Dear Professor Rose:

We sincerely appreciate your allowing us to revise our MS (bg-2022-177) again. Our responses to Anonymous Referee #3 and you are as follows:

The comments of anonymous Referee #3:

Line 30: SVs are divided into two groups, i.e., ≧ and < 10 mmol mol^{-1} H_2S (Tarasov, 2006).

These are the White Vents and Yellow Vents? Are there any relationship between Tarasov, 2006 and the qualitative description with White and Yellow which follows?

Reply:

White vents and yellow vents both contain high concentrations of H_2S (up to 172.4 mmol mol^{-1}). We rewrote the sentence in the revised MS in Line 34-35. We also list it as follows.

SVs off Kueishan (KS) Islet in Taiwan belong to the group of high H_2S concentrations (up to 172.4 mmol mol^{-1}, Chen et al., 2016).

Line 59-60: However, such heterogeneity resulting from temporal or spatial is unknown.

Not clear, heterogeneity of what?

Reply:

The corrected sentence is in the revised MS Line 66-67. We also list it as follows.

However, it is unclear whether the heterogeneous isotopic results are associated with vent environments or vent types.

Line 69: (*X. testudinatus*)…

Add reference for the authors which defined this species of crab. Also add info about their diet. A picture with the crab investigated is also missing.

Reply:

The corrected paragraph is in the revised MS Line 47-58, and the photos of vent crabs are in Fig. 1. We also list them as follows.

The vent crabs (*X. testudinatus* Ng, Huang & Ho, 2000; Family Xenograpsidae) are reddish to greyish-brown in color with quadrate carapace and sexual dimorphism (Fig. 1; Ng et al., 2000; Tseng et al., 2020). The wet weight and chela length of males (6.87 ± 2.90 g and 1.37 ± 0.40 cm; n = 831) are significantly larger than females (4.17 ± 1.25 g and 0.80 ± 0.16 cm; n = 274) (Tseng et al., 2020). They inhabit the pits, fissures, and crevices of sulfur chimneys and forage in vent areas during slack water (Jeng et al., 2004; Chang et al., 2018; Allen et al., 2020). They were more abundant at 5 and 35 m from the vent mouth and less at 20 m (Tseng et al., 2020). To avoid larval contact with toxic plumes, ovigerous females migrate to the vent periphery to release their offspring

and return to chimneys (Hung et al., 2019). The detection of high proteolytic enzyme activities in the midgut gland of male crabs indicated they are adaptive to irregular food availability (Hu et al., 2012). Based on the structure of mouthparts and gastric mills, vent crabs are scavengers (Jeng et al., 2004). Through 16S ribosomal RNA gene amplicon pyrosequencing of crab's midgut, its diets included eukaryotes (i.e., algae, fishes, bivalves, copepods, and anthozoans), prokaryotes (i.e., Rodobacteraceae, Oscillatoriphycidae, Mycoplasmataceae and Helicobacteraceae) and symbiotic epsilonproteobacteria and/or gammaproteobacteria (Ho et al., 2015; Yang et al., 2016).

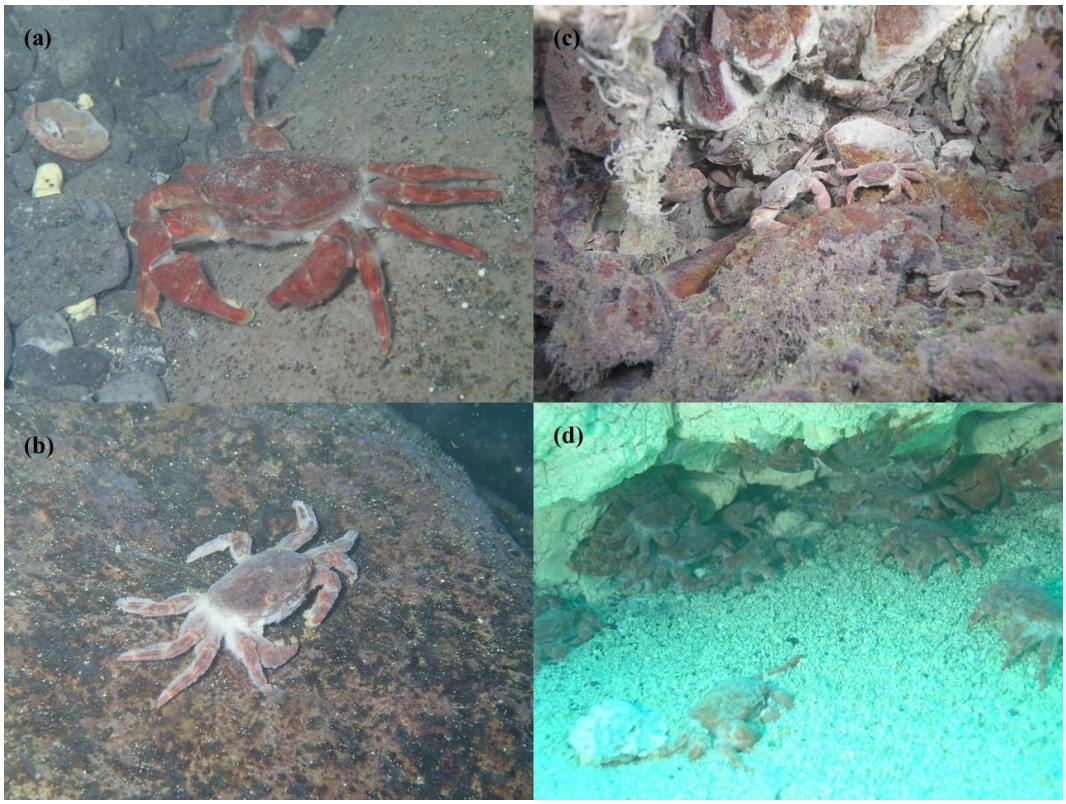


Figure 1: The photos of vent crabs (*Xenograpsus testudinatus*) inhibiting in SVs. (a) Male crab; (b) female crab; (c) vent crabs in a white vent; (d) vent crabs in a yellow vent.

Line 76-77: In the east of this Islet, there is a cluster of over 30 vents within an area of 0.5 km2 at depths of 5 to 30 m (Chen et al., 2005a).

(In the east of this Islet) should be deleted and revised as Eastward.

Reply:

The corrected sentence is in the revised MS Line 82-83. We also list it as follows.

Eastward there is a cluster of over 30 vents within an area of 0.5 km^2 at depths of 5 to 30 m (Chen et al., 2005a).

Line77-78: A summary of their environmental characteristics is △ in Table 1.

△ (the missed word) should be added displayed.

Reply:

The corrected sentence is in the revised MS Line 83-84. We also list it as follows.

A summary of their environmental characteristics is displayed in Table 1.

Line 99-119: **2.3 Preparation of vent crabs for isotope niche width and proteomic studies…**

2.4 Determination of isotope niche width of vent crabs from the WV and YV

2.3 and 2.4 should be together when presenting isotopes

Reply:

The primary purpose of paragraph 2.3 was sample collection for both isotopic and proteomic experiments. So we modified the paragraph to explain this in the revised MS Line 105-110. We also list them as follows.

2.3 Sampling of vent crabs from the WV and YV

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. 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). The specimens used in the isotope niche width and proteomic studies differed in samples of July but were the same in August.

Line 109: Those data were reported in the studies by Wu et al. (2021a, b).

Please add to the stable isotope analysis at least to what the sample are standardized and the standard error of analysis.

Reply:

The corrected paragraph is in the revised MS Line 115-121. We also list it as follows.

Stable isotope abundances were performed in conventional delta (δ) notation and unit in per thousand (‰) relative to the Pee Dee Belemnite for carbon and atmospheric N_2 standards for nitrogen, respectively (Sharp 2005). During analysis, there were interspersed several standard samples from at least two different laboratories, e.g., nylon and USGS40 (L-glutamic acid) with certified $\delta^{13}C$ of -27.8 and -28.9 ‰ and $\delta^{15}N$ of -9.8 and -4.3 ‰, respectively. Analytical accuracy was obtained by comparing measured values for the known values of reference materials (e.g., acetanilide), i.e., 0.2 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$. Analytical precision for both $\delta^{13}C$ and $\delta^{15}N$ was < 0.2 ‰ based on the standard deviation of internal standards.

Line 120-132: **2.5 Determination of protein expression patterns of vent crabs from the WV and YV**

When presenting protein expression than insert the text from 2.3 and 2.5 together.

Reply:

The primary purpose of paragraph 2.3 was sample collection for both isotopic and proteomic experiments. So we modified the paragraph in the revised MS Line 105-110. We also list them as follows.

2.3 Sampling of vent crabs from the WV and YV

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. 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). The specimens used in the isotope niche width and proteomic studies differed in samples of July but were the same in August.

Line 190-225: **4.2 The isotopic niche width of vent crabs from the WV and YV**

I suggest a final paragraph in which you briefly show which is the diet of these crabs and how this can be correlate with the measured isotopic signature (for example see Bojar et al., 2023 doi.org/10.1016/j.chemosphere.2023.138258 and references inside.

Reply:

We did not add another paragraph to explain the food sources of vent crabs. Instead, we discussed it in the same section as in the revised MS Line 217-234. We also list it as follows.

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). 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. Food of the vent crabs included dead zooplankton (-19.6 ± 1.3 ‰ and 7.2 ± 1.0 ‰; n = 13), bacteria (-22.2 ± 0.7 ‰ and 5.8 ± 2.5 ‰; n = 12), green algae and benthic deposited particulate organic matters (-19.9 ± 2.5 ‰ and 5.2 ± 1.6 ‰; n = 84), and algae film (-10.2 ‰ and -0.5 ‰; n = 1), respectively. The contribution of the above food items varied from vent center to periphery, i.e., 34, 13, 18, 39 %; 14, 8, 23, 14 %; 26, 58, 25, 31 %; and 26, 21, 34, 26 %, respectively. Dead zooplankton was more critical to crabs from the vent center than those peripheral ones. 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. The food of vent crabs in this study included dead zooplankton (-21.0 ± 0.2 ‰ and 6.1 ± 1.0 ‰, n = 20), vent particulate organic matter (-18.2 ± 1.1 ‰ and -1.7 ± 0.4 ‰; n = 2), and epibenthic crustaceans (including Amphidpoda, Mysida, and Euphausiacea) (-19.9 ± 0.1 ‰ and 6.0 ± 0.6 ‰; n = 2), respectively. The above food items' contribution differed between the center and periphery, i.e., 6–38 vs. 16–42 %; 11–87 vs. 6–31 %; and 7–53 vs. 46–61 %, respectively. In this case, the importance of dead zooplankton was similar in the two sites.

Line 224-225: The ~~discrepant~~ results among ~~different~~ studies indicate explicit state sampling information, including size, date, and location, is essential.
The word of “discrepant” is revised to a word of “different”; deleted the word of “different”
Reply:
The corrected sentence is in the revised MS Line 249-250. We also list it as follows.
The different results among studies indicate explicit state sampling information, including size, date, and location, is essential.

Line 249: 1.84–6.96 vs. 1.52–6.32 (pH seawater scale, 25 °C)
In fact, the intervals of pH overlap for the two groups, yellow and white. Near pH 7 the measurement precision is low.
Reply:
The pH of SVs varies in a wide range due to local circulation patterns. So, we explained it in the revised MS Line 274-275. We also list it as follows.
The diffusion of vent fluid relates to local circulation. Therefore, fluctuations in fluid temperature and pH reveal diurnal and bimonthly cycles (Chen et al., 2005b).

Line 259-260: 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).
Remove "and" from the beginning of the phrase.
Reply:
The corrected sentence is in the revised MS Line 285-286. We also list it as follows.
But 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).

Line 269: The dwelling crabs were associated with their resident vent, and within-vent variability is more dramatic in YV compared to WV.

more dramatic, I suggest "larger"

Reply:

The corrected sentence is in the revised MS Line 304-305. We also list it as follows.

The dwelling crabs were associated with their resident vent, and within-vent variability is larger in YV compared to WV.

Table 2: n: sample size.

n means that you analyzed 32 crabs? (q1) Is there any correlation between Carapace with and isotopic composition? (q2) For one crab how many times was a measurement repeated? (q3) Which is the standard deviation of measurements? (q4) If standard deviation of measurements is like ±0.1 permil, you should round your numbers, as the second decimal has no meaning.

Reply:

Yes, n means the number of crabs we analyzed. q4: The corrected Table 2 (a) with one decimal is in the revised MS, and related text is in Line 165-167, 179-180. The changed text for q1-q3 is in Line 112, 136-138, 122-124, and 164, respectively. We also list them as follows.

Line 112: About 0.3 g of leg muscle from one crab was taken, freeze-dried, and homogenized to powders.

Line 136-138: About 0.1 g leg muscle from one crab was taken and homogenized with 1 ml lysis buffer (7 M Urea, 2 M Thiourea, 4 % CHAPS, and protease inhibitor cocktail of two tablets per 100 ml) for proteomic sample preparation.

Line 122-124: Here, we used SPSS Statistics to analyze the published data by Pearson correlation tests between carapace width, wet weight, and the $\delta^{13}C$ and $\delta^{15}N$ values, and two-way multivariate analysis of variance (MANOVA) to test the effects of vent type and sampling month on the $\delta^{13}C$ and $\delta^{15}N$ values of crabs.

Line 164: There was no correlation between carapace width or wet weight and $\delta^{13}C$ or $\delta^{15}N$ values (Pearson correlation, $p > 0.05$).

Line 165-167: For WV crabs, the mean values were -17.6 ± 0.2 ‰ and -16.6 ± 0.3 ‰ for $\delta^{13}C$, and 7.8 ± 0.2 ‰ and 7.7 ± 0.4 ‰ for $\delta^{15}N$, respectively. For YV crabs, the data were -16.5 ± 0.4 ‰ and -16.2 ± 0.2 ‰ for $\delta^{13}C$, and 6.4 ± 0.8 ‰ and 7.0 ± 0.3 ‰ for $\delta^{15}N$, respectively.

Line 179-180: 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 -15.0 and 8.6 ‰ vs. -16.8 and 7.2 ‰, respectively (Fig. 3).

Table 2.

(a)

Crab group	n	Carapace width (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
W0702	32	22.17 ± 0.51 (14.70 ~ 27.50)	-17.6 ± 0.2 (-19.7 ~ -13.7)	7.8 ± 0.2 (4.0 ~ 9.2)
W0804	9	25.30 ± 0.81 (19.55 ~ 27.33)	-16.6 ± 0.3 (-17.5 ~ -15.0)	7.7 ± 0.4 (4.7 ~ 8.9)
Y0702	6	21.62 ± 0.53 (20.45 ~ 23.58)	-16.5 ± 0.4 (-18.0 ~ -15.0)	6.4 ± 0.8 (3.9 ~ 8.6)
Y0824	7	22.01 ± 0.89 (17.84 ~ 24.44)	-16.2 ± 0.2 (-17.0 ~ -15.2)	7.0 ± 0.3 (5.4 ~ 8.0)

Figure 2: The coverage and abundance of benthos in the white and yellow vents. (a) The coverage of attached organisms; (b) the abundance of low-mobility macrobenthic fauna. Mean ± S.E.M.

What about the crabs?

Reply:

We did not count vent crabs because they are fast-moving objects. We explained it in the revised MS Line 99-100. We also list them as follows.

Vent crabs were not quantified due to the difficulty of counting fast-moving objects.

Figure 3 and 4

I suggest putting together Fig. 3 and 4. Fig 4 is to complicated, display the fields for the yellow and white vents.

Reply:

The corrected Figure 4 is in the revised MS, which combined the information from Figs. 3 and 4 together. We also list it as follows.

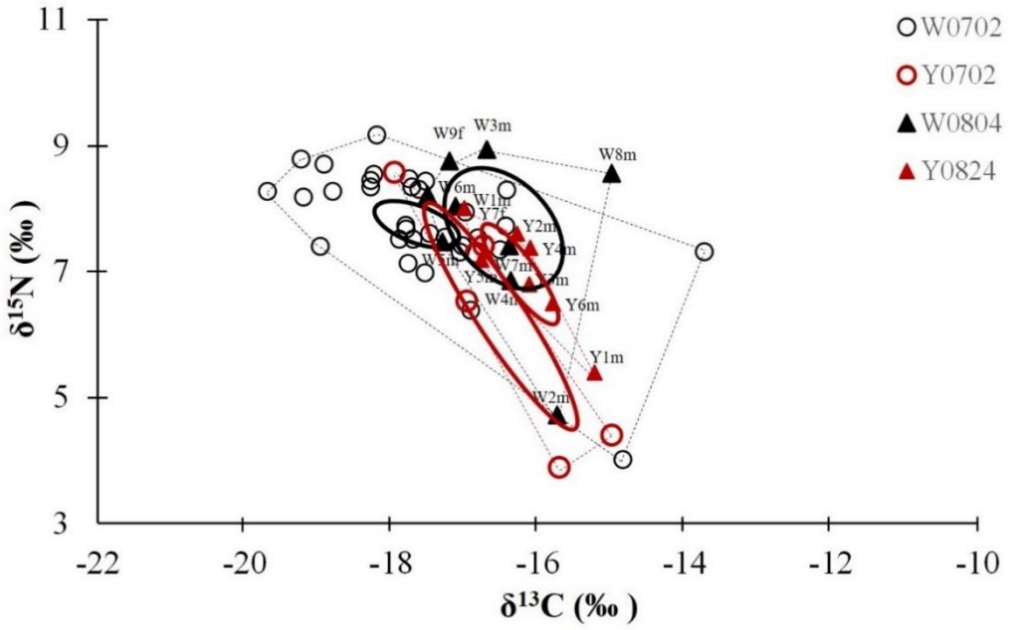


Figure 4: Plot of the Convex hull and standard ellipses areas based on the $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. Dot lines: convex hull areas; solid lines: standard ellipses areas (SEAc); W: white vent; Y: yellow vent; sampling date: July 2 (0702), August 4 (0804), and August 24 (0824); m: male; f: female; the crabs with label: same individuals for the stable isotope and proteomic experiments.

The remaining comments by Professor Rose:

(1) Add a clear statement of the research question(s) you want to answer (in the paragraph at line 70). The question is almost stated in places but it needs to be very clear and stated in the beginning of the manuscript. How the data collection and statistical analyses then relates to the question should be very clear or a few sentences added to make this link.

Reply:

The corrected sentences are in the revised MS Line 77-79. We also list them as follows.

Comparative studies on the feeding ecology of vent crabs over time or different vent types are rare. Therefore, we investigated the benthic community of a WV and a YV at a distance of 100 m and the feeding habits of vent crabs from both sites by analyzing isotopic niche width and protein expression patterns collected in July and August 2010.

(2) Better explanation of why and how the statistical analyses were done. Presently, the statistical methods are 2 sentences (line 135). Explain why BCS and PCA were used (relates to what specific questions) and how the data were treated when used in these analyses. For example, sites, months, individual measurements, etc.

Reply:

The corrected paragraph is in the revised MS Line 146-152. We also list it as follows.

The Bray Curtis similarity (BCS) measure is frequently used by ecologists to quantify differences between samples based on abundance or count data. A cluster analysis of the BCS indices was employed to quantify the differences in expressed protein bands of each vent crab from different vents and sampling months. We applied a square-root transformation on the protein bands and then ran a cluster analysis of BCS indices in the Primer 6.0 software (Clarke and Warwick, 2001). In addition, the contribution of each protein band was further determined by principal component analysis (PCA) in the Primer 6.0 software. The purpose of this analysis was to obtain the contribution of each protein band to the quantified differences of vent crabs by BCS (Paukert and Witting, 2002).

(3) Add a paragraph to the Discussion about the strengths and weaknesses of the data collection and analyses. What would you do next to better answer the question - for example, sample more crabs?, sample more sites within vent type?, sample different vents of the same type? use other isotopes? , different tissues?

Reply:

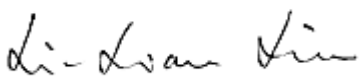
The added paragraph is in the revised MS Line 291-300. We also list it as follows.

The isotopic niche and proteomic studies linked the physiological states of vent crabs to SV environments. Suggestions for further studies include more replicates of different vent types, collecting crab samples simultaneously, increasing sample size, and considering genders. More stable isotopes from other tissues will also help better understand nutrition sources and tissue-specific isotopic incorporation rates of vent crabs. Such as the study of snails *Alviniconcha* sp. and *Ifremeria nautilei* from deep-sea vents in Vienna Woods, Manus Basin, isotopes of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ on foot and chitin shells allow determining the isotopic discrimination between inorganic water compounds used by organisms as nutritional sources and symbiont/host itself (Bojar et al., 2023). In a laboratory study on a freshwater shrimp *Macrobrachium borellii*, the time course of incorporating the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of muscle and hepatopancreas is evaluated to understand how an animal uses resources over time (Viozzi et al., 2021). If this also combines with proteomic analysis, we can elucidate more thoroughly how the physiological states of vent crabs cope with different vent types.

Thanks again for all of your help on this MS.

With my best regards,

Sincerely Yours,



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