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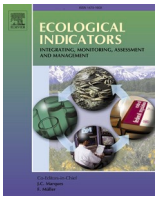

The use of radiocarbon to evaluate the trophic role of geothermal bacteria in shallow hydrothermal water ecosystem

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ABSTRACT

Stable isotopes such as $\delta^{13}C$ and $\delta^{15}N$ are routinely used in trophic ecology as isotope values are derived from diet and recorded subsequent fractionation in consumer tissue. However, this approach necessitates to estimate *a priori* the fractionation between food source and consumer leading to potential uncertainties. In this context, the development of additional biomarkers enables to have better resolution on feeding habits. Dissolved Inorganic Carbon (DIC) from volcanic activity and bacteria using this DIC presents a strongly depleted natural radiocarbon abundances ($\Delta^{14}C$). Through its activity, the geothermal plant of Bouillante in Guadeloupe (French West Indies) releases sulfur bacteria in shallow environment of the Bay. A previous study reveals ingestion of those bacteria by opportunist species (sea urchin and fish species). In the present study, ten species with different diet and feeding mode were sampled close to the release channel of the geothermal plant and in a control station in order to simultaneously determine their stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon compositions. Compared to models using $\Delta^{14}C$ data, models using $\delta^{13}C$ and $\delta^{15}N$ data underestimate the role of bacteria in diet of urchin and fish species whereas this role is overestimated for all other species. Compared to previous models, radiocarbon would give more realistic and reliable results than the classical use of stable isotope. This study confirms the utility of radiocarbon in food web ecology, particularly at the ecosystems having food sources with contrasting $\Delta^{14}C$ values.

1. Introduction

Since more than three decades, stable carbon and nitrogen isotope measurements ($\delta^{13}C$ and $\delta^{15}N$ values) are routinely used to determine trophic interactions in marine and terrestrial environments (Peterson and Fry, 1987). They constitute the most used tool in trophic ecology as isotope values are derived from diet, and are recorded in consumers tissues (Fry, 2006). Fractionation is the selective retention of heavy isotopes during metabolic reactions within the body of consumers. Difference in $\delta^{13}C$ signature between food sources and consumers is usually small (<1‰), allowing accurate determination of diet composition (McCutchan et al., 2003) whereas $\delta^{15}N$ in consumer are usually enriched by about 3 ‰ relative to their diet allowing identification of food resources and trophic level (Minagawa and Wada, 1984; Post, 2002). The fractionation needs to be estimated *a priori* in order to quantify food web relationships. However, this fractionation can vary in consumers according to their phylogenic group (*e.g.* vertebrates, invertebrates *etc.*), their diet (*e.g.* herbivores, carnivores *etc.*), their physiological states and environmental conditions (Vander Zanden and Rasmussen, 2001; Caut et al., 2009; Cabanillas-Terán et al., 2019). All those uncertainties can result in error of source contribution estimated by model hindering the use of stable isotope in food-web studies (Vander Zanden and Rasmussen, 2001). Isotopic pools with differing origins, such as body reserves, muscle protein, and direct food intake can have non-equal contributions to tissue formation complicating determination of trophic role of sources (Fry et al., 1999; Ayliffe et al., 2004). Stable isotope can also present limitations when isotope ratios of potential sources are overlapping and/or when they present high variabilities over short spatial or temporal scales.

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In order to increase discrimination between potential food sources, stable isotope can be used as tracers during experiments with prey artificially enriched in heavy isotopes. This approach is adapted to evaluate ingestion of small size food items such as bacteria (van Oevelen et al., 2006; Pascal et al., 2014), microphytobenthos (Moodley et al., 2002; Como et al., 2018) and meiofauna (Pascal et al., 2019). Enrichment experiments can be conducted *in situ* even in remote environment such as deep sea (Moodley et al., 2002; Nomaki et al., 2006). However, they are limited to small size preys and can present bias due to potential modification of feeding behavior in artificial conditions of experiments. Other approach to face shortcoming of stable isotopes is to use fatty acid biomarkers in addition to stable isotopes (Jaschinski et al., 2008; Leduc et al., 2009; Lebreton et al., 2011). The use of fatty acid can be challenging as it requires several criteria such as the unique and high abundance of fatty acids in potential food sources and the absence of neo synthesis of fatty acid in consumers (Chamberlain et al., 2005). All methods conventionally used in trophic ecology and mentioned above presents potential shortcomings and limitations according to environments and organisms studied. The best way to increase reliability of results from food web studies is consequently to use simultaneously several methods. In this context, the development of additional biomarkers will help to answer different research question in trophic ecology.

Radiocarbon (^{14}C) is the radioactive isotope of carbon continually produced in the earth's upper atmosphere by cosmogonic radiation. Due to the radioactive decay with the half-life of 5730 years, the concentration of ^{14}C ($\Delta^{14}C$) vary among the earth's different carbon reservoir and often presents a wider dynamic range than $\delta^{13}C$ in certain ecological contexts (Larsen et al., 2018). Unlike $\delta^{13}C$ and $\delta^{15}N$ values, the $\Delta^{14}C$ constitute a direct reflection of a specific source and is not affected by variable fractionations according to metabolic pathways. Due to this absence of discrimination, radiocarbon can constitute robust tracer of food source with an advantage over stable isotopes (Ishikawa et al., 2012; Larsen et al., 2018; Nishida et al., 2020). Historically, measurements of ^{14}C require substantial sample mass (Williams et al., 1981) but recent technical improvements of analysis (e.g. Yokoyama et al., 2019; Yokoyama et al., 2022) allow measurements in small size organisms like meiofauna (Nomaki et al., 2019) and in selective compounds such as microbial lipid (Pearson et al., 2005) and amino acid (Blattmann et al., 2020). Radiocarbon analyses are more time consuming and complicated than stable isotopes ones, though there are several advantages including *i*) it requires less replications as ^{14}C is less variable (Ishikawa et al., 2012), *ii*) it requires less precision than in ^{14}C archeological studies and *iii*) it becomes gradually more affordable and for those different reasons, radiocarbon will likely be more used in trophic ecology in coming decade (Larsen et al., 2018).

In marine environment, variation of radiocarbon according to geographic location allow the study of the role of currents in food availability (Ishikawa et al., 2021) and feeding behavior of migrating marine life from fish (Yokoyama and Ohkouchi, 2017) to whale (Eisenmann et al., 2017). ^{14}C slowly decays over time with a half-life of 5730 years and allow evaluation of trophic role of *i*) recent food items from surface ocean and for bottom dwelling fauna (Rau et al., 2001; Purinton et al., 2008; Guillemette et al., 2017) and *ii*) trophic role of old food items from oil reservoir (Chanton and Lewis, 2002; Wilson et al., 2016), upwelling (Berkman and Forman, 1996) and deep-sea hydrothermal vent activities (Williams et al., 1981). Deep sea environments are isolated from the contemporary atmosphere and are depleted in ^{14}C relative to surface ocean (e.g. Honda et al., 2000). In deep sea hydrothermal vent, chemoautotrophic microbes typically exhibit depleted ^{14}C concentrations as their inorganic carbon source comes from ^{14}C-depleted bottom-water (Stuiver et al., 1983) and/or ^{14}C depleted CO_2 or CH_4 released through volcanic activity (Bruns et al., 1980; Williams et al., 1981). In such chemosynthesis-based ecosystems, radiocarbon can be used more efficiently to evaluate trophic role of chemolithoautotroph microbes in deep sea (Nomaki et al., 2019).

The aim of the present study is to evaluate the potential of radiocarbon as tracer of chemoautotroph bacteria in coastal tropical environment. In Guadeloupe Island (French West Indies), a plant pumps geothermal water rich in H_2S from deep reservoir in order to produce electricity. This water is then cooled with sea water before being released into the sea. Environmental conditions in the discharge channel support development of sulfur-oxidizing bacteria. A previous study using *i*) fish counts according to plant activity and *ii*) stable isotope compositions (C, N, S) of potential consumers of bacteria, reveals an ingestion of sulfur bacteria by sea urchin and fish species whereas this trophic role was not detected for suspension feeders and omnivorous predators (Pascal et al., 2017). Sulfur-oxidizing bacteria presents high concentration of Hg and bacterial consumers (urchin and fish) bioaccumulate more Hg in Bouillante Bay than in adjacent environment confirming their active ingestion of bacteria (Ruiz et al., in prep). In order to evaluate the potential of radiocarbon as a tracer in food web, we tested 10 species previously analyzed with various feeding mode and different contribution of sulfur bacteria in their diet, where sampled close to the release channel of the geothermal plant and in a control station. The simultaneous determination of stable isotopes (^{13}C and ^{15}N) and radiocarbon (^{14}C) compositions of organisms allowed to compare results of modeled contribution of bacteria in their diet and the utility of radiocarbon tracer.

2. Material and method

2.1. Study area

The study area is located on the west coast of Basse Terre Island in Guadeloupe in French West Indies, which belongs to the active Lesser Antilles volcanic arc (Fig. 1). The multiple hot springs, steaming grounds

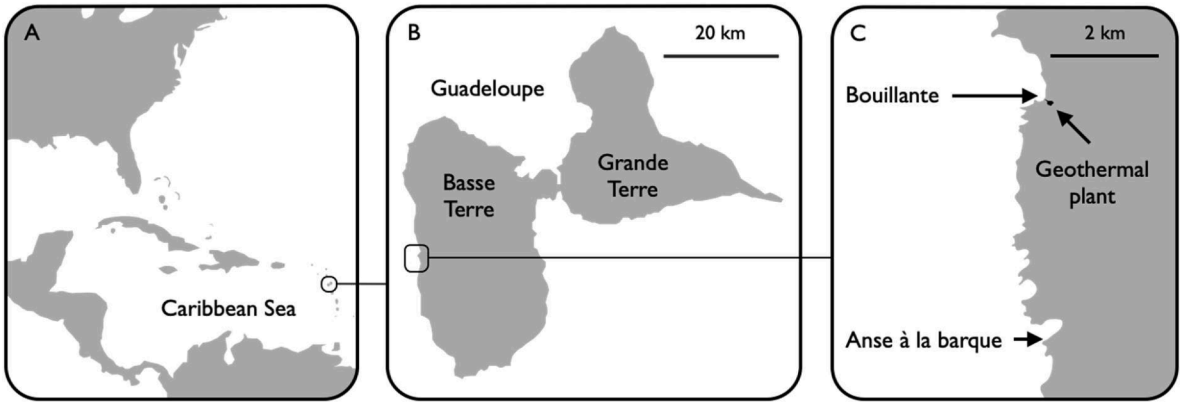

Fig. 1. (A) Location of Guadeloupe Island in the Caribbean Sea, (B) location of study area in Guadeloupe and (C) location of sampling stations in Bouillante bay (geothermal plant) and Anse à la barque (control).

and fumaroles characterizing this region explain the name of the town "Bouillante", which means "boiling" in French. Infiltration of rainwater (40 %) and sea-water (60 %) fills several fractured zones between 150 and 1100 m forming a reservoir of 30 million m^3 with a temperature of 250–260 °C (Lachassagne et al., 2009). To exploit this source of energy, a geothermal plant without reinjection started producing electricity in 1986 exploiting water coming from a 340 m deep well (Jaud and Lamethe, 1985). Additional deeper wells (1000–1200 m) are actually used to produce 6–7 % of Guadeloupe electricity consumption (Demarcq et al., 2014). All residual water is mixed with pumped seawater in order to reduce the temperature to 45 °C before being dumped at sea through discharge channel (Fig. 1). Geothermal fluid is characterized by a high concentration of dissolved H_2S reaching 35–45 mg l^{-1} in the steam condensate (Mas et al., 2006). Chemical and temperature conditions are favorable for bacteria forming a thick white mat (6–7 mm) visible with naked eye and covering pebbles at the bottom of discharge channel. This bacterial mat is constituted by cyanobacteria (*Pectonema sp*) covered by white sulfur-oxidising bacteria of the genus *Thiomicrospora* (Pascal et al., 2017). Due to strong current outflow (2.5 m^3 s^{-1}), bacteria are regularly ripped off from pebbles, carried by current and used as food resource by urchin and fishes at the discharge channel outlet (Pascal et al., 2017). Anse à la barque (AALB) was used as control station located 4.5 kms at the South of Bouillante Bay (Fig. 1) where no effects of such hydrothermal fluid and bacterial mat was recorded.

2.2. Sampling

Samples were collected in June 2020 while geothermal plant was active since a long period. For water sampling, 1) geothermal fluid was collected in Bouillante geothermal plant before its dilution with sea water and 2) surficial sea water was collected in AALB, 50 m away from the shore. Water samples were kept sealed and poisoned with $HgCl_2$ before their analyze. Pebbles were collected from Bouillante discharge channel in order to scrap and sample their bacterial biofilm for $\Delta^{14}C$ analyses. Stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of bacterial biofilm, sampled with similar protocol, over a long time period (2014–2015) and previously analyzed were averaged for present study. All other samples were collected through free diving in Bouillante close to the water discharge outlet (with a distance from the outlet <5 m) and in AALB. Echinoderm (*Diadema antillarum*), polychaeta (*Hermodice carunculata*), bivalves (*Spondylus tenuis*), crustacean (*Balanus* sp.), cnidarian *Pseudopterogorgia* sp. and sponges (*Aplysina fistularis* and *Iotrochota birotulata*) were collected by hand. Fishes (*Acanthurus bahianus* and *Abudefduf saxatilis*) were speared. Four specimens of each species were taken from each site. A dissecting microscope was used to isolate muscles of the echinoderm, polychaeta, bivalves and fishes. For cnidarian, crustacean and sponges coarse tissue were sampled. Samples were frozen, freeze-dried and then acidified with 0.1 M HCl to remove calcium carbonate and then weighed into precleaned silver capsules before analyse.

2.3. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$)

Carbon and nitrogen isotopic composition were determined using an isotope ratio mass spectrometer coupled to an elemental analyzer (Flash EEA 1112-DELTA V Advantage ConFloIV System, Thermo Fisher Scientific). Isotope ratios were conventionally expressed as δ values in ‰ (Coplen, 2011) relative to C and N international standards (respectively Vienna PeeDee Belemnite and atmospheric N_2). For the machine calibration of the IRMS, NBS19 and Sucrose ANU were used for $\delta^{13}C$ and IAEA N1 and IAEA N2 were used for $\delta^{15}N$ measurements. Every 10 to 15 samples, isotopic compositions of working standards for $\delta^{13}C$ C (Alanine: −19.6 ‰; Histidine: −10.7 ‰; Glycine: −33.8 ‰) and $\delta^{15}N$ (Alanine: 1.58, 9.97 ‰, and 20.6 ‰) were measured.

2.4. Radiocarbon ($\Delta^{14}C$)

Corrected water samples were treated to extract Dissolved Inorganic Carbon (DIC) using the bubbling method (McNichol et al., 1994). Namely, 4 ml of phosphoric acid were added to 250 ml of water, and bubbling was conducted for 15 min in a glass vessel filled with 60 mPa of helium gas to strip CO_2 liberated from DIC. Water vapor is released along with the CO_2 gas during this process, so 2 cryogenic traps containing ethanol cooled to –120 °C were used to remove the water vapor. The CO_2 was captured in a liquid nitrogen trap (Servettaz et al., 2018). The CO_2 collected was processed under the protocol described in Yokoyama et al. (2007, 2022): CO_2 was injected into a quartz tube together with 4 mg of iron powder pre-reduced at 450 °C and graphitized by heating at 630 °C for 6 h. The samples were measured using a single-stage accelerator mass spectrometer at the University of Tokyo Atmosphere and Ocean Research Institute (Yokoyama et al., 2019).

Decalcified, dried samples were placed in pre-combusted glass cups. The samples were further graphitized according to the modified methods of (Yokoyama et al., 2010). Briefly, dried samples (3.14 to 13.35 mg, typically 10 mg) were combusted in an evacuated quartz tube with copper oxide at 500 °C for 30 min and at 850 °C for 2 h. The CO_2 gas was cryogenically purified in a vacuum line and reduced to graphite with hydrogen and an iron catalyst at 550 °C for 10 h. We measured the $\Delta^{14}C$ values with an accelerator mass spectrometer (AMS) at the Atmosphere and Ocean Research Institute (Yokoyama et al., 2019). The $\Delta^{14}C$ (‰) value was defined as follows (Stuiver and Polach, 1977): $\delta^{14}C$ (‰) $= \delta^{14}C - 2(\delta^{13}C + 25)(1 + \delta^{14}C/1000)$. The $\Delta^{14}C$ value of the international standard (oxalic acid) takes into account radioactive decay since 1950 (Stuiver and Polach, 1977). The 1 sigma analytical precision of the $\Delta^{14}C$ measurements was within 5 ‰, typically within 3 ‰ (Yokoyama et al., 2022). Cross contamination amongst samples were avoided after careful treatment of samples as well as preparations (Yamane et al., 2019). Rigorous background assessments was conducted to ensure reliability of results (Yokoyama et al., 2010; Yamane et al., 2019).

2.5. Data analyses

The nonparametric Kruskal-Wallis test was used to examine differences in isotopic composition ($\delta^{13}C$, $\delta^{15}N$ and $\Delta^{14}C$) of consumers. All statistical analyses were performed using R. Values are presented as means with standard deviations (SD).

Bayesian isotopic mixing models were employed to determine contributions of bacteria to diets of consumers. SIAR (Version 4.2; (Parnell et al., 2010)) incorporates the variability of consumer and trophic enrichment factors (TEFs, i.e. the net isotopic composition change in a consumer and its ingested food sources) to produce the percent contribution of each source to a consumer's diet. TEFs are key factors when it comes to evaluate contributions of food sources to animal diets. TEFs used were 1.1 ± 0.5 ‰ for $\delta^{13}C$, 2.2 ± 0.5 ‰ for $\delta^{15}N$ and 2.0 ± 0.7 ‰ for $\delta^{34}S$ (McCutchan et al., 2003). These TEFs are typically used in isotope studies and are appropriate when consumers are not starved (Vandenberg et al., 1994; Lefebvre and Dubois, 2016). Variations of 20 % in these TEFs do not change the conclusion of the present study as they induce only small variations of 2.3 ± 1.9 % in model results.

A 2-step procedure was performed to run the SIAR modelling. First, TEFs (McCutchan et al., 2003) were subtracted from isotopic compositions of consumers caught in control bay in order to determine the isotopic compositions of bulk diets of each consumer in an environment without bacteria. Average values and standard deviations of those results were then used as 'food sources' in models using isotopic compositions of consumers from Bouillante bay in contact with bacteria. For each consumer, 2 potential food sources were considered: *i*) chemoautotrophic bacteria and *ii*) average isotopic composition of consumers' bulk food sources in control Bay and three different models were run with *i*) ^{13}C and ^{15}N, *ii*) ^{14}C and *iii*) ^{13}C, ^{15}N and ^{14}C. This evaluation of

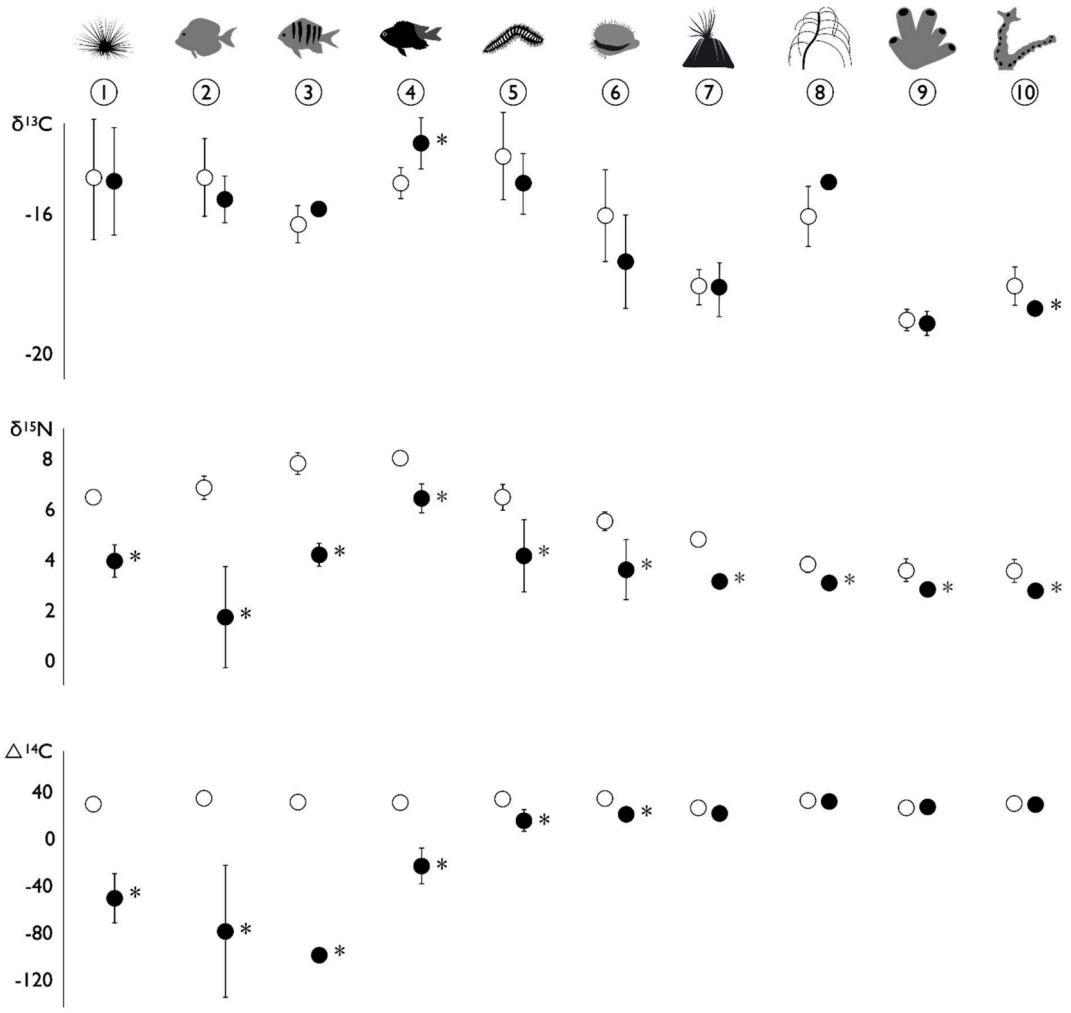

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ (± SD, n = 4) and $\Delta^{14}C$ (± SD, n = 3) in Anse à la barque (control) in white and Bouillante (geothermal plant) in black for 1) *D. antillarum*, 2) *A. bahianus*, 3) *A. saxatilis*, 4) *S. partitus*, 5) *H. carunculata*, 6) *S. tenuis*, 7) *Balanus* sp. 8) *Pseudopterogorgia* sp., 9) *A. fistularis* and 10) *I. birotulata*. *: significant difference between each station (Kruskal-Wallis test, *p < 0.05).

the contribution of bacteria to diets is based on the assumption that bacteria are the only food item whose contribution to faunal diets changes between Bouillante and control bays.

The model was run with 106 iterations and burn-in size was set as 105. Model solutions are presented using credibility intervals of probability density function distributions (Parnell et al., 2010).

3. Results

3.1. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$)

Bacteria released by the geothermal plant of Bouillante presented a $\delta^{13}C$ of −17.3 ± 1.5 ‰ and $\delta^{15}N$ of −4.7 ± 1 ‰. Among potential consumers from control site, highest $\delta^{15}N$ was found in the three fish species: *S. partitus* ($\delta^{15}N = 7.9 \pm 0.2$), *A. saxatilis* ($\delta^{15}N = 7.7 \pm 0.4$) and *A. bahianus* ($\delta^{15}N = 6.8 \pm 0.5$), fireworm *H. carunculata* ($\delta^{15}N = 6.5 \pm 0.5$) and the sea urchin *D. antillarum* ($\delta^{15}N = 6.4 \pm 0.2$). Except for *A. saxatilis* ($\delta^{13}C = -16.3 \pm 0.5$), all those consumers from control site also present highest $\delta^{13}C$: *S. partitus* ($\delta^{13}C = -15.1 \pm 0.5$), *A. bahianus* ($\delta^{13}C = -15.6 \pm 0.7$), *H. carunculata* ($\delta^{13}C = -14.4 \pm 1.2$) and *D. antillarum* ($\delta^{13}C = -15.1 \pm 0.5$) (Fig. 2).

Between the Bouillante and control stations, studied organisms do not present a significantly different $\delta^{13}C$ values (Kruskal-Wallis tests, $p > 0.05$) except *S. partitus* and *I. birotulata* (Fig. 2). In Bouillante, all studied organisms present significantly lower $\delta^{15}N$ than in the control station (Kruskal-Wallis tests, $p > 0.05$) (Fig. 2).

3.2. Radiocarbon ($\Delta^{14}C$)

Water released by the geothermal plant is composed by pumped geothermal water mixed with surrounding sea water from the bay and presented a Dissolved Inorganic Carbon (DIC) $\Delta^{14}C$ of −682 ‰ ($n = 1$) whereas the water from the control site presented a mean DIC $\Delta^{14}C$ of 25 ± 2 ‰ ($n = 2$). Sulfur bacteria released by the geothermal plant of Bouillante through the discharge channel presented a mean $\Delta^{14}C$ of −159 ± 32 ‰ ($n = 2$).

Among potential consumers from control site, highest $\Delta^{14}C$ was found in *A. bahianus* (35.5 ± 2.0) whereas the lowest was found in *A. fistularis* (25.0 ± 1.1) (Fig. 2). Between the Bouillante and control station, all studied organisms presented a significantly lower $\Delta^{14}C$ except *Balanus* sp., *Pseudopterogorgia* sp., *A. fistularis* and *I. birotulata* (Kruskal-Wallis tests, $p > 0.05$). Most important decrease in $\Delta^{14}C$ were observed for *A. saxatilis, A. bahianus* and *D. antillarum* and *S. partitus* (Fig. 3).

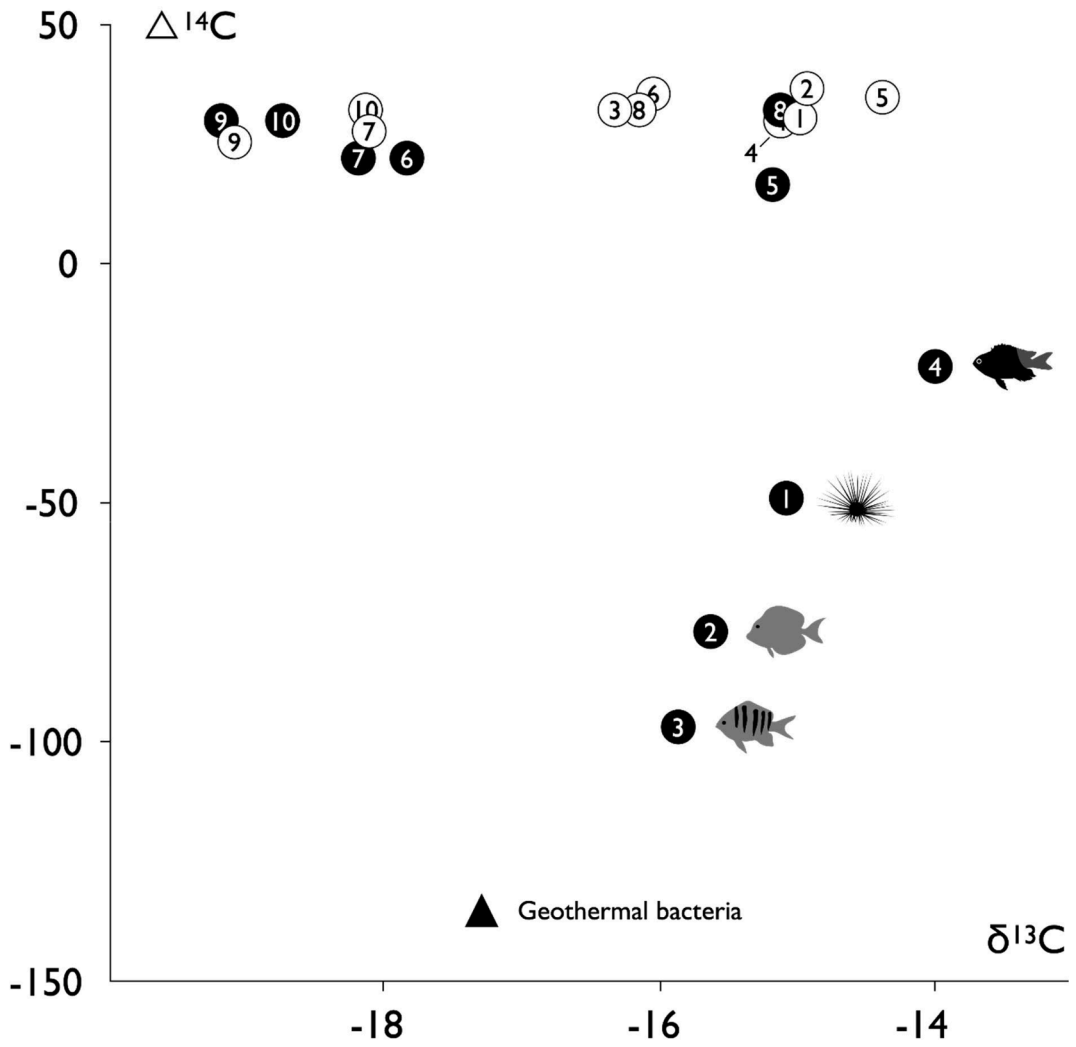

Fig. 3. Carbon composition ($\Delta^{14}C$ according to $\delta^{13}C$) of geothermal bacteria (triangle shape) and organism (round shape with 1) *D. antillarum*, 2) *A. bahianus*, 3) *A. saxatilis*, 4) *S. partitus*, 5) *H. carunculata*, 6) *S. tenuis*, 7) *Balanus* sp. 8) *Pseudopterogorgia* sp., 9) *A. fistularis* and 10) *I. birotulata*)) in Anse à la barque (control) in white and Bouillante (geothermal plant) in black.

3.3. Model SIAR

SIAR model simulations using $\delta^{13}C$-$\delta^{15}N$, $\Delta^{14}C$ and combined ^{13}C-$\delta^{15}N$-$\Delta^{14}C$ predict different estimates of chemoautotrophic bacteria contributions to studied organisms (Fig. 4). Mean contribution of bacteria in diet (%) were higher with models using $\delta^{13}C$-$\delta^{15}N$ *versus* models using $\Delta^{14}C$ for urchin and fish: *D. antillarum* (27 *vs* 44), *A. bahianus* (47 *vs* 54), *A. saxatilis* (37 *vs* 63) and *S. partitus* (18 *vs* 32) (Fig. 5). Mean

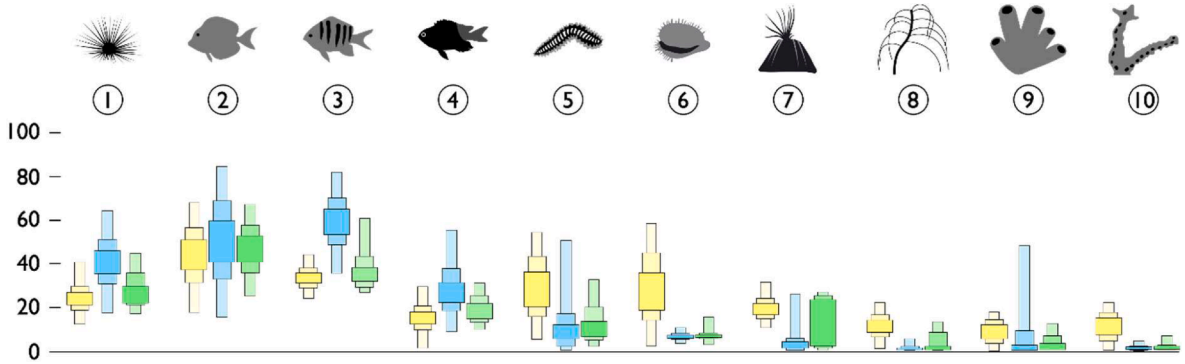

Fig. 4. Contribution (% by weight) of bacteria to the diet of 1) *D. antillarum*, 2) *A. bahianus*, 3) *A. saxatilis*, 4) *S. partitus*, 5) *H. carunculata*, 6) *S. tenuis*, 7) *Balanus* sp. 8) *Pseudopterogorgia* sp., 9) *A. fistularis* and 10) *I. birotulata*. Results were obtained from the stable Isotope Analysis in R mixing model with two food sources (bacteria and average diet of each consumer evaluated at the control station) with $\delta^{13}C$ and $\delta^{15}N$ (in yellow), $\Delta^{14}C$ (in blue) and $\delta^{13}C$, $\delta^{15}N$ and $\Delta^{14}C$ (in green). 95, 75 and 25% credibility intervals of probability distributions are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

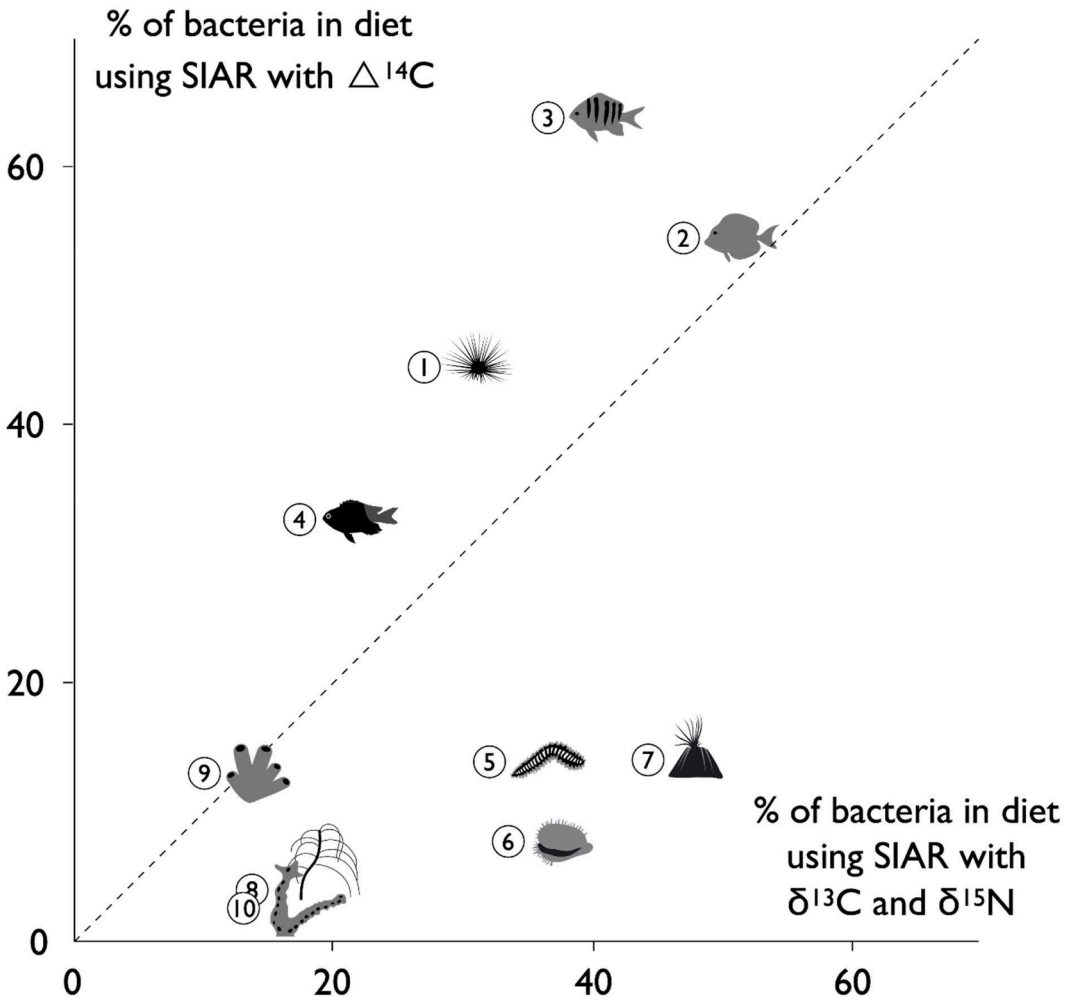

Fig. 5. Contribution (% by weight) of bacteria to the diet of each organism evaluated using SIAR mixing model with $\Delta^{14}C$ according to contribution of bacteria evaluated using similar model using $\delta^{13}C$ and $\delta^{15}N$.

contribution of bacteria in diet (%) were similar between both models for *A. fistularis* (10 *vs* 12) and lower with models using $\delta^{13}C$-$\delta^{15}N$ *versus* models using $\Delta^{14}C$ for all other organisms: *H. caruncalata* (32 *vs* 13), *S. tenuis* (34 *vs* 7), *Balanus* sp. (45 *vs* 14), *Pseudopterogorgia* sp. (13 *vs* 3), and *I. birotulata* (13 *vs* 2) (Fig. 5). Mean bacterial contributions (%) estimated with SIAR models using combined ^{13}C-$\delta^{15}N$-$\Delta^{14}C$ values (with lower and higher 95 % credibility intervals) to the diets of *D. antillarum*, *A. bahianus*, *A. saxatilis*, *S. partitus*, *H. caruncalata*, *Balanus* sp., *Pseudopterogorgia* sp., *A. fistularis* and *I. birotulata* were respectively 32 (20–48), 50 (27–73), 42 (29–65), 21 (10–23), 15 (1–35), 8 (3–12), 15 (0–27), 4 (0–14), 5 (0–15) and 2 (0–7) (Fig. 4).

Mean values of the interval between lower and higher 95 % credibility interval for all studied organisms was 40 ± 22 % for $\delta^{13}C$-$\delta^{15}N$ models, 39 ± 24 % for $\Delta^{14}C$ models and 24 ± 12 % for ^{13}C-$\delta^{15}N$-$\Delta^{14}C$ models but those values were not significantly different (Kruskal-Wallis tests, NS).

4. Discussion

4.1. Radiocarbon as a powerful tracer to understand trophic relationship

The use of radiocarbon to evaluate the trophic role of a food resource necessitates a specific $\Delta^{14}C$ composition of this food source compared to other items available. Chemoautotrophic microbes using ^{14}C depleted carbon releases by volcanic activity (CO_2 or CH_4) exhibit depleted ^{14}C concentrations compared to atmospheric carbon (Bruns et al., 1980; Williams et al., 1981). Bouillante Bay is an appropriate study site as the source of geothermal bacteria is artificial and consequently restricted spatially. Opportunist species such as fish and urchin ingest bacteria from geothermal water as it has been shown using C, N and S stable isotopes (Pascal et al., 2017) and heavy metal tracers (Ruiz et al., in prep). The present study reveals a specific radiocarbon content of geothermal bacteria changing the radiocarbon composition of their consumers in Bouillante Bay confirming the utility of this tracer in food web ecology.

In order to evaluate the potential advantages of radiocarbon, we compared $\Delta^{14}C$ data and stable isotope compositions ($\delta^{13}C$ and $\delta^{15}N$) simultaneously measured in Bouillante and Control stations. Similar analyses in a control station located 400 m away from the discharge channel were realized in a previous study and revealed analogous results concerning $\delta^{13}C$ of urchin, fireworm and sponge (Pascal et al., 2017). Results were also similar for $\delta^{15}N$ with highest values for fish, urchin and polychaeta confirming the higher trophic levels of those species. Similar results indicate a relatively stable structure of the food web allowing comparison between study despite the different sampling period. Differences in isotopic composition of potential consumers between stations from control and close to geothermal plant were not totally similar according to studies. None $\delta^{13}C$ composition was different in the present study, whereas a lower $\delta^{13}C$ was previously observed close to the geothermal plant for two species (*D. antillarum* and *A. saxatilis*) (Pascal et al., 2017). Contrariwise all species from the station close to the plant present a lower $\delta^{15}N$ in the present study whereas this difference was

only observed for three species previously (*D. antillarum*, *A. saxatilis* and *S. partitus*). In both studies, average isotopic compositions of potential consumers from control stations were used to evaluate contribution of geothermal bacteria to the diet of consumers found close to the release channel. The present model using $\delta^{13}C$ and $\delta^{15}N$ suggest an ingestion of bacteria by all consumers including filter feeders and omnivorous predators whereas importance of bacteria was previously shown to be limited in their diets using stable isotope (Pascal et al., 2017).

Water sample dissolved inorganic carbon (DIC) and fauna from the control site present a $\Delta^{14}C$ fluctuating between 25 and 35 ‰ reflecting atmospheric CO_2. Stream water can contain DIC from weathered carbonates and old organic matter with lower $\Delta^{14}C$ than contemporary atmospheric CO_2 (Fellman et al., 2015). In the control site, the influence of freshwater discharge would be limited due to its estrangement with river mouths and since rivers of Basse Terre do not contain carbonate bedrock due to the volcanic origin of the island. Several species from Bouillante present similar $\Delta^{14}C$ composition suggesting that the bias due to freshwater contribution can be reasonably set apart.

The DIC of water released by the plant present a $\Delta^{14}C$ of −682 ‰. The geothermal water pumped by the plant is diluted with seawater from the bay in order to reach a temperature lower than 45 °C before its release. $\Delta^{14}C$ of the DIC of geothermal water would consequently be lower than −682 ‰ with a composition characteristic of DIC from magmatic activity ($\Delta^{14}C = -1000$ ‰ or "dead carbon") (Corliss et al., 1979). Bacteria are synthetizing organic matter in their cells by using carbon available in their surrounding environment (Hansman et al., 2009; Nishida et al., 2020). Bacteria covering pebbles of the discharge channel should consequently present a $\Delta^{14}C$ similar to $\Delta^{14}C$ of DIC of water release by the plant. However, a difference of −159 *versus* −682 was observed between carbon of bacteria and water respectively. Bacteria would integrate carbon over a long time period and the punctual measurement done in water in this study is not necessarily representative of water composition at longer time scale. Despite this discrepancy, geothermal bacteria present a distinct radiocarbon composition compared to atmospheric CO_2 allowing the use of radiocarbon as a trophic tracer.

4.2. Ecological relations revealed from SIAR model

SIAR model using radiocarbon data quantify the role of geothermal bacteria in the diet of each organism. Results are similar to previous SIAR modelling using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ stable isotopes (Pascal et al., 2017): fish and urchin ingest more bacteria than others organisms. The 3 mobile fish ingest bacteria as it was previously shown with *i*) SIAR modelling, *ii*) evolution of isotopic composition of fish after the temporal stop of bacterial production, *iii*) higher abundance of fish when the plant was active (Pascal et al., 2017) and Hg composition of liver and muscle of each fish species (Ruiz et al., in prep). For the urchin *D. antillarum*, sulfur bacteria represent an important part of the diet (32 %) but lower than previously modelled (66 %). This difference can be due to the mobility of this species and its ability to selectively ingest food (Tuya et al., 2001; Cabanillas-Terán et al., 2019) changing diet composition between each study. The opportunist omnivorous scavenger fireworm *Hermodice carunculata* (Jumars et al., 2015) does not ingest important amount of sulfur bacteria as previously shown (Pascal et al., 2017). All other grazers are filter feeders with different strategy to extract their food from a dilute environment (Riigsård and Larsen, 2010). None of them ingest significant amount of geothermal bacteria as previously shown in Bouillante for passive suspension-feeder (Pascal et al., 2017) with the gorgonian *Pseudopterogorgia* sp. and active suspension-feeders such as bivalves (*S. tenuis* using gills and labial palps), barnacle (*Balanus* sp. using cirri feeding legs) and sponges (*I. birotulata* and *A. fistularis* pumping water to choanocyte chambers where particles are retained). *A. fistularis* is a bacteriosponge with a diet relaying on large amount of symbiotic bacteria using dissolved carbon resource (Reiswig, 1981; Hentschel et al., 2006). Radiocarbon does not allow to detect the influence of geothermal water in *A. fistularis* suggesting that the plant would have a limited influence on the total amount of dissolved carbon. The vent mussel *Bathymodiulus thermophiles* host chemosymbiotic bacteria giving the mussel the ability to perform CO_2 fixation (Ponnudurai et al., 2017). Due to the fixation of volcanic DIC, *B. thermophiles* present a depleted $\Delta^{14}C$ close to deep sea vent whereas 8 m away from this vent the influence of geothermal DIC was not detectable anymore in mussel (Williams et al., 1981) suggesting a rapid dilution of dissolved carbon from geothermic activity at decimeter to meter scale (Nomaki et al., 2019). Similar process can occur in the present study where all biological samples were collected as close as possible to the water discharge outlet with a distance lower than 5 m (according to the availability of species). However, this distance may be too large to detect influence of DIC in sponge at really small spatial scale due to rapid DIC dilution.

Mixing model using simultaneously $\delta^{13}C$, $\delta^{15}N$ and $\Delta^{14}C$ suggest an ingestion of bacteria limited to opportunist and moving fauna (fish and urchin) whereas fixed filter feeders were unaffected as revealed by a previous study in Bouillante (Pascal et al., 2017). Similar restriction of the trophic role of geothermal bacteria according to feeding mode has been observed in shallow vent of California (Trager and De Niro, 1990).

Food web of vents in deep sea are mainly supported by geothermal bacteria (Lonsdale, 1977) whereas in shallow vent, the energy is supplied by both chemosynthesis and photosynthesis (Tarasov et al., 2005). In intertidal vent of California, geothermal bacteria are ingested by grazers and not by filter feeders (bivalves) as in Bouillante Bay (Trager and De Niro, 1990). In Italy, most organisms ingest bacteria including filter feeders such as bivalves but not sponges (Southward et al., 1996; Southward et al., 1997). In Taiwan, grazers, predators and filter feeders represented by sea anemone, slipper limpet and coral ingest bacteria even if they do not represent the majority of their diet (Wang et al., 2022).

$\delta^{13}C$ and $\delta^{15}N$ values are routinely used to track organic matter moving through food web (Peterson and Fry, 1987). In the present study, the simultaneous measurement of those classical isotope and radiocarbon leads to results slightly different. Compared to modelled diet using radiocarbon, the use of $\delta^{13}C$ and $\delta^{15}N$ data lead to underestimate the role of geothermal bacteria for diadema urchin and 3 fish species whereas this role is overestimated for all other studied organisms. Radiocarbon leads to results closer to result previously modelled (Pascal et al., 2017). Most probable explanation for this difference would be due to trophic discrimination as $\delta^{13}C$ and $\delta^{15}N$ are affected by metabolic activity during the production of organic matter (Laws et al., 1997). Uncertainty associated with factor of discrimination can results in error higher than 30 % of source contribution estimated in mixing model (Vander Zanden and Rasmussen, 2001; Medina-Contreras et al., 2022). On the contrary radiocarbon are not affected by fractionation associated with trophic level, productivity and composition of the prey. Potential biases are reduced and radiocarbon consequently constitute a more robust tracer of carbon (Ishikawa et al., 2012) more sensitive in detecting supplementary feeding events (Eisenmann et al., 2017).

Radiocarbon analyses are more expensive than stable isotopes ones but it becomes gradually *i*) more affordable and *ii*) less demanding about sample biomass. As a result radiocarbon will probably be more used in food web study in coming decade (Larsen et al., 2018). The utility of radiocarbon is limited to food web containing a food item with a specific depleted value compared to others. Such depletion can be due to food items from geothermal activity but also in food items from the decay of old organic matter such as hydrocarbon and methane. In such situation, the present study confirms the utility of this tracer giving more reliable result than the classical use of stable isotope ($\delta^{13}C$ and $\delta^{15}N$).

CRediT authorship contribution statement

Pierre-Yves Pascal: Conceptualization, Investigation, Resources, Visualization, Writing – original draft. **Hidetaka Nomaki:** Investigation, Resources, Writing – review & editing. **Yosuke Miyairi:**

Investigation, Resources, Writing – review & editing. **Yusuke Yokoyama:** Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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