Marine Biology (2018) 165:150
https://doi.org/10.1007/s00227-018-3404-1

ORIGINAL PAPER

Trophic ecology of the neustonic cnidarian *Velella velella* in the northern California Current during an extensive bloom year: insights from gut contents and stable isotope analysis

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Received: 30 November 2017 / Accepted: 13 August 2018

Abstract
Aggregations of the neustonic hydrozoan *Velella velella* occur periodically in the northern California Current. Despite the regular occurrence of notable bloom events in this productive upwelling zone, little is known about their trophic ecology. We used gut content and stable isotope analyses (SIA) to elucidate *V. velella* prey selectivity and trophic niche to address their potential impacts on the marine ecosystem. The dominant prey items ingested by *V. velella* colonies were non-motile prey including cladocerans and northern anchovy (*Engraulis mordax*) eggs, though copepods were also common in gut contents. Removal rates of northern anchovy eggs could be magnified in bloom years and in areas of high spawning biomass. Stable isotope analysis revealed differences in isotopic niche width and overlap among *V. velella* based on latitudinal gradients and to a lesser extent on *V. velella* size and demonstrates the need for continued work to fully understand the trophic ecology of this unique neustonic organism.

Introduction

Velella velella, by-the-wind-sailors, are inverted hydroid colonies with a characteristic blue-hued float and chitinous sail extending above the sea surface (Kirkpatrick and Pugh 1984). Their presence at the sea surface makes them a conspicuous component of the pleuston (Cheng 1975), particularly when high abundances occur in tropical and temperate oceans. Mass strandings of colonies and huge rafts at sea have been reported in many of the world's oceans (Evans 1986; Flux 2009; Purcell et al. 2015; Pires et al. 2018). In the NE Pacific, these blooms and distribution patterns depend on a variety of factors including wind, food availability, growth, and mortality (Mackie 1962; Bieri 1977). These strandings can deposit substantial amounts of nitrogen and carbon to beaches with nutrient loads for 1 year approximated at 3.7 kg C m^{-1} $year^{-1}$ and 1.0 kg of N m^{-1} $year^{-1}$ for a beach in Oregon, USA. *V. velella* organic input to Oregon's sandy beaches may be less than coastal zones with adjacent kelp beds, but deposition events are significant relative to annual inputs (Kemp 1986). Their patchiness at sea makes sampling difficult, but during bloom years their ubiquitous occurrence facilitates capture of hundreds of individuals in a single surface net (or neuston) tow. With maximum standing stock in the Eastern Pacific reported as "millions upon millions", the impact of these colonies on marine food webs may not be trivial (Evans 1986).

Velella velella captures and ingests prey in the large central gastrozooid and the surrounding smaller gastro-gonozooids (Kirkpatrick and Pugh 1984). Gut contents of the colonies collected off the California coast (USA) in the 1950s were mostly fish eggs (48%) and euphausiid eggs (78%) at different sampling stations. Concurrently collected plankton

Responsible Editor: J. Purcell.

Reviewed by N. E. C. Fleming and undisclosed experts.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00227-018-3404-1) contains supplementary material, which is available to authorized users.

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Published online: 29 August 2018

data showed colonies preferentially selected these less abundant non-motile prey items and were less efficient at capturing actively swimming copepods or euphausiids (Bieri 1966). Prey selection has also been documented in the Celtic and Mediterranean seas. In the Celtic Sea, *V. velella* associated with algal rafts ingested raft epifauna, harpacticoid copepods, and planktonic prey such as calanoid copepods and fish eggs (Purcell et al. 2012). In the Mediterranean Sea, vertical migration of prey affected capture rates, with euphausiid larvae being selected at night whereas copepods were selected in the daytime (Purcell et al. 2015).

In addition to food supply through active prey capture, algal symbionts associate with *V. velella* tissue in gonophore and medusa cells (Banaszak et al. 1993; Lopes et al. 2016). In adult colonies, these symbionts are found in the mantle surrounding the chitinous float (Bouillon et al. 2006) and may provide supplementary nutrition to the colonies. Predation on *V. velella* has been noted though observations are relatively sparse. Fish, sea turtles, and birds capitalize on the opportunistic buffet provided by these extensive blooms (Arai 2005; Phillips et al. 2017). Considering their high abundance, widespread distribution, potential to prey on ichthyoplankton and compete with pelagic fishes, *V. velella* feeding should be examined in more detail.

In contrast to the readily available gut content data of *V. velella*, there are few studies that have analyzed its isotopic composition to establish their dietary niche (Lepoint et al. 2016). Stable isotope analysis has become an increasingly common and useful tool in ecological studies for examining trophic relationships and animal diets within and among communities. For many aquatic animals, the isotopic composition of consumers is similar to their diet, with carbon ratios ($^{13}C/^{12}C$) remaining stable during trophic transfer while nitrogen ratio values ($^{15}N/^{14}N$) become enriched by 2–5‰ from prey to predator (Peterson and Fry 1987). By plotting a species isotopic values in δ-space, we can quantity a population's isotopic niche width and examine variances in resource use and potential ecological position (Bearhop et al. 2004; Newsome et al. 2007).

The northern California Current System (NCCS) is an eastern boundary current with seasonal upwelling creating a productive coastal ecosystem. A substantial bloom of *V. velella* colonies occurred off the coast of Oregon in the summer of 2015 (unpublished data) and we were able to sample these colonies to examine their feeding ecology. Gut content and stable isotope analysis was employed to quantify *V. velella* trophic ecology. Gut contents provide a short-term integration of feeding patterns and reveal prey-capture trends and prey selectivity. Ratios of nitrogen and carbon stable isotopes can account for assimilated nutrients over time and are linked to a consumer's isotopic niche (Bearhop et al. 2004). Our objectives were to (1) determine gut contents from *V. velella* sampled in the NCCS, (2) calculate feeding selectivity based on complimentary plankton samples; and (3) examine *V. velella* trophic niche width in relation to colony size and sampling location.

Methods

Sample collection

Colonies of *V. velella* ($N = 87$) were opportunistically collected from the sea surface with long-handled dip nets at 13 stations from 30 May to 8 June 2015 aboard the R/V Bell M. Shimada (Fig. 1; Table 1). The most southern station was off Gold Beach, OR (42°24′26.3″N 124°25′18.3″W) and the most northern station was Willapa Bay, WA (46°22′23″N 123°57′32″W). The majority (76%) of the colonies were collected at offshore slope stations beyond the shelf break, defined as the 200 m isobath (Barth et al. 2000; Fig. 1). Individual colonies were placed in bags filled with filtered seawater and frozen in a − 80 °C freezer. Wherever colonies were collected, potential prey were sampled at the surface with a neuston net (1.5 × 0.5 m mouth; 300 µm mesh). The neuston net was towed at the surface for approximately 5 min at 3.7 km h^{-1} (~ 1.0 m s^{-1}). After sunset, subsurface bongo samples were collected at each station where *V. velella* were also collected. Paired bongos with a 60-cm diameter mouth opening and 333 µm mesh nets were fished as a continuous oblique tow from ~ 100 m (or within 5 m of the bottom at stations < 100 m) to the surface at a retrieval rate of 33 m min^{-1} and a ship speed of 1.0–1.5 m s^{-1}. A depth recorder and flowmeter were placed in the net during each bongo tow to determine tow depth and volume of water filtered. All plankton samples were immediately frozen at in a − 80 °C freezer.

Gut analysis and plankton sorting

Prior to dissection, frozen colonies were thawed for 12 h at 5 °C to allow for easier removal of prey items without damaging the colony. Each colony was dissected into two parts: the dactylozooids and the mantle covering the sail, and the central gastrozooid plus the gastro-gonozooids underneath the sail (Fig. 2). Ingested prey were removed from feeding zooids (gastrozooid and gastro-gonozooids) under a dissecting microscope. Prey were counted and identified to the lowest taxonomic level possible following Gardner and Szabo (1982) and Shanks (2002). Plankton samples collected from neuston samples at the same station as *V. velella* colonies were processed following Postel et al. (2000). Subsamples to quantify prey selection were taken with a Stempel pipette and organisms were counted and identified to lowest possible taxonomic level. The major and minor axes of the colony's ellipsoidal float were measured to calculate the area of each colony. By

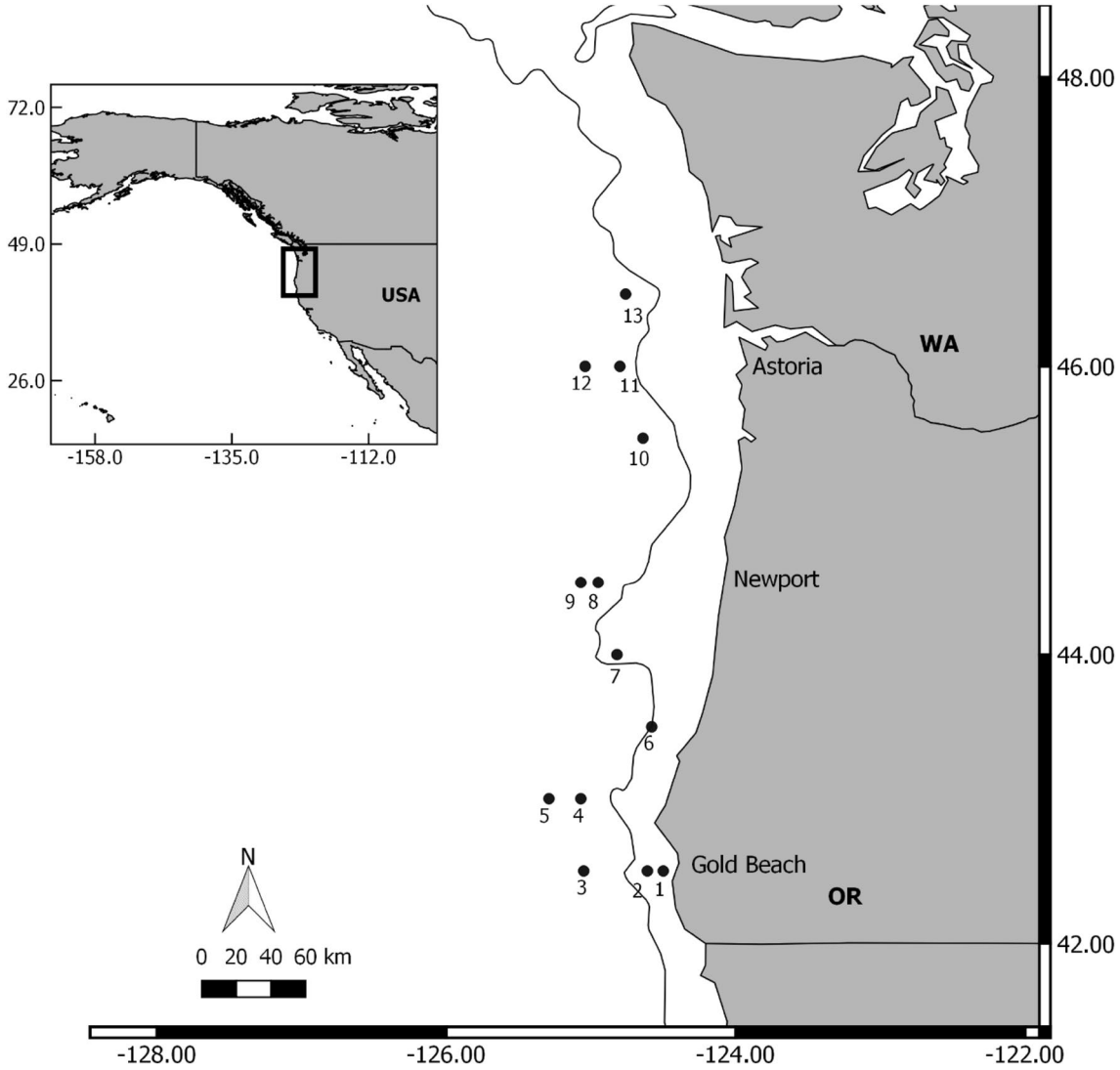

Fig. 1 Map of sampling stations where *V. velella* and plankton samples were collected in May and June 2015. The thin line represents the 200-m isobath

Table 1 Summary of *Velella velella* sampling stations from summer 2015 with the total number of prey found for each colony

Date	Colony (no.)	Station	Location	Transect	Colony area ($cm^2 \pm SD$)	Total prey
May 31	2	1	42.5°N, 124.61°W	Gold Beach	6.08 ± 2.26	7
May 31	2	2	42.5°N, 124.61°W	Gold Beach	6.85 ± 3.49	41
May 31	2	3	42.5°N, 124.94°W	Gold Beach	6.16 ± 2.65	6
June 1	10	4	43.0°N, 125.29°W	Bandon	2.48 ± 0.91	16
June 1	10	5	43.0°N, 125.18°W	Bandon	2.37 ± 1.16	103
June 2	10	6	43.5°N, 124.58°W	Coos Bay	3.93 ± 1.90	137
June 3	1	7	43.0°N, 124.82°W	Heceta	4.8	8
June 4	10	8	44.5°N, 124.07°W	Newport	3.79 ± 0.96	324
June 4	2	9	44.5°N, 124.59°W	Newport	2.43 ± 1.04	43
June 6	10	10	45.5°N, 124.64°W	Tillamook	4.32 ± 1.89	604
June 7	8	11	46.0°N, 124.8°W	Columbia River	2.33 ± 1.20	50
June 7	10	12	46.0°N, 125.04°W	Columbia River	4.17 ± 2.37	57
June 8	10	13	46.5°N, 124.76°W	Willapa Bay	3.88 ± 1.06	159

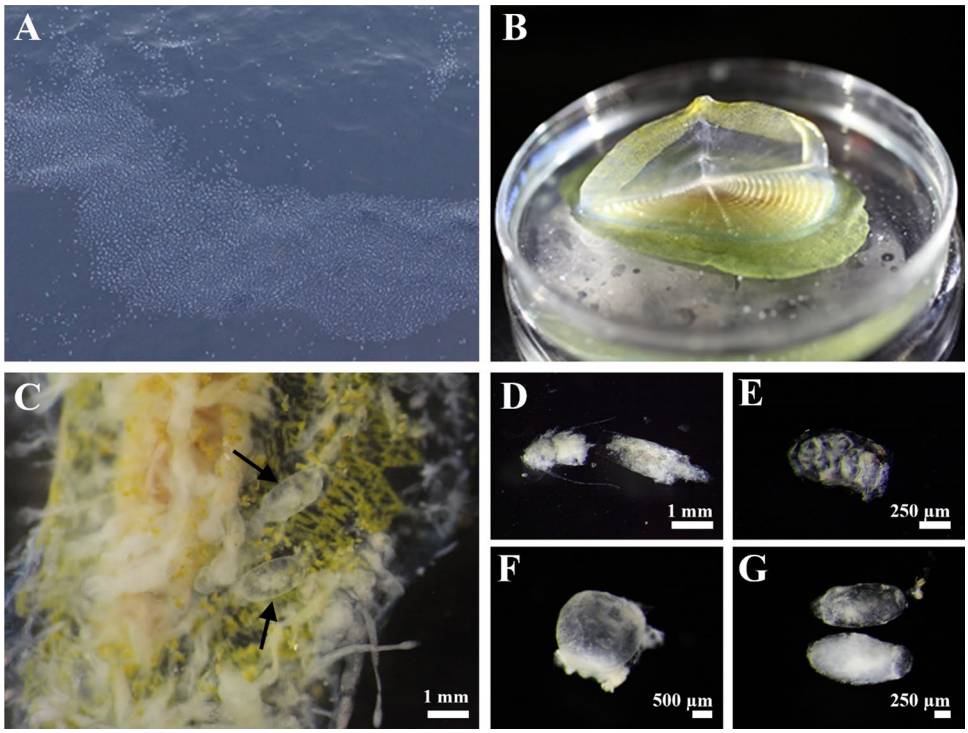

Fig. 2 **a** Aggregation of *Velella velella* off Oregon in June 2015 (photograph by Amanda Gladics/OSU). **b** *V. velella* colony. The green coloring on the sail and mantle and dark brown near center of the float correspond to symbiotic zooxanthellae. **c** Dissected *V. velella* with captured anchovy eggs (arrows). Ingested prey removed from *V. velella* zooids. **d** *Epilabidocera* spp., **e** *Evadne* sp., **f** Pacific saury egg, **g** anchovy eggs

dividing the size range by three, we assigned *V. velella* colonies into small (0.61–2.55 cm^2), medium (2.6–5.8 cm^2), and large (6.56–8.40 cm^2) size groupings.

Prey consumption patterns

Non-metric multidimensional scaling (NMDS) was employed to examine prey assemblages in *V. velella* guts at different sites. To facilitate comparisons, stations were grouped into cross-shelf transects (Table 1). We used percent prey ingested as the common metric among sites and the proportional data were transformed with an arcsine transformation. NMDS reduced dimensionality of the prey data and allowed for visual representation of patterns in *V. velella* prey consumption. We used permutational multivariate analysis of variance (PERMANOVA) to test whether the percentage of prey consumed changed when comparing different sampling transects (Anderson 2001). PERMANOVA is a non-parametric test used to compare and test for significant dispersion between groups. Simple linear regressions were performed to examine the effect of colony size on prey ingestion. Student's *t* tests were used to further examine general comparisons of gut contents and stable isotope values.

Ingestion rate and prey selection

For prey groupings in individual *V. velella* colonies, ingestion rate (IR, prey consumed day^{-1}) was calculated as:

$$\mathrm{IR} = G/\mathrm{DT} \times 24, \quad (1)$$

where G is the number of prey in guts and DT is prey digestion time. Prey digestion times were obtained from published *V. velella* and scyphomedusae digestion experiments (Purcell et al. 2015; Martinussen and Båmstedt 2001).

Prey selectivity for individual colonies was quantified using Pearre's (1982) selectivity index, C,

$$C = \pm(\chi^2/n)^{0.5}, \quad (2)$$

where,

$$\chi^2 = (a_d b_e - b_d a_e)^2 n/abde, \quad (3)$$

with "a" as the prey species of interest inside the guts (a_d) and plankton (a_e), "b" as all other species in diet (b_d) or plankton (b_e), "d" and "e" are total prey in diet and plankton, respectively, and "n" is the total number of prey in guts and zooplankton subsample, after adjusting for differences in digestion rate of different prey items (Sullivan et al. 1997). The C value is dimensionless and ranges from -1 to $+1$, with zero values representing no selection.

Stable isotope values

In the laboratory, frozen *V. velella* colonies ($n = 52$) were rinsed gently with deionized water. Colonies were previously dissected to remove and enumerate ingested prey items. Tissue used for stable isotopes included the chitinous sail, float and some residual mantle material. Conspicuous tissue with algal symbionts was removed.

Colonies were placed in individual weighing boats and placed in a drying oven at 55–60 °C until constant weight was maintained.

Plankton from the neuston and bongo tows were processed for stable isotope analysis. Using different mesh sieves, neuston and bongo samples were sorted to separate prey types encountered in *V. velella* guts. Prey types included: (1) calanoid copepods, of multiple genera, with total length greater than 2.5 mm, (2) eggs of Pacific saury (*Cololabis saira*) and northern anchovy (*Engraulis mordax*), and 3) a small plankton category, between 200 and 500 µm, consisting of a mix of euphausiid eggs, cladocerans, copepods, and barnacle nauplii and cyprid larvae. Individual prey did not provide enough material for analysis, so each prey category could contain up to a hundred individuals. Groups of prey were dried at 55–60 °C until a constant mass. Individual colonies and prey were homogenized with a mortar and pestle. Pulverized *V. velella* samples with a mean weight (± SD) of 1.45 ± 0.03 mg and plankton samples with a mean weight of 1.04 ± 0.08 mg were placed into tin cups. The optimal weight ranges were derived for *V. velella* (1.5 ± 0.05 mg) and bulk plankton (1.0 ± 0.05 mg) using C:N values.

To determine the contribution of algal symbionts to *V. velella* isotopic signatures, colonies were homogenized in deionized water with a 60-ml glass tissue grinder. These colonies were collected from neuston tows during separate NCC surveys in August and November 2015. The *V. velella* homogenate was centrifuged at 1600 RPM for 3 min. The host supernatant was removed, 2 mL of deionized water was added and the pellet resuspended. This process was repeated two times until an obvious layer of *V. velella* sail tissue settled beneath a dark-green algal layer. The pellet was resuspended and centrifuged at 200 rpm for 3 min to separate the loose algal layer and sail tissue. Sail tissue was then examined with a compound microscope to verify separation of tissue and algae. Host and algal samples were placed into separate 1.5 mL tubes and dried at 55–60 °C. Once dried, isolated sail and algal tissue was pulverized into a fine powder with a mortar and pestle and placed into tin cups. There was only enough material to process a single algal pellet.

Nitrogen and carbon isotope compositions were analyzed by continuous-flow isotope using a Carlo Erba elemental analyzer (EA) connected to a Thermo DeltaPlus ratio mass spectrometer (IRMS) at the Oregon State University Stable Isotope Laboratory in Corvallis, OR. Carbon isotope data were calibrated against Vienna Pee Dee Belemnite (VPDB) using the international standard USGS40 and internal lab standard SIL Sucrose. USGS40 and IAEA-N2 were used as standards for nitrogen. An international standard, caffeine, was used as check standard against VPDB and N_2. Typical standard error is ± 0.1 for $\delta^{13}C$ and ± 0.2 for $\delta^{15}N$. Isotopic ratios are expressed as delta (δ) values in parts per thousand relative to international measurement standards.

Isotopic niche analysis

Plotting stable isotope ratios in δ-space can serve as a proxy for community or population tropic niche. We used statistical methods outlined in the Stable Isotope Bayesian Ellipses in R (SIBER; R Core Team 2017) package to quantify niche width and niche overlap within *V. velella* colonies (Jackson et al. 2011). The Bayesian method uses standard ellipse area (SEA) as a metric for isotopic width that is insensitive to small sample sizes and accounts for uncertainty in the sampled data. This metric allows for robust comparisons across communities. The SIBER approach fits Bayesian multivariate normal distributions to isotope ratio data and uses an MCMC algorithm (10^4 iterations) to obtain posterior estimates of ellipses. A corrected SEA (SEA_C) was calculated to remove the bias associated with small sample sizes. Pairwise tests compared posterior draws to determine significant differences in SEA_C between groups. Using the point metric SEA_C, niche overlap was calculated as the area ($‰^2$) of overlap between two or more ellipses.

Results

Gut contents and background prey

A total of 1559 prey items were found in gastrozooids and gonozooids of 87 *V. velella* colonies. Only 6% of the colonies collected were empty. The two predominant prey groups were cladocerans (43%) and fish eggs (28%) (Figs. 2, 3). Cladocerans were exclusively *Evadne* spp. and 97% of fish eggs were identified as *E. mordax* (northern anchovy). Less common fish eggs were *C. saira* (Pacific Saury). One *E. mordax* larva was also encountered. Copepods were split by Order into Calanoida and Cyclopoida, which represented 13 and 6% of the overall diet, respectively. *Epilabidocera* spp. and *Acartia* spp. dominated Calanoid copepods and Cyclopoid copepods were exclusively *Corycaeus* spp. Invertebrate eggs, identified mainly as euphausiid eggs (~ 400 µm), were 5% of the overall diet. The 'Other' category included unidentified items such as copepod egg masses, Cirripedia, Appendicularia, and fish larva (listed in decreasing order of abundance).

The NMDS plot highlights the consistency of prey assemblages captured by *V. velella* within transects ($k = 3$, stress = 0.07, Fig. 4). The plot showed clear separation of transects near the Columbia River and the most southern site at Gold Beach, OR. There is also a clear separation between *V. velella* prey contents, especially fish eggs and cladocerans. Overall, colonies collected in the Coos Bay and Newport transects had ingested mostly invertebrate eggs and copepods, the ones from Bandon and Willapa Bay lines ingested mostly cladocerans, and

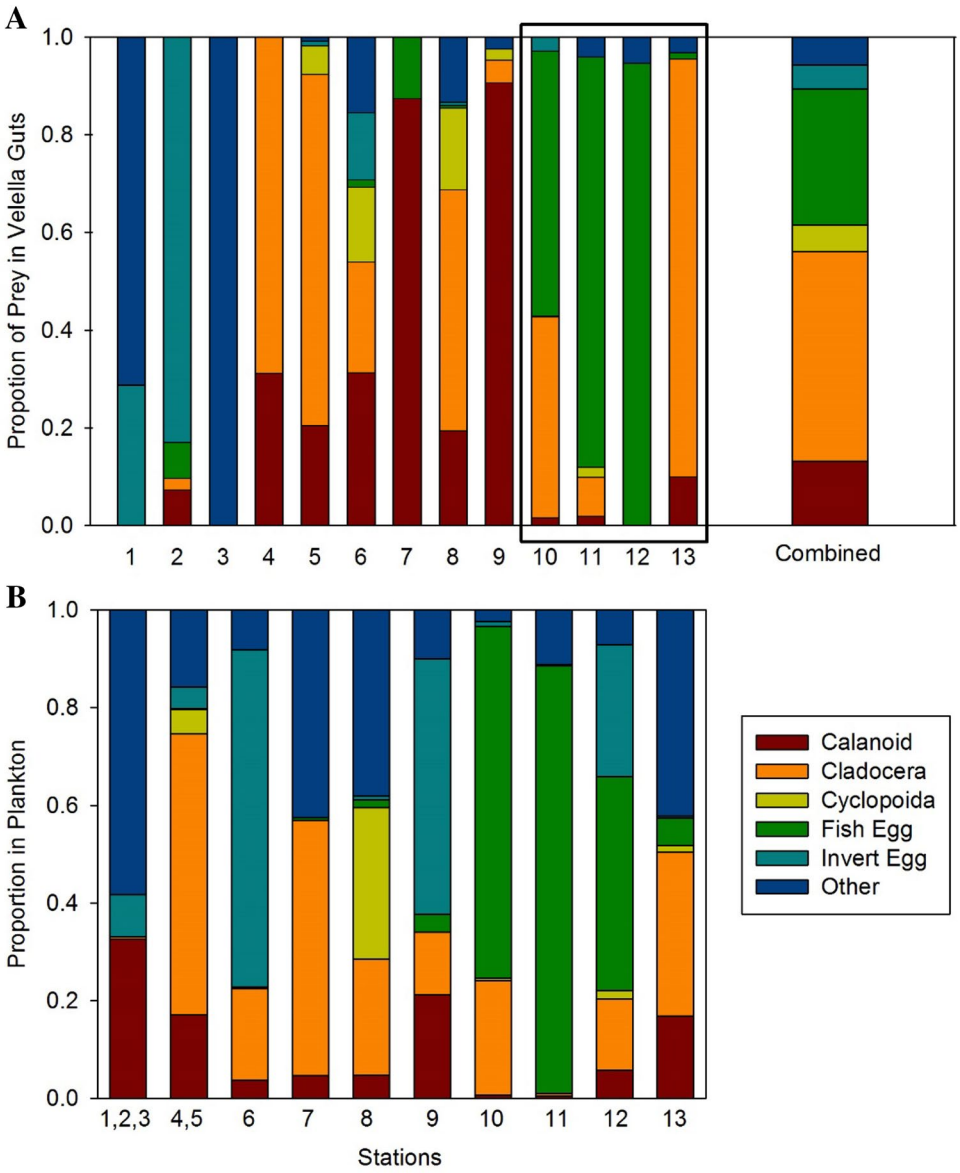

Fig. 3 Plots of prey and plankton proportions from sampling stations 1–13. **a** Proportion of major prey categories identified in *V. velella* gastrozooids and gonozooids. The stations in the black box are near the Columbia River plume. The larger bar plot is prey proportions combined among all *V. velella*. **b** Proportion of plankton in neuston tows from *V. velella* collection sites. For those collection sites without a corresponding neuston tow, the nearest station was used

colonies collected at the Columbia River and Tillamook lines ingested fish eggs (Fig. 4). PERMANOVA analysis confirmed a significant difference in prey assemblages by transect (pseudo-$F = 24$, $p = 0.001$). We used this information to divide stations into those near the Columbia River plume (10–13) from the stations south of the plume (1–9).

Fish eggs were ingested in greater proportions in the northern stations near the plume (Table 2). Larger *V. velella* contained a greater quantity of prey items [$F(1,71) = 12.1$, $p < 0.005$; Fig. 5]. Colonies at station 10 had the highest average ingestion rates for fish eggs and cladocerans (248 eggs day^{-1}; 162 cladocerans day^{-1}). Colony area did not significantly relate to calanoid [$r^2 < 0.001$, $F(1,43) = 0.03$, $p = 0.86$] or fish egg ingestion [$r^2 = 0.11$, $F(1,30) = 3.81$, $p = 0.06$], but area showed a significant relationship to cladoceran ingestion [$r^2 = 0.18$, $F(1,45) = 9.78$, $p = 0.003$; Fig. 5].

For most prey categories, there was a general trend of positive selection, especially for calanoid copepods and cladocerans (Fig. 6). There was also a trend of positive selection for fish eggs at non-plume stations. Both negative selection and no selection were seen at plume stations with large abundances of fish eggs in neuston samples (Fig. 6).

Stable isotope signatures and trophic niches

Carbon-to-nitrogen (C:N ± SD) values of *V. velella* were 4.4 ± 0.18. *V. velella* colonies had less negative $\delta^{13}C$ values (± SD) (− 18.48 ± 0.59‰) than those measured for prey categories (Fig. 7). *V. velella* $\delta^{15}N$ signatures

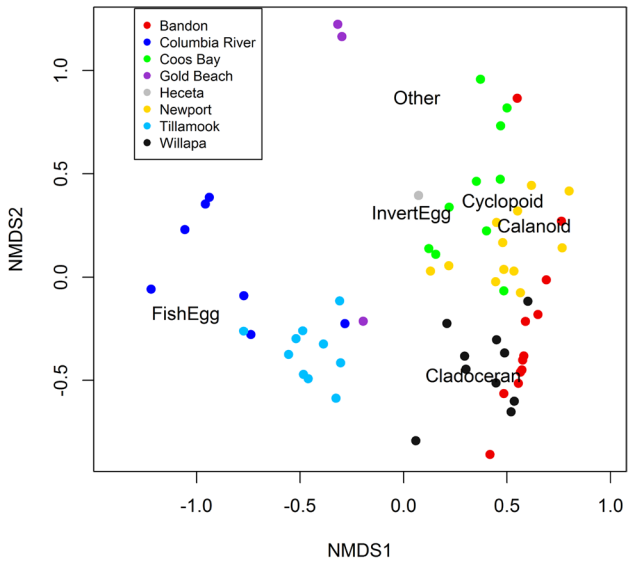

Fig. 4 NMDS plot with points representing individual *V. velella* colonies. Colors represent transects where colonies were collected and labels represent major prey categories dissected in *V. velella* guts

Table 2 Average ingestion rates (±SD) by station for major prey categories

Station	Prey ingestion (prey consumed day^{-1})		
	Calanoid	Cladoceran	Fish egg
2	9.23 ± 4.35	7.06	24.00
4	6.15	19.41 ± 16.67	na
5	14.35 ± 7.54	61.41 ± 21.89	na
6	29.40 ± 22.35	31.26 ± 21.89	8.00
7	43.08	na	8.00
8	23.38 ± 19.66	88.94 ± 73.96	8.00
9	120.00 ± 21.76	14.12	na
10*	43.95 ± 54.04	161.88 ± 143.49	248 ± 197.33
11*	6.15	9.41 ± 4.08	42 ± 24.47
12*	6.15	7.06	56 ± 43.16
13*	14.07 ± 10.48	96 ± 67.72	8.00

Digestion time of 3.9 h was used for calanoid copepods (Purcell et al. 2015), 3.3 h for fish eggs (Fancett 1988), and 3.4 h for cladocerans (Sullivan et al. 1997). Colonies at stations 1 and 3 did not contain any of the major prey categories. Stations marked with an asterisk are plume stations

(9.65 ± 0.38‰) were similar to copepods (9.63 ± 0.35‰) and small plankton (10.54 ± 0.24‰). Fish eggs had the largest $\delta^{15}N$ values (13.5 ± 1.01‰). *V. velella* colonies collected at plume stations had significantly lower $\delta^{13}C$ values (− 19.01 ± 0.39‰) than *V. velella* collected at non-plume stations (− 18.12 ± 0.51‰; $t_{49} = 7.08$, $p < 0.001$). Carbon and nitrogen signatures were not significantly different in colonies collected from offshore slope sites versus nearshore shelf sites ($t_{14} = -0.91$, $p = 0.3795$; $t_{23} = -0.09$, $p = 0.932$, respectively).

Table 3 shows $\delta^{13}C$ and $\delta^{15}N$ values of *V. velella* whole colony, sail tissue, and isolated algal symbionts. The $\delta^{13}C$ values of whole colonies and algae-free tissue were not significantly different ($t_7 = -0.63$, $p = 0.55$), but there was a highly significant difference in $\delta^{15}N$ values between tissue types ($t_7 = -5.97$, $p < 0.001$). The single algal sample that was analyzed had a distinctly lower $\delta^{15}N$ signature (6.96‰) than other sample types.

Colonies collected at non-plume stations had larger SEA_c than colonies collected along the plume transects (0.67 vs 0.30‰^{2}, respectively; Fig. 8). Using Bayesian SEA (SEA_B) calculations, non-plume stations' SEA_B was larger than plume stations in 99% of posterior draws (Fig. 9). There was no overlap between ellipses of plume and non-plume colonies (Fig. 8).

The SEA_C showed less marked differences among the size classes of *V. velella* (Fig. 8). Medium-sized colonies (0.67‰^{2}) were smaller than large and small colonies (1.00 vs. 1.05‰^{2}; Fig. 9). SEA_C overlap was highest between large and small individuals (0.61‰^{2}), but there was also substantial overlap between medium-sized individuals and the other sizes (small: 0.54‰^{2}, large: 0.57‰^{2}; Fig. 8).

Discussion

Our gut content analysis showed that NE Pacific *V. velella* colonies consumed primarily small zooplankton but were also substantial predators of northern anchovy eggs (Figs. 2, 3; Table 2). Similarly, high ingestion of fish eggs was noted in colonies collected off California and in the Celtic Sea (Bieri 1961; Purcell et al. 2012). The vertical distribution of fish eggs is determined by a variety of physical and biological factors, but their positive buoyancy allows for increased abundance at the surface (Coombs et al. 2004). As obligate surface feeders, *V. velella* could take advantage of these high energy content packets, especially in areas of substantial spawning events such as the Columbia River Plume (Fig. 4), as previously shown for another common gelatinous carnivore, *Chrysaora fuscescens*, in the NCC (Zeman et al. 2016). The northern stations were within the Columbia River plume, which is a major spawning site for the northern subpopulation of anchovy (Parnel et al. 2008) and neuston samples collected within the plume are known to show high densities of anchovy eggs compared to non-plume waters (Brodeur and Morgan 2016). Anchovies are one of the few fish species that spawn in the summer off Oregon (Brodeur et al. 2008), with most of the remaining mainly demersal species spawning in the winter and spring months. However, due to anomalously warm winter ocean conditions associated with the extensive warm 'blob' temperature anomaly

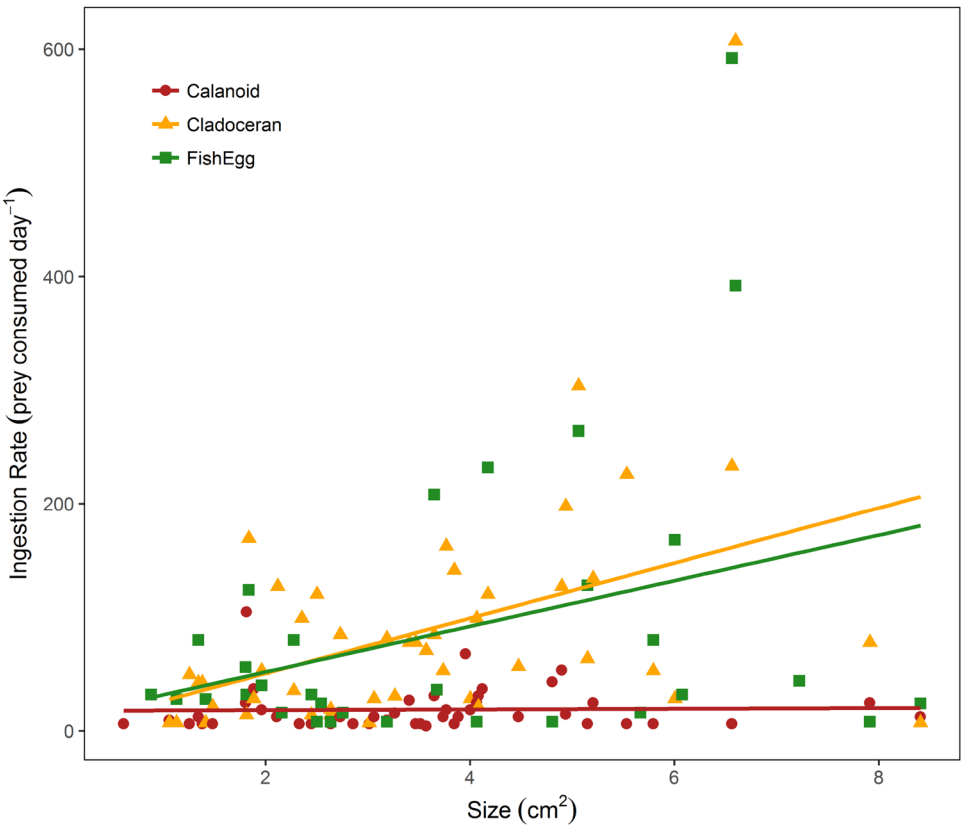

Fig. 5 Linear regression of ingestion rate of major prey categories versus *V. velella* area. Red and green points are calanoid copepod and fish egg ingestion, respectively. Cladoceran ingestion (yellow) is significantly related to colony area (ingestion = size × 24.3 + 1.97)

(Bond et al. 2015) throughout most of the preceding winter, northern anchovy had a much more prolonged and widespread spawning distribution than normal (Auth et al. 2018), and anchovy eggs and larvae may have been available to *V. velella* colonies during other seasons over much of the NCC (Fig. 4). Buoyant fish eggs serving as a predominant source of food for *V. velella* could explain why colonies are more abundant in the winter and spring months (Bieri 1977). At sea, *V. velella* are often encountered in large rafts with thousands of individuals. Ingestion and predation rates within these large aggregations would be greatly amplified relative to non-raft areas.

In the southern stations, *V. velella* was positively selected for fish eggs. In the northern stations, fish eggs were the most dominant prey type but were negatively selected (Fig. 6). Selection patterns at northern stations could be confounded by *V. velella* satiation at such high prey levels. Copepods, another abundant zooplankton in neuston tows, were positively selected at most stations, except for negative selection at some stations. These data contrast with previous *V. velella* gut analyses, in which passive prey were positively selected and motile prey were negatively selected (Bieri 1961; Purcell et al. 2015). However, the closely related pleustonic species *Porpita porpita* consumes mostly active prey, such as carnivorous copepods (Bieri 1970). Gelatinous predators that do not have an active 'cruising behavior' rely on prey behavior to initiate capture events (Costello et al. 2008). Since *V. velella* is not an active swimmer, encounter rates with motile prey may be higher than encounters with non-motile prey (Madin 1988) leading to positive selection for copepods. Encounter rates with prey are expected to increase as predator size increases (Kiørboe 2008) thus it is reasonable that larger colonies were able to ingest more non-motile prey (e.g., cladocerans, fish eggs; Fig. 5).

Stable isotope signatures and isotopic niche

Nitrogen ratio values of *V. velella* colonies were lower than expected considering their role as secondary consumers (Minagawa and Wada 1984). Previous stable isotope analyses (SIA) work with *V. velella* in the Mediterranean also demonstrated a lower than expected trophic level (Lepoint et al. 2016). The potential input from symbiotic zooxanthellae could explain these isotopic values as *V. velella* colonies could be incorporating nutritional input from symbionts. Since our sampling methods did not remove zooxanthellae, our carbon and nitrogen ratios may reflect some algal signature that is still present. With uncertainties surrounding jellyfish trophic enrichment factors, we did not transform the carbon and nitrogen isotopic values to account for trophic enrichment between predator and prey (Fleming et al. 2015). Even with this limitation, a qualitative examination of

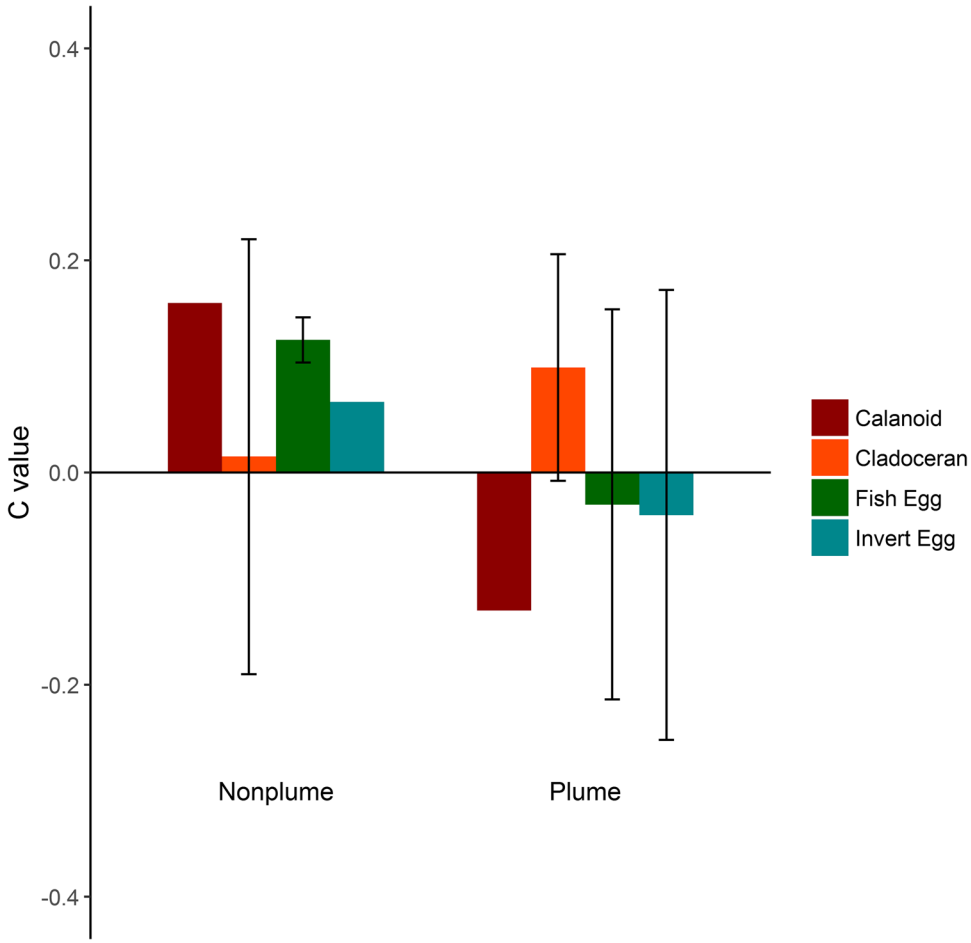

Fig. 6 Average *C* values for four major prey categories encountered in *V. velella* guts. Values are organized by non-plume stations (1–9) and plume stations (10–13). Error bars represent standard deviation

isotopic variability between *V. velella* and zooplankton prey (Fig. 7) suggests that our sources may not accurately represent *V. velella* diet. For instance, fish eggs were the second highest prey group encountered in guts (Fig. 3), but the *V. velella* tissue may not have reached an isotopic steady state with a predominantly fish egg diet (D'Ambra et al. 2014). Interestingly, Purcell et al. (2014) noted a significant lack of digestion of fish eggs by ephyrae of the scyphozoan, *Pelagia noctiluca*. *V. velella* may capture a substantial amount of fish eggs, but if the eggs are indigestible then ichthyoplankton may not be an important component of their diet. Generally, *V. velella* are associated with more oceanic central water masses, and their appearance in coastal waters is associated with prevailing onshore winds (Bieri 1977; Pires et al. 2018). As gut contents represent a recent snapshot of an animal's diet, fish eggs and other nearshore zooplankton may constitute an ephemeral proportion of *V. velella* diets. Much of their nutrition and associated biomass accumulation may have occurred in an open-ocean, low-nutrient environment (Bieri 1977) associated with lower $\delta^{13}C$ values than prey collected nearshore (Fig. 7; Miller et al. 2008).

There was no trophic niche overlap between colonies collected from southern Oregon and colonies near the Columbia plume (Fig. 8). Colonies collected from southern Oregon also have a broader isotopic niche than northern colonies. Gut contents corroborate a more 'generalist' niche, as *V. velella* colonies collected further from the plume had a greater diversity of prey in their guts (Fig. 3). The timing of onshore transport is crucial as isotopic signatures at time of sample collection represent dietary sources assimilated weeks prior (D'Ambra et al. 2014). As neustonic organisms, *V. velella* passively accumulate in response to wind and current patterns (Pires et al. 2018). Subsequent transport of *V. velella* aggregations may occur as distinct events and isotopic values could represent transient resource pools or isotopic variability of baseline producers they encounter as they move across the NE Pacific Ocean. *V. velella* colonies could also be experiencing diverse isotopic baselines as they are transported alongshore. The Columbia River plume represents a unique region in the NCCS, with multiple water mass convergences and frontal zones. The retention and transport time of colonies near the Columbia River plume is unknown, but the differing carbon and nitrogen values near the Columbia plume could also affect the isotopic values of primary producers and thus the isotopic values of consumers (Hill and Wheeler 2002). The difference in $\delta^{13}C$

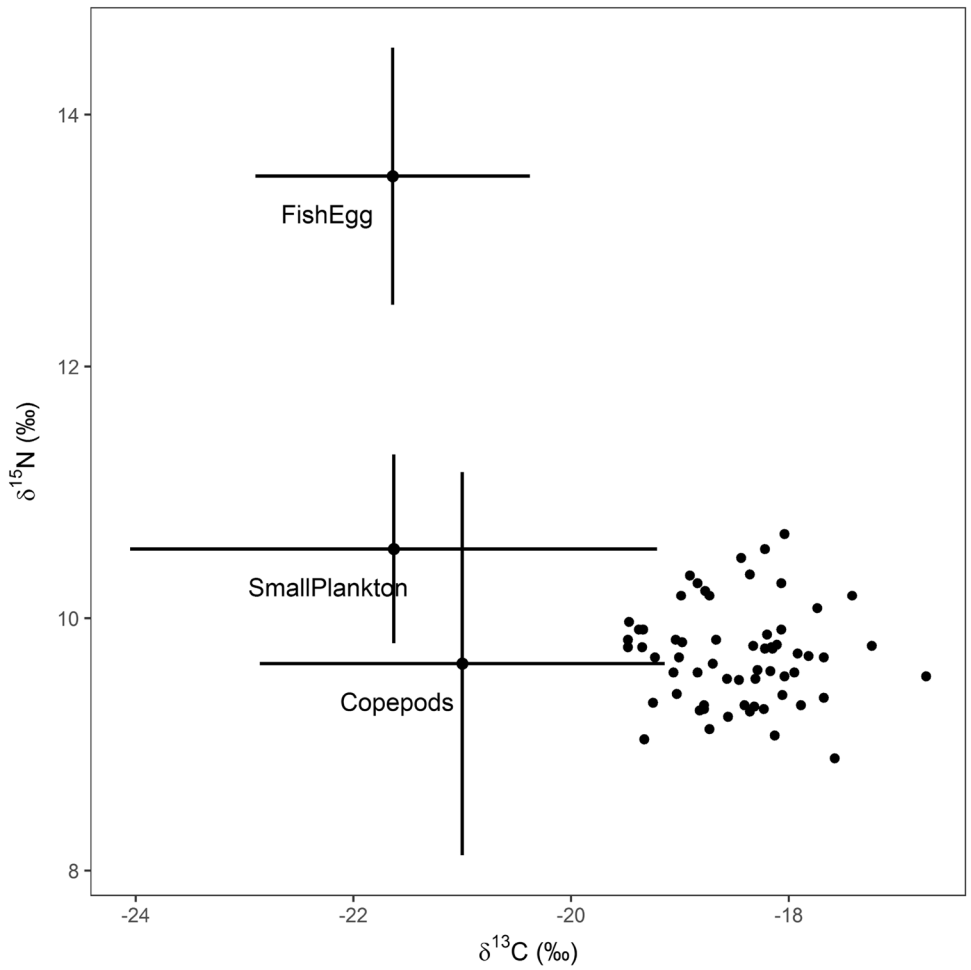

Fig. 7 Biplot of δ^{13}C and δ^{15}N (± SD) values for prey categories and individual *V. velella* colonies (black dots)

values between plume and non-plume stations also suggests that enough time has passed during transport to note subtle differences in isotopic signatures (Figs. 8a, 9a). A more thorough understanding of *V. velella* distribution patterns and concurrent sampling of baseline resources, for instance particulate organic matter (POM), could elucidate latitudinal dietary trends and explain the possible resource pools.

Isotopic variability within size classes as a response to possible diet and trophic shifts has been noted in *V. velella* and other jellyfish (Lepoint et al. 2016; Fleming et al. 2015). The size ranges in our study have large overlapping niche areas (Figs. 8b, 9b), suggesting similar resource use. Most of the organisms in our study were of a similar size, with 86% of the colonies in the small (< 2.5 cm^3) and medium-size range (2.5–6 cm^3). Future studies should consider whether isotopic niche width varies within a broader set of size classes than the ones observed in this study (Graham and Kroutil 2001).

Removing symbionts from host tissue showed that whole colonies had a higher δ^{15}N value (10.37 ± 0.06‰) than *V. velella* tissue with algal symbionts removed (9.77 ± 0.08‰; Table 3). The different δ^{15}N values between *V. velella* tissue types could be related to different turnover rates, which can be mediated by factors such as growth and metabolism, or variable fractionation values among tissues (D'Ambra et al. 2014). For instance, sail tissue may grow more slowly than the softer, mantle tissue and its isotopic values may represent

Table 3 Average δ^{13}C and δ^{15}N (± SD) values for whole *V. velella* colonies, *V. velella* tissue without algal symbionts, isolated algae, and plankton

	δ^{15}N (‰)	δ^{13}C (‰)	*n*	C:N values
V. velella				
Whole colony	10.37 ± 0.06	− 18.29 ± 0.11	3	4.2 ± 0.14
Sail tissue	9.77 ± 0.08	− 18.40 ± 0.13	7	4.4 ± 0.08
Algae	6.96	− 21.03	1	10.6
Plankton				
Copepods	9.63 ± 0.35	− 21.00 ± 0.43	19	9.1 ± 1.4
Fish egg	13.51 ± 0.42	− 21.64 ± 0.51	6	6.3 ± 1.6
Small plankton	10.54 ± 0.24	− 21.63 ± 0.76	10	7.2 ± 0.7

V. velella colonies were collected in August and November of 2015 and plankton were collected in June 2015

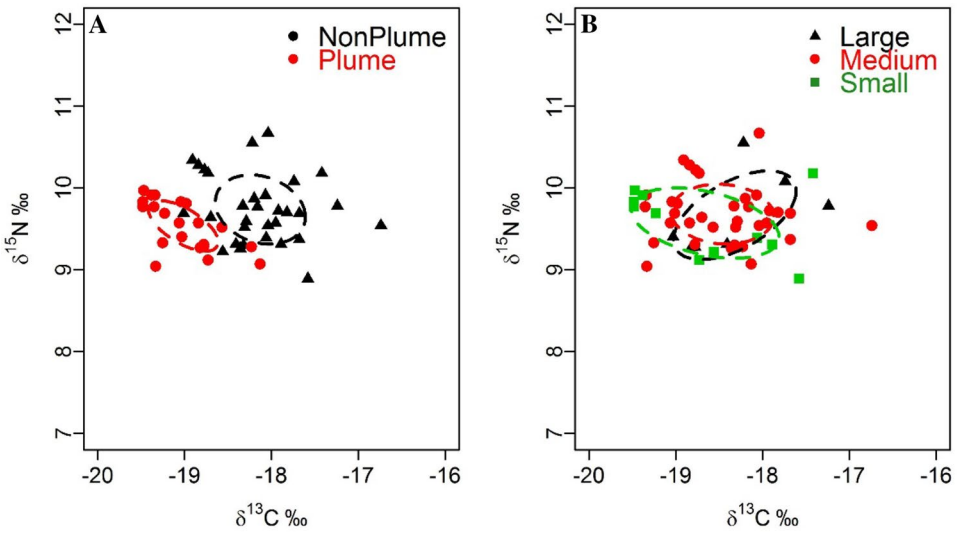

Fig. 8 Isotopic values of *V. velella* colonies with respective small sample size corrected standard ellipse SEA_C (dotted lines). Colonies are separated by **a** station location and **b** size (large: > 6.5 cm^2, medium: 2.5–6.0 cm^2, small: < 2.5 cm^2)

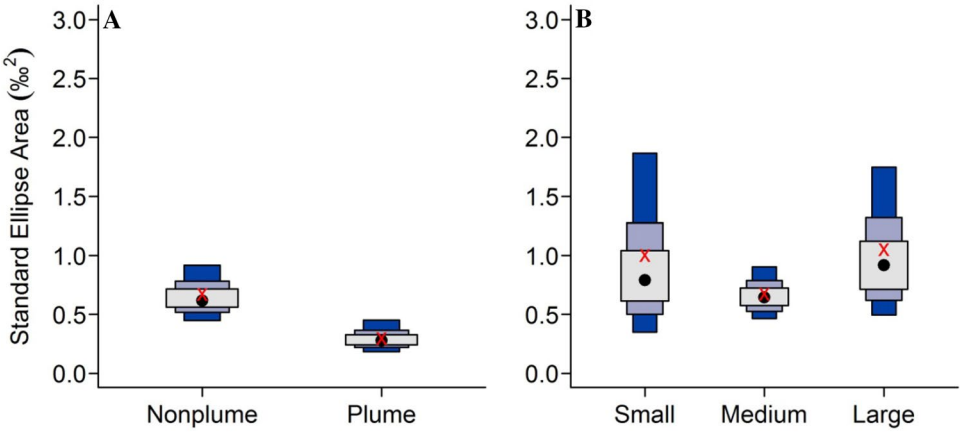

Fig. 9 Posterior estimates of isotopic niche widths (SEA_B) from stable isotope Bayesian ellipses in R (SIBER; Jackson et al. 2011). Blue-colored boxes represent 50, 75, and 95% (in order of decreasing width) credibility intervals from posterior distributions fitted to **a** station location and **b** size ranges of *V. velella* colonies. Black dots represent the modes of these distributions and red Xs are SEA_C point estimates

assimilation over a longer time period. The $\delta^{15}N$ value of a single symbiont sample was lower than the whole body *V. velella* samples, which we expected considering higher trophic levels should be enriched in nitrogen (Minagawa and Wada 1984).

Using carbon isotope signatures, we examined the potential carbon contributions of zooxanthellae to host nutrition (Bergschneider and Muller-Parker 2008). *V. velella* carbon ratios should be similar to the main source of carbon. Interestingly, $\delta^{13}C$ values for whole colonies were lower than both algae and most zooplankton categories (Table 3, Fig. 7). Stable isotope studies on zooxanthellate corals demonstrate the difficulty in teasing out heterotrophic and autotrophic contributions, which can be confounded by season, symbiont species and abundance, nutrient recycling, and source pools (Reynaud et al. 2009; Ferrier-Pagès et al. 2011; Yellowlees et al. 2008). Careful isotopic analysis of the various tissue types to determine the importance of zooxanthellae for *V. velella* nutrition should be examined from detailed laboratory studies with controlled diet proportions.

When interpreting isotopic concentrations it is important to understand the limitations of the technique. Consistency in biochemical preparation of jellyfish material is of utmost importance to have reliable data and uniform results. For instance, freezing jellyfish is known to elevate $\delta^{15}N$ value values by ~ 2‰ (Fleming et al. 2011). This enrichment of nitrogen values could have implications for estimating trophic positions and building food webs. Freezing and washing samples may increase nitrogen values but this may prevent inconsistencies in isotopic values, as these methods remove nitrogenous compounds that may make results more variable (MacKenzie et al. 2017). Furthermore, freezing samples could influence the relationship between size and $\delta^{15}N$ values. Oven drying at 60 °C has also been shown to enrich $\delta^{15}N$ values, in certain scyphozoans, by altering amino acid pools (Kogovšek et al. 2014). Freeze drying may be a preferred technique, but oven drying was a cost-effective way to dry samples and is a common method used in jellyfish SIA. Even if our nitrogen values were elevated because of possible protein denaturing, our colonies still

occupied a lower than expected trophic level. The inconsistencies in isotopic values due to variable sample processing of faunal groups are an important consideration and understanding these limitations is relevant for future SIA work on pleustonic predators and cnidarians with algal symbionts.

In the northern California Current, colonies of *V. velella* form extensive blooms during some years, creating large aggregations of opportunistic carnivores. In general, *V. velella* are efficient predators on non-motile prey and our diet analysis work has shown that cladocerans and copepods were fed upon along the entire coast Oregon coast. Fish eggs became a predominant prey item in northern stations close to the Columbia River and the potential predation impact on these eggs could be significant in large *V. velella* aggregations. This study represents the first use of stable isotope analysis to describe *V. velella* trophic ecology in the NCC. Trophic niche analysis suggests that colony distribution affects niche overlap and future work examining isotopic baselines, distribution patterns, and importance of algal symbionts will further elucidate dietary trends of *V. velella.*

Acknowledgements We acknowledge Curtis Roegner for providing the neuston samples and Toby Auth for assistance in identifying fish eggs and larvae, along with members of the Weis lab at Oregon State University, especially Jack Koch, for guidance and laboratory work with *V. velella* symbionts. For stable isotope direction, we would like to thank Jennifer McKay at the Oregon State University Stable Isotope Facility for her expertise. We thank Curtis Roegner, Elizabeth Daly, Rich Zabel, Jennifer Purcell and two anonymous reviewers for comments on the manuscript.

Funding We acknowledge the support of Oregon Sea Grant (R/ECO-33-Sutherland). We also appreciate the NOAA Fisheries Northwest Fisheries Science Center for providing ship, logistic, and salary support.

Compliance with ethical standards

Ethical approval All national and institutional guidelines for the use of animals were followed.

Conflict of interest All authors declare that they have no conflict of interest.

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