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Quantitative estimates of isopod resource utilization using a Bayesian fatty acid mixing model

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ABSTRACT: Herbivorous primary consumers are a key intermediate trophic linkage between primary production from microalgae, macrophytes, and vascular plants and higher-level consumers. Fatty acid (FA) biomarkers are useful for evaluating trophic interactions in aquatic ecosystems because of clear phylogenetic separation of algal group FA signatures. We used a FA-based Bayesian mixing model (FASTAR) to generate quantitative diet estimates of 3 algal phyla for an intertidal herbivorous isopod, *Idotea wosnesenskii*, at 6 sites in Puget Sound, Washington, USA. We generated a 'resource library' of FA signatures of isopods fed diverse algal diets in 10-wk feeding trials and used these empirical data to parameterize FASTAR, thus accounting for isopod modification of dietary FA. The FA profiles of fast-growing juvenile *Idotea* were closely related to the signatures of their diets, and several polyunsaturated FA (PUFA) were highly correlated between diet and consumer (e.g. $\Sigma C_{18}\omega6 + C_{18}\omega3$, 20:4ω6, and 20:5ω3). We used the model to characterize individual isopod diet variability within sites and to test whether isopods utilize specific algal phyla preferentially or in similar proportions to algae available in the field. The results identified both variation in resource utilization among individual isopods within certain sites, and site level similarities with total available algal cover. Body mass index of wild isopods was highest at sites where the model indicated high utilization (e.g. >30%) of both green and brown algae and low support from red algae. This novel FA-based mixing model approach demonstrated the potential for quantitative diet estimations of fast-growing aquatic herbivorous consumers.

KEY WORDS: Fatty acids · *Idotea wosnesenskii* · Feeding trial · Biomarker trophic enrichment · Bayesian mixing model · Quantitative diet estimation

INTRODUCTION

Marine food webs are supported by energy from photosynthetic organisms, including phytoplankton, seagrasses, and benthic micro- and macro-algae. The value of these basal resources as food for heterotrophs depends upon many factors, including ingestibility, digestibility, and biochemical composition (Brett et al. 2009). Which resources are important for a given consumer depend upon their availability in the environment and the food quality parameters that enable the consumer to maximize fitness (Stephens & Krebs 1986). Macroalgae and seagrasses (collectively macrophytes) account for a large proportion of nearshore primary production (e.g. Mann 1973, Duggins 1980, Duarte & Cebrian 1996) in the form of fresh biomass or detached detritus (Krumhansl & Scheibling 2012). Detrital resources are known to provide important subsidies to secondary production in intertidal and beach fringe habitats (Hagen et al. 2012).

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Benthic herbivorous crustaceans represent an important trophic pathway for the contribution of nearshore primary production to upper trophic level consumers, such as fish (Edgar & Shaw 1995). Isopods in the globally distributed genus *Idotea* are key grazers in marine littoral systems, and sometimes impart top-down control to algal communities (Engkvist et al. 2000, Leidenberger et al. 2012). Despite the functional importance of primary herbivores in food webs, little is known about which resources actually support particular invertebrate grazers in the field. Tracking the relative contribution of disparate primary producer groups into consumers is challenging because stomach content analyses may be both biased towards indigestible remnants of prey (Dalsgaard et al. 2003) and unfeasible for smaller bodied consumers (~1–5 mm) of small (~2–500 µm) food particles. For primary consumers, aside from diagnostic siliceous diatom frustules in gut contents, it is generally possible to only identify very coarse primary producer categories (e.g. 'detritus'; Cranford & Grant 1990, Steinarsdottir et al. 2009). To overcome these limitations, stable isotope (SI) ratios and fatty acid (FA) signatures are commonly used as biochemical markers for tracing sources of basal production into consumer diets (reviewed by Dalsgaard et al. 2003, Fry 2006, Kelly & Scheibling 2012). A benefit of the biomarker approach is that FA and SI in consumer tissues represent time-integrated dietary information about both what consumers are eating over weekly to monthly scales and which dietary sources are most valuable, and hence selectively retained in tissues.

The FA composition of macrophytes and microalgae differ phylogenetically among orders (Galloway et al. 2012) and classes (Taipale et al. 2013), respectively. These differences in basal resource FA can be tracked into many marine and freshwater consumers (Brett et al. 2009). For example, experiments have demonstrated strong correlations ($R^2 > 0.9$) and ~1:1 relationships between certain algal polyunsaturated FA (PUFA) and resulting consumer PUFA in invertebrate consumer tissues (Brett et al. 2006, Milke et al. 2006). Because of the large number of FA identified in any given sample (e.g. >30), such datasets are typically analyzed using multivariate techniques that incorporate the complex 'signature' (Iverson 1993) of many FA proportions. These multivariate approaches can be used to ask whether FA signatures are different among species, seasons, or habitats (Richoux & Froneman 2008, Guest et al. 2010) or to evaluate qualitative patterns. Rarely, however, can we make quantitative estimates of resource contributions to consumer diets because such efforts require that diet-to-consumer biomarker modification is known and accounted for. Such modification occurs when invertebrates selectively catabolize, or modify FA molecules from dietary precursor FA (Taipale et al. 2011, Kelly & Scheibling 2012). For example, animals cannot synthesize long-chain (e.g. $\geq C_{20}$) ω3 or ω6 PUFA de novo, but some can bioconvert them through modification of related short carbon chain precursor FAs (Brett & Muller-Navarra 1997, Dalsgaard et al. 2003).

Unlike the generally qualitative approach used with FA, SI are often used in quantitative mixing models (Moore & Semmens 2008, Parnell et al. 2010) to generate estimates of consumer diets based on the SI ratios of their potential prey and expected 'trophic enrichment factors' (TEF; Parnell et al. 2010). An appealing aspect of the SI-based modeling approach is that sample processing is relatively cheap compared to other biomarkers and analyses can be contracted on a fee-for-service basis. Another major reason why SI-based modeling has become so common is that there are relatively few SI variables used in these models (^{2}H, ^{13}C, ^{15}N, and ^{34}S) and significant effort has been put into empirically measuring TEF for isotopes of C and N for a wide variety of consumers. Researchers therefore commonly utilize published TEFs (e.g. ~0.4–1‰ for $\delta^{13}C$, and ~3–4‰ for $\delta^{15}N$; Post 2002) or conduct experiments designed to measure enrichment (Yokoyama et al. 2005). However, application of SI TEFs from the literature without knowing whether such values are relevant for the species of study is still common (Martínez del Rio et al. 2009) and problematic because TEFs vary widely among individuals and groups (Caut et al. 2009) and can have very significant effects on mixing model analyses (Bond & Diamond 2011).

Several limitations to the strictly SI-based mixing model approach can theoretically be addressed through the use of additional biomarkers. SI ratios are not taxon 'specific' and often do not differentiate many of the likely basal resources that are supporting consumers (Vuorio et al. 2006), whereas primary producer FA signatures differ phylogenetically and are sometimes entirely unique to a group. For example, there are group-specific C_{16} PUFAs in chlorophytes (16:2ω6, 16:3ω3, and 16:4ω3) and bacillariophytes (16:2ω7, 16:3ω4, and 16:2ω4; Taipale et al. 2013), and distinctive C_{16} monounsaturated FAs (MUFAs) in methane oxidizing bacteria (Taipale et al. 2012). In addition, there are frequently too few available SI tracers relative to the number of potential dietary sources, which can lead to mathematically 'underdetermined' mixing problems (Fry

2013a). Modern Bayesian mixing models can be applied to any number of sources (Ward et al. 2011, Semmens et al. 2013), but the potential underdetermined limitation remains unresolved and debated (Fry 2013b). Analysis of a theoretical consumer biomarker dataset showed that increasing the number of variables (by adding FA to the otherwise SI-based models) enhances the performance of a Bayesian mixing model to resolve expected consumer diets (Dethier et al. 2013).

Here, we used a combination of experimental feeding trials, field sampling, and a FA-based mixing model to estimate dietary contributions of key primary producers to the intertidal herbivorous isopod *Idotea wosnesenskii* (hereafter *Idotea*). We tested 3 general hypotheses: (1) that isopod FA profiles would reflect the FA composition of their macroalgal diets in controlled feeding trials; (2) that wild isopod algal consumption at diverse sites with differing algal availability could be explained quantitatively with a FA based mixing model; and (3) that the modeled algal consumption by wild isopods would vary among sites and individuals depending on algal standing stock at the field sites and according to expected isopod preferences. We used the feeding trials to identify FA variables that were correlated between diets and isopods, generated a 'resource library' of FA signatures of isopods fed these algal diets, and analyzed the FA signatures of wild-collected isopods using FASTAR (FA Source Tracking Algorithm in R; Galloway et al. 2014) — a Bayesian mixing model adapted from MixSIR (Moore & Semmens 2008) for estimating consumer diets. To ask whether wild isopods feed selectively according to expected preferences published for other isopods (e.g. Bell & Sotka 2012), we compared diet estimates from FASTAR with the measured available algal cover at each field site.

MATERIALS AND METHODS

Feeding trials

We conducted 2 feeding trials (summer 2012 and 2013) with 7 different dietary treatments from 3 macroalgal phyla (Table 1). We used fast-growing juvenile *Idotea* in long (10 wk) feeding trials so that diet-to-consumer FA modification was measured in organisms that had accrued the large majority of their tissues while consuming the treatment diets. We collected adult female *Idotea* with broods from the intertidal zone at Eagle Cove (EC), San Juan Island, Washington, on 6 June 2012 and 9 June 2013. Adults were transferred to aerated 2 l aquaria with 0.3 µm filtered seawater in a climate-controlled room with a natural diel light cycle. Juvenile *Idotea* were removed from brooding females, distributed randomly (n ≈ 100 per replicate) into 3 replicate aquaria per treatment diet, and starved for 2 d before the start of the feeding trials. Feeding trial diets were selected because these macroalgae are readily consumed by isopods in the laboratory (M. Eisenlord & M. Dethier unpubl. data) and are locally abundant representatives of algae consumed by other temperate isopods (e.g. Bell & Sotka 2012). Previous work has shown that these algae differ in their FA signatures at the phylum level (Galloway et al. 2012).

Algae for use in feeding trials were collected from the subtidal (*Nereocystis*) or intertidal (other taxa) zones in the vicinity of Friday Harbor Laboratories (FHL), except for the epiphytic *Smithora naiadum*, which was offered to isopods on blades of its host surfgrass *Phyllospadix scouleri*, collected from EC. In the feeding trial, isopods clearly did not consume *Phyllospadix* itself. For both experiments, we changed the water and provided new food at 48–72 h intervals, carefully removed unconsumed algae, and added sufficient (ca. 4 cm^2 pieces) fresh algae to allow ad libitum feeding. Three replicate fresh algal tissue (diet) samples were prepared as if for feeding and then frozen for

Table 1. Constituents of the isopod 'resource library' and their species codes. Each of the algal species was fed to isopods for 10 wk to establish the FA signatures of animals fed diets from each of 3 phylum-level sources, prior to evaluation of the wild isopods using the FASTAR mixing model. Two diets (*Fucus* and *Ulva*) were offered in 2 different feeding trials and these additional signatures are therefore pooled (see 'Materials and methods'). The *Smithora* treatment included host seagrass blades (which were not consumed by isopods)

Phylum (no. of taxa)	Type	Species	Trial no.	Replicates	Code
Ochrophyta (3)	Brown	*Nereocystis luetkeana*	1	3	1
		Fucus distichus	1, 2	5[a]	2
		Saccharina sessilis	2	3	3
Chlorophyta (1)	Green	*Ulva* sp.	1, 2	6	4
Rhodophyta (3)	Red	*Mazzaella splendens*	1	3	5
		Porphyra sp.	2	3	6
		Smithora naiadum	2	3	7

[a]Only 2 *Fucus* replicates could be analyzed from Trial 2

future FA analyses at roughly the beginning, middle, and end of each feeding trial. The mean daily experimental temperature throughout the 10-wk trials (which ran from mid-June through August 2012 and 2013) was 13.7 ± 1.4°C (mean ± SD), and is representative of summer sea surface temperatures in the vicinity (A. W. E. Galloway unpubl. data). Trial duration was based upon pilot studies showing that juvenile *Idotea* more than double in size during this time frame. At the end of the trial, animals were measured from tip of the head to the end of the pleotelson under a dissecting microscope, starved for 24 h to purge the digestive tract of algal diets, and frozen.

Wild animal collection and site characterization

We collected adult male *Idotea* (22.5 ± 4.6 mm, mean ± 1 SD) between 24 June and 6 July 2013 for FA analysis and quantitative diet modeling from 6 sites in the Salish Sea with diverse site characteristics: FHL, EC, Cattle Point (CP), Ledgewood (LG), Richmond Beach (RB), and Magnolia (MN) (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m507p219_supp.pdf for site coordinates and descriptions). We selected sites that had populations of *Idotea* but varied in available algal cover and dominant substrate type (see Fig 3). Five replicate animals were taken from each site to FHL and starved for 48 h to clear their digestive tracts. At each site, we surveyed 2 perpendicular transects for algal and substrate composition. The first (alongshore) transect was positioned at mean lower low water (MLLW), and a vertical transect ran from this line up to the top of the algal zone. The MN alongshore transect was approximately 2 m horizontally above MLLW due to logistical constraints. Along each transect, 10 haphazardly located quadrats were used to quantify percent cover of algae and underlying substrate type, following Dethier et al. (1993). Algal data are presented as percent cover normalized to 100% (to control for differences in total cover) for comparison with estimates of wild isopod resource utilization from the mixing model analyses.

Fatty acid extraction

Samples were stored at −20°C for <2 mo, lyophilized for 48 h and ground with an acetone prewashed stainless steel mortar and pestle prior to extraction. FA methyl esters (FAME) were extracted (following Taipale et al. 2011, Galloway et al. 2013) from 5–10 mg of dried tissue using a 4:2:1 chloroform:methanol:water mixture. These samples were then sonicated and vortexed 2 times, and the organic phases removed and pooled, and FA were transmethylated at 50°C for 16 h using 1% sulfuric acid as a catalyst. Extracted FAME were ultimately dissolved in 1.5 ml hexane for analysis using gas chromatography (GC). We analyzed FAME with GC-Flame ionization detection (FID; HP 6958, Agilent DB-23 column), and an 85-min temperature program (Taipale et al. 2011) designed to separate C_{16} and C_{18} MUFA and PUFA. Peak identification was achieved primarily using GC-FID and a 40 FA standard (Nu-chek Prep standard 569B), and the identity of unknown peaks was determined by lining up retention times of putative peaks previously identified (Galloway et al. 2013) with GC-Mass spectrometry (MS) using the same column and identical temperature program.

Analyses

Feeding trials

We evaluated total log-transformed *Idotea* growth (in mm) across treatments using univariate ANOVA with Welch's test of equality of means and Hochberg's GT2 post-hoc tests (due to unequal sample size within treatments). We calculated the mean proportion of each FA across all samples from both feeding trials and excluded rare FAs that comprised <0.1% of the total FAs, resulting in a dataset of 28 FAs that were renormalized to 100%. We further reduced this dataset to 8 PUFAs for FASTAR modeling. The FA signatures of the algal diets and the experimental *Idotea* fed those diets (28 FA and 8 PUFA datasets) were compared separately using permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) followed by pairwise tests. PERMANOVA is a powerful alternative to traditional MANOVA for analysis of FA data because it does not require that data conform to multivariate normality or that the number of variables does not exceed the number of sampling units (Anderson 2001). Multivariate FA data were visualized using non-metric multidimensional scaling (NMDS), using the 'ordiplot' and 'ordihul' functions in the Vegan library in R (R Development Core Team 2013). We used a correlation analysis to compare FAs in algal diets and to isopods fed those diets for 8 FA categories or individual FAs that were selected *a priori* (following Brett et al. 2006). FA categories and abbreviations used are reported in Table 2.

Wild animals

We calculated a body mass index (BMI) for the wild isopods — (dry weight in mg / [length in mm]^3) × 100 (Jormalainen & Tuomi 1989) — and compared BMI among sites with 1-way ANOVA with Tukey's LSD post-hoc tests. We calculated the mean, normalized proportion of the same 28 FAs for all wild samples (n = 30 isopods) used above. Prior to modeling analyses, we reduced the analytical dataset to 8 PUFAs, due to a lack of general correlations between diet and isopod saturated FA (SAFA) and MUFA (see 'Results'). We compared wild isopod FA signatures from each site using 1-way PERMANOVA on both FA data sets, followed by post-hoc pairwise tests. All univariate ANOVAs, coefficients of determination, and associated Pearson correlation p-values were calculated with SPSS v. 19.0 for Mac. PERMANOVA tests used fixed factors and Type III SS, and significance was determined using unrestricted permutation of the raw data (9999 permutations), using Monte-Carlo (MC) generated p-values when the number of unique permutations was <200. Multivariate significance tests were performed using PRIMER v.6.0 and PERMANOVA+ (PRIMER-E; Anderson et al. 2008).

Modeling

We used the feeding trial data to generate a 'resource library' of FA signatures of *Idotea* fed 7 primary producer diets from all 3 macroalgal phyla available in the wild (Table 1). The resource library therefore inherently accounts for FA modification across the trophic interface from algal diets to isopod consumers and does not utilize unidirectional fractionation or enrichment factors (e.g. as commonly used for stable isotopes) because 'source' FA profiles entered into the FASTAR model are actually signatures of isopods experimentally fed those pure monoculture source diets. This approach further accounts for the likely event of diet-specificity in biomarker modification for consumers (Budge et al. 2012, Prado et al. 2012, Rosen & Tollit 2012, McLeod et al. 2013) because diet-to-consumer biomarker modification is directly determined in feeding trials. The *a priori* rationale for the 3-source, phylum-level library is based on our previous work showing that primary producer FA signatures at the phylum level differ (Galloway et al. 2012) and that seasonal variation in macroalgal FA signatures does not overwhelm these phylum-level differences (Dethier et al. 2013). The 8 FAs used for modeling (16:4ω3, LIN, ALA, SDA, ARA, EPA, 22:5ω3, and DHA) were those PUFAs that had a mean value of >1% among all experimental animals, or, if the mean was <1%, had a coefficient of variation in all animals >1. This approach provided a repeatable method by which we could select PUFA that were both relatively abundant and also variable (and therefore potentially important for discriminating) among samples.

We used the recently developed FASTAR mixing model (Galloway et al. 2014) to determine the proportional contribution of the basal resources in our library to wild isopod consumers based upon the consumer FA signatures. The FASTAR model is adapted from the SI mixing model MixSIR (Moore & Semmens 2008). The model iteratively assesses potential combinations of the possible food sources to select combinations that best reflect the FA profiles found in the consumers. This is done in a Bayesian context (see Moore & Semmens 2008); thus, FASTAR provides a posterior distribution that describes the probability of each potential food source's proportional contribution to the biomass of the individual. In the present application, FASTAR performs this analysis for each wild isopod individual replicate (n = 1) and then summarizes the posterior distribution for a group (all individuals from a given site, n = 5) as simply the sum of the individual posterior distributions. We used a multivariate uniform prior for the proportions (Dirichlet) and residual error is estimated directly from the varia-

Table 2. Definitions of FA categories and abbreviations used and results of bivariate correlations of individual FA or sums of FA within categories (see 'Materials and methods') between the diets and the *Idotea* fed those diets in the feeding trials. Each comparison reports the coefficient of determination (R^2), associated Pearson Correlation p-values (n = 26 in each correlation), and the linear relationship ($y = a + bx$) between the FA content of isopods and their diets

FA category (definition)	Abbreviation	Diet to *Idotea* correlation		
		R^2	p-value	Relationship
Saturated	SAFA	0.007	0.676	$y = 26.0 + 0.02x$
Monounsaturated	MUFA	0.038	0.339	$y = 25.6 - 0.09x$
18:2ω6 (LIN)+18:3ω6 (GLA)	$\Sigma C_{18}\omega6$	0.615	<0.001	$y = 0.86 + 0.45x$
18:3ω3 (ALA)+18:4ω3 (SDA)	$\Sigma C_{18}\omega3$	0.792	<0.001	$y = 0.34 + 0.47x$
LIN+GLA+ALA+SDA	$\Sigma C_{18}\omega6+C_{18}\omega3$	0.757	<0.001	$y = 0.93 + 0.47x$
20:4ω6 (ARA)	ARA	0.837	<0.001	$y = 2.74 + 0.73x$
20:5ω3 (EPA)	EPA	0.859	<0.001	$y = 12.4 + 0.47x$
ARA+EPA	ΣARA+EPA	0.781	<0.001	$y = 16.4 + 0.51x$

tion among the potential sources. Results are presented graphically as probability densities or as the 5%, 50% (median), and 95% (i.e. 90% Bayesian credibility interval, BCI) of the posterior distributions, which were estimated using a Markov Chain Monte Carlo (MCMC) Gibbs sampling algorithm implemented using the open source Just Another Gibbs Sampler (JAGS) software (Plummer 2003) within R. For each of the 6 sites, we compared the proportion of each source to the consumer derived from FASTAR (using the median of the group posterior distribution as the best point estimate with the 90% BCI) and the proportional cover of that algal group in the field. The FASTAR code used is freely available at www.ecologybox.org (search term: 'FASTAR').

RESULTS

Feeding trials

Growth

Juveniles were harvested at the same time each year and did not differ in initial size between years (combined initial size of 3.1 ± 0.1 mm; hereafter mean ± 1SD). In both feeding trials and all treatments, juvenile *Idotea* increased in size by 5.7 ± 2.5 mm over 10 wk, an average increase of 182 ± 78%. *Idotea* growth across algal diet treatments differed overall (ANOVA, $F_6 = 651.99$, $p < 0.0001$) and growth differed between all treatments tested (Hochberg's GT2, $p < 0.001$), except between the *Saccharina* and *Ulva* treatments, with the rankings of (highest to lowest % size increase): *Smithora* > *Porphyra* > *Ulva* = *Saccharina* > *Fucus* > *Mazzaella* (Fig. 1).

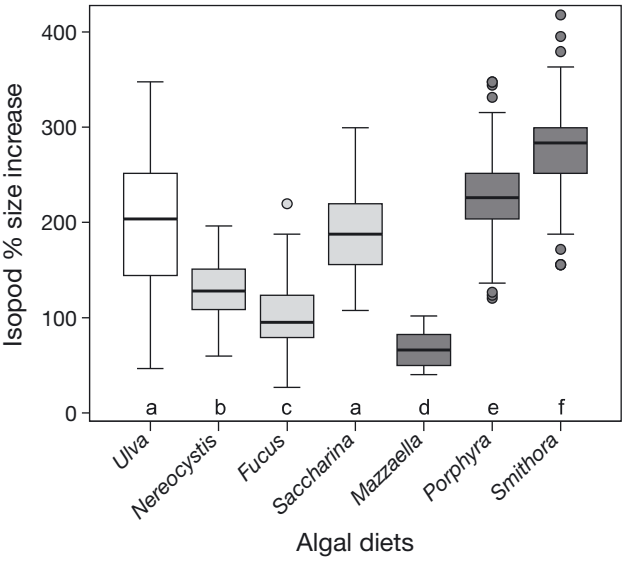

Fig. 1. Boxplot (median, quartile range and 95% CI whiskers, and outlier points) of percent size (mm) increase of juvenile *Idotea* during two 10-wk feeding trials, shaded according to 3 phyla used: Chlorophyta, white; Ochrophyta, light grey; Rhodophyta, dark grey. Growth measurements were taken on all isopods in each of the 3 replicate aquaria for all diets (total of 1727 isopods). Two diets (*Ulva* and *Fucus*) were offered in both feeding trials, and therefore there are 6 replicate aquaria for these treatments (see Table 1). Letters indicate which treatments differed in a log-transformed growth ANOVA with Hochberg's GT2 post-hoc tests

Fatty acids

Total proportional SAFA (Fig. 2a) and MUFA (not shown) were not correlated between diet and consumer (Table 2 reports all 8 calculated bivariate correlations). All ω3 and ω6 PUFA categories and individual PUFAs evaluated were significantly and positively correlated between the algal diets and *Idotea* fed those diets, and diets were generally enriched in the C_{18} ω3 and ω6 FAs relative to isopods with intercepts close to zero (Table 2, Fig. 2b). There was an approximate 1:1 relationship between diets and isopods for the longer-chain C_{20} PUFA, particularly for ARA, which also had a low intercept (Fig. 2c). Isopods were clearly enriched in EPA relative to the diets with low EPA (i.e. intercept ~12%), but slightly depleted relative to the high EPA content red algal diets (Fig. 2d, Table 2).

The reduction of the datasets from 28 FAs to 8 PUFAs for modeling (see 'Materials and methods') did not cause any changes in multivariate treatment-level or site-level significance tests. The FA signatures of isopods fed different phyla of algae strongly differed overall (PERMANOVA; 28 FAs, Pseudo-$F_2 = 57.72$, $p = 0.0001$; 8 PUFAs, Pseudo-$F_2 = 85.16$, $p = 0.0001$) and between each other in post-hoc pairwise tests (both 28 FAs and 8 PUFAs, $p = 0.0001$ for all comparisons).

Wild animals

Site characteristics

The BMI of wild *Idotea* differed overall among sites (ANOVA, $F_5 = 3.67$, $p = 0.013$). The mean BMI (with 95% confidence interval [CI] of the mean) for each site, in order from highest to lowest was: MN = 1.09 (0.83–1.35), EC = 0.95 (0.82–1.06), FHL = 0.94 (0.82–1.06), RB = 0.77 (0.56–0.98), CP =

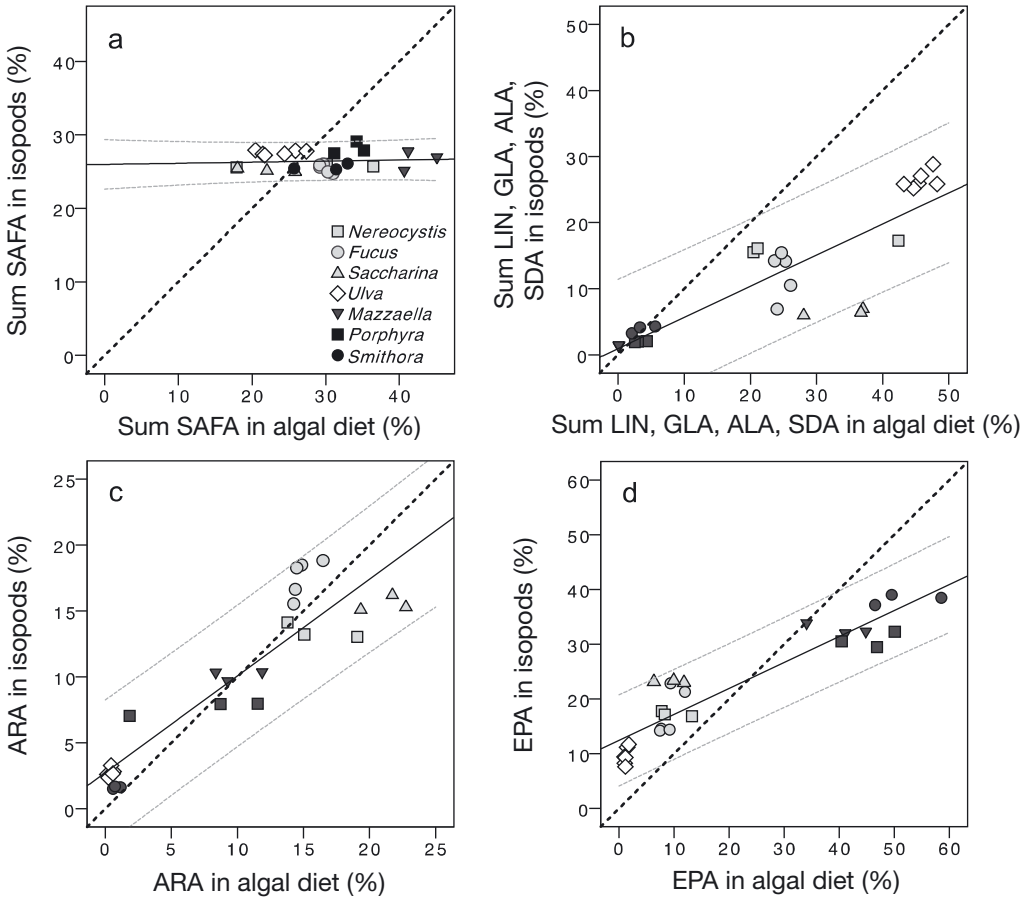

Fig. 2. Bivariate plots of correlations between FAs in the algal diets and in the *Idotea* fed those diets. Panels show different FA categories or individual FAs: (a) SAFA, (b) sum of $C_{18}\omega6$ and $C_{18}\omega3$ FAs (LIN, GLA, ALA, and SDA), (c) ARA, and (d) EPA. All plots also show the 1:1 relationship (black dashed reference lines). The grey dotted lines are 95% CI of the individuals. See Table 2 for details

0.77 (0.60–0.93), and LG = 0.75 (0.54–0.97). Post-hoc tests (Tukey's LSD) identified that the differences were all between the MN site and the CP (p = 0.004), RB (p = 0.004), and LG (p = 0.003) sites. The 6 study sites varied in composition of underlying substrate (Fig. 3a), total percent algal cover, and algal composition (Fig. 3b).

Fatty acids

The FA signatures of wild isopods differed overall (PERMANOVA; 28 FAs, Pseudo-F_5 = 13.47, p = 0.0001; 8 PUFAs, Pseudo-F_5 = 14.57, p = 0.0001) and between all pairwise site comparisons (28 FAs p < 0.01; 8 PUFAs p < 0.05) except for the CP and LG sites (28 FAs p = 0.222; 8 PUFAs p = 0.195). The FA signatures (8 PUFAs) of both wild isopods and the experimental isopods comprising the source library for FASTAR modeling were visualized as a 3-source multivariate resource polygon in Fig. 4. Summary FA data for all isopod FA analyzed in all treatments and sites are presented in Table S2 in the Supplement.

FASTAR analysis

The FASTAR analysis of wild isopods identified considerable variation in proportional contributions of potential sources among individuals within certain sites (i.e. FHL, CP, MN), and more uniform resource utilization at other sites (i.e. EC, RB; Fig. 5). The range and central tendency of the posterior probabilities of each source contribution at each site is summarized in Table 3 as the median and 5th to the 95th percentile range of mixture solutions. Visual comparisons between the median FASTAR diet estimate for an algal group and the proportional algal cover at each site in the field (Fig. 6) showed a range of similarities between modeled algal use by isopods and availability across sites. The LG and RB sites had small differences (<10% for all algal groups), FHL, EC, and CP exhibited moderate differences (~5–20% and variable among algal groups), and MN had large differences (>30% for green and brown algal groups; Fig. 6). The differences for green and brown algae were not unidirectional across sites, but the model consistently indicated lower resource utilization of

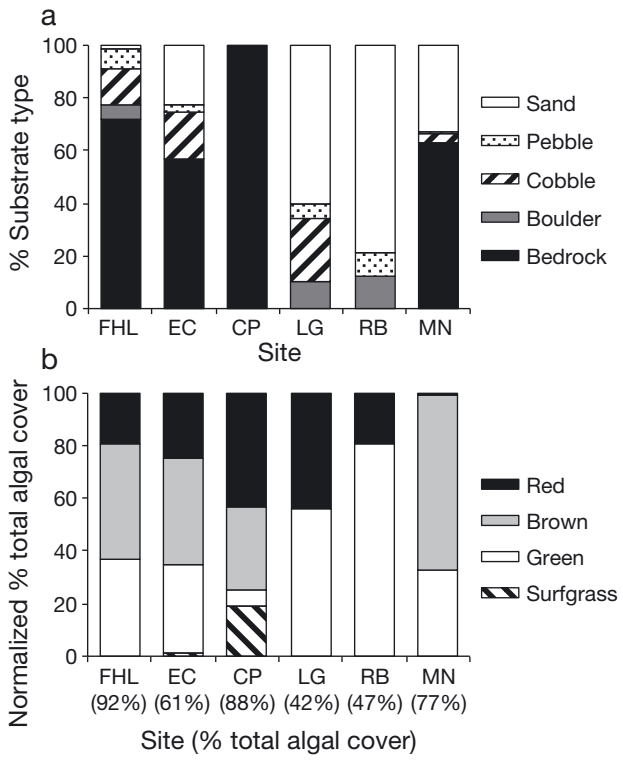

Fig. 3. Site characteristics of the 6 *Idotea* collection sites: Friday Harbor Labs (FHL), Eagle Cove (EC), Cattle Point (CP), Ledgewood (LG), Richmond Beach (RB), and Magnolia (MN) (see also Table S1 in the Supplement at www.int-res.com/articles/suppl/m507p219_supp.pdf). (a) Percent cover of substrate type, normalized to 100%. (b) Summary of percent macrophyte cover of 4 macrophyte categories, normalized to 100% to control for site differences in total % algal cover (in parentheses under site codes) among sites

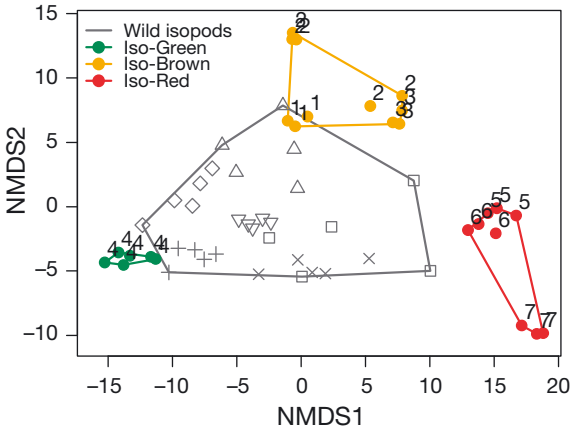

Fig. 4. NMDS plot of the 8 common PUFA datasets in wild and experimental *Idotea* (Iso) fed each of the algal diets (see codes in Table 1) with convex polygons defining each group by the algal phylum it was fed on in multivariate space. The NMDS uses Euclidean distance, and the 2D stress for the plot is 0.03. Each colored circle represents the signature of isopods in replicate aquaria fed known diets for 10 wk. The source library thus accounts for diet-specific trophic modification of PUFA by isopods (see 'Materials and methods'). Grey symbols denote the FA signatures of the wild isopods from 6 sites (upward triangle = FHL, downward triangle = EC, square = CP, cross = LG, plus sign = RB, and diamond = MN; site abbreviations are defined in Fig. 3)

red algae relative to their availability in the field at all sites (Fig. 6).

DISCUSSION

We have demonstrated an approach for generating quantitative estimates of resource utilization by a wild herbivorous consumer based on the FA compositions of individuals that were fed known diets. The critical steps involve (1) identifying that the biomarker signatures are different in the basal resource groups (Galloway et al. 2012), (2) clarifying the basal resolution of these biomarkers once source 'variation' is included (Dethier et al. 2013), (3) measuring the transfer of biomarkers across the plant–animal interface and developing a relevant source library of FA signatures of a consumer fed these resources in controlled feeding trials, and (4) using this information to model what conspecific wild consumers are eating based on their biomarker signatures. We followed these steps and used the FASTAR mixing model to generate resource use of wild isopods based on their independently collected FA signatures. This 4-step approach has also recently been used to model assimilation by cladoceran zooplankton of 7 basal resource groups, including phytoplankton, bacteria, and terrestrial organic material, in large boreal lakes (Galloway et al. 2014). Our analyses here indicate that the differences in FA content in individual isopods, like cladocerans, can be quantified and attributed to individual isopod-level resource utilization.

The FASTAR model results identified considerable variation among individual isopods with respect to resource use within a given population, and showed that this variability differed among sites. Individual consumer biomarker analyses have been used to describe variability in trophic niche width for large terrestrial carnivores at the scale of multiple populations (Semmens et al. 2009). Our analytical scale of resolution is novel for invertebrate herbivores, and was possible because of the diet specificity of the sources in our library and design of our model to solve for diets of individual consumers. Differences between sites in the among-individual variability of resource use were likely due to isopod food prefer-

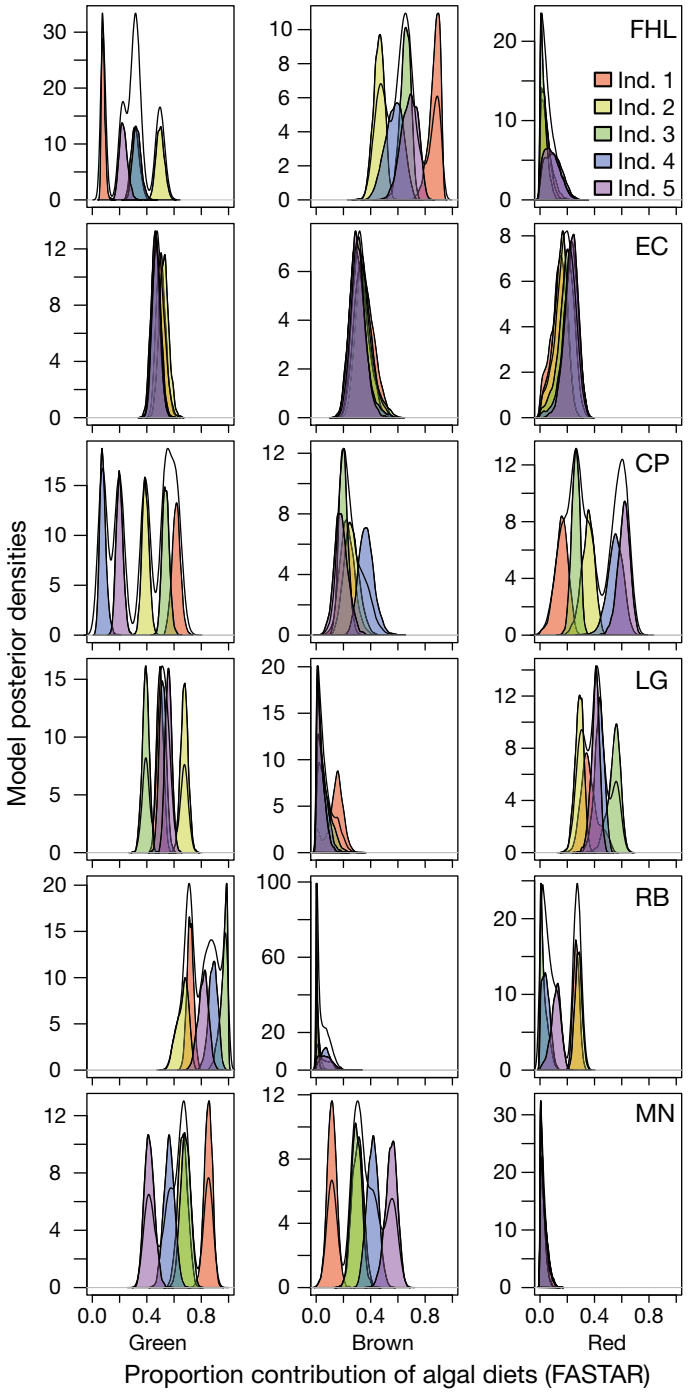

Fig. 5. FASTAR estimates of the proportional utilization of algal resources by wild isopods from 3 phyla — green (Chlorophyta), brown (Ochrophyta), and red (Rhodophyta) — at 6 sites in Puget Sound. The model was run using 8 PUFAs and the source library was assembled from independent feeding trials (see Table 1, 'Materials and methods'). Site abbreviations are defined in Fig. 3. Each plot shows the posterior density (*y*-axis) of results from the Bayesian mixing model FASTAR for a given dietary source (*x*-axis). The FASTAR solution for each individual isopod (n = 5 replicates from each site) is reported (colored distributions) along with the 'group-level' solution compiled from all replicates (non-colored distribution)

ences and several potentially interacting additional factors including the degree of beach protection (exposure), tidal height at collection location, food patchiness, and substrate characteristics (Fig. 3). For example, the Cattle Point (CP) site had the largest individual diversity (i.e. non-uniform distributions) in FASTAR diet solutions (Fig. 5). CP also had the lowest proportion of green algal cover of any site, yet the model indicated that this resource was a significant contributor to the group (90% BCI = 6–64), particularly to certain individuals. Because FASTAR did not misattribute green algae as a source to animals actually fed brown or red algal diets (see Table S3, Fig. S1 in the Supplement), it is unlikely that the significant proportions estimated for green algae at this site are erroneous. We hypothesize that diet diversity of CP is due to relatively high exposure and wave energy at that site (100% bedrock, long SW fetch); isopods may stay in individual tide pools and thus experience small-scale differences in prey availability, including patchiness in the distribution of preferred food (such as *Ulva* sp.). In contrast, the FASTAR diet predictions at Eagle Cove (EC) were very uniform across individuals. The EC site characteristics (semi-protected, diverse substrate cover, diverse algal cover) may have allowed isopods greater mobility and access to the diversity of algal diets present, thereby leading to more consistent utilization of the preferred diets within each algal phylum.

The novel site level diet estimates for wild isopods using FASTAR showed that despite among-individual variation within each site, isopods were generally supported by the most abundant resources in their local habitats. Because previous work on species within this isopod genus (*Idotea balthica*) has shown that isopods often prefer (Bell & Sotka 2012), and grow faster on green algal diets (e.g. *Ulva* sp.) relative to other foods (Wernberg et al. 2013), we hypothesized that the model would indicate preferential utilization of available green algae. Growth data from our laboratory experiments indicated that the percent size increase of juvenile isopods varied among diets but was highest in animals fed *Smithora* (an epiphyte of seagrass) and *Porphyra* (both red), as well as *Ulva*, *Saccharina*, and *Fucus* (brown) (Fig. 1). At the site level, the results showed that the median model estimates for brown algal consumption by wild isopods at EC, CP, RB and MN were very

Table 3. FASTAR results summary (using 8 PUFA dataset) of wild isopods from 6 Puget Sound sites (Fig. 3). Data represent the 5th, 50th, and 95th percentile (i.e. 90% Bayesian credibility interval, BCI) of the FASTAR estimates of proportional assimilation of 3 algal diets by isopods at each of the sites, gathered post-hoc from the individual replicate-based analysis (see 'Materials and methods')

Site	n	Green		Brown		Red	
		Median	90% BCI	Median	90% BCI	Median	90% BCI
FHL	5	0.30	(0.07–0.52)	0.64	(0.43–0.90)	0.05	(0.00–0.17)
EC	5	0.48	(0.42–0.56)	0.32	(0.23–0.45)	0.19	(0.06–0.29)
CP	5	0.39	(0.06–0.64)	0.23	(0.14–0.41)	0.35	(0.12–0.65)
LG	5	0.52	(0.38–0.69)	0.05	(0.00–0.18)	0.41	(0.26–0.58)
RB	5	0.81	(0.63–0.98)	0.03	(0.00–0.13)	0.12	(0.01–0.30)
MN	5	0.65	(0.39–0.87)	0.33	(0.09–0.58)	0.02	(0.00–0.07)

close (i.e. within 10%) to the brown algal cover at those sites. At sites with no brown algal cover (LG and RB) the median FASTAR estimates for contributions of brown algae to isopod diets were correspondingly very low (5 and 3%, respectively; Table 3). The model results identified that isopods consumed a higher proportion of brown algae at FHL, and a lower proportion of brown algae at MN than expected based on algal cover. The algal community data, which was collected at the genus level, indicated that the red algal diets which resulted in the highest growth in our experimental animals (*Smithora* and *Porphyra*) were available only at LG and CP, respectively, corresponding to the sites for which FASTAR predicted the highest contributions of red algae to the diets of wild isopods (Figs. 5 & 6; Table 3).

The site with the highest BMI of wild isopods was MN, which was dominated by the 2 diets that experimental animals grew fastest on (*Ulva* sp. and *Fucus*). The median FASTAR estimates of resource utilization for animals at this site (65% green, 33% brown, 2% red) may therefore indicate an ideal dietary mixture for isopod physiological requirements. The 3 sites with the relatively higher BMI (MN, FHL, and CP) generally grouped together in the upper left portion of the wild isopods polygon in the NMDS plot (Fig. 4), and while the FASTAR solutions from these sites were not characterized by one particular diet mixture of the 3 resource groups, all 3 sites had both abundant brown algal cover (Fig. 3b) and relatively high model estimates of brown algal consumption by isopods (Fig. 6). Isopod BMI was also likely driven by other factors than diet (as estimated by FA composition); for example, wild isopods with the lowest BMI were from either sandy sites with relatively low total algal cover

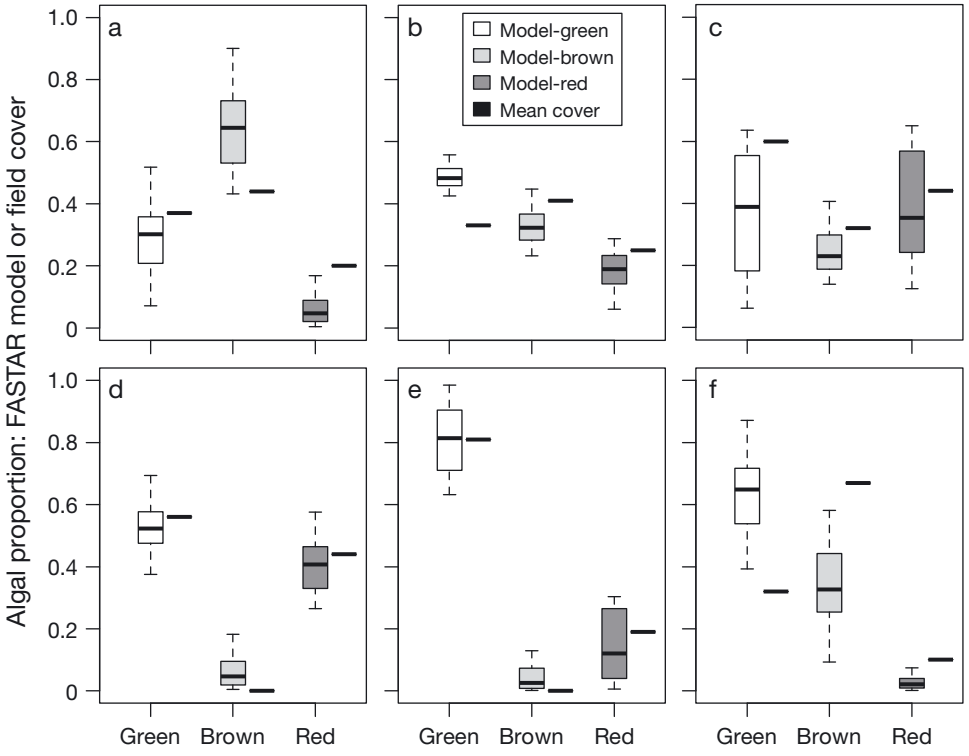

Fig. 6. Differences between the FASTAR model prediction of isopod resource utilization for each algal group (box plots show median as bold internal line, and 25th–75th and 5th–95th percentiles as box boundaries and whiskers, respectively), and the mean% normalized algal cover available (as independent black reference mark adjacent to each box plot) at each site. Panels: a) FHL, b) EC, c) CP, d) LG, e) RB, f) MN (see Fig. 3)

(42% at LG and 47% at RB) or from the highly exposed CP site, which was dominated by bedrock (Fig. 3) and had few interstitial hiding places. The FASTAR results have thus generated several hypotheses about the role of cover and habitat complexity in isopod foraging behavior, which could be evaluated in future experimental field studies.

Analyses of the 2 FA datasets (28 FA and 8 PUFA) showed that *Idotea* fed algae with distinct FA signatures were readily distinguished (Fig. 4). In addition, species-specific algal FA signatures were clearly reflected as differences among experimental isopods (e.g. brown algal species code 1–3 in Fig. 4). The phylum-level resolution of our resource library was developed based on previous work (Dethier et al. 2013) which showed that seasonal variation in FA signatures in macrophytes may be greater than the order-level, but not phylum-level, resolution. With the exception of the green algal biomarker 16:4ω3, other PUFAs utilized in our mixing model analyses were present in some amount in all resource library phyla. The presence of taxon-specific FAs or clear differences in proportions of FAs among taxa greatly strengthens the applicability of an FA-based mixing model approach for estimating consumer diets. For example, *Ulva* sp. had uniquely high levels of 16:4ω3 (~18% of total FA vs. 0% in other diets); and animals fed *Ulva* sp. retained this green algal marker (16:4ω3 = 3.4 ± 0.4% compared with 0% in *Idotea* fed other diets). In contrast, stable isotopes could not have been used to resolve this question, as even 3 SI signatures cannot separate green algae ($\delta^{13}C$ = −15.0 ± 1.8; $\delta^{15}N$ = 6.9 ± 0.1, $\delta^{34}S$ = 20.2 ± 0.6; n =12 from 3 sites and 2 seasons) from the brown macroalgal genera used in this study ($\delta^{13}C$ = −14.1 ± 2.1; $\delta^{15}N$ = 6.5 ± 0.8, $\delta^{34}S$ = 21.0 ± 0.5; n = 53 from 3 species, 3 sites and 2 seasons; means and SDs calculated from Dethier et al. 2013).

Our experimental data showed that while the proportions of several PUFAs in macroalgae were correlated with those PUFAs in the isopods (Table 2), the relationship between the modifications of FAs from dietary sources to consumer tissues depended on the food source. Generally, C_{18} PUFAs, which animals may bioconvert to metabolically active long-chain ω3 and ω6 highly unsaturated FAs (see Dalsgaard et al. 2003), were relatively enriched in the algae (Fig. 2b), while C_{20} PUFAs were enriched in the isopods. For example, *Idotea* maintained relatively high levels of ARA (~2.5%) and EPA (~10.5%), even on the *Ulva* sp. diet, which had very low levels of these FAs (~1.5%). Proportions of ARA increased in nearly a 1:1 relationship (Fig. 2c), and EPA was also strongly linearly correlated between algal diet and *Idotea* tissue (Table 3, Fig. 2d), indicating the potential usefulness of these 'essential' PUFAs as biomarkers for isopod diets. The greater than zero intercepts for the lines of C_{20} ω6 and ω3 FAs (Table 2, Fig. 2c,d) can also be used to quantify this pattern. EPA and ARA were also identified as excellent dietary biomarkers in the freshwater zooplankter *Daphnia pulex* and the juvenile spat of the scallop *Argopecten irradians* (Brett et al. 2006, Milke et al. 2006, respectively), and EPA was an important predictor of growth in *Daphnia* (Muller-Navarra et al. 2000) and the freshwater isopod *Asellus aquaticus* (Lau et al. 2013). Such diet-to-consumer relationships should not be assumed to exist in unstudied taxa, and may not be apparent in short-term feeding trials (e.g. McLeod et al. 2013) or for slow growing invertebrates that have not had sufficient time to turn over biochemical components of their tissues (Taipale et al. 2011). A similar caution applies to the application of experiments designed to measure trophic fractionation of stable isotope biomarkers (Martínez del Rio et al. 2009, Bond & Diamond 2011, Layman et al. 2012).

The use of FAs as biomarkers for quantitative diet estimation of consumers was first advanced by Iverson et al. (2004) in the form of quantitative fatty acid signature analysis (QFASA). It is well understood by QFASA's practitioners and critics that experimental data describing trophic enrichment of biomarkers from diets to consumer tissues (referred to as 'calibration coefficients', CC, in the QFASA literature) is critical to the appropriate use and interpretation of the model (Iverson et al. 2004, Budge et al. 2012, Rosen & Tollit 2012). QFASA has generally been applied to predators (Thiemann et al. 2008), which are difficult to keep in controlled, replicated laboratory conditions under diverse diets (Rosen & Tollit 2012). Our approach does not make the assumption that trophic modification of biomarkers is consistent among taxa or tissues; the library signatures (and associated variation) used for modeling wild isopod diets were independent and measured in the experimental isopods themselves. Future experimental work could compare FA trophic enrichment measured here with other aquatic isopods (e.g. *Idotea balthica* and the freshwater isopod *Asellus aquaticus*) to test the phylogenetic specificity of FA modification for related crustaceans.

The Bayesian-based approach used here differs substantially from the QFASA model, which uses a distance-minimizing approach to select the proportion of prey items that constitute the best fit for the predator diet. Distinct from a frequentist analytical approach, which treats the parameters as fixed and the data as random, a Bayesian analysis assumes that

the parameters are unknown and that the data are fixed, and describes the parameters probabilistically based on the observed data (Semmens et al. 2013). The Bayesian model results characterize the range of highly likely mixing solutions given the data and model assumptions, and therefore provide a probabilistic picture of the contributions of several potential resources to a consumer. Few studies have measured FA diet-to-consumer modification systematically for a diverse array of diets and for a consumer that has grown significantly on these diets (but see Galloway et al. 2014). Such studies are critical for future biomarker-based approaches involving both SI and FA. Indeed, the diversity of slopes and intercepts we documented in the diet to isopod FA correlations shows that trophic modification of dietary FA is not consistent for all FAs among these diverse diets.

Experimental evaluation of the model performance on a subset of the library resources indicated that for certain brown algae (e.g. *Saccharina*) FASTAR may underestimate the actual contribution of brown algal diets and misclassify a portion (e.g. median ~16%) of the diet to red algae. FASTAR did not misattribute sources for animals fed 100% green or red algal diets (see Table S3, Fig. S1 in the Supplement). This misattribution error between *Saccharina* and red algae is probably due to the fact that isopods fed *Saccharina* had higher levels of EPA (~22%) than other brown algal sources, making this diet closer to the high EPA content (~30% of total FA) found in all red-algal fed isopods (see Table S2 in the Supplement). This bias likely did not confound our wild isopod diet estimates because *Saccharina* was not generally abundant at our study sites. For consistency, the resource library was based upon isopods raised on freshly collected algal diets. However, isopods in the field may consume fresh and detrital algal material in varying stages of decay. Because senescent algal detritus is likely colonized by microbial communities (Sosik & Simenstad 2013) and aged and fresh algal diets may differ with respect to FA composition (Galloway et al. 2013), future research could evaluate the effects of diet decay on isopod growth and biochemical composition (sensu Dethier et al. 2014, Raymond et al. in press). While not used in the resource library here (due to slight differences in experimental methods), the PUFA profiles of *Idotea* raised in a pilot study on aged *Nereocystis* diets overlapped in multivariate space with the animals raised on fresh *Nereocystis* (A. W. E. Galloway and M. E. Eisenlord unpubl. data), indicating that the phylum-level variation captured in our resource-library here is likely to be robust to the degradation state of algal diets.

We have shown how a series of feeding trials can be used to build a resource library of FA signatures of a consumer eating unique diets, and how this library can be used in a Bayesian modeling framework to generate quantitative estimates of resource consumption in wild herbivores. The PUFA content of isopods was generally highly correlated with their algal diets, so we focused modeling efforts on these FAs. It is important to note that our modeling approach did not require 1:1 relationships between diet and consumer FAs; all FAs could have theoretically been included in the model regardless of the degree of trophic modification because our resource library is based upon the signatures of the isopods fed those diets. Our results showed that individual isopods within a local population can be expected to vary with respect to their resource utilization or preferences, but also identify that the general site-level resource assimilation by isopods is still related to the algal cover available. Two particular strengths of the FA mixing model approach used here that set it apart from previous SI-based mixing model analyses are that the FA signatures of algal resources are distinctive and there are more FA variables than there are resources in the model, thus bypassing potential complications of 'underdetermined mixing problems' (Fry 2013a,b, Semmens et al. 2013). Our FA-based mixing model estimates of wild isopod resource utilization provide a novel example of a multi-step methodology that can be applied to other fast-growing herbivorous aquatic consumers for which biomarker trophic modification has been measured.

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