



Flipping for Food: The Use of a Methane Seep by Tanner Crabs (*Chionoecetes tanneri*)

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Methane seep habitats are widespread chemosynthesis-based ecosystems that span continental margins and interact with surrounding marine systems. With many of these habitats occurring below 200 m, seeps can serve as an important source of nutrients in otherwise food limited deep-sea environments. However, the potential for marauding megafauna to assimilate seep derived nutrition has been difficult to quantify. Here, we provide the first evidence of a commercially harvested species, *Chionoecetes tanneri* (tanner crab), assimilating chemosynthetic production. Although bulk isotope analysis of *C. tanneri* tissue indicated no quantifiable incorporation of seep-derived carbon or sulfur (mean $\delta^{13}\text{C}$, -18.5‰ ; mean $\delta^{34}\text{S}$, 19.5‰), depletions in ^{13}C ($\delta^{13}\text{C}$ as light as -38.8‰) were noted in fatty acid (FA) compounds. In addition, diagnostic biomarkers for seep bacteria, including 16:1 ω 6 and 18:1 ω 8c FA's, were found to have been assimilated by *C. tanneri*. Further supporting a trophic link between the seep and the *C. tanneri*, seep associated bacteria and archaea were, in certain cases, the dominant taxa in the gut contents of the crabs. This work provides the first insights into a link between seep production and deep-sea ecosystem services, specifically fisheries production. In addition, it reveals a methodological bias that could exist in some trophic studies where bulk isotopes under-represent the role of seep nutrition in the diet of marauding animals.

Keywords: stable isotopes, fisheries, methane seep, microbiology, compound specific, fatty acid, chemosynthesis, trophic subsidy

INTRODUCTION

Deep-sea habitats provide a range of ecosystem goods and services to society (Armstrong et al., 2010; Thurber et al., 2014). These habitats are believed to be energy-limited ecosystems, with a slow rain of food from the surface driving community structure across all size classes (Rex et al., 1993; Wei et al., 2010). Microbially facilitated chemosynthetic production in deep-sea habitats, such as methane seeps can produce an abundant supply of food and support dense faunal communities (Levin, 2005; Cordes et al., 2010). However, the role these habitats serve for surrounding ecosystems and society remains largely unknown (Levin et al., 2016). Here we identify the unexpected use of chemosynthetic production by a commercially harvested species, *Chionoecetes tanneri* (tanner crab), identifying a novel link between seep ecosystems, fisheries, and humans. Through this we discover unexpected methodological biases that could have obscured quantification of export production from seep ecosystems in previous studies.

Methane seeps are increasingly becoming recognized as abundant areas of productivity along margin habitats, with over 1,000 methane seep sites discovered in the past 2 years along the Cascadia subduction zone alone (Bell et al., 2017; Riedel et al., 2018; Seabrook et al., 2018). Non-seep organisms have been observed at seeps, including commercially important species, such as longspine thornyheads (*Sebastolobus altivelis*; Grupe et al., 2015) and the Patagonian toothfish (*Dissostichus eleginoides*; Sellanes et al., 2012), however, the nature of these interactions are unclear. In 2012, a dense aggregation of *C. tanneri* were found inhabiting Clayoquot Slope, a methane seep off the coast of British Columbia. The crabs were observed actively foraging, with the crabs sifting through the sediment at actively bubbling regions, feeding both on and around microbial mats at the site, and in some cases “flipping” off the seafloor from bubble activity (Figures 1a–d; Supplementary Videos 1, 2). In 2014, an additional dense crab aggregation, of various size classes, around the nearby Barkley Canyon seep was also seen (Figure 1c). The observations of active foraging behavior by the *C. tanneri* specimen suggested that they may be using the seep as a source of trophic support. *Chionoecetes tanneri* are a commercially harvested species in the NE Pacific (Somerton and Donaldson, 1996; Kruse et al., 2005; Keller et al., 2012) that migrate across the margin (Keller et al., 2012, 2016). As a result, if they are harvesting seep production they could be exporting it to non-seep ecosystems and potentially to humans.

Quantification of the transfer of chemosynthetic production to the surrounding ecosystem has often led to enigmatic results, showing little to no use of chemosynthetic production by mobile organisms (MacAvoy et al., 2002, 2008). As microbial production can be difficult to quantify, biomarker analysis, including stable isotope and fatty acid analysis are often employed to measure the transfer of bacterial and archaeal production to higher trophic levels. Stable isotope analysis makes use of incorporations of carbon (C) and sulfur (S) from an organism's diet and can be used to disentangle food webs, if the food sources differ in their stable isotopic composition (Fry and Sherr, 1989; Van Dover, 2007; Boecklen et al., 2011). At seep systems, chemosynthetic bacteria and archaea have unique isotopic signatures compared to non-seep food sources. For example, methanotrophically-derived carbon ranges from -40 to -100‰ in $\delta^{13}\text{C}$, and thiotrophically-derived sulfur ranges from -25 to -35‰ $\delta^{34}\text{S}$ (Levin and Michener, 2002; MacAvoy et al., 2003, 2005; Levin, 2005; Boecklen et al., 2011). This contrasts with carbon and sulfur compounds derived from photosynthesis, which range from -15 to -25‰ $\delta^{13}\text{C}$ and 18 to 22‰ $\delta^{34}\text{S}$, respectively (Fry and Sherr, 1989; Boecklen et al., 2011). To get a more high-resolution view of an organism's diet, fatty acid (FA) analysis can be used, as certain FA compounds as well as branching and double bond patterns can be linked to specific types of producers (e.g., Thurber et al., 2013). Seep associated production can be identified through diagnostic biomarkers for methanotrophy and sulfate reduction, including the bacterial produced 16:1 ω 6, 16:1 ω 8, and 18:1 ω 8 FA's (Bowman et al., 1991; Thurber et al., 2012). The assimilation of photosynthetically produced food can be tracked with the essential FA's 20:5 ω 3 (eicosapentaenoic

acid), 22:5 ω 3 (docosahexaenoic acid), and 20:4 ω 6 (arachidonic acid) (Copeman et al., 2002; De Troch et al., 2012).

Molecular analysis of stomach contents provides an additional tool within trophic studies, as it allows the identification of what an organism has most recently consumed, including microbial taxa (*sensu*; Niemann et al., 2013). At seep sites, molecular gut content analysis can identify consumption of bacteria and archaea that dominate biogeochemical cycling at seeps including: sulfate reducing, sulfide oxidizing, and methanotrophic bacteria and methane oxidizing archaea (Knittel and Boetius, 2009; Valentine, 2011). With this method, Niemann et al. (2013) was able to identify a dominance of sulfide-oxidizing Epsilonproteobacteria in the guts of a lithoid crab, linking the microbial community of a microbial mat at a methane seep to the crabs diet. When used in conjunction with fatty acid and stable isotopic analysis, a holistic view of an individual's diet can be obtained. Each of the methods integrate an animals diet over different temporal scales and have the potential to parse out otherwise hard to constrain trophic linkages between highly mobile animals and chemosynthesis-based ecosystems. Here, we use bulk stable isotope, compound specific isotope analysis of Fatty Acid Methyl Esters (FAME), and molecular gut content analysis, to test whether *C. tanneri* is incorporating chemosynthetic production into their biomass. Further, we compare how each of these three methods reflect the diet of *C. tanneri* differently.

METHODS

Study Site and Sampling

Samples were collected from the methane seep habitats at Clayoquot Slope ($48^{\circ}40'15''$ N, $126^{\circ}50'52''$ W; depth: 1250 m), a site within the long-term seafloor monitoring sites of Ocean Networks Canada's (ONC) NEPTUNE cabled observatory (Barnes et al., 2010). Sampling occurred during two of ONC's yearly maintenance cruises with ROV Jason aboard R/V TG Thompson (Cruise TN328, Sept 2015), and ROV Hercules aboard E/V Nautilus (Cruise NA071, May–June 2016). Crab traps were deployed at Clayoquot Slope during both of these expeditions to collect *C. tanneri*. Once the traps were received on deck the *C. tanneri* specimens ($n = 11$ for 2015 collection; $n = 12$ for 2016 collection) were frozen at -80°C until further processing. Push cores were collected during these two expeditions, as well, from seep sites at Clayoquot Slope and the nearby Barkley Canyon, where a mass migration event of *C. tanneri* was observed in 2014, and non-seep sites at Cascadia Basin within the observatory network (site information in Supplementary Table 1). These cores were vertically sectioned at 0–1, 1–2, 2–3, and 3–5 centimeter intervals and preserved at -80°C until further processing.

Isotope Analysis

Bulk Isotope Analysis

The muscle tissue from the walking legs of the *C. tanneri* specimens was extracted from the specimen in the laboratory to measure $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ signatures. Walking leg tissues of

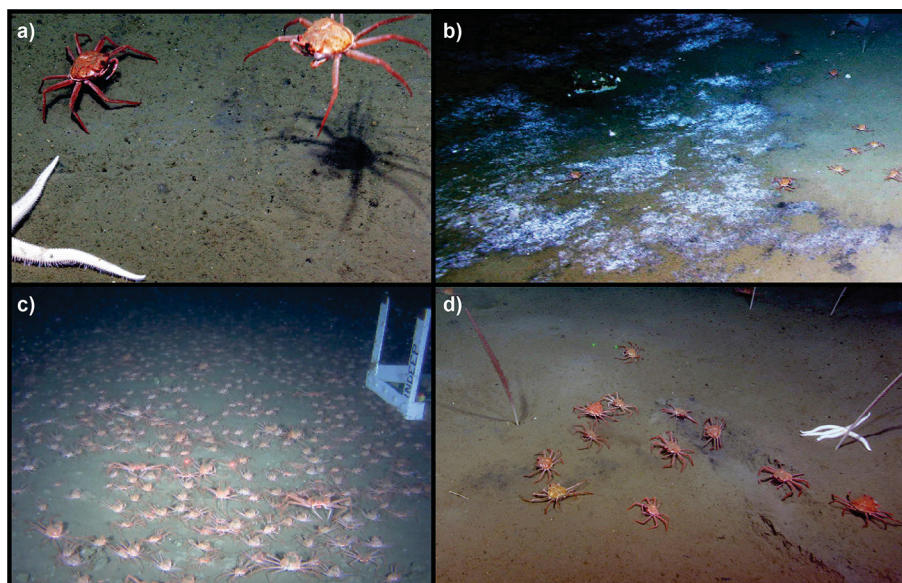


FIGURE 1 | (a,b,d) *Chionoecetes tanneri* at Clayoquot Slope seep showing active foraging behavior in the sediment amongst bubbles and on microbial mats **(a,b,d)** and being lifted off the seafloor by methane hydrates building up on its abdomen **(a)**. **(c)** A migration event of hundreds (estimated) of tanner crabs of multiple size classes at 1000 m depth in the nearby Barkley Canyon, in January 2015. Photos courtesy of Ocean Networks Canada.

C. tanneri specimens were dried at 60°C for 48 h. We additionally isolated tissue from the gut wall and gills of a subset of *C. tanneri* specimens. All tissues were dried at 60°C for 48 h, and acidified with 12 μ l of 1 M HCl (Thurber et al., 2010). Sediment samples were freeze dried and treated with 2N phosphoric acid to remove carbonates.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis

Dried tissue samples ($n = 23$; walking leg tissue, guts, and gills) and sediment samples (Clayoquot Slope: 4 cores; Barkley Canyon: 3 cores; Cascadia Basin: 4 cores) were packaged in tin capsules (0.3–0.7 mg dry weight) and analyzed using a Costech (Valencia, CA USA) elemental analyzer interfaced with a continuous flow Micromass (Manchester, UK) Isoprime isotope ratio mass spectrometer (EA-IRMS) for $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios at Washington State University. Measurements are reported in δ notation [per mil (‰) units] using ovalbumin as a routine standard. Precision for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was generally ± 0.2 and ± 0.4 ‰. Isotopic ratios are expressed relative to standards (Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen gas for $\delta^{15}\text{N}$).

$\delta^{34}\text{S}$ analysis

Dried walking leg tissue samples ($n = 10$, 5 from 2015 and 5 from 2016; 3–5 mg dry weight) were packaged in tin capsules for mass spectrometry and sent to UC Davis Stable Isotope Laboratory for $\delta^{34}\text{S}$ analysis. Samples were measured following their standard procedures on an Elementar vario ISOTOPE cube interfaced to a SerCon 20–22 IRMS (Sercon Ltd., Cheshire UK). The precision for the method employed at this facility is ± 0.4 ‰.

Compound Specific Isotope Analysis

To determine the quantity, type, and source of lipids assimilated into the biomass of *C. tanneri*, the Fatty Acid Methyl Esters (FAME) and associated C isotope signatures were analyzed. A direct transesterification procedure was performed to extract the FAME from the freeze-dried walking leg tissue of the *C. tanneri* specimens ($n = 23$; following Thurber, 2015). FAMES were quantified and identified using retention times on a Thermo Fisher Scientific TRACE 1310 gas chromatography—flame ionization detector (GC-FID) with a Thermo TR5 (length: 60 m, I.D.: 0.32 mm) column. Identification of compounds was in comparison to known standards (Supelco 37 Component FAME mix, Sigma Aldrich). GC-Mass spectrometer analysis on a Hewlett Packer (HP) 6890 gas chromatography system with a DB1 (length: 30 m, I.D.: 0.25 mm) column and a HP 5973 mass selective detector was used to confirm peak identify. Dimethyl disulfide (DMDS) adducts [derived following (Nichols et al., 1986)] were used to identify mono-unsaturated bond locations and also run on this instrument. Blanks were run with all batches, and all solvents were ACS grade or better. Fatty-Acids (FA) under 14 carbon lengths, and non-FA peaks were not included in analysis.

Compound specific isotope analysis on three selected FAME extracts (selected based on the corresponding gut contents that had high sequencing success) were analyzed with a GC-c-IRMS at the UC Davis Stable Isotope Laboratory on an Agilent 6890 gas chromatograph, coupled to Thermo MAT 253 through a GC-C-III combustion interface on a BPX-70 SGE (length: 60 m, O.D.: 0.25 mm) column. Isotopic concentrations were corrected for the C used in the methanol as part of the transesterification.

Gut Content Analysis

The gut contents of the *C. tanneri* specimens ($n = 11$, 2015 collection only) and the sediment cores were sequenced to identify the microbial taxa present at the time of collection. The gut contents were separated from the gut tissue, and DNA was extracted from both the separated gut contents and the gut wall and residue (pieces of the gut tissue that residue from the gut contents remained on). DNA was extracted from sediment cores and gut contents using the DNeasy PowerSoil Kit (Mobio/Qiagen) following manufacturer's instructions. Extracted DNA was PCR-amplified using 515f and 806r (Caporaso et al., 2012; Apprill et al., 2015) primers as in Seabrook et al. (2018). PCR products were sequenced on the Illumina MiSeq platform at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. Resulting sequences were processed using a custom pipeline (**Supplementary Materials**) that involved mothur v39, Usearch7 (64 bit), and QIIME v. 1.9.1 (Edgar, 2010; Schloss et al., 2011). Sequences were clustered at 97% Operational Taxonomic Unit ($OTU_{0.03}$), and assigned by comparison to the Silva v123 database formatted for QIIME (<https://www.arb-silva.de/download/archive/qiime/>). Aligned sequences were rarefied to the least abundant quality-filtered sequences per sample, after omitting those with uncharacteristically low sequencing success (3690 sequences per sample for this project; resulting in $n = 3$ gut contents, $n = 6$ gut wall and residue). The 16S rRNA gene sequences are archived in the National Center for Biotechnology Information public database under BioProject ID PRJNA494176.

Bray-Curtis similarity comparisons on log transformed rarefied $OTU_{0.03}$'s were used to generate non-metric multidimensional scaling (NMDS) plots and cluster diagrams which were used to visualize patterns in the community structure. PERmutation Multivariate ANalysis Of VAriance (PERMANOVA; McArdle and Anderson, 2012) was used to identify significant differences between sites and crab gut contents. The taxa responsible for differences among groups and the level of similarity between sites and gut contents were measured using the SIMilarity PERcentage Analysis (SIMPER; Clarke and Warwick, 2001). All multidimensional analyses were performed using the software package PRIMER v7 with the PERMANOVA+ add on (Clarke and Gorley, 2015).

RESULTS

Bulk Isotopes

Bulk tissue stable isotope results indicated that *C. tanneri* relied on photosynthetically derived food sources as its main form of nutrition. The $\delta^{13}C$ values of the crab tissue ranged between -17.4 and -20.5‰ , and $\delta^{15}N$ values ranged between 10.7 and 16.1‰ (**Figure 2A**; **Supplementary Table 2**). Of the three different tissue types analyzed, the only significant differences were between the $\delta^{13}C$ values of the muscle and gill tissues (paired t -test, $p = 0.005$), and the $\delta^{15}N$ values of the gill and gut tissues (paired t -test, $p \leq 0.005$). A false discovery rate (0.05) correction was applied to correct for multiple comparisons. There was a significant difference in the bulk ^{13}C of mature vs. juvenile *C. tanneri* (determined from carapace width; <8.5

and >8.5 cm; t -test, $p = 0.044$; **Supplementary Table 2**; Keller et al., 2016). Clayoquot Slope and Barkley Canyon sediment $\delta^{13}C$ values averaged -22.1 ± 0.1 and $-22.3 \pm 0.2\text{‰}$, respectively, and Cascadia Basin $\delta^{13}C$ values averaged $-21.6 \pm 0.2\text{‰}$. Values were averaged across vertical fraction of sediment and then averaged across replicates within site, standard error is reported across replicates within site. $\delta^{34}S$ values for the *C. tanneri* tissues ranged between 17.9 and 20.6‰ , reflecting background (non-seep) seawater sulfur (**Figure 2B**; **Supplementary Table 1**). There was no indication of the assimilation of matter derived from methanotrophic or thiotrophic sources in the bulk isotopic values measured, as would be indicated by significantly lower isotopic values.

Compound Specific Isotopes and FAME Identification

Diagnostic biomarkers for seep bacteria were found in all three crabs, including 16:1 ω 6 and 18:1 ω 8c (aerobic methanotrophs), and 16:1 ω 5 and 18:1 ω 7c (sulfate reducing bacteria). These later FAs are not specific to sulfate reducing bacteria but are common biomarkers for sulfate reducing bacteria. Essential FAs 20:5 ω 3 (eicosapentaenoic acid), 22:5 ω 3 (docosahexaenoic acid), and 20:4 ω 6 (arachidonic acid) were very abundant in all three crabs measured and had $\delta^{13}C$ values associated with photosynthetic sources. The three crab specimens analyzed had fatty acids that were ^{13}C depleted relative to photosynthetic sources (**Figure 3**). In crab 3, the fatty acids that showed ^{13}C depletion were 16:1 ω 6 ($\delta^{13}C = -38.8\text{‰}$), 18:3 FAs ($\delta^{13}C = -38.6$ and -34.7‰), 19:0 ($\delta^{13}C = -33.1\text{‰}$), and 19:1 ω 12 ($\delta^{13}C = -33.7\text{‰}$). Additionally, in crab 7, the 17:1 ω 6 FA was depleted ($\delta^{13}C = -33.2\text{‰}$). The more depleted $\delta^{13}C$ values were not found across all specimen studied, indicating that this depletion is not due to isotopic fractionation during synthesis of the FA within the organisms, but its isotopic composition is sourced from the diet.

Gut Content Analysis

Seep-associated bacteria and archaea were found in the gut contents of *C. tanneri* specimen (**Figures 4A,B**). Seep associated bacterial taxa comprised $>80\%$ of the stomach contents in crab 7 and were found in smaller numbers ($\sim 3\%$) in crabs 3, 2, and 9 as well. These bacterial taxa included: sulfate reducing *Desulfobulbus*, *Desulfocapsa*, Seep-SRBs 1, 2, and 4; methanotrophic Methylococcales; and sulfide oxidizing *Sulfurovum* and Thiotrichales (mat-forming). Anaerobic methane oxidizing archaea (ANME) comprised up to 100% of the archaeal taxa found in the guts of the *C. tanneri* specimen. The seep-associated bacteria and archaea that were found in the *C. tanneri* gut contents were found in the sediment from both the Barkley Canyon and Clayoquot Slope methane seep sites, but were rare or absent in the Cascadia Basin non-seep sites (Seabrook et al., 2018; **Supplementary Tables 5, 6**). The seep associated bacteria and archaea that were found in *C. tanneri* guts comprised 4.34% of all sequences found in the seep sediment, while only comprising 0.05% of the sequences found in the non-seep sediment. Two non-seep associated bacterial clades, Mycoplasmatales and Entomoplasmatales, were also dominant in the crab gut contents and gut walls with Mycoplasmatales

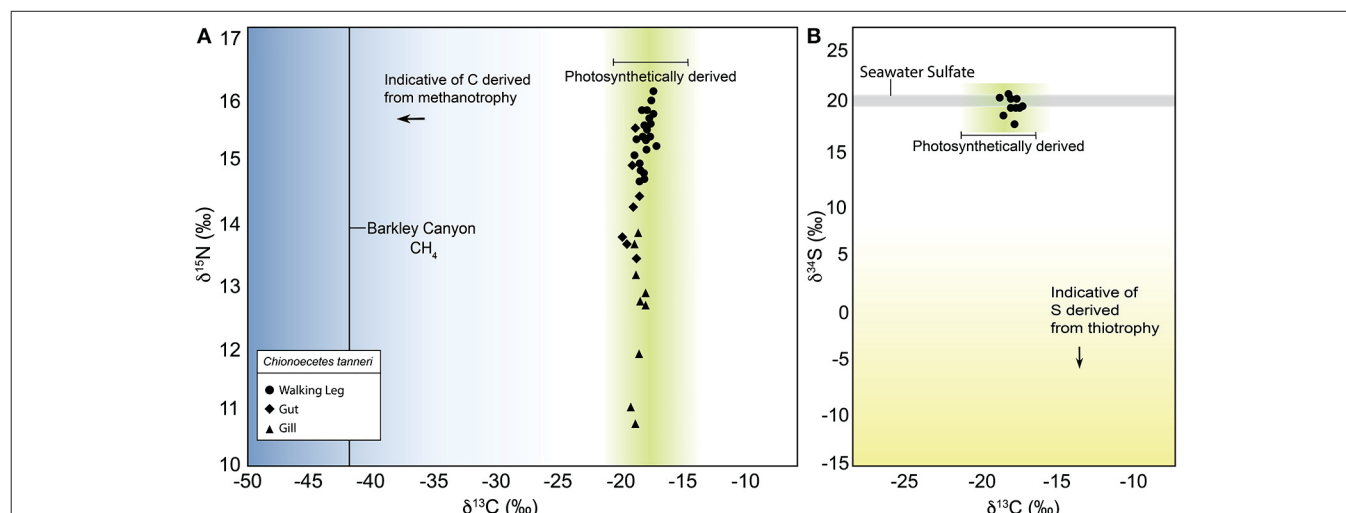


FIGURE 2 | Bulk stable isotope signatures of the different *C. tanneri* tissue analyzed. In (A) $\delta^{13}\text{C}$ $\delta^{15}\text{N}$ indicating the values of *C. tanneri* in relation to expected values of photosynthetic production. The black line indicates the $\delta^{13}\text{C}$ value of methane at the nearby Barkley Canyon methane seep, and the blue gradient indicates the range that methane derived carbon would be expected in. (B) $\delta^{13}\text{C}$ in relation to $\delta^{34}\text{S}$ for *C. tanneri* where the gray line indicates the $\delta^{34}\text{S}$ range for background (non-seep) seawater sulfate, the yellow gradient indicates the range that seep derived sulfide compounds would be expected in. In both (A,B) the green gradient is the range photosynthetically derived carbon is expected in.

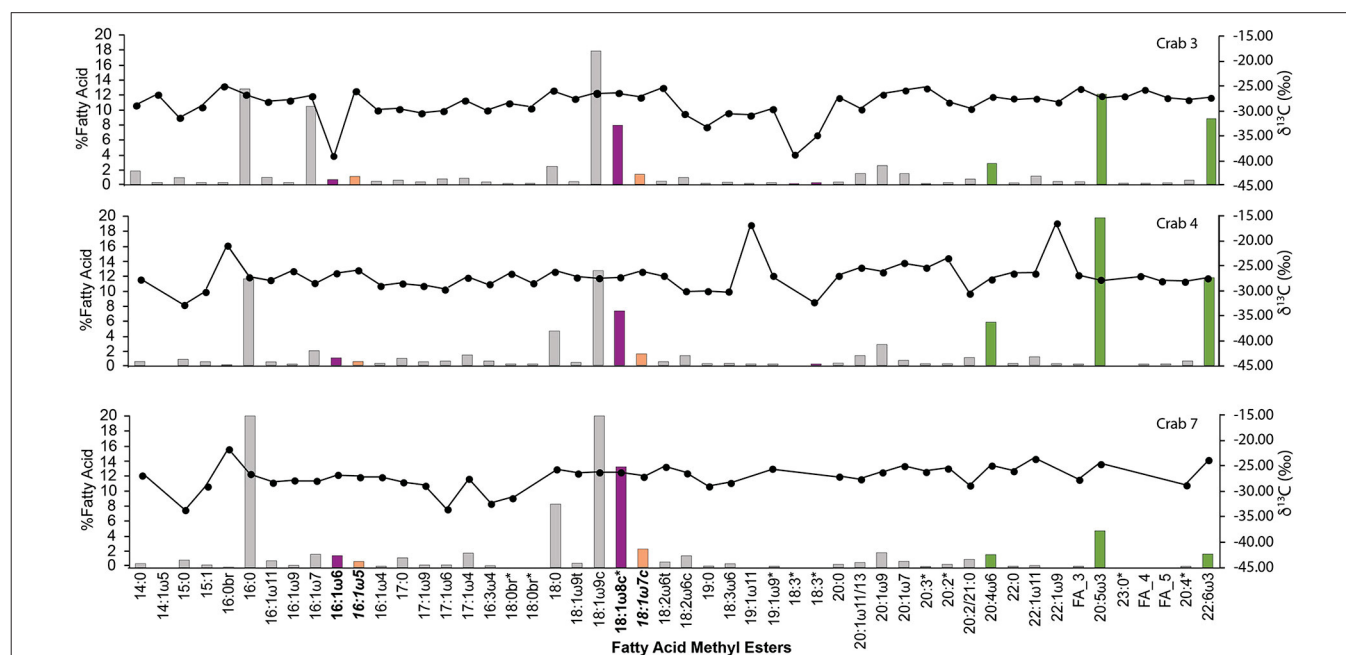


FIGURE 3 | The fatty acid (FA) distribution and carbon isotope composition of FAs extracted from *C. tanneri* muscle tissue. Left y-axis and bars represent percentage of FA, while right axis and points are the isotopic composition. Pink bars with FAs in bold have been associated with methanotrophic bacteria, orange bars with FAs in italic bold have been associated with sulfate reducing bacteria, and green bars are essential fatty acids linked to photosynthetic sources. Asterisks represent instance where FA compounds were inferred from mass spectra.

abundant in all but Crab 4's gut microbiome; in this crab Entomoplasmatales was instead the dominant bacterial group. These groups were very common in the gut contents but rare or absent in the seep sediment. SIMPER analysis revealed that these two taxonomic groups were dominant drivers in the observed dissimilarity between the microbial community in the *C. tanneri*

guts and the seep and non-seep sediments (responsible for up to 1.16% of observed dissimilarity between the *C. tanneri* gut and sediment samples; **Supplementary Tables 7–10**).

The seep, non-seep, gut content, and gut wall microbial communities were all distinct from each other (**Supplementary Figure 1**, PERMANOVA; pseudo-F:8.323,

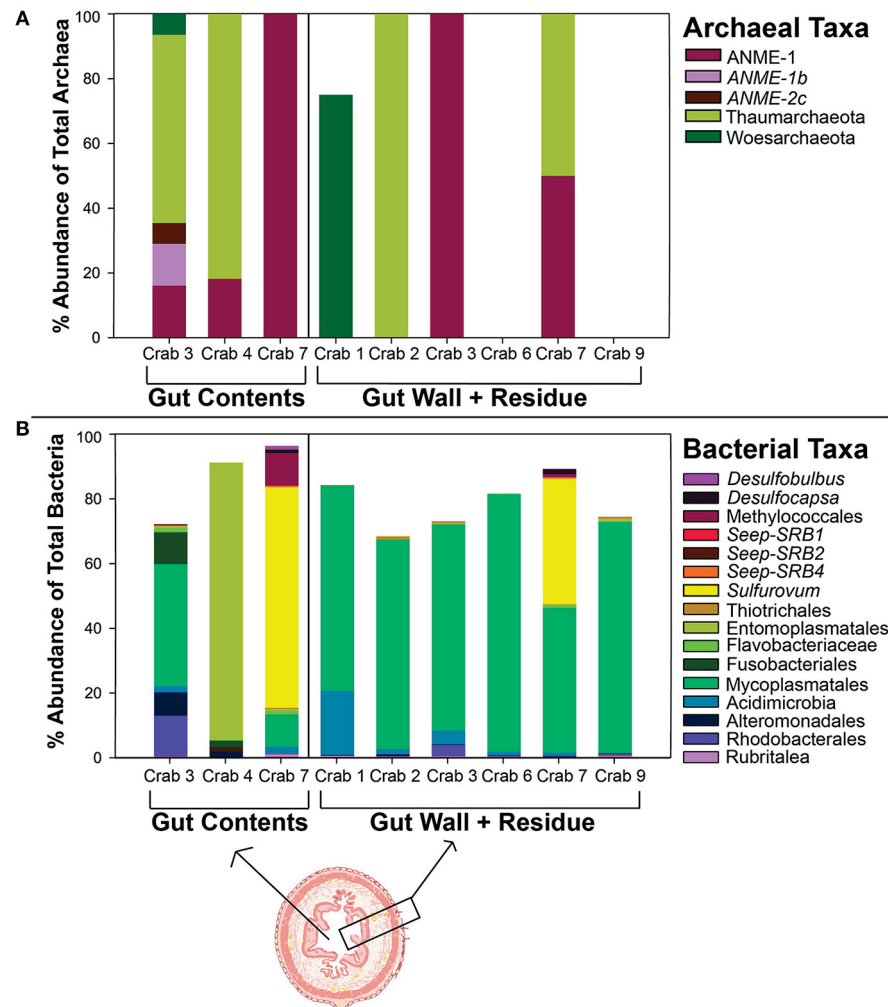


FIGURE 4 | The percent abundance of the (A) archaea and (B) bacteria isolated from the gut contents and gut wall and residue of *C. tanneri*. In (A) only the five most dominant archaeal groups are shown, and in (B) the 16 most dominant bacterial groups are shown. The order is given for the bacterial and archaeal groups, except in italicized instances when genus is given for clarity.

p -value = 0.001). Similarity numbers provided are a result of this PERMANOVA test. The gut wall microbial community across the specimen analyzed were 25.3% similar to each other. The gut wall microbial community shared more similarity with the gut content microbial communities (at 21.9% similarity) than was observed within the gut content microbial community itself (at 16.3% similarity). The dissimilarity among the microbial communities isolated from the gut contents was largely driven by Crab 4's gut community which was dominated by the Entomoplasmatales group. It was possible to compare the microbial community isolated from the gut contents and gut wall in only two of the *C. tanneri* specimen (Crab 7 and Crab 3). The microbial community isolated from Crab 7's gut contents and gut walls clustered together, sharing >40% similarity, whereas the microbial community isolated from Crab 3's gut content was more distinct from the microbial community associated with the gut wall, sharing only 25% similarity (Supplementary Figure 2).

DISCUSSION AND CONCLUSIONS

We found multiple lines of evidence that demonstrated that the *C. tanneri* were grazing upon seep nutrition, although it was not reflected in bulk isotopic measurements. Although the FA signatures revealed a dominance of photosynthetically derived carbon in the crab tissue, there were also multiple bacterial FAs associated with chemosynthetic processes and instances of depletion in ^{13}C associated with methane-derived carbon within particular FAs. The high abundance of seep-associated microbes in some *C. tanneri* guts provides more resolution to these conflicting results, showing the direct ingestion of seep associated bacteria and archaea. This is strengthened by the increased abundance of these specific seep-associated bacteria and archaea in the seep sediment compared to the non-seep sediment.

While greater sample size could have provided a better quantification of the variability among crabs that occur at

these seeps, the samples analyzed provided concrete evidence of methane seep usage by *C. tanneri*. Although the gut contents from all crabs collected in 2015 were extracted and sequenced, only the gut contents of three specimen and the gut wall and residue of 6 specimen passed our quality control and sequencing depth cut offs. In many cases, amplification was repeatedly attempted on the other individuals with no increased success. Gut contents can be difficult to sequence, especially in trap caught organisms. This is due to a combination of the crabs being trapped for a period of time during which they may have evacuated their gut and not had the ability to feed or digestive enzymes degrading present (Symondson, 2002; King et al., 2008; Liu et al., 2015). Durand et al. (2010) showed that 72 h or less was enough time for the guts of *Rimicaris exoculata* to be completely cleared of transient bacteria, so the time between some of the *C. tanneri* specimen entering the traps and on ship preservation may have been long enough for this to occur. Despite sample size limitations, the molecular gut content analysis and compound specific isotopes did reveal the ingestion of seep associated microbes and the assimilation of seep-derived nutrition by some of the *C. tanneri* specimen. Behavioral observations also indicate a link between *C. tanneri* and the seep habitat that suggest our findings based on a few specimens are likely common within the species. The persistent dense population of *C. tanneri* at Clayoquot Slope contrasts with the highly migratory nature of this species (Keller et al., 2012, 2016) and provides an additional indication that *C. tanneri* use the seep habitat. In addition, *C. tanneri* are normally separated geographically by size classes and sex yet there were multiple size classes and both sexes present at Clayoquot Slope (Supplementary Table 2). Mass migration events have also been observed near the seep, including a mass migration event at the nearby Barkley Canyon seep (Figure 1c; Doya et al., 2017). The sustained high density of *C. tanneri* at the Clayoquot Slope seep is coupled with observations of the crabs feeding amongst the seep sediment and microbial mats. Considered with the compound specific isotope and molecular gut content results, it is evident that the crabs are getting some trophic support from the seep site, despite their dominant nutritional pathway appearing to be sourced from photosynthetic-detritus.

The variability within and among the microbial communities isolated from the gut contents and gut walls illustrates natural heterogeneity in the environment, the differences in time since last meal and capture, and potential variability in the resident communities of the *C. tanneri* gut microbiome. Although we were only able to compare the gut contents and gut walls of two of the specimens, it was interesting that Crab 7 had similarity between the two gut portions while Crab 3 did not. This is potentially a result of Crab 7's gut walls having more gut content residue left on the gut wall. The two non-seep associated bacterial clades that were dominant in the crab gut contents (Mycoplasmatales and Entomoplasmatales) are commensal or pathogenic bacteria found in many animal species including crustaceans and are likely not from the diet (Wang et al., 2004; Ku et al., 2013; Terahara et al., 2017). These two groups were also shown to be two of the biggest drivers of the dissimilarity between the microbial communities in the gut contents and in

the sediment from the seep and non-seep sites, supporting that these groups are resident and not transient in the *C. tanneri* guts. Similarly, Durand et al. (2010) found these two groups to be prominent components of the resident gut microbiome in *Rimicaris exoculata* specimens, and hypothesized that they are ubiquitous members of crustacean guts. The proportion of Mycoplasmatales and Entomoplasmatales varied among the crabs studied, with Mycoplasmatales dominant in all samples except the gut contents of Crab 4 where Entomoplasmatales comprised ~90% of the bacterial community present. Stark differences between the microbial communities within mobile consumers, such as *C. tanneri* may be a result of ecosystem and community influence on the composition of animal gut microbiomes. These differences may be an indication of new arrivals vs. longer term site residents that have had more time for genetic exchange to occur between specimens. Similar to the microbial exchange that occurs between human populations, the aggregation of the highly mobile *C. tanneri* at the Clayoquot Slope seep could be leading to genetic dispersal between formerly distinct communities and habitats (Pehrsson et al., 2016).

The compound specific isotope analysis of FAMES extracted from *C. tanneri* muscle tissue revealed mixed signals, but a clear incorporation of specific compounds that are derived from chemosynthetic biomass is evident. Specifically, in the 16:1ω6 and 18:3 FAs ($\delta^{13}\text{C} = -38.8$ and -38.6‰ , respectively). Although these signatures are not as depleted in ^{13}C as has been noted previously, they are around 10‰ more depleted in ^{13}C than would be expected from photosynthetic sources and are similar to values (i.e., $\delta^{13}\text{C} = -37$ to -40‰ $\delta^{13}\text{C}$) that have been linked to chemosynthetic bacterial biomass in similar studies (Boshker and Middelburg, 2002; Niemann et al., 2013). In addition the methane that is present at these sites has been previously identified as thermogenic in origin based on its heavy carbon isotopic signature ($\delta^{13}\text{C} = -30$ to -43‰ ; Pohlman et al., 2009, 2011) which would also lead to a heavier $\delta^{13}\text{C}$ signal from methane-derived carbon. It is important to note that, due to more similar isotopic values between thermogenic methane and photosynthetic production, the ability to easily quantify the role of seeps in the diet of migratory grazers is further hampered at seeps fueled by this heavier methane.

A long-standing debate is whether or not seeps act as oases on continental margins, providing habitat and trophic subsidies to non-seep organisms (MacAvoy et al., 2005; Jørgensen and Boetius, 2007; Guilini et al., 2012). An important aspect of this is whether vagrant species (i.e., non-seep endemic) that are observed at chemosynthesis-based ecosystems consume food produced from either methanotrophic or thiotrophic processes. Bulk isotopes have been used as a one tool to track sources of primary production in consumers, however, isotopic mixing between chemosynthetic and photosynthetic sources can confound results and complicate interpretation of trophic linkages. Seeps are composed of multiple habitats with their own microbial taxa and biomarkers (Levin and Michener, 2002; Boetius and Suess, 2004; Bernardino and Smith, 2010) many of which have non-distinct, or contrasting, bulk SI signatures that reflect the heterogenous nature of seep habitats. This is even clear in the bulk isotopic analysis of sediment presented here,

with $\delta^{13}\text{C}$ values of -22.1 and -22.3‰ that fall within the isotopic range typically indicative of photosynthetic production (Fry and Sherr, 1989; Levin and Michener, 2002). This may be a result of photosynthetic detrital (including refractory) inputs overwhelming the seep signature, microbial fixation pathways that result in heavy isotopic signatures (Hügler and Sievert, 2011), or that thermogenic methane fuels this particular seep. Yet, similar values have been reported in near-seep sediments (e.g., Demopoulos et al., 2018 measured $\delta^{13}\text{C}$ of -23.3‰) and even seep macrofaunal from within areas of very active seepage often have values that are not depleted in ^{13}C (e.g., Levin and Michener, 2002 measured $\delta^{13}\text{C}$ of -22.2‰ ; Levin et al., 2013 measured $\delta^{13}\text{C}$ as low as -22.6‰ ; Thurber et al., 2010 measured $\delta^{13}\text{C}$ as low as -18.5‰). Quantifying the assimilation of chemosynthetic production with bulk isotopes has proved challenging in previous studies, as well. The incorporation of bacterial chemoautotrophic production by grazing gastropods and deposit-feeding sipunculids in the GoM was obscured by more enriched isotopic values that ranged from -32 to -20‰ $\delta^{13}\text{C}$ and -14 to -1‰ $\delta^{35}\text{S}$ due to the mixing with photosynthetic production (MacAvoy et al., 2005). In a key paper, (MacAvoy et al., 2003) found significant input of chemosynthetic production into *Scorasterias cf. tanneri* (an Asteroid), *Eptatretus* sp. (a hag fish), and *Rochina crassa* (a spider crab) in the GoM using compound specific analysis of fatty acids however this was only reflected in the bulk $\delta^{13}\text{C}$ of the less mobile sea star *S. tanneri*, while the more mobile *Eptatretus* sp. and *R. crassa* were both enriched in ^{13}C (-20.9 and -18.4‰ , respectively). Interestingly, an *Eptatretus* sp. that was caught in a non-seep environment was slightly more enriched in ^{13}C than the *Eptatretus* sp. caught at the seep site (by 1‰), potentially reflecting a small signal from seep derived carbon. Similarly, in this study Crab 3, which had the most depleted compound specific $\delta^{13}\text{C}$ signals, was $\sim 1\text{‰}$ more depleted in ^{13}C than the other crabs measured; this may also reflect a slight signal from seep derived carbon. Complementing the results in MacAvoy et al. (2003, 2005), while compound specific isotope analysis of fatty acids and gut content analysis indicated that *C. tanneri* specimen were assimilating seep derived carbon, the bulk isotope data did not. Our results suggest that bulk isotopes may be under-representing the assimilation of seep production in some fauna.

A contributing factor to the under-representation of seep derived nutrition in bulk isotope data in our study may also be the use of C from different food sources for anabolic vs. catabolic processes, likely driven by the quality vs. quantity of food sources. On margins, various food pools exist including particulate organic matter from surface productivity, organic falls, and localized chemoautotrophic production at seeps and vents (Levin and Dayton, 2009; Levin and Sibuet, 2012; Sweetman et al., 2017). Each of these habitats and food pools contribute to margin ecosystems in a variety of ways and are different in terms of quantity (seep production) and quality (fresh phytodetritus). Previous work has indicated that in habitats with multiple trophic pathways, food quantity and quality can interact to lead to preferential assimilation of high-quality food types, even if it is in lower quantity (Persson et al., 2007; Hessen, 2008; Marcarelli et al., 2011). Mobile consumers have been shown to selectively

feed on more high-quality matter when both high-quality and low-quality matter was offered, while sedentary consumers did not show such a discrimination (Cruz-Rivera and Hay, 2008). Detrital matter has been shown to be of higher quality, or more nutritious, than the direct production in habitats, such as coral reefs and kelp forests, and thus often assimilated in a higher proportion than less nutritious direct production (Campanyà-Llovet et al., 2017). The same could be true in methane seep habitats, where the base of production is facilitated by bacteria, which do not produce the essential FAs shown to be critical in a metazoans diet (Copeman et al., 2002; Zuo et al., 2012). The enriched $\delta^{13}\text{C}$ and $\delta^{35}\text{S}$ signatures in the bulk isotopes may be reflecting a preferential assimilation of higher quality detrital matter by the *C. tanneri* specimen studied. Supporting this, we do see a higher abundance of the essential FAs (20:5 ω 3, 22:5 ω 3, and 20:4 ω 6) relative to the bacterial produced 16:1 and 18:1 FAs in the crab tissues. Food quantity and quality effects, such as these may disproportionately affect ecosystem processes and skew food web analysis that is based on those compounds assimilated (i.e., C, N, S isotopes, and FAs; Marcarelli et al., 2011).

Another major factor that could be influencing the diet of species as resolved by biomarker techniques is the temporal resolution of the different techniques employed. Bulk stable isotopes have turnover times of >400 days in some invertebrate heterotrophs (MacAvoy et al., 2001), which could be leading to an underrepresentation of seep nutrition due to the highly mobile and migratory nature of the *C. tanneri*. Individual fatty acids have differing turnover times, but have been shown to in general be on the order of a few days to a few weeks (Ruess and Chamberlain, 2010). As our analytical techniques increase in power and precision, we are becoming more able to resolve mixed signals in trophic studies that are due to spatial and temporal factors, particularly through the integration of multiple techniques. Analysis of fatty acids allowed us to separate out specific isotope signatures from the bacterial produced FAs and the more abundant essential FAs sourced from photosynthetic production. These compound specific isotopes of FAs can also reveal more short-term assimilation than possible with bulk isotopes due to the shorter turnover times of FAs, potentially allowing us to capture short term use of a new food source by an organism (MacAvoy et al., 2001; Ruess and Chamberlain, 2010; Bec et al., 2011). The analysis of the gut contents of the crabs allowed us to observe the most recent food source consumed by *C. tanneri* and dietary connections that were missed in the bulk isotopic methods with longer turnover times.

The implications of mobile species like *C. tanneri* being able to use seep habitats extends beyond the habitat provisions that the crabs derive directly from the seep itself. The use of a seep by highly mobile consumers like *C. tanneri* could intensify cross-boundary movements of methane sourced carbon and genetic material. This directly contributes to the “sphere of influence” of a seep habitat on the surrounding margin ecosystem (sensu; Levin et al., 2016). The ecological significance of energy transfers between habitats has been highlighted in other systems, such as with the annual returns of anadromous fishes to rivers shown to bring marine-derived nutrients that sustain the productivity of freshwater food webs (Chaloner et al., 2002). Interactions are

considered ecologically important when cross-boundary trophic subsidies are assimilated by the consumers in the respective habitats (Carpenter et al., 2005). Here, we show that *C. tanneri* are assimilating cross-boundary subsidies and thus have the potential to be agents in the dispersal of nutrients due to their highly mobile and migratory nature. This could benefit background (non-seep) ecosystems by delivering allochthonous food, as well as distributing genetic material (seep bacteria and archaea in their gut) across the margin and potentially linking disparate seep systems.

Climate change is predicted to reduce the total input of photosynthetic production to the deep sea, with direct impacts on the biomass of deep-sea fauna (Jones et al., 2014; Sweetman et al., 2017). As a result, the use of alternative sources of production may become increasingly important to mitigate impacts of shortages of surface production (Smith et al., 2008; Levin and Sibuet, 2012). In the often food limited deep-sea, the ability for chemosynthetic production to serve as a trophic subsidy for non-seep species, as it has now been shown in the shallow ocean (Higgs et al., 2016), could help support the health and productivity of ocean environments. Thus, the ability for non-seep organisms to use chemosynthetic production as a trophic subsidy could have implications as we seek to spatially manage ecosystems in a way that promotes sustainable and productive ocean environments. This study provides the first evidence of seep derived food sources contributing to the diet of commercially exploited non-seep species, highlighting the potential importance of these environments for providing a provisioning ecosystem service. More robust analysis on the trends presented herein will aid in further understanding cross-boundary assimilations of chemosynthetic production. However, those studies will be faced with the challenges of dealing with the methodological limitations we highlight, which may preclude the application of mixing models to quantify the role of seeps in margin ecosystems, among other things. The use of multiple methods, as presented here, may prove critical to parsing out discrete signals from mixed settings and understanding complex food webs with both photosynthetic and chemosynthetic food sources. While we may still debate whether seeps act as oases in the deep, this study makes it clear that certain lines of evidence could under-represent the role of seeps and highlights the potential for seeps to act as critical oases to non-seep endemic fauna in the future. In the case of *C. tanneri*, seep

production may serve as a trophic subsidy that could help to buffer the impact of climate change on a commercially harvested marine species.

AUTHOR CONTRIBUTIONS

SS, FDL, and AT conceived and designed the experiments. SS performed the experiments. SS and AT analyzed the data. SS, FDL, and AT contributed reagents, materials, analysis tools. SS, FDL, and AT wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00043/full#supplementary-material>

Supplementary Videos 1 and 2 | Remotely Operated Vehicle video footage of tanner crabs 'farming' on methane bubbles at Clayoquot Slope seep (**Supplementary Video 1**), and an areal overview showing the patched nature of bacterial mats and methane bubbling at Clayoquot Slope seep (**Supplementary Video 2**). Both video clips have been edited increasing its original frame rate to 150% in some instances. Original footage from ROPOS R1555 dive, on June 3, 2012, is available through Ocean Networks Canada's free data web portal <https://data.oceannetworks.ca/SeaTube?resourceType=1000&resourceId=1001&divId=219&time=2012-06-03T14:00:00.000Z>, and time stamp ranges from 15:51:34 to 16:03:02 UTC.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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