Dear Professor Rose:

We sincerely appreciate for allowing us to submit our revised MS (bg-2022-177). This version, entitled "Differential feeding habits of the shallow-water hydrothermal vent crab *Xenograpsus testudinatus* correlate with their resident vent types at a scale of meters" has been extensively revised. Thanks for all the valuable comments by the anonymous referee and Dr. Wang. Our responses are as follows:

Anonymous referee #1, 02 Nov 2022

This manuscript investigated stable isotopic compositions and protein expression patterns of the vent crabs inhabiting the yellow vents and the white vents in famous shallow-water hydrothermal vents located off Kueishan Island, Taiwan. This manuscript provided little isotope and protein expression data (n=16) compared to previous literature. It does not match the general data quality/quantity for Biogeosciences. Due to the limited data, the calculated isotopic niche width for the vent crabs from different vent types may not be representative. The discrimination of protein expressions may also be problematic.

Reply:

We actually conducted the isotopic and proteomic studies twice in 2010. We collected vent crabs on July 2 at both vents, August 4 at WV, and 24 at YV. The specimens used in the isotope niche width and proteomic analyses differed in samples of July but were the same in August. Our primary aim was to compare the feeding habits of crabs from different vent types. So, we decided to report the results from August samples only to avoid potential influence by inter-individual variations. Although the data were not too many, we thought the results were still significant.

Here, we combined the two sets of data to investigate spatial and temporal variations in the feeding habits of the vent crabs. We found that crabs' $\delta^{13}C$ and $\delta^{15}N$ values significantly differed spatially and temporally (MANOVA test, $p < 0.05$). The niche width of vent crabs from YV-Aug (0.88 ‰^{2}) narrowed substantially compared to other groups (i.e., YV-July (2.94 ‰^{2}), WV-July (2.88 ‰^{2}), and WV-Aug (3.62 ‰^{2})) ($p<0.05$), respectively. The protein expression patterns of the crabs exhibited three groups, i.e., WV-July & YV-July, WV-Aug, and YV-Aug, respectively. Our results indicated that the dwelling crabs were associated with their living vent, and within-vent variability was more noticeable in YV compared to WV. The primary corrected results in the revised MS included L145–164, Tables 2 and 3, Figure 6. And the main reanalyzed results are as follows.

L145–156: **3.2 Isotopic niche width of vent crabs from the WV and YV**

Table 2 and Fig. 3 showed the size ranges of vent crabs and their $\delta^{13}C$ and $\delta^{15}N$ values from the two vents in July and August 2010. For WV crabs, the mean values were -17.58 ± 0.21 ‰ and -16.59 ± 0.27 ‰ for $\delta^{13}C$, and 7.77 ± 0.16 ‰ and 7.66 ± 0.43 ‰ for $\delta^{15}N$, respectively. For YV crabs, the data were -16.54 ± 0.43 ‰ and -16.18 ± 0.22 ‰ for $\delta^{13}C$, and 6.35 ± 0.75 ‰ and 6.98 ± 0.32 ‰ for $\delta^{15}N$, respectively. The analysis of two-way MANOVA on $\delta^{13}C$ and $\delta^{15}N$ isotopes revealed significant effects of vent type and sampling month (Pillai's trace, $p < 0.05$), and there was no interaction between the two factors.

The isotopic niche width of crabs from YV-Aug was significantly narrower than those of YV-July, WV-July, and WV-Aug., respectively. Their SEAc areas were 0.88 ‰^{2} vs. 2.94, 2.88, and 3.62 ‰^{2} ($p < 0.05$), respectively (Table 3 and Fig. 4). The overlapped SEAc area between the two vents was 1.47 ‰^{2} in July, while it was 0.86 ‰^{2} in August (Table 3). In July, the overlap percentage was similar in both WV and YV (51.02 vs. 50.03 %). In contrast, the overlap percentage in WV was low (23.68 %) compared to YV (97.87 %) in August. These results indicate that temporal variations of food sources in YV were more significant than in WV.

L157–164: **3.3 Protein expression patterns of vent crabs from the WV and YV**

A total of 27 protein bands were selected for BCS analysis (Fig. 5). Vent crabs were clustered into three groups, i.e., WV-July & YV-July, WV-Aug, and YV-Aug, respectively (Fig. 6). The first to the fifth principal components accounted for 42.9, 22.4, 9.9, 7.4, and 5.4 % of the total variance, respectively. The first principal component (PC1) mainly contributed to the separation, i.e., bands 5, 7, 23, 26, and 27. August samples with the lowest and highest PC1 values were crabs W8m and Y5m, which corresponded to their $\delta^{13}C$ and $\delta^{15}N$ values of -14.99 and 8.55 ‰ vs. -16.77 and 7.18 ‰, respectively (Fig. 3). Further identification of specific protein bands can characterize their structures and functions. In brief, as the isotopic results, the vent crabs exhibited temporal and spatial variations in protein expression patterns.

Table 2. The isotopic data and statistical results of vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents in July and August 2010. (a) The $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs; (b) results of the two-way multivariate analysis of variance (MANOVA, Pillai's trace). W: white vent; Y: yellow vent; black bold: p<0.05; sampling date: July 2 (0702), August 4 (0804), and August 24 (0824); n: sample size.

(a)

Crab group	n	Carapace width (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
W0702	32	22.17 ± 0.51 (14.70 ~ 27.50)	-17.58 ± 0.21 (-19.69 ~ -13.73)	7.77 ± 0.16 (4.02 ~ 9.16)
W0804	9	25.30 ± 0.81 (19.55 ~ 27.33)	-16.59 ± 0.27 (-17.50 ~ -14.99)	7.66 ± 0.43 (4.72 ~ 8.94)
Y0702	6	21.62 ± 0.53 (20.45 ~ 23.58)	-16.54 ± 0.43 (-17.96 ~ -14.99)	6.35 ± 0.75 (3.89 ~ 8.57)
Y0824	7	22.01 ± 0.89 (17.84 ~ 24.44)	-16.18 ± 0.22 (-17.00 ~ -15.22)	6.98 ± 0.32 (5.39 ~ 8.00)

(b)

MANOVA ($\delta^{13}C$, $\delta^{15}N$)	*df*	Pillai's trace	F	Num *df*	Sig.
Site	2	0.14	4.04	49	**0.02**
Month	2	0.14	4.04	49	**0.03**
Site * Month	2	0.02	0.55	49	0.58
Residuals	50				

Table 3. The ellipses analyses of vent crabs (*Xenograpsus testudinatus)* from the white and yellow vents in July and August 2010. (a) Comparisons of the SEAc areas among crab groups using Layman metrics based on the posterior distribution (95% credited intervals) of the modes ($p<0.05$, A>B); (b) the overlapping percentage of ellipses area among groups. W: white vent; Y: yellow vent; SEAc: standard ellipse area corrected; sampling date: July 2 (0702), August 4 (0804), and August 24 (0824).

(a)

Crab group	SEAc (‰2)	95% confidence interval	Comparisons ($p<0.05$, A>B)
W0702	2.88	1.95–3.96	A
Y0702	2.94	1.35–8.63	A
W0804	3.62	1.48–6.18	A
Y0824	0.88	0.40–2.24	B

(b)

Crab group		Overlap SEAc (‰^{2})	Overlap in A (%)	Overlap in B (%)
A group	B group			
W0702	W0804	2.05	71.30	56.71
W0702	Y0702	1.47	51.02	50.03
W0702	Y0824	0.76	26.23	86.22
W0804	Y0702	1.89	52.19	64.35
W0804	Y0824	0.86	23.90	97.87
Y0702	Y0824	0.72	24.60	82.47

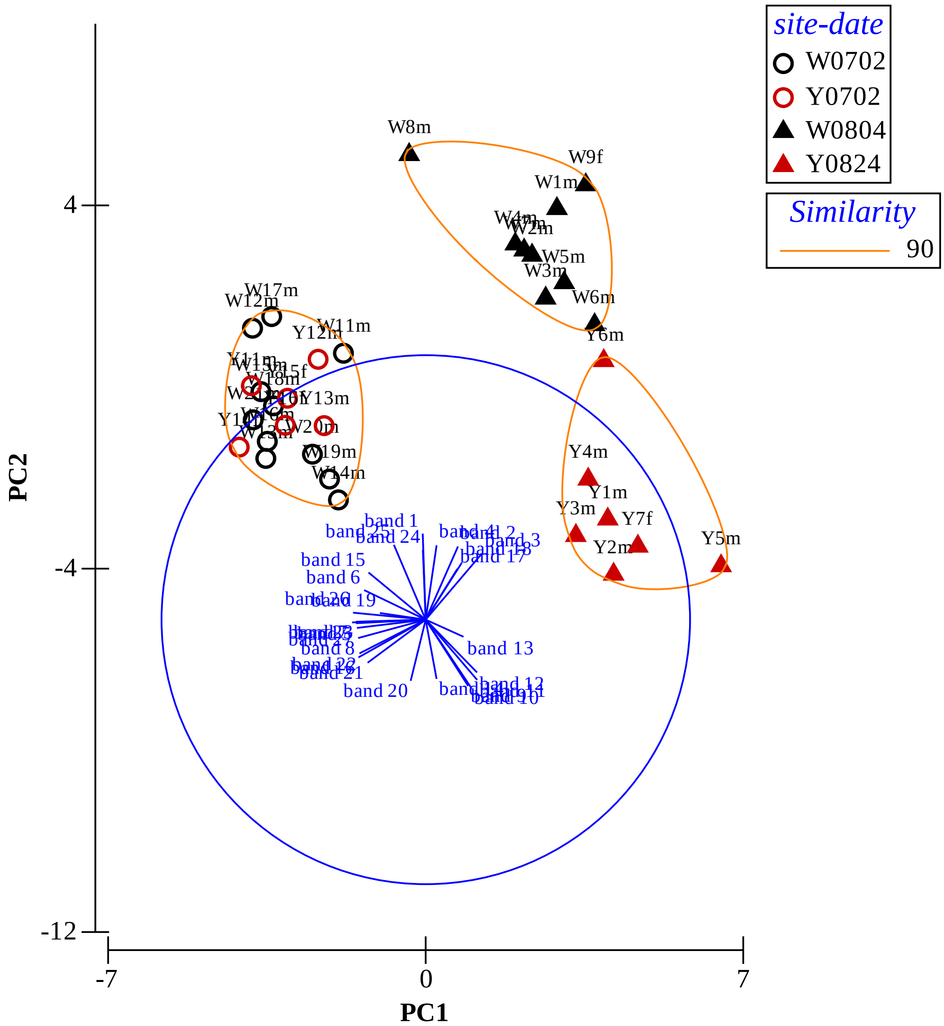


Figure 6: Results from the combined principal component analysis (PCA) and cluster analysis of Bray–Curtis similarity (BCS) indices using standardized overall protein expressions. W: white vent; Y: yellow vent; m: male; f: female; band 1–27: variable of protein bands; 0702: July 2; 0804: August 4; 0824: August 24.

The discussion should have discussed the data more thoughtfully. I can only get more details of related data suggesting the consistency among various studies (in 4.1, 4.2). Or there are more summaries of similar observations demonstrating this work is not unique (in 4.2, 4.3). It's also difficult to follow the logic (in 4.4). Such criticism may result from the author's writing skills, preventing my understanding.

Reply:

We extensively revised our discussion. The corrected sections (4.2, 4.3, and 4.4) in the revised MS are in L190–225, L226–245, and L246–264, respectively. We also list them as follows.

L190–225: **4.2 The isotopic niche width of vent crabs from the WV and YV**

Wu et al. (2021a) and Hung et al. (2019) reported that the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs significantly differed between WV and YV. However, both studies combined specimens from two sampling months. Wu et al. conducted their experiments in July and August 2010, with the values of -17.4 ± 0.2 ‰ (WV; n = 44) and -16.3 ± 0.2 ‰ (YV; n = 17) for $\delta^{13}C$ and 7.8 ± 0.14 ‰ (WV) vs. 6.7 ± 0.3 ‰ (YV) for $\delta^{15}N$, respectively (Wu et al., 2021a). Hung et al. gathered their samples in April and July 2010. They found male crabs from YV differed from all other groups, i.e., YV-female, WV-male, and WV-female, respectively (sample size and data not shown) (Hung et al., 2019).

Within-vent variability in $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs was also documented in several studies. Hung et al. collected their samples in April and July 2010, and the $\delta^{13}C$ and $\delta^{15}N$ values of both male and female crabs exhibited no difference between the center and edge of a WV (sample size and data not shown) (Hung et al., 2019). In Wang et al., crabs from one site influenced by both WV and YV and three peripheral groups (150–300 m) presented a wide range of $\delta^{13}C$ (-20.5 to -14.3 ‰) and $\delta^{15}N$ (3.2 to 9.8 ‰) values sampled in June and July 2014 (Wang et al., 2022). And, there was no significant difference in the isotopic data among the four groups ($p > 0.05$), i.e., -16.9 ± 0.77 ‰ and 8.1 ± 0.94 ‰ (n = 6); -17.2 ± 1.34 ‰ and 7.5 ± 1.01 ‰ (n = 40); -16.6 ± 1.03 ‰ and 7.2 ± 1.43 ‰ (n = 156); -16.9 ± 0.66 ‰ and 8.3 ± 1.17 ‰ (n = 10), respectively. Further isotopic niche analysis demonstrated that the contribution of dead zooplankton as a food source to those crabs ranged from > 34 % (vent center) to ≤ 18 % (peripheral sites). We also analyzed the isotopic data published by Chang et al. for comparison (Chang et al., 2018). They gathered vent crabs from a WV along the southwest transect in August and September 2015. The $\delta^{13}C$ and $\delta^{15}N$ values were significantly different between the center and periphery (70–100 m) (MANOVA, $p = 0.01$), i.e., -16.20 ± 2.49 ‰ and 5.33 ± 4.06 ‰ (n = 4); -17.55 ± 0.74 ‰ and 8.85 ± 0.79 ‰ (n = 10), respectively. Dead zooplankton as a food source for those crabs were 6–38 % vs. 16–42%, respectively.

In this study, the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs significantly differed between vent types and sampling months (MANOVA test, Table 2). Our results showed that the crabs' isotopic niche width (shown as the SEAc area) was considerably narrower in YV-Aug (0.88 ‰^{2}) than those in YV-July, WV-July, and WV-Aug (2.94, 2.88, and 3.62 ‰^{2}) ($p < 0.05$), respectively (Table 3). In the southwest Mediterranean, seasonal variations in the $\delta^{13}C$ and $\delta^{15}N$ values of the sally lightfoot crab *Percnon gibbesi* ranged from -18.33 to -13.08 ‰ and from 3.71 to 8.2 ‰ in 2016 (Bada et al., 2022). The isotopic niche width of *P. gibbesi* varied from 1.4 ‰^{2} in winter to 4.5 ‰^{2} in autumn, while the data were 1.5 and 2 ‰^{2} in spring and summer, respectively. It showed that the diets of *P. gibbesi* in autumn had the widest niche (food variability) linked to the local variability in algal resources. In the Pechora Sea, the isotopic niche width in scavenger hermit crab *Pagurus pubescens* varied between sites of 4N and 9N with a distance of 13 km because of a significant difference in their macrobenthic abundance (Gebruk et al., 2021). The isotopic niche width for the hermit crab was 0.15 ‰^{2} at 4N and 0.27 ‰^{2} at 9N, with 0.05 ‰^{2} overlapped. Differences in diet sources were correlated with local macrobenthic clams as shown at 4N, characterized by low *Astarte montagui* (32 g m^{-2}), in contrast to the high biomass of *A. borealis* and *Macoma calcarea* (500 g m^{-2}) at 9N. The niche width of this hermit crab had an even smaller overlapping SEAc area than our between-vent comparisons, i.e., 1.47 ‰^{2} in July and 0.86 ‰^{2} in August 2010. In brief, our study clearly shows that the isotopic signatures of the resident vent crabs reflected temporal and spatial heterogeneities. The discrepant results among different studies indicate explicit state sampling information, including size, date, and location, is essential.

L226–245: **4.3 Protein expression patterns of vent crabs from the WV and YV**

Our proteomic results indicated that vent crabs were distinguishable as groups of WV-July & YV-July, WV-Aug, and YV-Aug, respectively. In the case of dove snails, *A. misera* inhabiting in WVs of KS Islet, their protein expression patterns were related to the diffusion of locally emitted vent fluids (Chen et al., 2015). The naturally acidified seawater in the southward sampling site had pH ranges from 7.78 to 7.82, while it was 7.31–7.83 in the east, southwest, and northwest locations. Based on the expressed protein profiles, the *Anachis* snails were classified into the south and another group. In a CO_2-SV off Vulcano Island in Sicily, sea anemones *Anemonia viridis* were collected at a distance of 350–800 m from a vent, where the pH values were 7.6, 7.9, and 8.2, respectively (Urbarova et al., 2019). Gene expression patterns of *A. viridis* revealed two clades, i.e., low pH group (pH 7.6) vs. high pH ones (pH 7.9 and pH 8.2). Overall, mobile vent crabs, slow-moving dove snails, and sessile sea anemones all performed adaptation abilities associated with their environments.

Organisms respond to environmental changes in a time-dependent manner. When the Chinese mitten crabs *E. sinensis* were transferred to high salinity (25 psu) for six days, the protein profiles of posterior gills were different from the control group (0 psu) (Yang et al., 2022). The nutrition value of linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, LNA) in the Chinese mitten crabs *E. sinensis* was evaluated in the laboratory for 107 days (Wei et al., 2018). A total of 186 proteins were expressed differentially in the hepatopancreas between the groups of LA and LNA. In the Teboulba fishing harbor in Tunisia, high levels of aliphatic and aromatic hydrocarbon pollutants were in the sediments (Jebali et al., 2014). The Mediterranean crabs *C. maenas* showed differential protein expression patterns in hepatopancreas between control (day 0) and exposed groups with 15, 30, and 60 days. These proteomic-based studies exhibited the earliest responses of tested crabs to environmental changes detected at least on day 6. In this study, the protein expression patterns of vent crabs changed in one month (Fig. 5), indicating the vent environments probably fluctuated often.

L246–264: **4.4 Association of crabs' feeding habits with vent types**

It has long been known that WVs and YVs in KS Islet differ in the color and composition of vent plumes (Chen et al., 2005b; Lebrato et al., 2019; Mei et al., 2022). A relatively low fluid temperature and high pH in WVs compared to YVs (30–65 vs. 54–121 °C and 1.84–6.96 vs. 1.52–6.32 (pH seawater scale, 25 °C) (Table 1). Recently, Lebrato et al. studied temporal biogeochemical changes in this SV system during 2009–2018 (Lebrato et al., 2019). Their principal findings are the catastrophic earthquake and typhoon Nepartak in 2016 shaped the seabed morphology, seawater chemistry, vent fluid composition and flow rate, and benthic ecology, then gradually recovered in 2018. In addition, the reduction in venting activity and fluid flow in YV was more severe than in WV. The feeding habits of vent crabs presented by isotopic and proteomic results did reflect the geochemical characteristics of vent types.

Previous studies reported that the movement of vent crabs reveals different spatial scales. The daily foraging movement is in the vent area (Jeng et al., 2004; Chang et al., 2018; Allen et al., 2020). During the reproductive season, ovigerous females move to the vent periphery, release their larvae, and then return to the chimneys (Hung et al., 2019). The migratory distance was about 100–200 m horizontally from the vent mouth. Besides, vent crabs were absent in the by-catch of nearby non-vent fisheries (Wang et al., 2013). And the holotype of this species was collected from a 15 m deep rocky reef in the Gengxin Fish Port, I-Lan, Taiwan (Ng et al., 2000). These investigations indicate that vent crabs can actively move and survive in vent and non-vent environments. However, how far and how often the crabs move around is unknown. Here, we demonstrated the vent crabs exhibited temporal and spatial variations in isotopic niche width and protein expression patterns (Table 3 and Fig. 6). Even with a distance of 100 m, the endemic vent crabs are strongly associated with their vent types. In addition, within-vent variability in food sources is more dramatic in YV compared to WV.

Additional references:

Bada, N., Da Ros, Z., Rindi, F., Busi, S., Azzurro, E., Derbal, F., and Fanelli, E.: Seasonal trophic ecology of the invasive crab *Percnon gibbesi* (Brachyura, Plagusiidae) in the southwestern Mediterranean: Insights from stomach contents and stable isotope analyses. Mar. Environ. Res., 173, 105513, https://doi.org/10.1016/j.marenvres.2021.105513, 2022.Lebrato, M., Wang, Y. V., Tseng, L. C., Achterberg, E. P., Chen, X. G., Molinero, J. C., Bremer, K., Westernstroer, U., Soding, E., Dahms, H. U., Kuter, M., Heinath, V., Johnck, J., Konstantinou, K. I., Yang, Y. J., Hwang, J. S., and Garbe-Schonberg, D.: Earthquake and typhoon trigger unprecedented transient shifts in shallow hydrothermal vents biogeochemistry, Sci. Rep., 9, 16926, https://doi.org/10.1038/s41598-019-53314-y, 2019.

Wu, J. Y., Lin, S. Y., Peng, S. H., Hung, J. J., Chen, C. T. A., and Liu, L. L.: Data on isotopic niche differentiation in benthic consumers from shallow-water hydrothermal vents and nearby non-vent rocky reefs in northeastern Taiwan. Data Br., 37, 107216, https://doi.org/10.1016/j.dib.2021.107216, 2021b.

Several specific comments are as followings.

1. Table 1 needs to be clarified and easier to read. The geochemical data for vent fluids from previous literature need to be synthesized more appropriately.

Reply:

The revised Table 1 is as follows.

Table 1. Location and environmental measurements of the study sites. (Mean ± S.E.); n: sample size.

Environmental parameters	WV (White vent)	YV (Yellow vent)	Sampling date	References
Shallow-water hydrothermal vents	WVs	YVs		
Vent plume				
Temperature (°C)	30–65 (50.7 ± 8.2, n = 109); 31–38	78–116 (106.00 ± 9.16, n = 115); 50–90	2000; 2017	Chen et al., 2005b; Mei et al., 2022
pH	1.84–6.96 (3.20 ± 1.17, n = 110)	1.52–6.32 (2.49 ± 0.72, n = 116)	2000	Chen et al., 2005b
H_2S (mmol mol^{-1})	2.3–21.0 (12.94 ± 4.55, n = 4)	7.6–114.7 (60.12 ± 19.57, n = 6)	"	"
CO_2 (mmol mol^{-1})	916–987 (n = 3)	976–992 (n = 2)	"	"
N_2 (mmol mol^{-1})	0.02–0.04 (n = 3)	0.11–2.23 (n = 2)	"	"
Sampling vent's geographic coordinates	24.83404° N, 121.96172° E	24.83553° N, 121.96361° E		
Vent plume				
Temperature (°C)	47–49 (48.00 ± 0.37, n = 6); 55 ± 4	115–116 (115.40 ± 0.22, n = 5); 106 ± 6	2010–2014; 2010–2011	Chen et al., 2016; Hung et al., 2019
	62	97	2010.07.02	Lin, 2011
	41	105	2010.08.03–05	Yang et al., 2012
	58	97	2010.08.24–27	"
		65;105; 121; 105; 54–63	2009; 2010.08.07; 2011; 2016.03; 2016.08–2017.08	Lebrato et al., 2019
pH	5.45 ± 0.65	2.48 ± 1.06	2010-2011	Hung et al., 2019; Lin, 2011
	5.06	2.81	2010.07.02	Lin, 2011
	4.83	2.82	2010.08.03–05	Yang et al., 2012
	5.74	2.22	2010.08.24–27	"
H_2S (mmol mol^{-1})	2.2–57.4 (18.4 ± 8.4, n = 6)	4.3–172.4 (90.8 ± 29.1, n = 6)	2010–2014	Chen et al., 2016

CO_2 (mmol mol^{-1})	161.7–760.6 (503.8 ± 78.7, n = 8)	731.0–881.6 (798.4 ± 23.8, n = 6)	"	"
N_2 (mmol mol^{-1})	109.5–633.7 (309.9 ± 72.4, n = 8)	33.4–140.9 (65.1 ± 17.0, n = 6)	"	"
Crab collecting site				
Distance to vent center (m)	~ 5	~ 5	2010.08	This study (WV: 0804; YV:0824)
Depth (m)	17	7	"	"
Temperature (°C)	25	26.7	"	"
pH	7.3	7.8	"	"
Deposited sulfur particles (diameter)	globules (~ 0.05–0.1 cm)	balls (> 2 cm)	"	"

2. The drawing quality of figures 3 and 4 need to be improved.

Reply:

Figure 3 is the output of Excel in Microsoft, and Figure 4 is the output of the analysis software SIBER v2.1.6 (Stable Isotope Bayesian Ellipses in R) package in R 4.2.2 software (R Development Core Team, 2013) and RStudio 2022.12.0-353. Both figures are already the best results we can get, which are as follows.

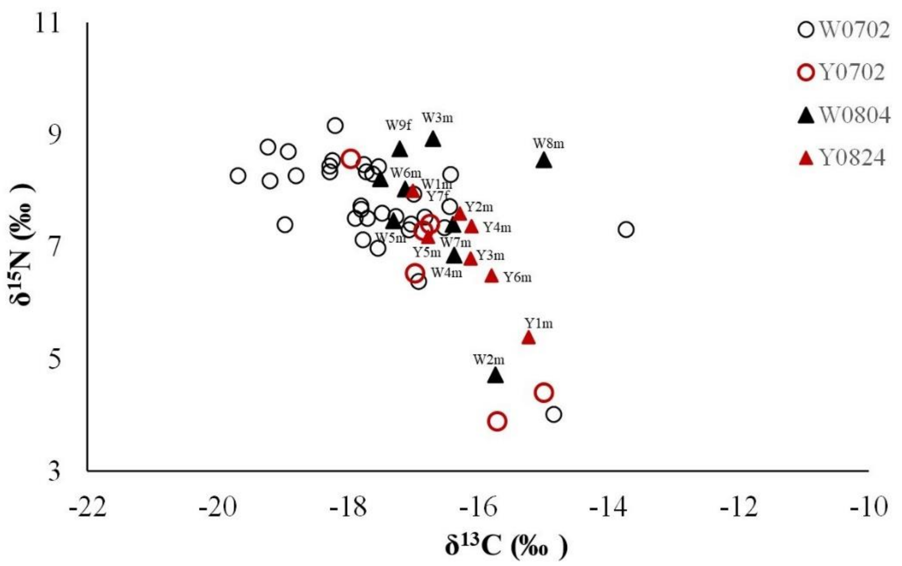


Figure 3: The $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. W: white vent; Y: yellow vent; sampling date: July 2 (0702), August 4 (0804), and August 24 (0824); m: male; f: female; the crabs with label: same individuals for proteomic experiments.

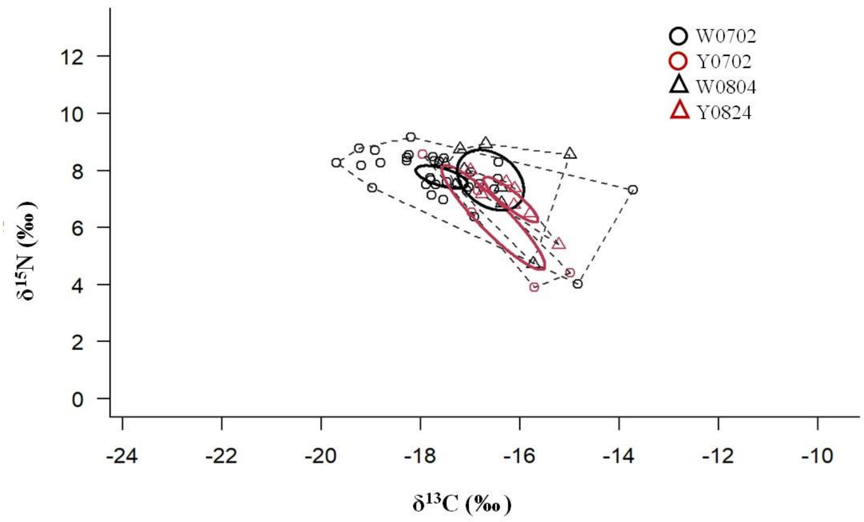


Figure 4: Convex hull and standard ellipses areas based on the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. Dot lines: convex hull areas; solid lines: standard ellipses areas (SEAc); W: white vent; Y: yellow vent; 0702: July 2; 0804: August 4; 0824: August 24.

3. Geochemical and isotopic data are mentioned repeatedly.

Reply:

We analyzed more data and did our best to avoid repetitive discussion in our revised MS.

4. The estimate of isotopic niche width for vent crabs need to include plenty of isotope data collected in previous studies (e.g. Wu et al., 2021).

Reply:

We have already included additional data in our revised MS, and the findings are in L145–156.

L145–156: **3.2 Isotopic niche width of vent crabs from the WV and YV**

Table 2 and Fig. 3 showed the size ranges of vent crabs and their $\delta^{13}C$ and $\delta^{15}N$ values from the two vents in July and August 2010. For WV crabs, the mean values were -17.58 ± 0.21 ‰ and -16.59 ± 0.27 ‰ for $\delta^{13}C$, and 7.77 ± 0.16 ‰ and 7.66 ± 0.43 ‰ for $\delta^{15}N$, respectively. For YV crabs, the data were -16.54 ± 0.43 ‰ and -16.18 ± 0.22 ‰ for $\delta^{13}C$, and 6.35 ± 0.75 ‰ and 6.98 ± 0.32 ‰ for $\delta^{15}N$, respectively. The analysis of two-way MANOVA on $\delta^{13}C$ and $\delta^{15}N$ isotopes revealed significant effects of vent

type and sampling month (Pillai's trace, $p < 0.05$), and there was no interaction between the two factors.
The isotopic niche width of crabs from YV-Aug was significantly narrower than those of YV-July, WV-July, and WV-Aug., respectively. Their SEAc areas were 0.88 ‰^{2} vs. 2.94, 2.88, and 3.62 ‰^{2} ($p < 0.05$), respectively (Table 3 and Fig. 4). The overlapped SEAc area between the two vents was 1.47 ‰^{2} in July, while it was 0.86 ‰^{2} in August (Table 3). In July, the overlap percentage was similar in both WV and YV (51.02 vs. 50.03 %). In contrast, the overlap percentage in WV was low (23.68 %) compared to YV (97.87 %) in August. These results indicate that temporal variations of food sources in YV were more significant than in WV.

Referee #2: Yiming Wang, ywang@shh.mpg.de, report 08 Jan 2023

General comments:

This study investigated stable isotope niche width, and protein expression for vent crab *Xenograpsus testudinatus* from the shallow water hydrothermal vents located off Kueishan Islet, Taiwan. To do this, authors provided total of 16 samples, nine from the white vent (WV) and seven from the yellow vent (YV) for comparison. In addition, authors also compared the benthic community between the two habitats using the quadrates along four transects. However, in my opinion, the scientific quality and rigors of this manuscript do not fulfil the requirement of *Biogeosciences* for the following reasons:

1. There is no clear hypothesis or research question. Specifically, the authors failed to demonstrate why it is important to investigate the endemic vent crabs in different vent types in this shallow-water hydrothermal vent environment. It is unclear why this would be interesting for the scientific community to gain this knowledge. In addition, since protein expression depends on a variety of factors such as physiological (e.g. stress) and environmental condition (e.g. pollution, pH, etc.), I would assume the protein results would be different because the YV and WV conditions are vastly different, so where do the authors go with this information?

Reply:

We actually conducted the isotopic and proteomic studies twice in 2010. The specimens used in the isotope niche width and proteomic analyses differed in samples of July but were the same in August. Related explanations and primary revised results are presented in reply to Referee #1 and our revised MS. Vent crabs can move to a distance of 100–200 m, as shown in ovigerous females. However, their regular moving range is unknown. If trans-vent movement is common, we expect to have no significant difference between WV and YV by the application of proteomic tools. We also revised

our abstract to point out the significance of our study. Here is the revised abstract.
The shallow-water hydrothermal vents (SVs) located off Kueishan (KS) Islet, Taiwan, are one of the world's most intensively studied vent systems. It has long been known that white vents (WVs) and yellow vents (YVs) differ in the color and composition of vent plumes. The endemic vent crabs (*Xenograpsus testudinatus*) are abundant in both vent types, and ovigerous females migrate to the vent periphery with a distance of 100–200 m to release their offspring. However, most research on the vent crabs was associated with WV or unspecified vent areas. To increase our knowledge of crabs dwelling in other vent types, we compared the feeding habits of vent crabs living in WV and YV with two sampling months. Specifically, we examined the benthic community of WV and YV, isotopic niche width, and protein expression patterns of the crabs from the two vents at a distance of 100 m and sampled in July and August 2010. The coverage of sessile organisms and low-mobility fauna in WV was more abundant than those in YV, based on the survey in August 2010. The $\delta^{13}C$ and $\delta^{15}N$ values of crabs significantly differed spatially and temporally (MANOVA test, $p < 0.05$). The niche width of vent crabs from YV-Aug (0.88 ‰^{2}) narrowed substantially compared to the rest, i.e., YV-July (2.94 ‰^{2}), WV-July (2.88 ‰^{2}), and WV-Aug (3.62 ‰^{2}) ($p<0.05$), respectively. Based on the protein expression patterns, the vent crabs exhibited three groups, i.e., WV-July & YV-July, WV-Aug, and YV-Aug, respectively. Our results indicated that the dwelling crabs were associated with their living vent, and within-vent variability was more noticeable in YV compared to WV. We suggested that vent crabs inhabit their resident vent. Even at a scale of meters, trans-vent movement is probably rare as an adaptation to minimize predation risk.

2. Sample sizes are too small for robust inference. Given that the main question in the paper is to examine whether the isotope niche widths of *X. testudinatus* from two vent types are similar, total of 16 samples are too small for this. Although authors used the corrected standard ellipse area (SEAc) to lessen the biases towards smaller sample sizes, I suspect additional data will change the SEAc and overlapped SEAc as well. The authors should discuss any potential biases due to the sample sizes and how the niche width may change when sample sizes are increased in the discussion.

Reply:

We added more data to investigate spatial and temporal variations in the feeding habits of the vent crabs. Our data indicate sample size and inter-individual variation are both critical factors. For comparative purposes, we present the isotopic and proteomic results with the same data sets grouped by vent types vs. vent types and sampling months as follows.

Isotopic results based on vent types.

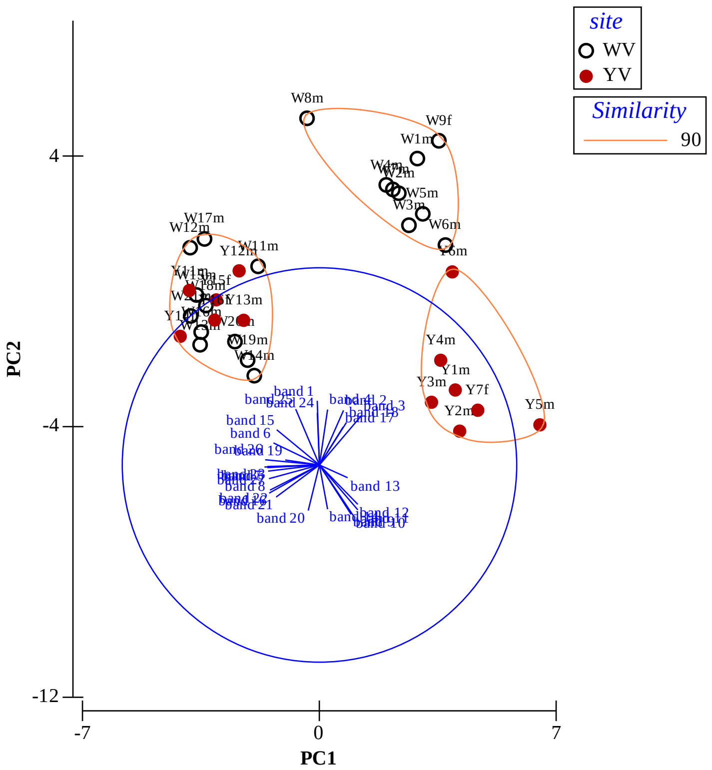
Crab group	n	Carapace width (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
WV	41	22.86 ± 048 (14.70 ~ 27.50)	-17.36 ± 0.18 (-19.69 ~ -13.73)	7.74 ± 0.17 (4.02 ~ 9.16)
YV	13	21.83 ± 0.52 (17.84 ~ 24.44)	-16.35 ± 0.31 (-17.96 ~ -14.99)	6.69 ± 0.30 (3.89 ~ 8.57)

MANOVA ($\delta^{13}C$, $\delta^{15}N$)	*df*	Pillai's trace	F	Num *df*	Sig.
Site	2	0.18	5.52	51	**0.01**
Residuals	52				

Crab group	SEAc (‰^{2})	95% confidence interval	Comparisons ($p<0.05$, A>B)
WV	3.32	2.34–4.40	A
YV	2.25	1.23–3.96	A

Crab group		Overlap SEAc (‰^{2})	Overlap in A (%)	Overlap in B (%)
A group	B group			
WV	YV	1.64	49.30	72.80

Proteomic results based on vent types.



■ Isotopic results based on vent types and sampling months.

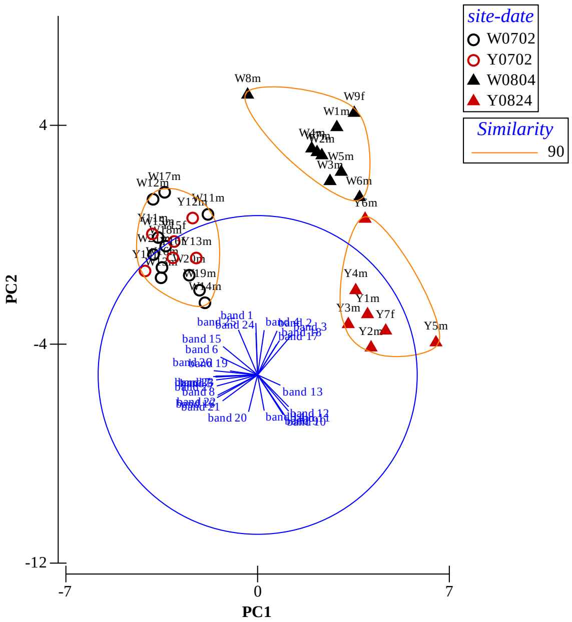
Crab group	n	Carapace width (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
W0702	32	22.17 ± 0.51 (14.70 ~ 27.50)	-17.58 ± 0.21 (-19.69 ~ -13.73)	7.77 ± 0.16 (4.02 ~ 9.16)
W0804	9	25.30 ± 0.81 (19.55 ~ 27.33)	-16.59 ± 0.27 (-17.50 ~ -14.99)	7.66 ± 0.43 (4.72 ~ 8.94)
Y0702	6	21.62 ± 0.53 (20.45 ~ 23.58)	-16.54 ± 0.43 (-17.96 ~ -14.99)	6.35 ± 0.75 (3.89 ~ 8.57)
Y0824	7	22.01 ± 0.89 (17.84 ~ 24.44)	-16.18 ± 0.22 (-17.00 ~ -15.22)	6.98 ± 0.32 (5.39 ~ 8.00)

MANOVA ($\delta^{13}C$, $\delta^{15}N$)	*df*	Pillai's trace	F	Num *df*	Sig.
Site	2	0.14	4.04	49	**0.02**
Month	2	0.14	4.04	49	**0.03**
Site * Month	2	0.02	0.55	49	0.58
Residuals	50				

Crab group	SEAc (‰^{2})	95% confidence interval	Comparisons (p<0.05, A>B)
W0702	2.88	1.95–3.96	A
Y0702	2.94	1.35–8.63	A
W0804	3.62	1.48– 6.18	A
Y0824	0.88	0.40–2.24	B

Crab group		Overlap SEAc (‰^{2})	Overlap in A (%)	Overlap in B (%)
A group	B group			
W0702	W0804	2.05	71.30	56.71
W0702	Y0702	1.47	51.02	50.03
W0702	Y0824	0.76	26.23	86.22
W0804	Y0702	1.89	52.19	64.35
W0804	Y0824	0.86	23.90	97.87
Y0702	Y0824	0.72	24.60	82.47

■ Proteomic results based on vent types and sampling months.



3. Data needs more thoughtful interpretation. Several paragraphs in the discussion were written like results. The authors listed some previous literature without properly connected to the interpretation of their own data. For example, section 4.2 paragraph 1: The isotope values between the YV and the WV from this study were not significant different, but a previous study by Wu et al., (2021) did show differences in isotope values between the YV and WV in different sampling year. The authors mentioned Wu et al.'s work, but never discussed what factors might have contributed the different outcomes between the two studies. The authors also mentioned another study that compared isotope values of vent crabs between different sex but also did not connect to their own study. Another example, in section 4.2 paragraph 3, the authors compared the isotope niche overlap percentages observed in the vent crabs with hermit crabs from a totally different environment (in Pechora Sea). However, it is difficult to see why the hermit crab is relevant to the study site in KS. This part should be better explained or deleted.

Reply:

We extensively revised our discussion as shown in our corrected MS (L190–225), and the updated section (4.2) is as follows.

L190–225: **4.2 The isotopic niche width of vent crabs from the WV and YV**

Wu et al. (2021a) and Hung et al. (2019) reported that the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs significantly differed between WV and YV. However, both studies combined specimens from two sampling months. Wu et al. conducted their experiments in July and August 2010, with the values of -17.4 ± 0.2 ‰ (WV; n = 44) and -16.3 ± 0.2 ‰ (YV; n = 17) for $\delta^{13}C$ and 7.8 ± 0.14 ‰ (WV) vs. 6.7 ± 0.3 ‰ (YV) for $\delta^{15}N$, respectively (Wu et al., 2021a). Hung et al. gathered their samples in April and July 2010. They found male crabs from YV differed from all other groups, i.e., YV-female, WV-male, and WV-female, respectively (sample size and data not shown) (Hung et al., 2019).

Within-vent variability in $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs was also documented in several studies. Hung et al. collected their samples in April and July 2010, and the $\delta^{13}C$ and $\delta^{15}N$ values of both male and female crabs exhibited no difference between the center and edge of a WV (sample size and data not shown) (Hung et al., 2019). In Wang et al., crabs from one site influenced by both WV and YV and three peripheral groups (150–300 m) presented a wide range of $\delta^{13}C$ (-20.5 to -14.3 ‰) and $\delta^{15}N$ (3.2 to 9.8 ‰) values sampled in June and July 2014 (Wang et al., 2022). And, there was no significant difference in the isotopic data among the four groups ($p > 0.05$), i.e., -16.9 ± 0.77 ‰ and 8.1 ± 0.94 ‰ (n = 6); -17.2 ± 1.34 ‰ and 7.5 ± 1.01 ‰ (n = 40); -16.6 ± 1.03 ‰ and 7.2 ± 1.43 ‰ (n = 156); -16.9 ± 0.66 ‰ and 8.3 ± 1.17 ‰ (n = 10), respectively. Further isotopic niche analysis demonstrated that the contribution of dead zooplankton as a food source to those crabs ranged from > 34 % (vent center) to ≤ 18 % (peripheral sites). We also analyzed the isotopic data published by Chang et al. for comparison (Chang et al., 2018). They gathered vent crabs from a WV along the southwest transect in August and September 2015. The $\delta^{13}C$ and $\delta^{15}N$ values were significantly different between the center and periphery (70–100 m) (MANOVA, $p = 0.01$), i.e., -16.20 ± 2.49 ‰ and 5.33 ± 4.06 ‰ (n = 4); -17.55 ± 0.74 ‰ and 8.85 ± 0.79 ‰ (n = 10), respectively. Dead zooplankton as a food source for those crabs were 6 – 38 % vs. 16 – 42%, respectively.

In this study, the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs significantly differed between vent types and sampling months (MANOVA test, Table 2). Our results showed that the crabs' isotopic niche width (shown as the SEAc area) was considerably narrower in YV-Aug (0.88 ‰^{2}) than those in YV-July, WV-July, and WV-Aug (2.94, 2.88, and 3.62 ‰^{2}) ($p < 0.05$), respectively (Table 3). In the southwest Mediterranean, seasonal variations in the $\delta^{13}C$ and $\delta^{15}N$ values of the sally lightfoot crab *Percnon gibbesi* ranged from -18.33 to -13.08 ‰ and from 3.71 to 8.2 ‰ in 2016 (Bada et al., 2022). The isotopic niche width of *P. gibbesi* varied from 1.4 ‰^{2} in winter to 4.5 ‰^{2} in autumn, while the data were 1.5 and 2 ‰^{2} in spring and summer, respectively. It showed that the diets of *P. gibbesi* in autumn had the widest niche (food variability) linked to the local variability in algal resources. In the Pechora Sea, the isotopic niche width in scavenger hermit crab *Pagurus pubescens* varied between sites of 4N and 9N with a distance of 13 km because of a significant difference in their macrobenthic abundance (Gebruk et al., 2021). The isotopic niche width for the hermit crab was 0.15 ‰^{2} at 4N and 0.27 ‰^{2} at 9N, with 0.05 ‰^{2} overlapped. Differences in diet sources were correlated with local macrobenthic clams as shown at 4N, characterized by low *Astarte montagui* (32 g m^{-2}), in contrast to the high biomass of *A. borealis* and *Macoma calcarea* (500 g m^{-2}) at 9N. The niche width of this hermit crab had an even smaller overlapping SEAc area than our between-vent comparisons, i.e., 1.47 ‰^{2} in July and 0.86 ‰^{2} in August 2010. In brief, our study clearly shows that the isotopic signatures of the resident vent crabs reflected temporal and spatial heterogeneities. The discrepant results among different studies indicate explicit state sampling information, including size, date, and location, is essential.

4. The writing needs an overhaul: A) The Introduction is unfocused and did not present a clear hypothesis. The stable isotope and proteome methods in the Introduction seem to be out of place. B) There are a lot of repetitive geochemical information for the KS vent region in the Introduction and Method. Some of these values (e.g. temperature ranges, etc) don't even match. Similarly, there are a lot of repetitive information in the Results and Interpretation for the isotope niche width. This information needs to be condensed and streamlined. C) There are numerous unclear sentences in the manuscript, e.g. line 102-104, 105-107, 200-204 etc. D) The authors cited some previous work, but required readers to go into the original references and figure out what they mean, e.g. line 207-209: 4N and 9N.

Reply:

We revised the whole MS. Major revised discussion is in comment 3. Some modified introduction parts are in the revised MS L53–73. These paragraphs are as follows.

L53–73: Stable isotope analysis is commonly applied in the study of animal feeding ecology. Through the processes of assimilation, consumers increase with stable isotope values of 0.0–1.3 ‰ for $\delta^{13}C$ and 1.4–5 ‰ for $\delta^{15}N$ in each trophic transfer (DeNiro and Epstein, 1978, 1981; Post, 2002; McCutchan et al., 2003). With the isotopic data, consumers' trophic position and niche width can be calculated (Layman et al., 2011). Trophic studies in SVs in KS Islet revealed that dead zooplankton killed by sulfur plumes (as plankton-derived production) is essential to scavengers and carnivores based on the $\delta^{13}C$ and $\delta^{15}N$ data (Wang et al., 2014; Chang et al., 2018; Wu et al., 2021a). The importance of dead zooplankton to vent crabs decreases from the vent center to the periphery (Wang et al., 2022). Furthermore, vent crabs collected from YV had significantly lower $\delta^{13}C$ and $\delta^{15}N$ values than those in WV (Wu et al., 2021a). However, such heterogeneity resulting from temporal or spatial is unknown.

Under changing environments, proteome analysis is also a helpful approach to gaining a better understanding of the physiological states of organisms (López-Pedrouso et al., 2020). For example, the variation of protein patterns of the dove snail *A. misera* was consistent with the diffusion of local vent fluids in KS Islet (Chen et al., 2015). Proteomic studies exhibited differential expression signatures in the Chinese mitten crab (*Eriocheir sinensis*) when treated with different feeds (Wei et al., 2018) or hyper-osmotic stress (Yang et al., 2022), in mud crab *Scylla olivacea* when exposed to heavy metals (Razali et al., 2019), and in Mediterranean crab (*Carcinus maenas*) from different harbors (Jebali et al., 2014). Similarly, we can extend our knowledge of the within- and between vents' variations of the physiological states of crabs living in SVs by applying proteomic tools.

Although the vent crab (*X. testudinatus*) is one of the most intensively studied species in SV systems, most research was associated with WV or unspecified vent areas. Studies on crabs dwelling in other vent types are rare. Therefore, spatial and temporal variations in the feeding habits of vent crabs were investigated in this study. Specifically, we examined the benthic community of WV and YV, isotopic niche width, and protein expression patterns of the crabs from two vents at a distance of 100 m and sampled in July and August 2010.

Specific comments:

Line 51: Change "more depleted" to "lower". You can say "something is depleted in 13C" but you can not say "depleted d13C values". The correct way is to say "lower d13C values"

Reply:

We corrected the usage according to your suggestion. For example, Line 57–58: Furthermore, vent crabs collected from YV had significantly lower $\delta^{13}C$ and $\delta^{15}N$ values than those in WV (Wu et al., 2021a).

Line 59: Reference Lopez-Pedrouso et al., is missing in reference list.

Reply:

We included the reference in our revised MS (L368–369).

L368–369: López-Pedrouso, M., Varela, Z., Franco, D., Fernández, J. A., and Aboal, J. R.: Can proteomics contribute to biomonitoring of aquatic pollution? A critical review, Environ. Pollut., 267, 115473, https://doi.org/10.1016/j.envpol.2020.115473, 2020.

Line 96: Unclear, please rephrase.

Reply:

The corrected paragraph is in L99–104 of the revised MS and is as follows.

L99–104: **2.3 Preparation of vent crabs for isotope niche width and proteomic studies**

Vent crabs have gathered 5 m away from the mouths of the WV and YV on sampling dates of July 2 (both vents), August 4 (WV), and 24 (YV) 2010, respectively. The specimens used in the isotope niche width and proteomic studies differed in samples of July but were the same in August. Each collected crab was covered with aluminum foil and kept in liquid nitrogen, then frozen at -80 °C for later use. Crab samples were examined for cleaning debris, and epibionts, then their carapace width and wet weight were measured before dissection (Fan et al., 2016).

Line 116: The standard ellipse area is SEA, the corrected standard ellipse area is SEAc. Please change.

Reply:

The corrected sentence in our revised MS (L112–114) is as follows.

L112–114: Measurements of isotopic niche width, proposed by Layman et al. (2007), were calculated for vent crabs, i.e., the corrected standard ellipse area (SEAc), which was a measure of the mean score of the isotopic niche occupied by all crab individuals in each group and their potential primary food sources in the $\delta^{13}C$ and $\delta^{15}N$ space (Jackson et al., 2011).

Line 151: Change "insignificantly different" to "not significantly different". This expression has been used in several places, e.g. line 18-19, line 187, please change them all.

Reply:

We corrected the usage according to your suggestion, as shown in the revised MS L197–199 and is as follows.

L197–199: Hung et al. collected their samples in April and July 2010, and the $\delta^{13}C$ and $\delta^{15}N$ values of both male and female crabs exhibited no difference between the center and edge of a WV (sample size and data not shown) (Hung et al., 2019).

Line 164, Remove "the first study". This is not the first study to investigate the feeding habits of vent crab (X. testudinatus) in the KS vent sites (see Wang et al., 2020 https://www.biorxiv.org/content/10.1101/2020.09.09.288985v1.full). Also, this kind of claim should be avoided in general.

Reply:

The corrected sentence is in the revised MS L165 and is as follows.

L165: This study compared the feeding habits of vent crabs (*X. testudinatus*) from a WV and a YV within 100 m.

Line 196: Change “insignificant differences” to “no significant differences”

We corrected the usage according to your suggestion as comment Line 151.

Line 200: Changed to “varied from 18-34%”

Reply:

The corrected sentence is in the revised MS L203–205 and is as follows.

L203–205: Further isotopic niche analysis demonstrated that the contribution of dead zooplankton as a food source to those crabs ranged from > 34 % (vent center) to ≤ 18 % (peripheral sites).

Line 205: I am unclear why this reference in Pechora Sea is relevant to the study. The environment is completely different.

Line 206: The 4N and 9 N are study sites from the cited reference, but readers have to find the original paper to get this information. Again, it is unclear how the study site in Pechora Sea is relevant to KS site.

Reply:

We revised the paragraph in the corrected MS (L210–225) to make comparisons among different studies. We also noted that providing detailed sampling information is essential, e.g., sample size, date, and location.

L210–225: In this study, the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs significantly differed between vent types and sampling months (MANOVA test, Table 2). Our results showed that the crabs’ isotopic niche width (shown as the SEAc area) was considerably narrower in YV-Aug (0.88 ‰^{2}) than those in YV-July, WV-July, and WV-Aug (2.94, 2.88, and 3.62 ‰^{2}) ($p < 0.05$), respectively (Table 3). In the southwest Mediterranean, seasonal variations in the $\delta^{13}C$ and $\delta^{15}N$ values of the sally lightfoot crab *Percnon gibbesi* ranged from -18.33 to -13.08 ‰ and from 3.71 to 8.2 ‰ in 2016 (Bada et al., 2022). The isotopic niche width of *P. gibbesi* varied from 1.4 ‰^{2} in winter to 4.5 ‰^{2} in autumn, while the data were 1.5 and 2 ‰^{2} in spring and summer, respectively. It showed that the diets of *P. gibbesi* in autumn had the widest niche (food variability) linked to the local variability in algal resources. In the Pechora Sea, the isotopic niche width in scavenger hermit crab *Pagurus pubescens* varied between sites of 4N and 9N with a distance of 13 km because of a significant difference in their macrobenthic abundance (Gebruk et al., 2021). The isotopic niche width for the hermit crab was 0.15 ‰^{2} at 4N and 0.27 ‰^{2} at 9N, with 0.05 ‰^{2} overlapped. Differences in diet sources were correlated with local macrobenthic clams as shown at 4N, characterized by low *Astarte montagui* (32 g m^{-2}), in contrast to the high biomass of *A. borealis* and *Macoma calcarea* (500 g m^{-2}) at 9N. The niche width of this hermit crab had an even smaller overlapping SEAc area than our between-vent comparisons, i.e., 1.47 ‰^{2} in July and 0.86 ‰^{2} in August 2010. In brief, our study clearly shows that the isotopic signatures of the resident vent crabs reflected temporal and spatial heterogeneities. The discrepant results among different studies indicate explicit state sampling information, including size, date, and location, is essential.

Line 212-220, See my general comments about interpretation.

Reply:

We revised section 4.3 in the corrected MS (L226–245), which is as follows.

L226–245: **4.3 Protein expression patterns of vent crabs from the WV and YV**

Our proteomic results indicated that vent crabs were distinguishable as groups of WV-July & YV-July, WV-Aug, and YV-Aug, respectively. In the case of dove snails, *A. misera* inhabiting in WVs of KS Islet, their protein expression patterns were related to the diffusion of locally emitted vent fluids (Chen et al., 2015). The naturally acidified seawater in the southward sampling site had pH ranges from 7.78 to 7.82, while it was 7.31–7.83 in the east, southwest, and northwest locations. Based on the expressed protein profiles, the *Anachis* snails were classified into the south and another group. In a CO_2-SV off Vulcano Island in Sicily, sea anemones *Anemonia viridis* were collected at a distance of 350–800 m from a vent, where the pH values were 7.6, 7.9, and 8.2, respectively (Urbarova et al., 2019). Gene expression patterns of *A. viridis* revealed two clades, i.e., low pH group (pH 7.6) vs. high pH ones (pH 7.9 and pH 8.2). Overall, mobile vent crabs, slow-moving dove snails, and sessile sea anemones all performed adaptation abilities associated with their environments.

Organisms respond to environmental changes in a time-dependent manner. When the Chinese mitten crabs *E. sinensis* were transferred to high salinity (25 psu) for six days, the protein profiles of posterior gills were different from the control group (0 psu) (Yang et al., 2022). The nutrition value of linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, LNA) in the Chinese mitten crabs *E. sinensis* was evaluated in the laboratory for 107 days (Wei et al., 2018). A total of 186 proteins were expressed differentially in the hepatopancreas between the groups of LA and LNA. In the Teboulba fishing harbor in Tunisia, high levels of aliphatic and aromatic hydrocarbon pollutants were in the sediments (Jebali et al., 2014). The Mediterranean crabs *C. maenas* showed differential protein expression patterns in hepatopancreas between control (day 0) and exposed groups with 15, 30, and 60 days. These proteomic-based studies exhibited the earliest responses of tested crabs to environmental changes detected at least on day 6. In this study, the protein expression patterns of vent crabs changed in one month (Fig. 5), indicating the vent environments probably fluctuated often.

Line 243: Remove “the first study”. This kind of claim should be avoided in general.

Reply:

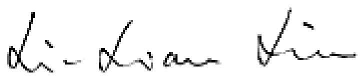
The corrected sentence is in the revised MS (L266–268) and is as follows.

L266–268: This study compared the benthic community, isotopic niche width, and protein expression patterns of the endemic vent crabs (*Xenograpsus testudinatus*) from different types of SVs at 100 m.

Thanks again for all of your help on this MS.

With my best regards,

Sincerely Yours,



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