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~~Here, two distinct vent types were identified as white vents (WVs) and yellow vents (YVs), based on the color, physical and chemical characteristics 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~~The endemic vent crabs (*Xenograpsus testudinatus*) are abundant in both WVs and YVs~~. 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~~Studies on crabs dwelling in other vent types are rare. Here, we investigated the feeding habit of crabs inhabiting YV and made a comparison with WV crabs~~. Specifically, we examined the benthic community of WV and YV, isotopic niche width, and protein expression patterns of the crabs from the two vent~~ types~~s at a distance of 100 m and sampled in July and August 2010. The coverage of sessile organisms and low-mobility fauna in WV ~~were~~ was more abundant than those in YV, based on the survey in August 2010. The δ^{13}C and δ^{15}N values of crabs significantly differed spatially and temporally (MANOVA test, p < 0.05)~~were insignificantly different between crabs from WV and YV~~. 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~~WV was significantly broader than those from YV, shown as SEAc areas of 3.62 ‰² vs. 0.88 ‰² (p < 0.05). The overlap percentages in WV and YV were 23.9 % and 97.9 %, respectively. The food sources of crabs in WV were more diverse than those in YV.~~ Based on the protein expression patterns, the vent crabs exhibited three groups, i.e., WV-July & YV-July, WV-Aug, and YV-Aug, respectively~~were classified into WV- and YV- dwellings~~. Our results indicated that the dwelling crabs

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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 riskOur results revealed that the feeding habits of the endemic vent crabs (*X. testudinatus*) are adapted to their vent types at a distance of 100 m, and the trans-vent movement is uncommon.

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; Parson et al., 1995). 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), Milos Island (4.9 mmol mol^{-1}, Greece, Dando et al., 1995), 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). On the contraryIn contrast, SVs off Kueishan (KS) Islet in Taiwan have the highest record of H$_2$S concentrations (up to 172.4 mmol mol^{-1}, Chen et al., 2016)high H$_2$S concentrations were only recorded in SVs off Kueishan (KS) Islet, Taiwan (as high as 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 54–12178–116 ℃, 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., 2021aChan et al., 2016; Chang, 2006; Chen et al., 2015; Jeng et al., 2004; Tang et al., 2013; Wang et al., 2014; Wang et al., 2015; Wu et al., 2021). In YV, the only macrofauna is the endemic vent crabs. The speciesSpecies 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 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

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m ~~, whereas it was 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 ~~vent~~male crabs indicated they are adapt~~ed~~ive to irregular food availability~~supply~~ (Hu et al., 2012).

65 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 δ^{13}C and 1.4–5 ‰ for δ^{15}N in each trophic transfer (DeNiro and Epstein, 1978, 1981; Post, 2002; McCutchan et al., 2003~~; Post, 2002~~). With the isotopic data, consumers' trophic position~~,~~ pathways, and niche width can be calculated (Layman et al., 2011). ~~The detection of high proteolytic enzyme activities in the midgut gland of vent crabs indicated they are adapted to irregular food supply (Hu et al., 2012). A~~ The detection of high

70 ~~proteolytic enzyme activities in the midgut gland of vent crabs indicated they are adapted to irregular food supply (Hu et al., 2012). T~~trophic studi~~es~~y 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 δ^{13}C and δ^{15}N data (Wang et al., 2014; Chang et al., 2018; Wu et al., 2021a)~~by δ^{13}C and δ^{15}N analysis reveals that dead zooplankton killed by sulfur plumes (as plankton-derived production) is essential to scavengers and carnivores (Chang et al., 2018; Wang et al., 2014; Wu et al., 2021)~~. The importance

75 of dead zooplankton to vent crabs decreases from the vent center to the periphery ~~accordingly~~ (Wang et al., 2022). Furthermore, vent crabs collected from YV had significantly lower~~more depleted~~ δ^{13}C and δ^{15}N values than those in WV (Wu et al., 2021a). However, such heterogeneity resulting from temporal or spatial is unknown.

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80 and Epstein, 1978, 1981; McCutchan et al., 2003; Post, 2002). With the isotopic data, consumers' trophic position, pathways, and niche width can be calculated (Layman et al., 2011).~~

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)~~The proteome is the set of expressed proteins in an organism, which varies with tissue, physiological condition, and environment where the organism lives. By proteomic tools, the

85 difference in protein expression profiles of the studied organisms can be characterized (Lopez-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

90 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.

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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~~Here, we investigated the feeding habit of crabs inhabiting in YV and crabs from WV was also studied for comparative purposes~~. Specifically, we examined the benthic community of WV and YV, isotopic niche width, and protein expression patterns of the crabs from ~~the~~ two vent ~~types~~ at a distance of 100 m and sampled in July and August 2010.

2 Materials and Methods

2.1 Sampling sites

KS Islet is located northeast of Taiwan (Fig. 1). In the east of this Islet, 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). The most common vent types are WV and YV. A summary of their environmental characteristics is in Table 1. ~~WVs show venting temperatures of 30–65 °C, whereas YVs exhibit 78–116 °C (Chen et al., 2005b; Mei et al., 2022).~~

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 ~~of vent plumes~~ varied between 41–62~~in the range of 47–59~~ °C in the WV and 54–121~~100–116~~ °C in the YV (Yang et al., 2012; Chen et al., 2016; Hung et al., 2019; Lebrato et al., 2019~~Chen et al., 2016; Hung 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$_2$S 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 are 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 °C, 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 cm^2) 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,~~While~~ it was ten in YV because~~due to~~ the distance to shore was only 7 m in the north direction. We photographed the quadrats, and the number of low-mobility fauna w~~as~~ere counted, i.e., amphipod, columbellid snail, sea anemone, calyptraeid snail, chiton, vermatid snail, polychaete, Sipuncula, and mytilid mussel. 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

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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), ~~Fauchald (1977), Okutani (2000), and Hooper et al. (2002)~~Hooper et al. (2002), Miyake (1982), and Okutani (2000).

2.3 Preparation of vent crabs for isotope niche width and proteomic studies

125 Vent crabs ~~from the WV and YV~~ have gathered 5 m away from the ~~vent~~ mouths of the WV and YV on sampling dates of July 2 (both vents), August 4 (WV), and 24 (YV)~~4 and 24 Aug.~~ 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 ℃ for later use. ~~There were nine crabs with eight males and one female from WV; 7 samples with six males and one female from YV.~~ Crab samples were examined to eliminate debris, and epibionts,

130 then their carapace width and wet weight were measured before dissection (Fan et al., 2016). ~~The carapace width and wet weight range of the crabs were 19.6–27.3 cm and 3.82–12.38 g from WV, while those from YV were 17.8–24.4 cm and 2.50–7.73 g, respectively.~~

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

About 0.3 g of crab's leg muscle~~The leg muscle of crabs, about 0.3 g,~~ was taken, freeze-dried, and homogenized to powders

135 ~~with a mortar and pestle~~. We weighed and encapsulated approximately 1 mg of 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. Those data were reported in the studies by Wu et al. (2021a, b). Here, we used SPSS Statistics to analyze the published data by a two-way multivariate analysis of variance (MANOVA) to test the effects of vent type and sampling month on the $\delta^{13}C$

140 and $\delta^{15}N$ values of crabs~~For carbon and nitrogen isotope analyses, approximately 1mg of powder was weighed and encapsulated in a tin capsule. Studies using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) were performed at the stable isotope laboratory at the University of California at Davis.~~

~~During analysis, several replicates of at least two different laboratory standards, e.g., nylon (certified $\delta^{13}C$ and $\delta^{15}N$ values of~~

145 ~~-27.8 and -9.8 ‰) and USGS40, L-glutamic acid (certified $\delta^{13}C$ and $\delta^{15}N$ values of -28.9 and -4.3 ‰), were interspersed with samples. Analytical accuracy was obtained by comparing measured values for the known values of the included laboratory 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 $\delta^{15}N$ based on the standard deviation of replicates of internal standards was < 0.2 ‰ (UC Davis Stable Isotope Facility). Finally, stable isotope abundances were expressed in conventional delta (δ) notation and deviations in parts per thousand (‰) relative~~

150 ~~to the analytical standards of Pee Dee Belemnite and atmospheric N_2 for carbon and nitrogen, respectively. Ratios were derived~~

~~from the following equation: δX (‰) = [(R~~_{~~sample~~}~~ / R~~_{~~standard~~}~~) − 1] × 10³, where X is ¹³C or ¹⁵N, and R is the corresponding ratio of ¹³C / ¹²C or ¹⁵N / ¹⁴N (Sharp, 2005).~~

~~We used SPSS to run the statistical analysis of multivariate analysis of variance (MANOVA) to compare the difference in isotopic values between WV and YV.~~ Measurements of isotopic niche width, ~~as~~ proposed by Layman et al. (2007), were calculated for <u>vent</u> crabs<u>, i.e., the corrected</u> ~~obtained from WV and YV. The~~ standard ellipse area (SEAc)<u>, which</u> 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). This metric corrects bias generated when sample sizes are small, represents a measure of 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 <u>also</u>~~allowed us to~~ calculate<u>d</u> the overlap area of the standard ellipses (and respective percentages) between <u>sites and months,</u>~~WV and YV habitats,~~ which was <u>used to</u>~~as measure~~ diet similarity. <u>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</u>~~Metrics including SEAc, an overlap of standard ellipses, and a Bayesian mixing model were calculated using the SIBER v2.1.6 (Stable Isotope Bayesian Ellipses in R) package in R 4.2 software (R Development Core Team, 2013).~~

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). <u>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 of crab's leg muscles was</u> ~~The leg muscle of crabs, about 0.1 g, were~~ taken and homogenized with 1 ml lysis buffer (7 M Urea, 2 M Thiourea, 4 % CHAPS, and protease inhibitor cocktail <u>of</u> two tablets per 100 ml) for proteomic sample preparation. <u>We centrifuged the h</u>~~H~~omogenates ~~were centrifuged~~ at 12000 g for 30 min at 4 ˚C <u>and collected t.</u> ~~The~~ <u>T</u>he supernatant ~~was collected, and t.~~ <u>T</u>he protein concentration was determined by Bradford assay, ~~using~~ <u>with</u> bovine serum albumin as the standard. <u>We prepared t</u>~~T~~he stacking and resolving gels ~~were prepared~~ with percentages of 5 and 12 % (Hoefer SEM 260 system, Amersham Pharmacia). ~~After loading 25 µg protein i~~<u>I</u>n each sample lane, electrophoresis was run for 30 min at 120 V <u>after loading 25 µg protein</u> and then for four h at 180 V. The gels were stained ~~with~~ <u>by</u> Coomassie blue G-250 (Candiano et al., 2004). Stained gels were scanned and transformed into digitalized images using Image Scanner (Amersham Pharmacia). <u>We utilized</u> ~~The~~ <u>the</u> Multi Gauge software v2.2 (Fujifilm) ~~was utilized~~ for protein quantification. The protein bands were assigned ~~with~~ band numbers, and their intensity levels were calculated as their relative area to the total protein area on the gel.

A cluster analysis of the Bray-Curtis similarity (BCS) indices (Primer 6.0) was employed to compare the expression of overall protein patterns among vent crabs (Clarke and Warwick, 2001). In addition, the contribution of each protein band was further examined by principal component analysis (PCA).

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3 Results

3.1 Composition of the benthic community in the WV and YV

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 ~~coverage~~ ~~we~~reas 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. 2). 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 surveyed area of YV, no low-mobility fauna was observed. In addition, fast-moving vent crabs were always
present ~~in~~ at both sites~~WV and YV~~.

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 δ^{13}C and δ^{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 δ^{13}C, and 7.77 ± 0.16 ‰ and 7.66 ± 0.43
‰ for δ^{15}N, respectively. For YV crabs, the data were -16.54 ± 0.43 ‰ and -16.18 ± 0.22 ‰ for δ^{13}C, and 6.35 ± 0.75 ‰ and
6.98 ± 0.32 ‰ for δ^{15}N, respectively. The analysis of two-way MANOVA on δ^{13}C and δ^{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~~The mean values of δ^{13}C and δ^{15}N in WV were -16.59 ± 0.27 ‰ and 7.66 ± 0.43 ‰, ranging from~~
~~-17.50 ‰ to -14.99 ‰ and 4.72 ‰ to 8.94 ‰, respectively (Fig. 3). While in the YV, they were -16.18 ± 0.22 ‰ and 6.98 ±~~
~~0.32 ‰, with the ranges of -17.00 ‰ – -15.22 ‰ and 5.39 ‰ – 8.00 ‰, respectively. The δ^{13}C and δ^{15}N values were~~
~~insignificantly different between crabs from WV and YV (MANOVA, $p > 0.05$).~~
~~The niche width of crabs from WV was significantly broader than the ones from YV, shown as SEAc areas of 3.62 ‰^{2} vs.~~
~~0.88 ‰^{2} ($p < 0.05$), respectively (Fig. 4 and Table 2). The overlapped SEAc area between the two vents was 0.86 ‰^{2}. The~~
~~overlap percentage in WV was low (23.9 %); in contrast, it was high in YV (97.9 %). This indicated that almost all the food~~
~~items in YV were present in WV. The food sources of crabs in WV were more diverse.~~

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~~Based on the protein expression results, 27 protein bands were selected for further BCS analysis (Fig. 5). Crabs from WV and YV fell into two different clusters (Fig. 6). The results further determined the contribution of each protein variable by PCA. The first to the fifth principal components accounted for 46.5, 20.4, 12.2, 9.8, and 4.2 % of the total variance, respectively. The separation was mainly contributed by the first principal component, i.e., bands 25, 10, 9, 2, and 1. Based on the cluster results, the vent crabs were categorized into WV and YV dwellings~~.

4 Discussion

This study compared the feeding habits of vent crabs (*X. testudinatus*) from a WV and a YV within 100 m. The benthic 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~~was the first study to compare the feeding habits of the endemic vent crabs (*X. testudinatus*) living in different types of SVs within 100 m. The benthic community of WV and YV, food sources, and protein expression patterns of the dwelling crabs were associated with their habitats. It is supported by the crabs in YV having a narrower niche width and highly overlapped isotopic SEAc area compared to the one in WV. And based on the analysis on 27 protein bands, the crabs were clustered into two groups relating to their vent types~~.

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.,

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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 ~~along a 50 m transect line from two sites near vent mouths of WV and YV,~~ that encrusting red algae *Hildenbrandia* spp. and three red turf algae occupied~~only~~ 5 % of the substrate ~~was covered with encrusting red algae *Hildenbrandia* spp. and three red turf algae. Wu et al. (2021) found three types of periphyton within the area of 15 m from the center of YV, while WV had two sponge species, two brown algae, two red algae, five green algae, and one type of periphyton.~~

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,~~In 2009 and 2010,~~ Wu et al. ~~(2021)~~ didn't record any low-mobility benthic fauna in YV in 2009 and 2010 (Wu et al., 2021a). W~~, w~~hile 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 $\delta^{13}C$ 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 (*X. testudinatus*) were insignificantly different between WV and YV. The values ranged from -17.50 ‰ to -14.99 ‰ ($\delta^{13}C$: -16.59 ± 0.27 ‰; n = 9) and 4.72 ‰ to 8.94 ‰ ($\delta^{15}N$: 7.66 ± 0.43 ‰; n = 9) in WV. In comparison, the values of YV crabs were from -17.00 ‰ to -15.22 ‰ ($\delta^{13}C$: -16.18 ± 0.22 ‰; n = 7) and 5.39 ‰ to 8.00 ‰ ($\delta^{15}N$: 6.98 ± 0.32 ‰; n = 7), respectively. Wu et al. (2021) reported significantly different $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs from WV and YV collected in 2009 and 2010, i.e., -16.3 ± 0.7 ‰ (n = 44) and -17.4 ± 1.1 ‰ (n = 17) for $\delta^{13}C$ and 6.7 ± 1.3 ‰ vs. 7.8 ± 1.0 ‰ for $\delta^{15}N$, respectively. Another study compared the genders of vent crabs collected in 2010 (Hung et al., 2019). They found male crabs from WV and YV differed significantly ($\delta^{13}C$: -17.2 ‰ vs. -15.8 ‰; $\delta^{15}N$: 7.1 ‰ vs. 5.0 ‰) ($p < 0.01$) but not in females (($\delta^{13}C$: -17.3 ‰ vs.17.0 ‰; $\delta^{15}N$: 7.3 ‰ vs. 7.6 ‰).~~

~~In 2014, crabs from one vent influenced by both WV and YV and three peripheral sites (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 (Wang et al., 2022). Insignificant differences were found in both $\delta^{13}C$ and $\delta^{15}N$ values of vent crabs among the four sites. Their means of $\delta^{13}C$ and $\delta^{15}N$ values and sample sizes were -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 vent crabs living in the center and periphery varied from > 34 % to ≤ 18 %. Our results showed that the crabs' SEAc area was significantly wider in WV (3.62 ‰²) than those in YV (0.88 ‰²). This indicated that crabs in WV had more food sources than in YV. The discrepant results among studies may partly result from the differences in sampling sizes and locations. In general, the isotopic signatures of the resident vent crabs reflected their habitat heterogeneities.~~

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~~In the Pechora Sea, the isotopic niche width in scavenger hermit crab *Pagurus pubescens* varied between stations because of a significant variation in macrobenthic abundance over the spatial scales (Gebruk et al., 2021b). 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 biomass of Astarte montagui (32 g m⁻²), in contrast to high biomass of A. borealis and Macoma calcarea (500 g m⁻²) at 9N (Gebruk et al., 2021a). This result had an even lower overlapping SEAc area than our study, i.e., 0.86 ‰².~~

4.3 Protein expression patterns of vent crabs from the WV and YV

Our proteomic results indicated that vent crabs ~~from WV~~ were distinguishable as groups of WV-July & YV-July, WV-Aug, and YV-Aug, respectively~~from those in YV and the vent crabs are adapted to their local environments~~. 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 ~~(Italy)~~, sea anemones *Anemonia viridis* were collected at a distance of 350–800 m from a vent~~away from a vent about 350–800 m~~, 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.

~~O~~rganisms ~~It is known that or~~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~~by feeding with LA or LNA diet 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~~Between the LA and LNA groups, 186 proteins from crabs' hepatopancreas were differentially expressed~~. 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

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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). 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~~The distribution of vent crabs is related to vent systems. The compositions of vent plumes influenced all the benthic assemblage in SV ecosystems, food availability, and metabolism of vent crabs (Chen et al., 2005b; Mei et al., 2022; Hu et al., 2012; Chang et al., 2018; Wang et al., 2014; Wu et al., 2021)~~.

P~~In p~~revious studies reported that~~,~~ the movement of vent crabs reveals~~shows~~ 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,~~It is also reported that~~ vent crabs were absent in the by-catch of nearby non-vent fisheries (Wang et al., 2013). And the~~The~~ holotype ~~and three paratype specimens~~ of this species wa~~sere~~ collected from a 15 m deep rocky reef in the Gengxin Fish Port, ~~Peikuan,~~ I-Lan ~~County~~, Taiwan (Ng et al., 2000). These investigations indicate that ~~the~~ 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~~Through the analyses of isotopic niche width and protein expression patterns, this study extended our knowledge to realize the metabolic performance of SV endemic crabs is strongly associated with vent types, and trans-vent movement at a scale of meters is uncommon~~.

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5 Conclusions

This ~~was the first~~ study ~~to~~ compared the benthic community, isotopic niche width~~food sources~~, and protein expression patterns of the endemic vent crabs (*Xenograpsus testudinatus*) from different types of SVs at 100 m. The $\delta^{13}C$ and $\delta^{15}N$ 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 more dramatic 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~~coverage of sessile organisms and low-mobility fauna in YV were fewer than in WV. It was found that narrower niche width and highly overlapped isotopic SEAc area of the crabs in YV compared to the one in WV. Moreover, based on the protein expression patterns, the crabs were clustered into two groups relating to their dwelling vent types. It is concluded that the crabs are faithful to their resident vent and the minimum movement range and timing outside the vent area are probably an adaptation to predation risk~~.

Author Contributions

Siou-Yan Lin and Shao-Hung Peng helped ~~for~~ collected 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$_2$S (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

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	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)	"	"

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~~The niche width analyses for vent crabs (*Xenograpsus testudinatus*) from different vent types. WV: white vent; YV: yellow vent; SEAc: standard ellipse area corrected. Comparisons between communities using Layman metrics were based on the posterior distribution (95 % credited intervals) of the modes (p < 0.05, a > b)~~.

(a)

Crab group	n	Carapace width (mm)	δ^{13}C (‰)	δ^{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 (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				

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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)~~Fig~~

(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

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| 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

40
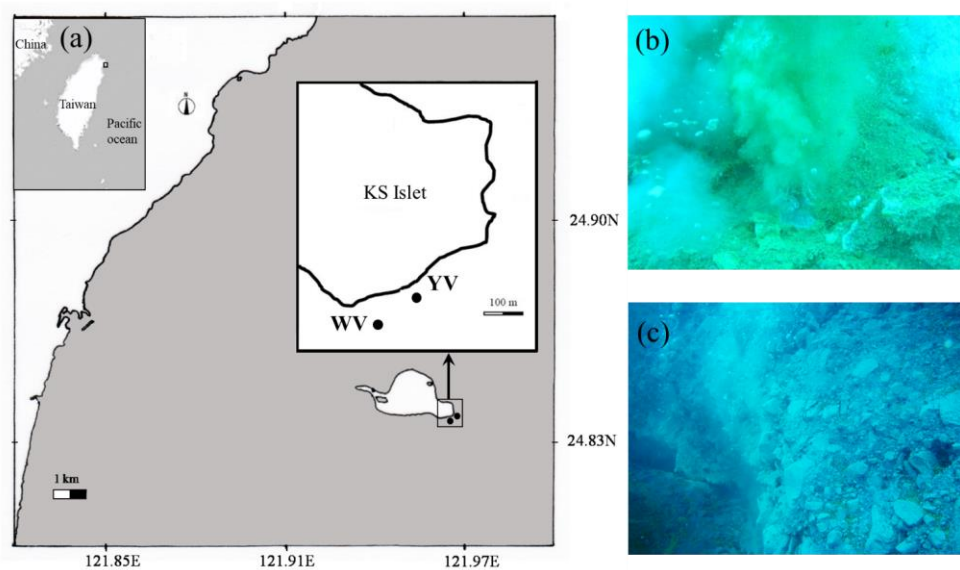
45

Figure 1: Map of the Kueishan Islet showing sampling sites. (a) The study sites ~~in white vent and yellow vent~~; (b) ~~p~~Photograph of the yellow vent; (c) ~~p~~Photograph of the white vent ~~(scale bar: 1 kilometer on the big map and 100 meters on the enlarger map);~~. KS Islet: Kueishan Islet; WV: white vent; YV: yellow vent.

(a)

(b)
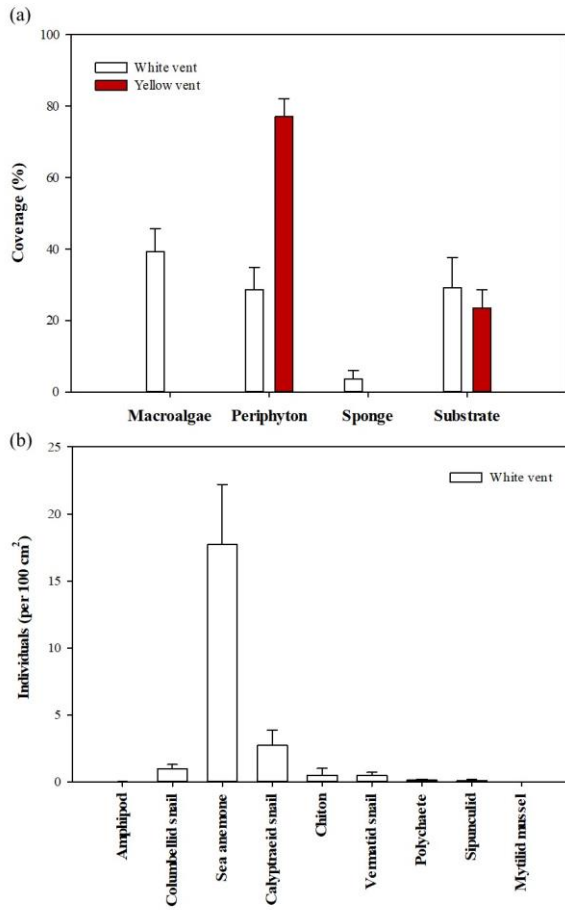
50

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

Figure 3: The δ^{13}C and δ^{15}N values of ~~the~~ vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. W~~V~~: white vent; Y~~V~~: 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.
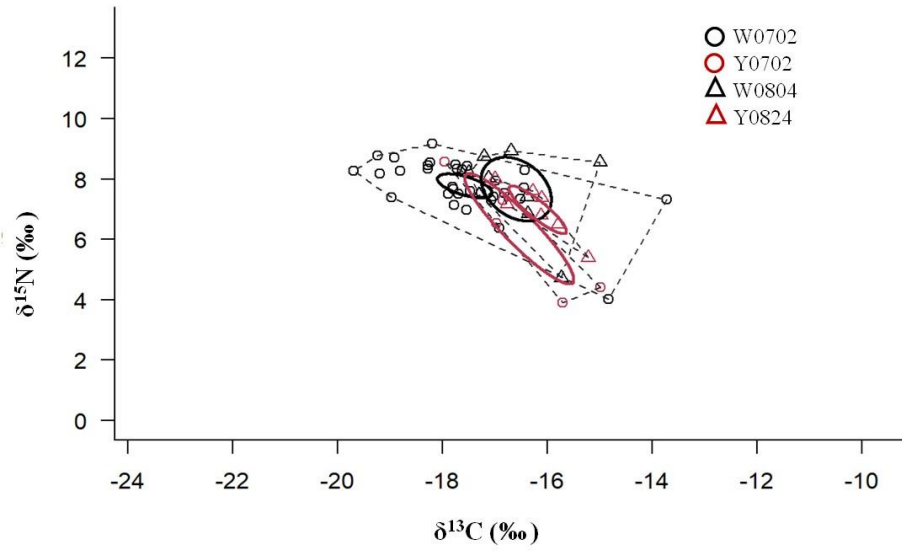
60

Figure 4: Convex hull ~~areas~~ and standard ellipses areas based on <u>the</u> $\delta^{13}C$ and $\delta^{15}N$ <u>values</u>~~data~~ of ~~the~~ vent crabs (*Xenograpsus testudinatus*) from <u>the</u> white and yellow vents. Dot lines: convex hull areas; solid lines: standard ellipses areas (SEAc); W~~V~~: white vent; Y~~V~~: yellow vent<u>; 0702: July 2; 0804: August 4; 0824: August 24</u>.
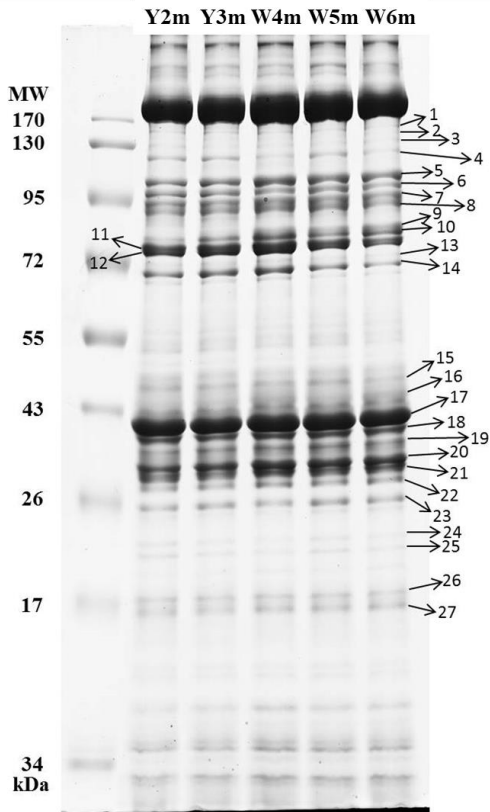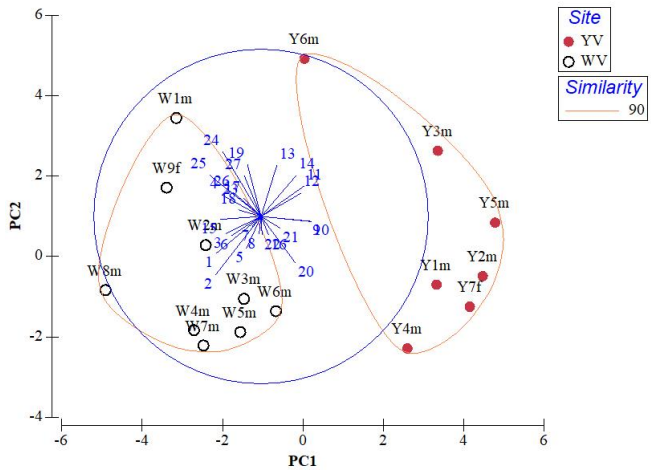
Figure 5: Gel electropherogram with molecular markers of ~~the~~ vent crabs (*Xenograpsus testudinatus*) from the white and yellow vents. Y2-3m: male individuals from the yellow vent; W4-6m: male individuals from the white vent; MW: molecular weight; unit: kDa; number: protein band serial number.
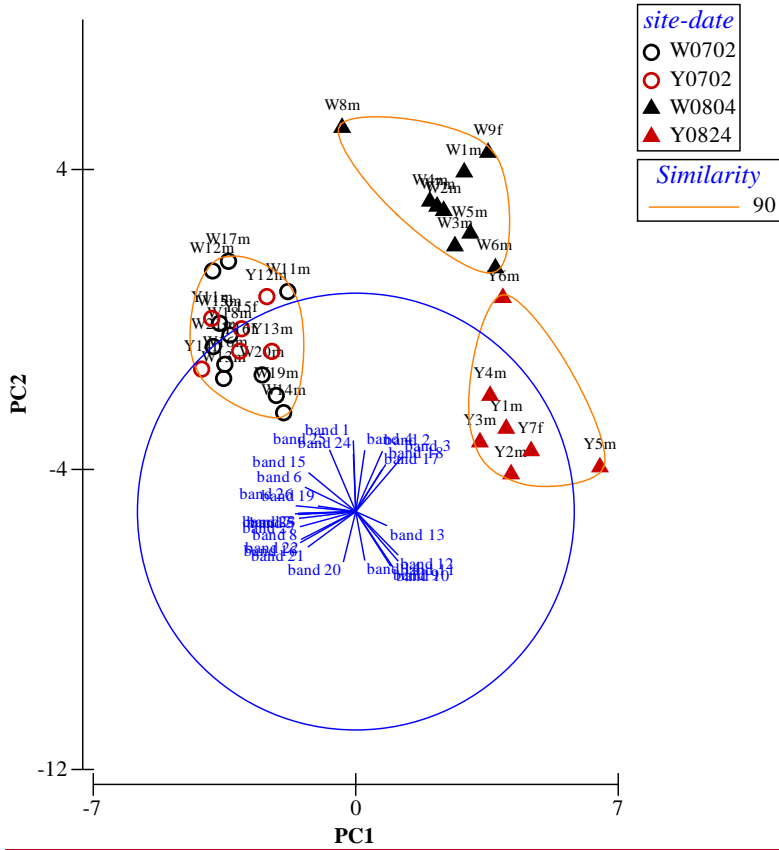
Figure 6: Results from the combined principal component analysis (PCA) and cluster analysis of Bray–Curtis similarity (BCS) indices using standardized overall protein expressions. W~V~: white vent; Y~V~: yellow vent; m: male; f: female; band 1–27: variable of protein bands; 0702: July 2; 0804: August 4; 0824: August 24.