triterpanes and steranes, underwent differential weathering. Combined with earlier biomarker observations, geographic weathering patterns were consistent with previous reports that weathering has been generally more rapid in PWS than in the GOA. Current weathering rates of biomarkers and PAHs appear negligible, thus explaining why biologically available PAHs were not elevated in passive samplers deployed along an oiled beach in 2015.

## Introduction

In 2015, intertidal beaches in western Prince William Sound that were extensively coated with *Exxon Valdez* oil (Neff et al. 1995) were examined to 1) fingerprint the oil, 2) determine oil sources, 3) report oil persistence and weathering over decades, and 4) determine biological availability. Nine of the worst case sites were revisited to continue a long term data set that tracks oil quantity and weathering composition in contaminated sediments. These long-term monitoring sites will be resampled every 5 years over the next 20 years (Figure 1 and Table 1). Sites with a history of persistent subsurface oil were prioritized for monitoring based on heavy subsurface oil surveyed in most recent years, a variety of shore types prone to oil retention, and those sites with a high probability of persistent oil (Michel et al. 2010). To assess the biological availability of polynuclear aromatic hydrocarbons (PAHs) (Carls et al. 2004), passive samplers were deployed for 10 days before disturbance along 1 beach.

Forensic analysis of the oil allowed us to understand contamination source(s) and how they might influence biota. Analytical measurement included PAHs, which were modeled for source composition (Carls 2006; Carls et al. 2015) and geochemical biomarkers (triterpanes, hopanes, and steranes), which are particularly useful in definitive oil identification (Wang and Stout 2007; Carls et al. 2015). The PAH and alkane sediment results from 2015 were incorporated into the long-term data analyzed in Chapter X. Here we report only the biomarker results, which were not incorporated into the earlier long-term biomarker assessment (Chapter Y; Carls et al. 2016) and PAH results from passive samplers.

## Methods

Nine beach segments were selected for the long-term monitoring of lingering subsurface oil (Table 1). Factors considered for prioritization were based on: initial oiling, shore types prone to oil retention (Michel and Hayes 1993; Hayes and Michel 1998; Michel et al. 2010), past oil surveys to aid our understanding of loss rates (Gibeaut and Piper 1998; Short et al. 2004; Short et al. 2006; Short et al. 2007), most recently observed oil in heaviest categories (HOR and MOR), and a high probability of oil

persistence (Nixon and Michel 2015). Six of these beaches were randomly selected for study by Short et al. (2007) and a seventh was adjacent to a beach in that study.

*Sediment collection*

A survey grid was established on each of nine beach segments to allow calculation of the site area and stratified random sampling. The number of random pits was dependent on beach length (0.5 to 0.8 quadrats per meter) but the actual number of hydrocarbon samples was dependent on the number of visibly oiled pits. The number of oiled samples per beach ranged from zero (EVANI) to 17 (ELEAI). The hydrocarbon samples were targeted to represent the oil encountered while digging. Samples were collected with hydrocarbon-free spoons, placed in hydrocarbon-free jars, and frozen pending analysis.

*Passive Samplers*

The bioavailability and composition of mobile oil constituents was assessed in ambient water with low-density polyethylene membrane sampling devices (PEMDs) (Carls et al. 2004). These passive samplers were polyethylene plastic strips (~98 µm × 4.9 cm × 50 cm) housed in aluminum canisters (11.5 cm diameter × 6.6 cm) with perforated aluminum endplates (3 mm holes spaced 4.8 mm apart). The PEMDs were placed on one oiled beach segment (KN0114; n = 18). The PEMDs were retrieved 10 days after deployment, sealed in ziplock bags, and frozen as soon as practical pending processing. Passive sampler air blanks, packed in jars, were opened at each beach segment for about 1 minute either during deployment or retrieval. Two unopened PEMDs served as trip blanks and two additional laboratory blanks were never shipped. Shortly after arrival at the laboratory, the aluminum canisters were opened and the PEMDs were transferred to hydrocarbon-free glass jars with Teflon lined lids and frozen for chemical analysis.

*Sample processing*

Sediment and oil samples were dried with anhydrous sodium sulfate and extracted with dichloromethane (DCM). Extract volumes were reduced to approximately 5 ml on a steam table. The exact total volume was recorded and an aliquot was archived. The DCM was evaporated from the remaining extract to determine the total mass of extracted oil. This information was used to calculate the application of 3 to 6 mg of oil from the archived aliquot to a 6 g silica column. The aliphatic fraction was eluted with 10 ml pentane and PAHs were subsequently eluted with 20 mL of 1:1 (v/v) pentane:DCM. 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 using the amount of oil applied to silica column as the divisor.

Aromatic fractions were analyzed for PAHs by gas chromatography – mass spectroscopy. Data were acquired in selected ion monitoring mode and concentrations were determined by the internal standard method (Short et al. 1996). Measured PAHs were naphthalenes (N0 to N4), biphenyl (BPH), acenaphthylene (ACN), acenaphthene (ACE), fluorenes (F0 to F4), dibenzothiophenes (D0 to D4) phenanthrenes (P0 to P4), anthracene (ANT), fluoranthene (FLU), pyrene (PYR), fluoranthene/pyrenes (FP1 to FP4) benzo(a)anthracene (BAA), chrysenes (C0 to C4), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(e)pyrene (BEP), benzo(a)pyrene (BAP), perylene (PER), indeno(1,2,3-cd)pyrene (ICP), dibenzo(a,h)anthracene (DBA), and benzo(ghi)perylene (BZP). Concentrations below

method detection limits were set to zero. A PAH source model was used to estimate origins of observed PAHs (Carls 2006; Carls et al. 2015).

Aliphatic fractions were analyzed for biomarkers by gas chromatography – mass spectroscopy. The data were acquired in SIM mode, and concentrations were determined by the internal standard method with response factors (RF) based on two representative compounds, 17α (H),21β(H)-hopane (H30) and 5α(H),14α(H),17α(H)-cholestane. The accuracy of the biomarker analyses was about ± 15% based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was about 20%, depending on the biomarker. Biomarker concentrations were not corrected for recovery; surrogate recovery averaged 92% (range 44 to 103%). Reported biomarkers and their abbreviations are listed in Table 2.

*Source oil samples*

Twenty-one source previous source oil sample measurements were used to determine analyte concentrations in fresh *Exxon Valdez* oil (Alaska North Slope crude oil, ANSCO) (Carls et al. 2015). Six additional source measurements were prepared and analyzed in 2015 as quality controls. Analytical measurements were completed as previously described.

*Pattern-matching forensics*

Biomarker composition in samples was compared to that in ANSCO (obtained from the T/V *Exxon Valdez* in 1989) to determine if they matched source oil composition (Carls et al. 2015). In brief, 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 ($H_i$ / ∑ $H_i$), where $H_i$ is the $i^{th}$ hopane concentration and ∑$H_i$ is the total hopanoid concentration. For each sample, the number of $H_i$ / ∑ $H_i$ 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. The probability that an unknown sample was consistent with ANSCO composition was assessed by reference to results of randomly permuting the source oil data set 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 three classes were > 0.6.

*Weathering*

Application of the forensic biomarker model provided evidence of biomarker weathering. Concentrations of some compounds in some samples were less than expected from the source oil. Depleted biomarkers included several triterpanes (TR28a through TR29b) and steranes (DIA27S through C27bbS). Depletion generally was not as obvious for hopanes, but in the more advanced cases some hopanes were involved (H28, GAM, H33R through H35R). Biomarkers in source oil samples analyzed with this data set were not depleted, thus providing quality assurance information.

The model was used to further explore biomarker weathering by increasing the relative concentrations of depleted compounds. This was accomplished by applying a multiplicative corrective factor to the composition in each sample. Each factor value was determined iteratively to minimize the sum residual

between each analyte in the source oil and the sample. This procedure was completed independently for triterpanes, hopanes, and steranes. The 2015 source oil samples analyzed with this data were used as the source in this procedure; results were highly similar when the older source samples were used as the reference.

Calculation of residuals (between analytes in the sample and mean source oil values) for compounds that were lost more rapidly (TR28a – TR29b, for example) was also completed as an alternative method to examine weathering. This approach was simpler and required no modeling assumptions. Furthermore, these residuals were correlated with the corrective factors; r = 0.991, 0.932, and 0.923 for triterpanes, steranes, and hopanes, respectively (n = 38).

## Results

*Sediment*

Biomarkers were present in all 38 oil samples collected from PWS sediment in 2015. Half of these (19) were definitive for ANSCO when the analysis was based on all three biomarker classes. Biomarkers evidently weathered in some of these samples and this degraded the ability of the model to identify oil. When estimated from hopanes alone, which were the least prone to weathering, 35 of 38 samples were definitive for ANSCO, and this oil was observed in every beach except EVANI where no oil was discovered.

Biomarkers were reduced or missing in some samples and this was interpreted as evidence of weathering. For example, the triterpanes TR28a – TR29b were reduced relative to other triterpanes and the steranes DIA27S – C27bbS were reduced relative to other steranes (Fig. 2). Reduction of these compounds was related; when triterpanes were depleted, so were the steranes; correlation between the independently determined correction factors was 0.972. In contrast, evidence of hopane loss was subtle or missing and hopane factors were not related to triterpane or sterane factors (r = 0.086 and 0.020, respectively).

*Passive Samplers*

Oil was not present in passive samplers, based on PAH source modeling and concentration (Table 3). Total PAH concentrations were statistically indistinguishable from concentrations in blanks ($P_{ANOVA}$ = 0.739) and composition was also indistinguishable ($P_{ANOVA}$ = 0.517). Observed concentrations were ≤ 77 ng/g device. These concentrations are within typical background levels for passive samplers (Carls and Masuda 2014).

## Discussion

Alaska North Slope crude oil spilled by the *Exxon Valdez* tanker vessel in 1989 was definitively present at each beach sampled in 2015. However, some triterpanes and steranes were reduced or missing in about half of the samples. This change from the source oil was apparently caused by biomarker weathering. In support of the hypothesis that biomarkers were missing due to weathering, the same loss patterns were evident in Cape Gull samples collected after 1989, a

location previously identified with rapid biomarker weathering (Irvine et al. 2006; Short et al. 2007; Irvine et al. 2014; Carls et al. 2016). Furthermore, no biomarker loss was observed in fresh oil samples processed with this data set, eliminating that possibility that unknown systematic measurement errors were causal. In addition, Carls et al. (2016) observed greater weathering rates for these compounds in samples collected between 1989 and 2014 using a different analytical approach. Thus, the biomarker loss patterns observed in 2015 are evidence of weathering and this evidence is consistent with previous observations.

Although these data provide evidence that biomarkers have weathered in sequestered oil, they were only collected during a narrow period of time (June 2015), thus do not provide evidence of current rates of change. Addition of previous data (128 samples, collected from 1989 to 2014; Carls et al. 2016) allowed examination of biomarker weathering over time. Triterpane and sterane residuals (for lost compounds TR28a – TR29b and DIA27S – C27bbS) were relatively low through 1992 and then became more variable (Fig. 3). This is evidence that the biomarkers were remained relatively for a few years but that later weathering progressed at differential rates among years. There is no clear evidence of time trends after 1992.

Biomarkers were generally more weathered in PWS than in the GOA except at CGULL (Fig. 4). This weathering distribution is consistent with the geographic PAH weathering distribution, which also indicates more rapid weathering in PWS (Chapter Y). Weathering at CGULL has previously been identified as unusually fast in the GOA (Irvine et al. 2006; Short et al. 2007). The consistency of this biomarker weathering result demonstrates that the biomarker source modeling method (Carls et al. 2015) is sufficiently accurate that it can reliably distinguish relative change among biomarker compounds and this change is due to the differential weathering of the more vulnerable biomarkers.

The absence of PAHs in passive samplers deployed in 2015, which are considerably more mobile than biomarkers, is evidence that the oil was not meaningfully biologically available outside the sediment in 2015. The lack of meaningful biological exposure is discussed in greater detail in Chapter X. In contrast, TPAH concentrations in some passive samplers deployed in previous SCAT projects (2002 to 2004) were orders of magnitude greater than observed in 2015 and composition was consistent with oil (Carls and Masuda 2014).

## Conclusions

Alaska North Slope crude oil spilled by the tanker vessel *Exxon Valdez* has remained in some western PWS beaches for > 25 years and it remains definitively identifiable with biomarker forensic modeling. Nonetheless, biomarkers, particularly some triterpanes and steranes, underwent differential weathering. Combined with earlier biomarker observations (Carls et al. 2016), geographic weathering patterns were consistent with previous reports that weathering has been generally more rapid in PWS than in the GOA (Irvine et al. 1999; Short et al. 2007; Irvine et al. 2014; Carls et al. 2016). Current weathering rates appear negligible, including the weathering of PAHs (Chapter Y), thus explaining why biologically available PAHs were not elevated in passive samplers deployed along an oiled beach.

**Table 1.** Long-term oil monitoring sites. Segment lengths and areas were determined in 2015.

| Location | Abbreviation | Segment | Initial oiling | Length (m) | Site Area ($m^2$) |
|---|---|---|---|---|---|
| Eleanor Is. | ELEAI | EL056C | Medium oil (1990-1993) | 90 | 13,212 |
| Eleanor Is. | ELEAI | EL058B | Heavy oil (1989) | 51 | 9,372 |
| Evans Is. | EVANI | EV039A | Heavy oil (1990-1993) | 109 | 26,716 |
| Greens Is. | GREEI | GR103B | Heavy oil (1990-1993) | 100 | 20,742 |
| Herring Bay | HERRB | KN0114A | Heavy oil (1990-1993) | 68 | 13,605 |
| Herring Bay | HERRP | KN0300A-2 | Medium oil (1990-1993) | 52 | 11,135 |
| Knight Island | KNIGI | KN0506A | Heavy oil (1990-1993) | 50 | 9,171 |
| Sleepy Bay | SLEEB | LA018A-1 | Heavy oil (1990-1993) | 100 | 15,722 |
| Smith Is. | SMITI | SM006B | Heavy oil (1990-1993) | 100 | 28,014 |

**Table 2.** Biomarkers and their abbreviations. Asterisks mark analytes used for pattern matching; the number of triterpanes, hopanes, and steranes used for modeling were 10, 20, and 15, respectively.

| Abbreviation | Biomarker: Isoprenoids | Target Ions |
|---|---|---|
| norprist | norpristane | 57 |
| prist | 2,6,10,14-tetramethylpentadecane (pristane) | 57 |
| phyt | 2,6,10,14-tetramethylhexadecane (phytane) | 57 |

| Abbreviation | | Biomarker: Triterpanes | Target Ions |
|---|---|---|---|
| TR23 | * | C23 tricyclic terpane | 191 |
| TR24 | * | C24 tricyclic terpane | 191 |
| TR25a | * | C25 tricyclic terpane (a) | 191 |
| TR25b | * | C25 tricyclic terpane (b) | 191 |
| TET24 | * | C24 tetracyclic terpane | 191 |
| TR26a | *[a] | C26 tricyclic terpane (a) | 191 |
| TR26b | | C26 tricyclic terpane (b) | 191 |
| TR28a | * | C28 tricyclic terpane (a) | 191 |
| TR28b | * | C28 tricyclic terpane (b) | 191 |
| TR29a | * | C29 tricyclic terpane (a) | 191 |
| TR29b | * | C29 tricyclic terpane (b) | 191 |

| Abbreviation | | Biomarker: hopanes | Target Ions |
|---|---|---|---|
| Ts | * | 18α(H),21β(H)-22,29,30-trisnorhopane | 191 |
| Tm | * | 17α(H),21β(H)-22,29,30-trisnorhopane | 191 |
| H28 | * | 17α(H),18α(H),21β(H)-28,30-bisnorhopane | 191 |
| NOR25H | | 17α(H),21β(H)-25-norhopane | 191 |
| H29 | * | 17α(H),21β(H)-30-norhopane | 191 |
| C29Ts | * | 18α(H),21β(H)-30-norneohopane | 191 |
| M29 | * | 17α(H),21β(H)-30-norhopane (normoretane) | 191 |
| OL | | 18α(H) and 18β(H)-oleanane | 191 |
| H30 | * | 17α(H),21β(H)-hopane | 191 |
| NOR30H | * | 17α(H)-30-nor-29-homohopane | 191 |
| M30 | * | 17β(H),21α(H)-hopane (moretane) | 191 |
| H31S | * | 22S-17α(H),21β(H)-30-homohopane | 191 |

| | | | |
|---|---|---|---|
| H31R | * | 22R-17α(H),21β(H)-30-homohopane | 191 |
| GAM | * | Gammacerane | 191 |
| H32S | * | 22S-17α(H),21β(H)-30,31-bishomohopane | 191 |
| H32R | * | 22R-17α(H),21β(H)-30,31-bishomohopane | 191 |
| H33S | * | 22S-17α(H),21β(H)-30,31,32-trishomohopane | 191 |
| H33R | * | 22R-17α(H),21β(H)-30,31,32-trishomohopane | 191 |
| H34S | * | 22S-17α(H),21β(H)-30,31,32,33-tetrakishomohopane | 191 |
| H34R | * | 22R-17α(H),21β(H)-30,31,32,33-tetrakishomohopane | 191 |
| H35S | * | 22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane | 191 |
| H35R | * | 22R-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane | 191 |

| **Abbreviation** | | **Biomarker: steranes** | **Target Ions** |
|---|---|---|---|
| S22 | * | $C_{22}$ 5α(H),14β(H),17β(H)-sterane | 217,218 |
| DIA27S | * | $C_{27}$ 20S-13β(H),17α(H)-diasterane | 217,218 |
| DIA27R | * | $C_{27}$ 20R-13β(H),17α(H)-diasterane | 217,218 |
| C27S | * | $C_{27}$ 20S-5α(H),14α(H),17α(H)-cholestane | 217,218 |
| C27bbR | * | $C_{27}$ 20R-5α(H),14β(H),17β(H)-cholestane | 217,218 |
| C27bbS | * | $C_{27}$ 20S-5α(H),14β(H),17β(H)-cholestane | 217,218 |
| C27R | * | $C_{27}$ 20R-5α(H),14α(H),17α(H)-cholestane | 217,218 |
| C28S | * | $C_{28}$ 20S-5α(H),14α(H),17α(H)-ergostane | 217,218 |
| C28bbR | * | $C_{28}$ 20R-5α(H),14β(H),17β(H)-ergostane | 217,218 |
| C28bbS | * | $C_{28}$ 20S-5α(H),14β(H),17β(H)-ergostane | 217,218 |
| C28R | * | $C_{28}$ 20R-5α(H),14α(H),17α(H)-ergostane | 217,218 |
| C29S | * | $C_{29}$ 20S-5α(H),14α(H),17α(H)-stigmastane | 217,218 |
| C29bbR | * | $C_{29}$ 20R-5α(H),14β(H),17β(H)-stigmastane | 217,218 |
| C29bbS | * | $C_{29}$ 20S-5α(H),14β(H),17β(H)-stigmastane | 217,218 |
| C29R | * | $C_{29}$ 20R-5α(H),14α(H),17α(H)-stigmastane | 217,218 |

[a]TR26a and TR26b cannot be resolved with current column settings at our laboratory, thus were combined for modeling.

**Table 3.** Total PAH concentration (ng/g device) and source model results for passive samplers. Source model values can range from −1 (pyrogenic) to +1 (petrogenic). Values near 0 indicate no definitive source.

| location | model | TPAH |
|---|---|---|
| KNO114A | 0.017 | 77.14 |
| KNO114A | -0.083 | 26.25 |
| KNO114A | -0.083 | 54.30 |
| KNO114A | -0.083 | 37.05 |
| KNO114A | -0.050 | 20.36 |
| KNO114A | -0.017 | 7.39 |
| KNO114A | -0.117 | 17.74 |
| KNO114A | -0.067 | 23.40 |
| KNO114A | -0.017 | 8.71 |
| KNO114A | -0.067 | 9.25 |
| KNO114A | -0.067 | 9.17 |
| KNO114A | -0.050 | 14.37 |
| KNO114A | -0.017 | 6.56 |
| KNO114A | 0.000 | 4.94 |
| KNO114A | -0.017 | 9.02 |
| KNO114A | -0.017 | 8.57 |
| KNO114A | -0.017 | 9.22 |
| KNO114A | 0.000 | 4.43 |
| Blank, field | -0.017 | 26.23 |
| Blank, field | -0.067 | 57.98 |
| Blank, lab | 0.000 | 0.00 |
| Blank, lab | 0.000 | 0.00 |
| Blank, trip | -0.033 | 51.41 |
| Blank, trip | -0.067 | 18.44 |

**Fig 1.** Sites surveyed during June 2015 in western Prince William Sound.

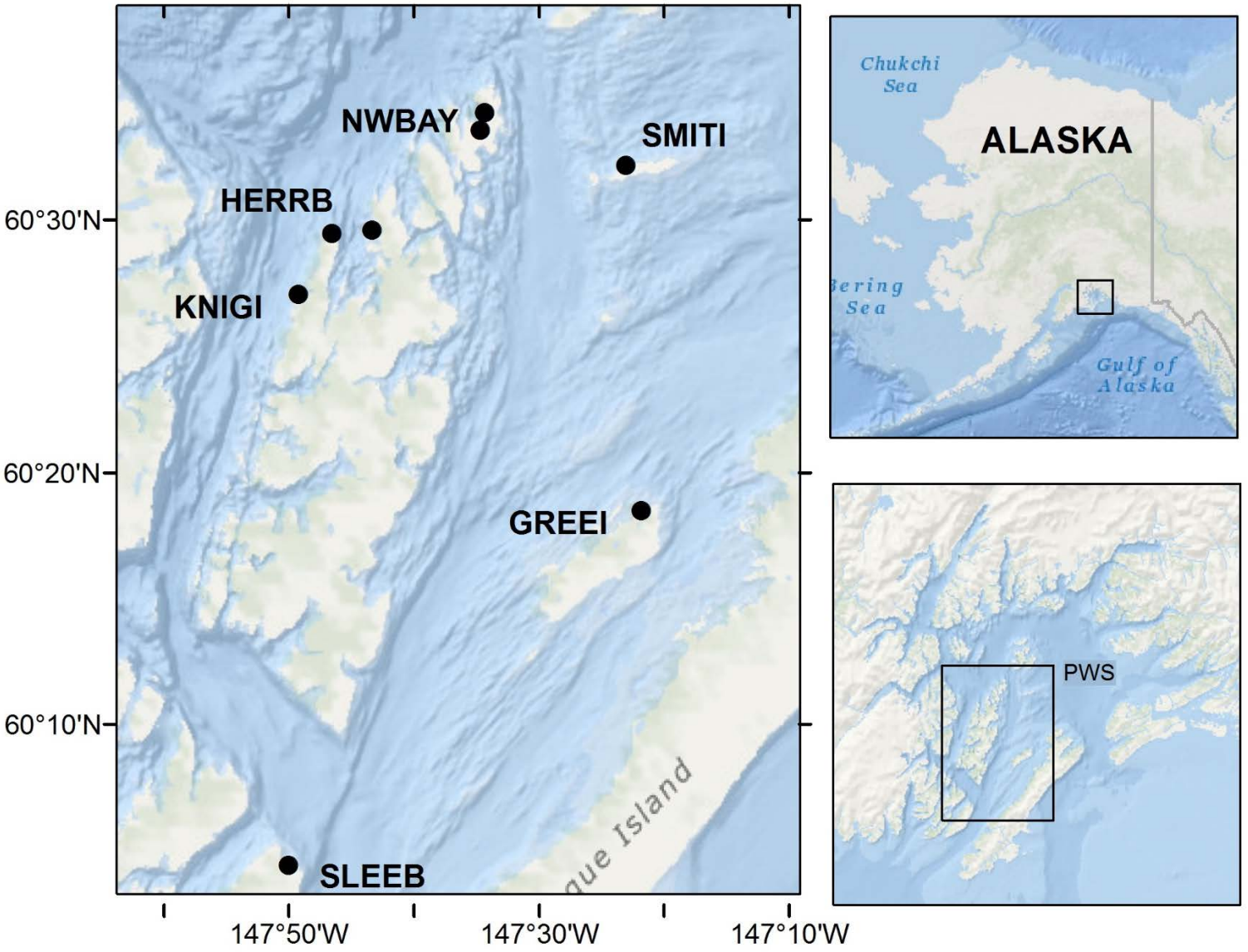


**Fig. 2.** Biomarker weathering example. Least weathered (top) to more weathered (bottom).

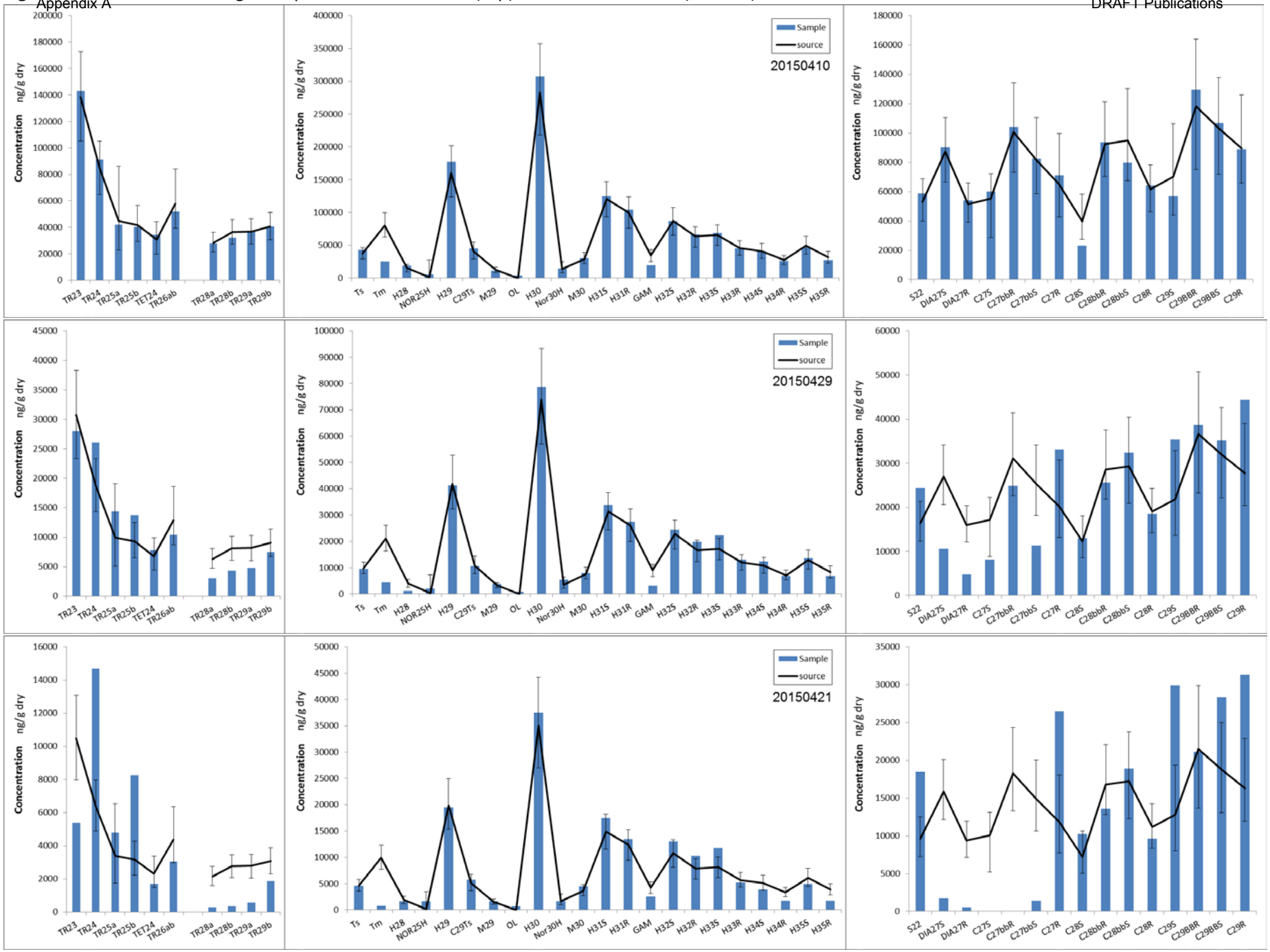


**Fig. 3.** Triterpane weathering residuals (top panel), calculated for TR28a – TR29b only and sterane weathering residuals (bottom panel), calculated only for DIA27S – C27bbS. *\\nmfs.local\AKC-ABL\RECA\Chemistry\Hydrocarbons\Projects\SCAT\SCAT_LTM_2015\Results & analyses\graphics\T & S residuals.eps .jpg. see All SCAT sediment v2.xlsm, sheet "Biomarker Stats" Illustrations were generated with Minitab.*

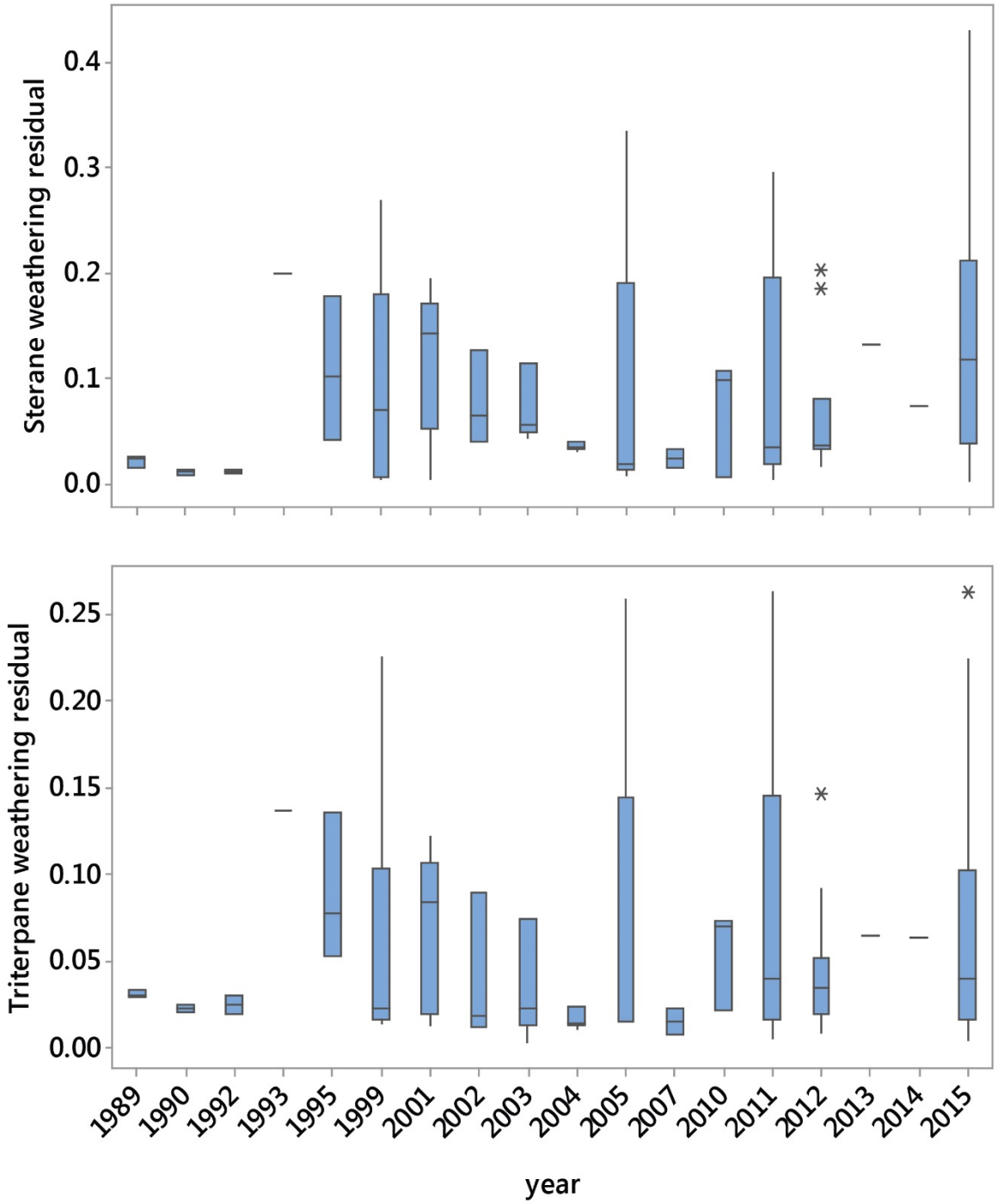
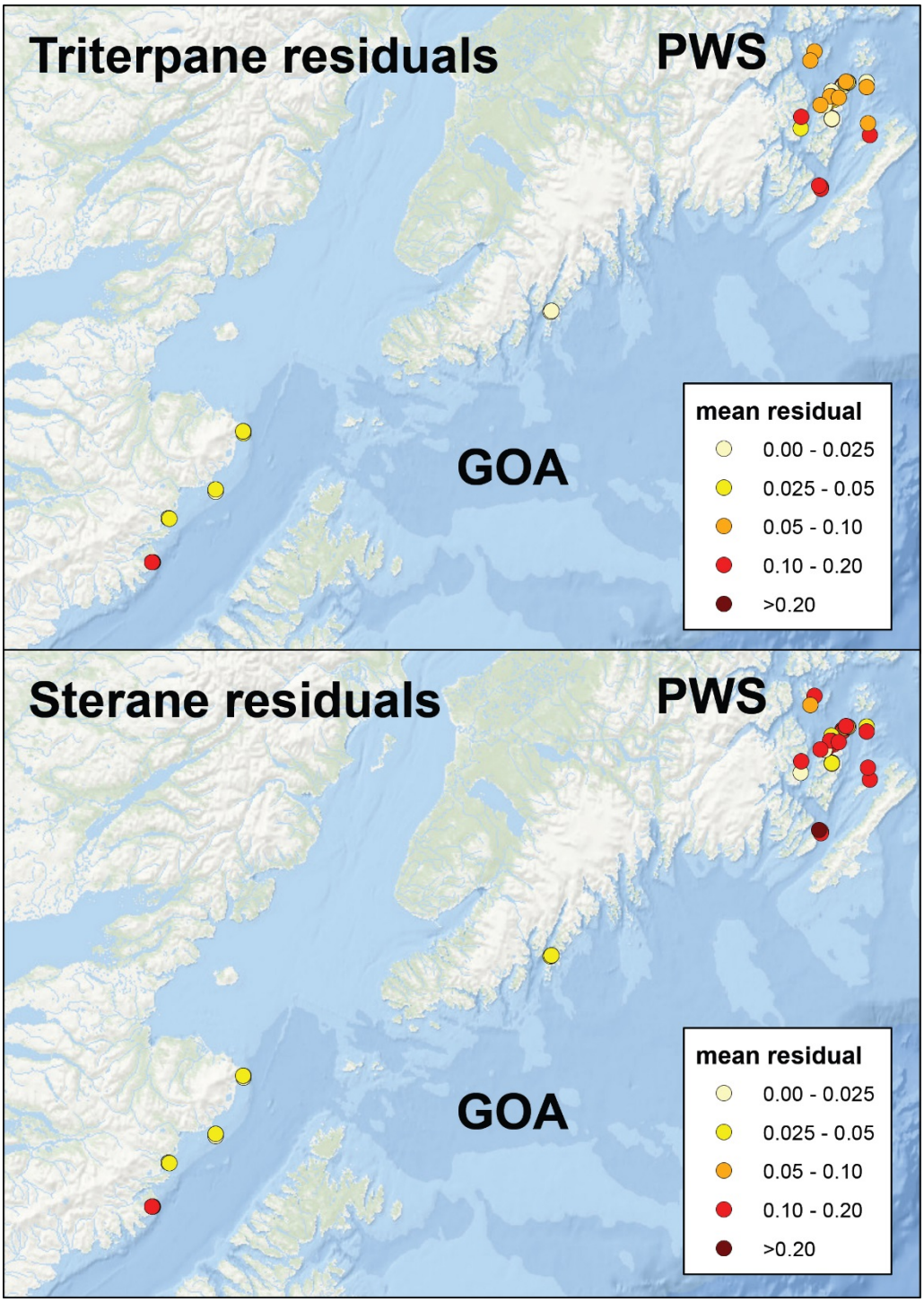


**Fig. 4.** Geographic and temporal distribution of the sum of the absolute residuals of variable triterpanes (TR28a-TR29b) and steranes (DIA27S-C27bbs) with respect to source oil. See SI 1 for further detail. *\\nmfs.local\AKC-ABL\RECA\Chemistry\Hydrocarbons\Projects\SCAT\SCAT_LTM_2015\Maps\T & S w residuals.eps (.jpg). These were generated from biomarker weathering.mxd. Source data are located in SCAT 2015 biomarkers.mdb. This all derives from "all SCAT sediment v2.xlsm" and associated spreadsheets.*



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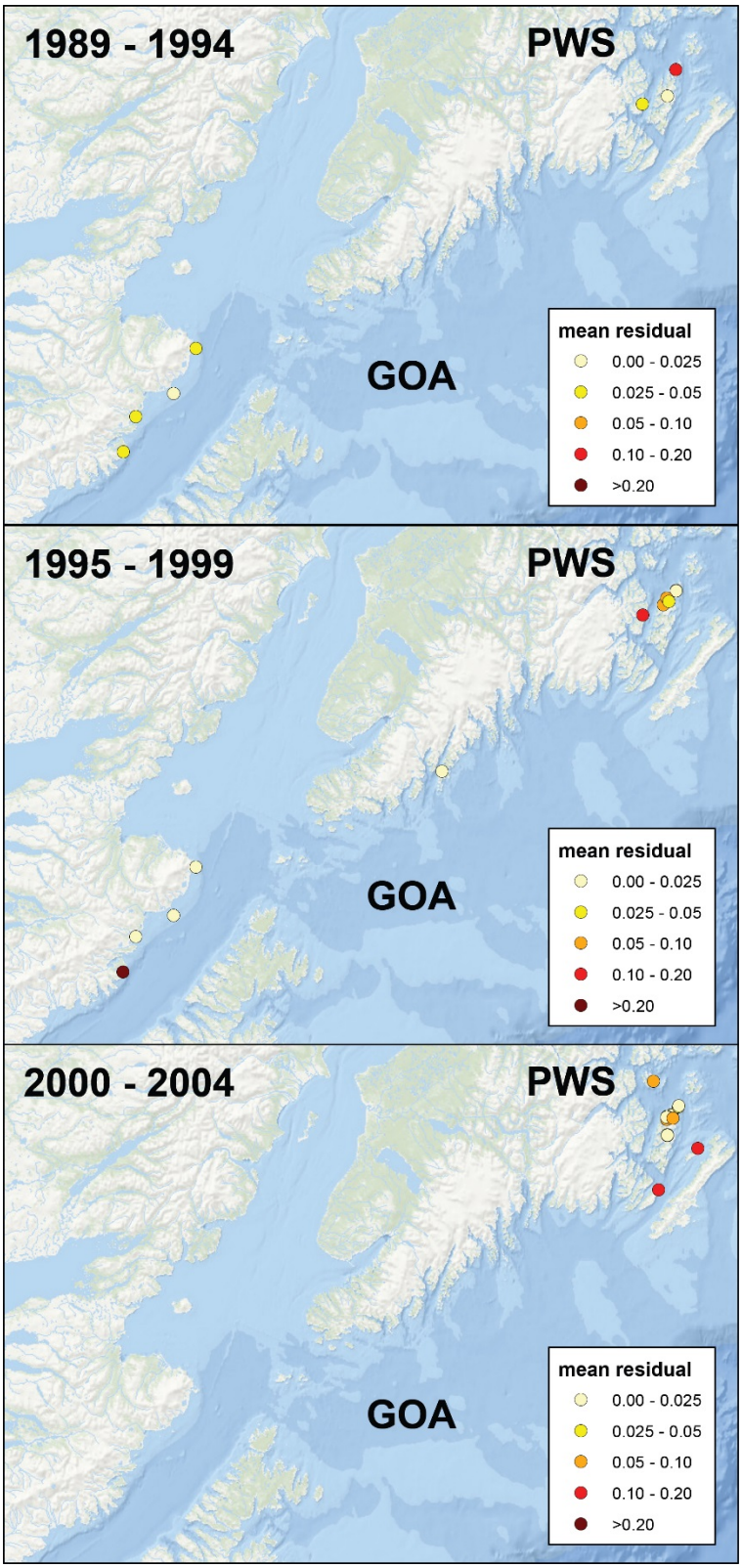
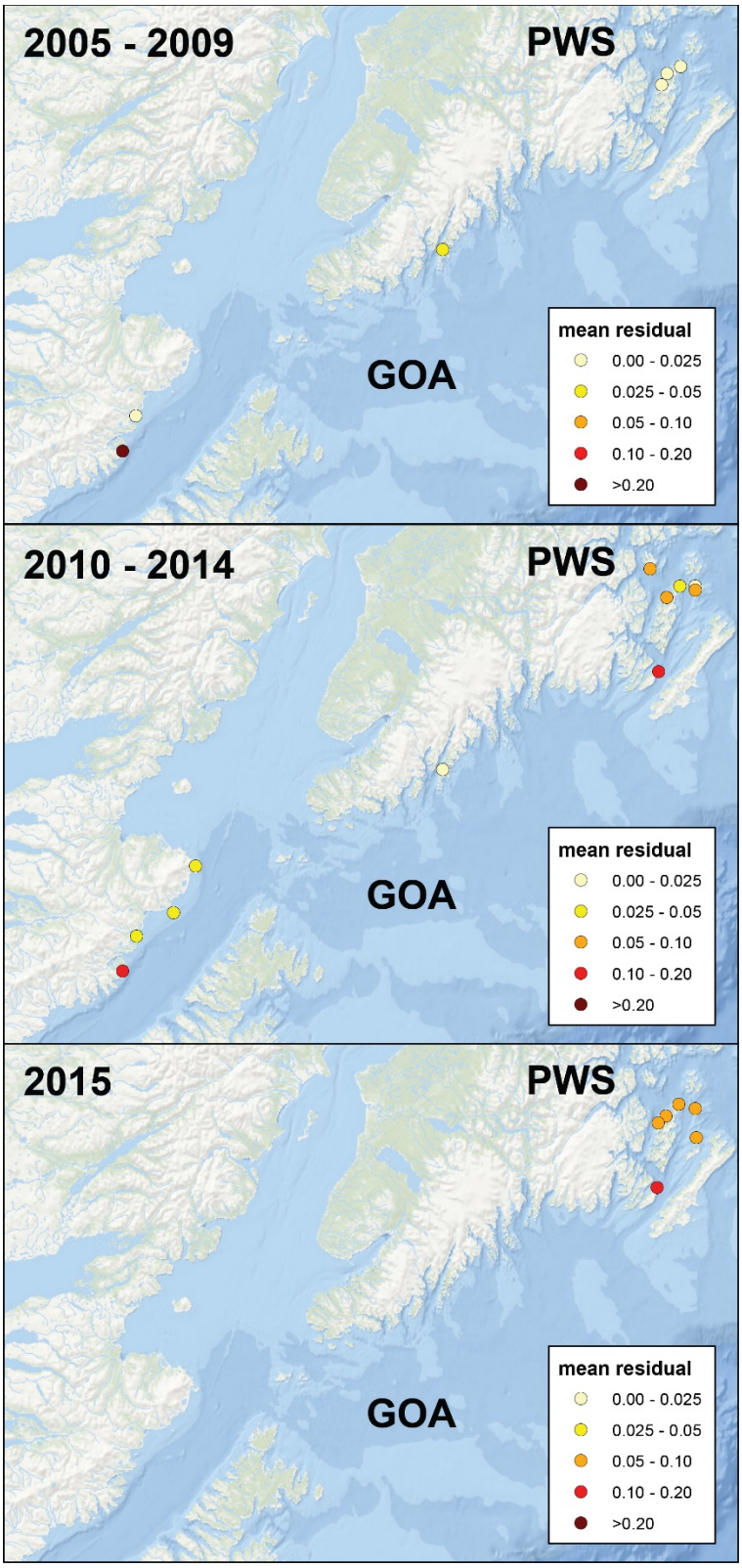
SI 1. Geographic and temporal distribution of the sum of the absolute residuals of variable triterpanes (TR28a-TR29b) with respect to source oil.




2005 - 2009
PWS
GOA
mean residual
0.00 - 0.025
0.025 - 0.05
0.05 - 0.10
0.10 - 0.20
>0.20
2010 - 2014
PWS
GOA
mean residual
0.00 - 0.025
0.025 - 0.05
0.05 - 0.10
0.10 - 0.20
>0.20
2015
PWS
GOA
mean residual
0.00 - 0.025
0.025 - 0.05
0.05 - 0.10
0.10 - 0.20
>0.20