Differential feeding habits of the shallow-water hydrothermal vent crab *Xenograpsus testudinatus* correlate with their resident vent types at a scale of meters

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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 in YV, based on the survey in August 2010. The $\delta^{13}C$ and $\delta^{15}N$ values of crabs 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.

1 Introduction

Hydrothermal vents distribute in shallow water (SV: < 200 m) and deep sea (DV: > 200 m), where they have unique physicochemical and geological characteristics and peculiar biological communities (Parson et al., 1995; Dando et al., 2000). The vents' plumes are rich in chemical compounds and gas, primarily reduced sulfur compounds, methane, and hydrogen gas. Based on hydrogen sulfide concentration, SVs are divided into two groups, i.e., $\geqq$ and < 10 mmol mol^{-1} H$_2$S (Tarasov, 2006). The low H$_2$S concentration group was reported in SVs of Milos Island (4.9 mmol mol^{-1}, Greece, Dando et al., 1995), Louise Harbor (8 mmol mol^{-1}, Papua New Guinea, Pichler et al., 1999a), Tutum Bay (< 0.3 mmol mol^{-1}, Papua New Guinea, Pichler et al. 1999b), Punta Mita (< 0.01 mmol mol^{-1}, Mexico, Prol-Ledesma et al., 2002), and Bahía Concepción (undetected, Mexico, Forrest et al., 2005). SVs off Kueishan (KS) Islet in Taiwan belong to the group of high H$_2$S concentrations (up to 172.4 mmol mol^{-1}, Chen et al., 2016). These SVs are further distinguished according to the color of plumes, e.g., white vents (WVs) and yellow vents (YVs). The concentrations of H$_2$S in WVs and YVs were 12.94 ± 4.55 and 60.12 ± 19.57 mmol mol^{-1}, respectively (Chen et al., 2005b). WVs have a relatively low fluid temperature of 30–65 ℃ and higher pH of 1.84–6.96; YVs have temperatures of 54–121 ℃, a minimum pH of 1.52 (pH seawater scale, 25 ℃) (Table 1) (Chen et al., 2005b; Lebrato et al.,

2019; Mei et al., 2022). The fluid temperatures revealed diurnal and bimonthly cycles, which reach a maximum of two to four hours after each high tide (Chen et al., 2005b).

In WV, benthic organisms include bacteria, red algae, hexacoral (*Tubastraea aurea*), sea anemones (*Anthopleura* spp.), snails (*Anachis misera* and *Nassarius* sp.), chitons, serpulid polychaetes, and vent crab (*X. testudinatus*) (Jeng et al., 2004; Chang, 2006; Tang et al., 2013; Wang et al., 2014; Chen et al., 2015; Wang et al., 2015; Chan et al., 2016; Wu et al., 2021a). In YV, the only macrofauna is the endemic vent crabs. The species richness of macrofauna increases with horizontal distance from the mouth regions of WV and YV to 2000 m away, indicating that the vent fluids may negatively impact several kilometers (Chan et al., 2016).

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).

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

Under changing environments, proteome analysis is also a helpful approach to better understanding 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. 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 Materials and Methods

2.1 Sampling sites

KS Islet is located northeast of Taiwan (Fig. 2). Eastward 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). The most common vent types are WV and YV. A summary of their environmental characteristics is displayed in Table 1.

In this study, a WV (24.83404° N, 121.96172° E) and a YV (24.83553° N, 121.96361° E) were sampled in July and August 2010, and the distance between the two vents was about 100 m. The vent plume temperature varied between 41–62 ˚C in the WV and 54–121 ˚C in the YV (Yang et al., 2012; Chen et al., 2016; Hung et al., 2019; Lebrato et al., 2019). The pH in the WV was 4.83–5.74, while the YV had a range from 2.22 to 2.82 (Lin, 2011; Yang et al., 2012; Hung et al., 2019). The H_2S concentration of the plumes was 18.4 ± 8.4 mmol mol^{-1} and 90.8 ± 29.1 mmol mol^{-1}, respectively (Chen et al., 2016). The gas compositions of the two vents were dominated by CO_2 (503.8 ± 78.7 and 798.4 ± 23.8 mmol mol^{-1}, respectively) and N_2 (309.9 ± 72.4 and 65.1 ± 17.0 mmol mol^{-1}, respectively) (Chen et al., 2016). The recorded environmental parameters of our sampling locations on August 4 and 24, 2010, in the WV and YV included temperature, pH, and depth, i.e., 25.0 and 26.7 ℃, 7.3 and 7.8, and 17 and 7 m, respectively (Table 1).

2.2 The survey of benthic communities in the WV and YV

The WV and YV benthic communities were investigated by SCUBA diving on 4 and 24 Aug. 2010, respectively. Three quadrates (15×15 cm²) were set at distances of 3, 8, and 13 m from the vent mouth in the north, east, south, and west directions. A total of twelve quadrats in WV were surveyed. In contrast, it was ten in YV because the distance to shore was only 7 m in the north direction. We photographed the quadrats, and the number of low-mobility fauna was counted, i.e., amphipod, columbellid snail, sea anemone, calyptraeid snail, chiton, vermatid snail, polychaete, sipunculid, and mytilid mussel. Vent crabs were not quantified due to the difficulty of counting fast-moving objects. The coverage of attached organisms was scored by ImageJ, i.e., macroalgae, periphyton, and sponge. Low-mobility fauna or attached organisms were collected by hand with hammers or shovels for identification purposes. The collected samples were brought to the laboratory and preserved in 75 % ethanol. Identifications of small crustaceans, mollusks, annelids, and other invertebrates were based on the references of Carlgren (1949), Fauchald (1977), Miyake (1982), Cutler (1994), Okutani (2000), and Hooper et al. (2002).

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 ℃ 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.

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

About 0.3 g of leg muscle from one crab was taken, freeze-dried, and homogenized to powders. We weighed and encapsulated approximately 1mg of the powder in a tin capsule for carbon and nitrogen isotope analyses. A PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) was used at the stable isotope laboratory at the University of California at Davis. 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 δ^{13}C of -27.8 and -28.9 ‰ and δ^{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 δ^{13}C and 0.3 ‰ for δ^{15}N. Analytical precision for both δ^{13}C and δ^{15}N was < 0.2 ‰ based on the standard deviation of internal standards. Those data were reported in the studies by Wu et al. (2021a, b). Here, we used SPSS Statistics to analyze the published data by Pearson correlation tests between carapace width, wet weight, and the δ^{13}C and δ^{15}N values, and two-way multivariate analysis of variance (MANOVA) to test the effects of vent type and sampling month on the δ^{13}C and δ^{15}N values of crabs.

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 δ^{13}C and δ^{15}N space (Jackson et al., 2011). This metric corrects bias generated when sample sizes are small, represents the total amount of niche occupied in the isotopic space, and allows for robust statistical comparisons between datasets with different sample sizes (Jackson et al., 2011, 2012). Moreover, this metric also calculated the overlap area of the standard ellipses (and respective percentages) between sites and months, which was as diet similarity. The metric was run by the 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.

2.5 Determination of protein expression patterns of vent crabs from the WV and YV

The protein expression profiles of vent crabs were determined by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1-D SDS-PAGE), followed by Chen et al. (2015) and Lin (2011). The sample sizes of vent crabs from WV-July, YV-July, WV-Aug, and YV-Aug were 11, 6, 9, and 7, respectively. 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. We centrifuged the homogenates at 12000 g for 30 min at 4 ˚C and collected the supernatant. The protein concentration was determined by Bradford assay, with bovine serum albumin as the standard. We prepared the stacking and resolving gels with percentages of 5 and 12 % (Hoefer SEM 260 system, Amersham Pharmacia). In each sample lane, electrophoresis was run for 30 min at 120 V after loading 25 µg protein and then for four h at 180 V. The gels were stained by Coomassie blue G-250 (Candiano et al., 2004). Stained gels were scanned and transformed into digitalized images using Image Scanner (Amersham Pharmacia). We utilized the Multi Gauge software v2.2 (Fujifilm) for protein quantification. The protein bands were assigned band numbers, and their intensity levels were calculated as their relative area to the total protein area on the gel.

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 Results

3.1 Composition of the benthic community in the WV and YV

155 The coverage of attached organisms and the abundance of low-mobility benthos in the two vents differed. In WV, the coverage of macroalgae, periphyton, and sponge was 39.2 ± 6.6 %, 29.0 ± 6.4 %, and 3.6 ± 2.4 % (n = 12), respectively. YV only had periphyton of 77.0 ± 16.2 % (n = 10) (Fig. 3). Dominant low-mobility fauna in WV were sea anemones (17.7 ± 4.5 individuals per 100 cm^2), calyptraeid snails (2.7 ± 1.1 individuals per 100 cm^2), and columbellid snails (1.0 ± 0.3 individuals per 100 cm^2), respectively. The rest species were quite a few, including polychaete, chiton, sipunculid, and mytilid mussel. While within the

160 surveyed area of YV, no low-mobility fauna was observed. In addition, fast-moving vent crabs were always present at both sites.

3.2 Isotopic niche width of vent crabs from the WV and YV

Table 2 and Fig. 4 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. There was no correlation between carapace width or wet weight and $\delta^{13}C$ or $\delta^{15}N$ values (Pearson correlation, $p > 0.05$).

165 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. 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.,

170 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.

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

180 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). 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.

4 Discussion

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

185 community of the two vents, stable isotope values, and protein expression patterns of the dwelling crabs were associated with their habitats and showed temporal variation. The $\delta^{13}C$ and $\delta^{15}N$ values significantly differed in vent crabs between sites and sampling month. The niche width of crabs from YV-Aug narrowed considerably than those of YV-July, WV-July, and WV-Aug, respectively. The protein profiles revealed three groups, i.e., WV-July & YV-July, WV-Aug, and YV-Aug, respectively.

Based on the results, we suggested that the endemic vent crabs inhabit by their vent types, and within-vent variability is more dramatic in YV compared to WV.

4.1 The benthic community in the WV and YV

In this study, within the area of 15 m from the center of WV, the coverage of attached benthos was 72 %, including macroalgae, periphyton, and sponge. In YV, it only had periphyton for 77 %. Our previous study found three types of periphyton in YV, while WV had two sponge species, two brown algae, two red algae, five green algae, and one kind of periphyton (Wu et al., 2021a). Along a 50 m transect line near the vent mouths of WV and YV, Chan et al. (2016) and Wang et al. (2022) reported that encrusting red algae *Hildenbrandia* spp. and three red turf algae occupied 5 % of the substrate.

Low-mobility fauna was absent in the 15 m area from the center of YV. In contrast, there were nine species in WV, including sea anemones (17.7 ± 4.5 individuals per 100 cm^2), calyptraeid snails (2.7 ± 1.1 individuals per 100 cm^2), and columbellid snails (1.0 ± 0.3 individuals per 100 cm^2). Polychaete, chiton, sipunculid, and mytilid mussels were all with density < 1 individual per 100 cm^2. By comparison, Wu et al. didn't record any low-mobility benthic fauna in YV in 2009 and 2010 (Wu et al., 2021a). While in WV, there were 14 species, including shrimp *Alpheus lobidens*, four snail species, chiton of *Ischnochiton comptus*, sea anemone of *Anthopleura* sp., coral of *T. aurea*, and four polychaete species. In 2014, Chan et al. (2016) reported along a 50 m transect line from the center of YV, sea anemone *Anthopleura* sp. occupied 5 ± 4 % of the substrate. The fauna in WV was more abundant, which included sea anemone (17.5 ± 28.9 %), immobile snails of *Bostrycapulus aculeatus* and *Dendropoma dragonella* (7–30 %), and other four snail species (1–3 individuals per 25 × 25 cm^2). Overall, the investigated results across studies were consistent with higher species diversity and abundance of benthic communities in WV than in YV.

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 δ^{13}C and δ^{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 δ^{13}C and 7.8 ± 0.14 ‰ (WV) vs. 6.7 ± 0.3 ‰ (YV) for δ^{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 δ^{13}C and δ^{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 δ^{13}C and δ^{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 δ^{13}C (-20.5 to -14.3 ‰) and δ^{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.

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 different results among studies indicate explicit state sampling information, including size, date, and location, is essential.

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, the sediments had high levels of aliphatic and aromatic hydrocarbon pollutants (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.

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 ℃ and 1.84–6.96 vs. 1.52–6.32 (pH seawater scale, 25 ℃)) (Table 1). 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). 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 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). 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). 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.

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 δ^{13}C, δ^{15}N, and δ^{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 (δ^{13}C and δ^{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.

5 Conclusions

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. The δ^{13}C and δ^{15}N values significantly differed in vent crabs between sites (WV and YV) and sampling months (July and August). The dwelling crabs were associated with their resident vent, and within-vent variability is larger in YV compared to WV. It is concluded that vent crabs are faithful to their inhabiting vent. Trans-vent movement, even at a scale of meters, is probably sporadic as an adaptation of minimizing predation risk.

Author Contributions

Siou-Yan Lin and Shao-Hung Peng helped collect samples and conducted the study; Jung-Fu Huang, Chen-Tung A. Chen, and Jia-Jang Hung were involved in the study design; Jing-Ying Wu and Li-Lian Liu designed the study, analyzed data and wrote the paper. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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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 (℃)	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 (℃)	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 (℃)	25	26.7	"	"
pH	7.3	7.8	"	"
Deposited sulfur particles (diameter)	globules (~ 0.05–0.1 cm)	balls (> 2 cm)	"	"

490

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 δ^{13}C and δ^{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)	δ^{13}C (‰)	δ^{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)

(b)

MANOVA (δ^{13}C, δ^{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

510

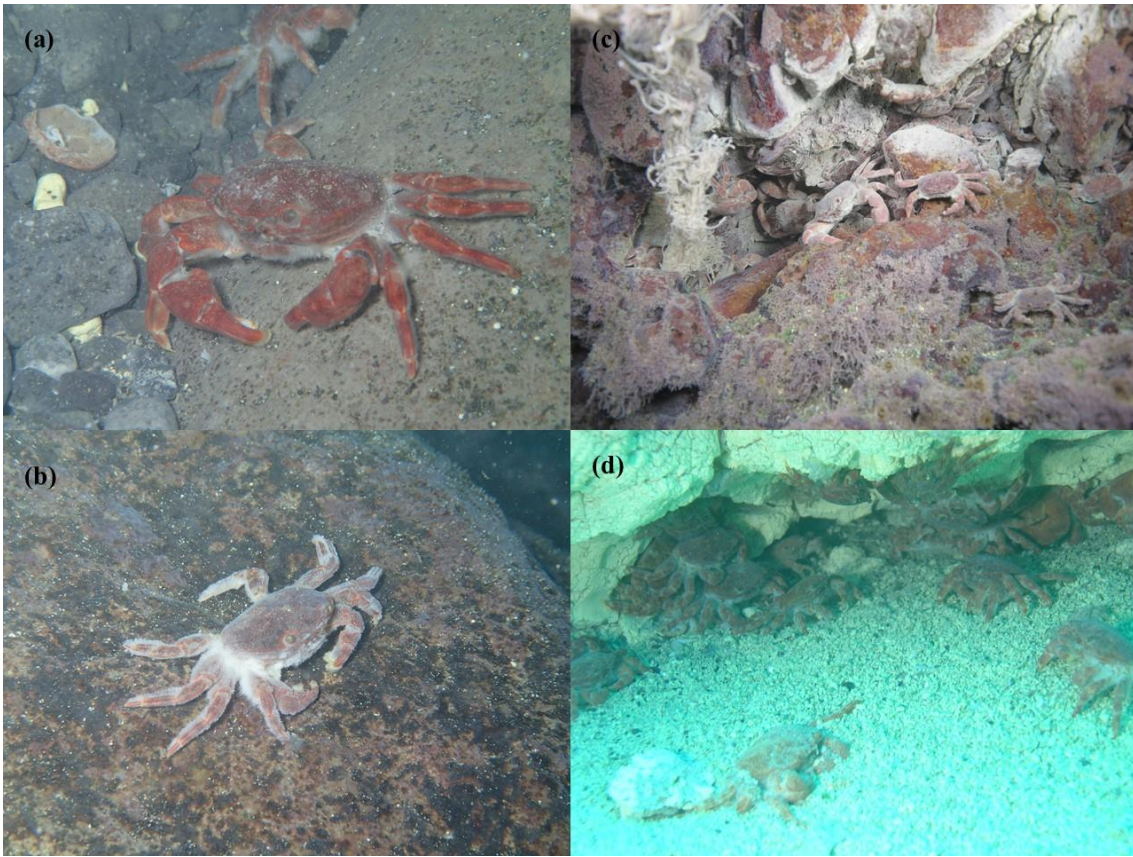



Figure 1: The photos of vent crabs (*Xenograpsus testudinatus*) inhibiting in SVs. (a) Male crab; (b) female crab; (c) vent crabs in a white vent; (d) vent crabs in a yellow vent.

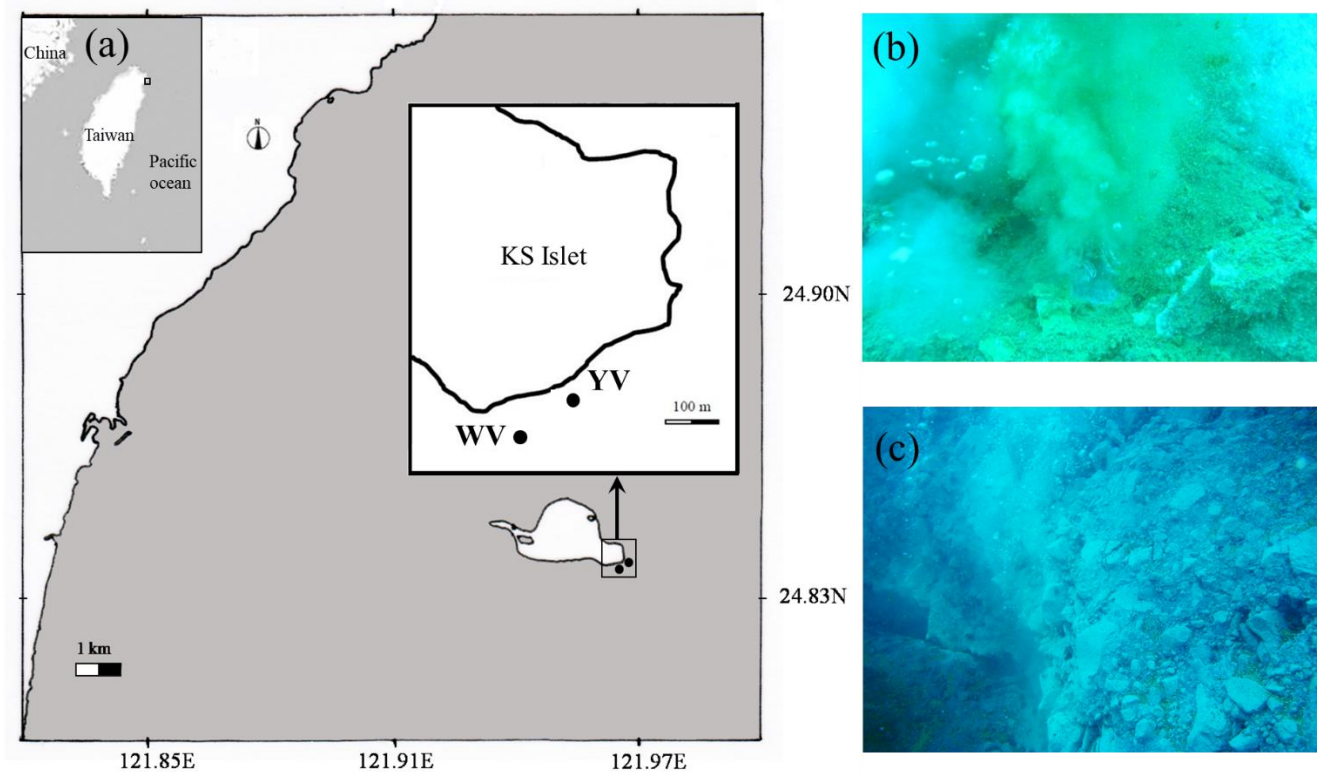



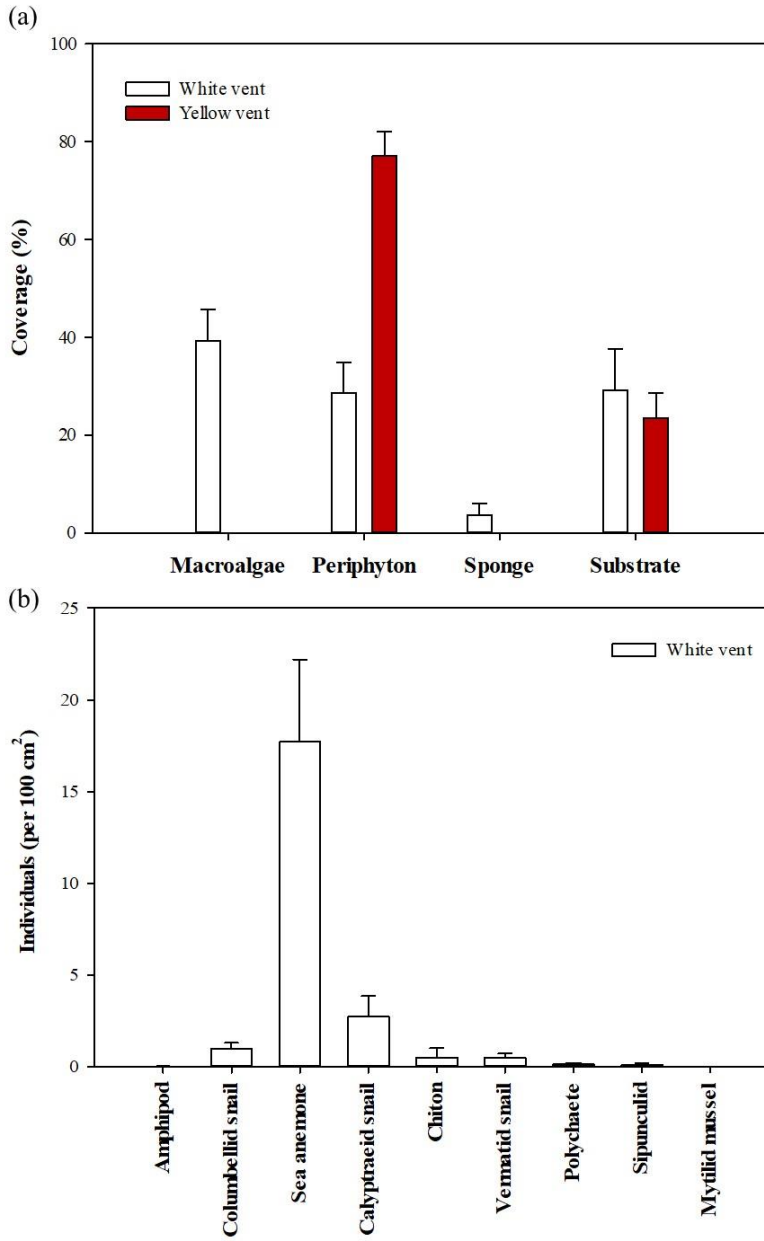
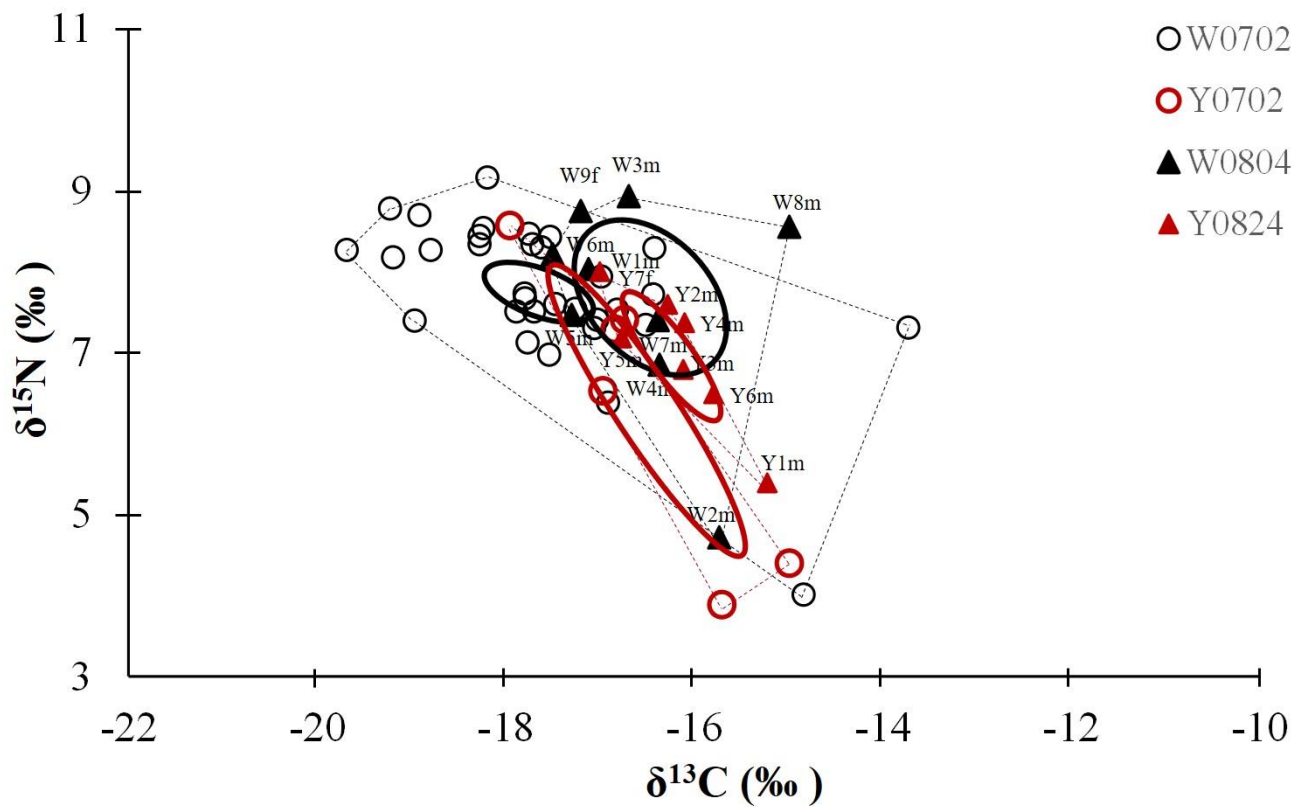
Figure 2: Map of the Kueishan Islet showing sampling sites. (a) The study sites; (b) photograph of the yellow vent; (c) photograph
520   **of the white vent. KS Islet: Kueishan Islet; WV: white vent; YV: yellow vent.**

525 **Figure 3: 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.**

530

Figure 4: Plot of the convex hull and standard ellipses areas based on the δ¹³C and δ¹⁵N 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.

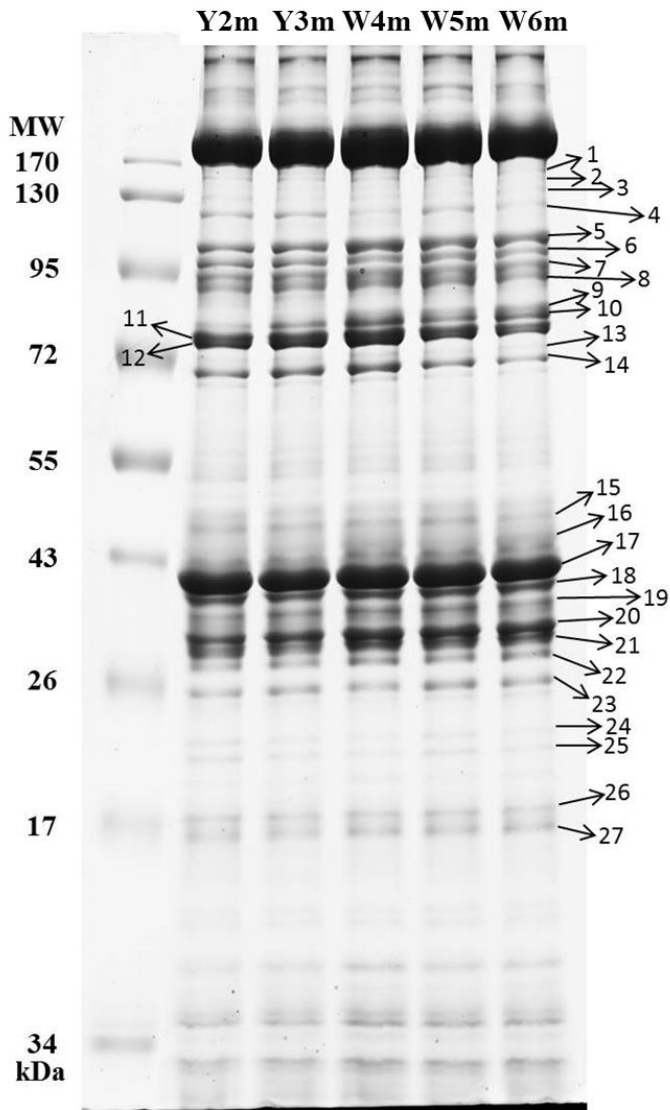



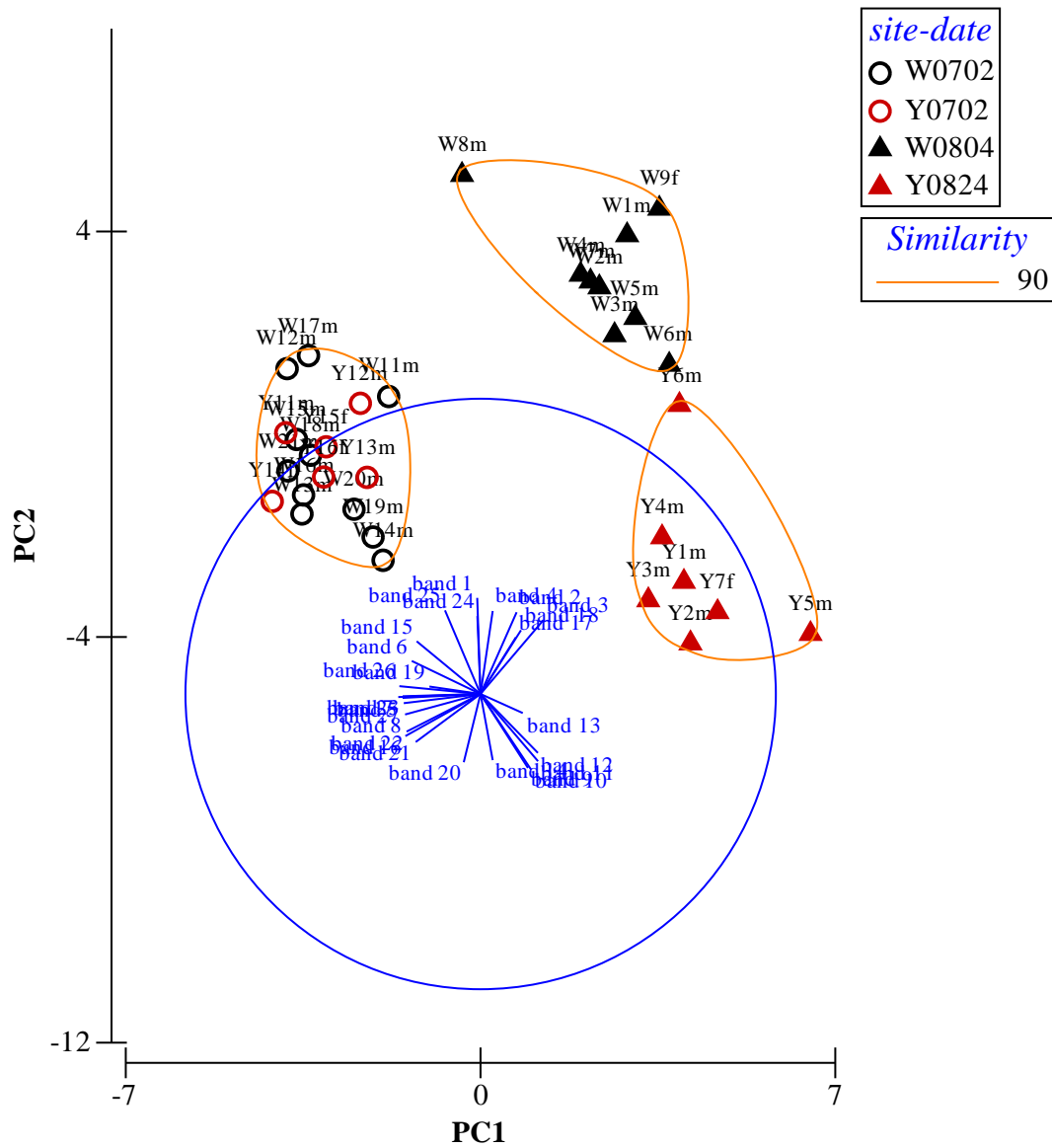
Figure 5: Gel electropherogram with molecular markers of vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. Y2-3m: male individuals from the yellow vent; W4-6m: male individuals from the white vent; MW: molecular weight; unit: kDa; number: protein band serial number.

540

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.**