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HORIZONS

Utility of salps as a baseline proxy for food web studies

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In recent years, pelagic tunicates (mostly salps, but potentially doliolids, appendicularians and pyrosomes as well) have been used in isotopic studies as a baseline consumer (trophic position 2) when recreating food web dynamics to overcome the challenges of using particulate organic matter (POM). While pelagic tunicates are continuous filter feeders, recent evidence has shown that they have selective feeding behaviors, and preferentially assimilate certain particles. In this review, we combine available stable isotope data for POM and pelagic tunicates and identify that trophic enrichment in ¹³C and ¹⁵N relative to POM is highly variable, and suggests tunicates prefer to consume smaller, heterotrophic organisms. Here we propose that it is not appropriate to consider pelagic tunicates as representative first level consumers in the classical pelagic food web in stable isotope analyses. Rather it needs acknowledgment that they are members of the microbial food web, and thus reflect an alternate food chain.

KEYWORDS: stable isotope; pelagic tunicate; baseline; food web; salp

INTRODUCTION

In recent decades, stable isotope analysis has developed into a powerful and increasingly used tool in food web studies across ecosystems as it can identify trophic interactions (Peterson and Fry, 1987; Pethybridge *et al.*, 2018). This is because both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of an

organism's tissue change predictably in relation to their food and thus the knowledge of the stable isotope values at the base of the food web allows reconstruction of the trophic pathways and energy sources of the related ecosystem (Peterson and Fry, 1987). $\delta^{15}\text{N}$ can be used to estimate the trophic position of an organism, as a mean enrichment of 3.4 ‰ in ¹⁵N generally occurs between

prey and predators (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 1999; Post, 2002), whereas $\delta^{13}\text{C}$ is more often used to identify dietary sources (Post, 2002).

In order to calculate the trophic position of an organism, a food-web baseline needs to be established. In aquatic research, suspended particulate organic matter (POM) is often used in stable isotope analysis as a proxy for phytoplankton and a baseline for trophic calculations (Post, 2002). However, the use of POM stable isotope values is not ideal as it usually represents an unknown mixture of the particles in the water that may consist of detritus, phytoplankton, bacteria and microzooplankton, and may thus encompass 2–3 trophic positions on its own. As a result, POM stable isotope values have been found to vary in relation to different oceanographic conditions (Stowasser *et al.*, 2012; Henschke *et al.*, 2015), depending on several factors including the size (Rau *et al.*, 1990) and species composition (Wong and Sackett, 1978) of organisms in the sample, as well as factors that influence species growth rates such as nutrient availability (Aberle and Malzahn, 2007), temperature and CO_2 concentration (Goericke and Fry, 1994). There are a few size-fractionated isotope studies for phytoplankton, and when combined they demonstrate that $\delta^{13}\text{C}$ ($r = 0.57$, $P = 0.03$) and $\delta^{15}\text{N}$ ($r = 0.6$, $P = 0.02$) isotope values increase significantly with increasing phytoplankton size (Fig. 1). However, size-fractionation does not distinguish autotrophs from heterotrophs and a detailed analysis of POM samples is needed to distinguish between autotrophic and heterotrophic organisms. Therefore, the complexity of bulk POM is an additional consideration when interpreting stable isotope values and trophic pathways.

One currently used method of combatting this uncertainty is to use the stable isotope values of primary consumers, such as pelagic tunicates (mostly salps, but potentially doliolids, appendicularians and pyrosomes as well), as a time-integrated baseline assumed to be representative of trophic position 2 (Cherel *et al.*, 2008; Richoux and Froneman, 2009; Stowasser *et al.*, 2012; Menard *et al.*, 2014). Carnivores tend to exhibit fairly consistent nitrogen fractionation, whereas assimilative and metabolic factors influence how nitrogen is fractionated in herbivores, resulting in highly variable $\delta^{15}\text{N}$ enrichment (Vander Zanden and Rasmussen, 2001). As a result, calculating trophic positions using primary producers as a baseline can create an error variance that is threefold greater as opposed to a primary consumer baseline (Vander Zanden and Rasmussen, 2001). In this study, we have used published and unpublished data sets to compare the bulk stable isotope values of POM, pelagic tunicates (88% salps) and copepods collected

concurrently across various marine ecosystems (Tables I and II). The main aim of this paper was to evaluate if pelagic tunicates robustly represent “first level consumers” *sensu* “the second trophic position animals” in marine food webs.

CHALLENGES IN STABLE ISOTOPE VALUES: SAMPLE PREPARATION AND TISSUE CHOICE FOR SALPS

Although powerful, stable isotope analysis comes with a suite of challenges. One of the main problems researchers face is correcting for lipid content in tissues as lipids have a more negative $\delta^{13}\text{C}$ value relative to other compounds (Logan *et al.*, 2008). Not correcting for lipid content can result in incorrect interpretations of an organism’s dietary or habitat shifts. Two methods for dealing with lipid content are used: (1) extraction of lipids from samples prior to stable isotope analysis and (2) correcting for lipid content through mathematical models using C:N mass ratios. Both of these methods have problems, as prior lipid removal can alter nitrogen stable isotope values, whereas using C:N corrections are often tissue, season and ecosystem specific (del Rio *et al.*, 2009). One reliable, albeit more expensive, approach is to run samples twice, once for nitrogen stable isotope values before lipid removal and another after lipids are removed for the carbon stable isotope values. Prior to comparing tunicate stable isotope values across different studies, it is important to determine the effect of lipid removal. Comparison of carbon and nitrogen stable isotope values in defatted and non-defatted salps using the Bligh and Dyer (1959) method found no significant difference and this was consistent across salp species (Fig. 2a and b).

Stable isotope values may also differ between body parts of the organism analyzed. In the case of salps, no significant differences were observed between stable isotope values of the whole body compared to only the tunic (stomach removed) or only the stomach for four different species (Fig. 2c and d). Salps naturally do not store high amounts of lipids (<1%; Hagen, 1988), and this is reflected in the stable isotope values of defatted and non-defatted salps. Hence, they do not generally require any prior treatment (e.g. lipid removal), or usage of particular body parts/organs for the stable isotope analyses. Therefore, the stable isotope values for salps are comparable across studies regardless of the preparation method, which makes them an attractive first consumer level surrogate.

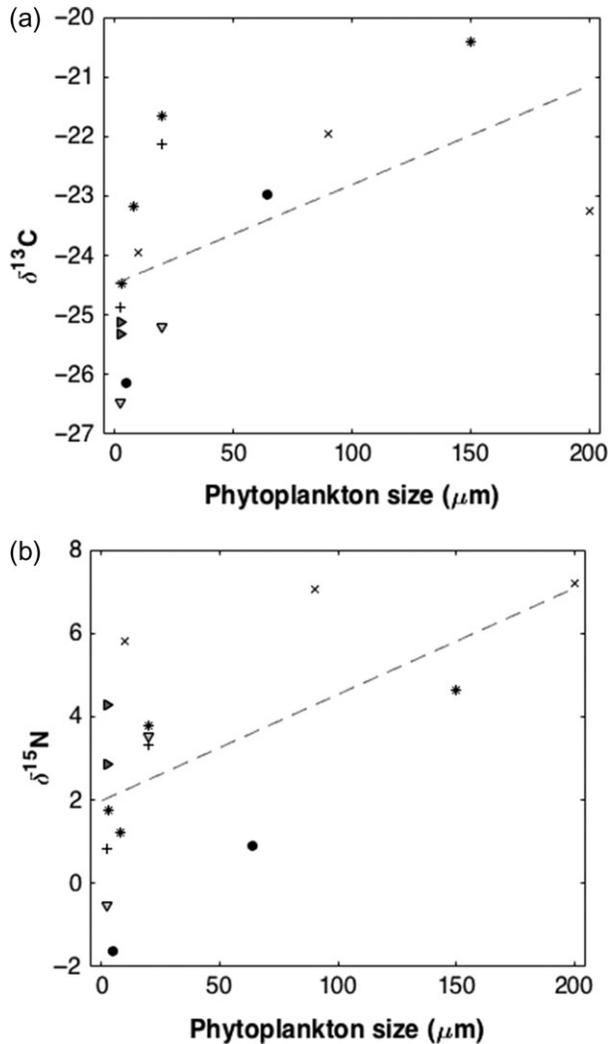


Fig. 1. Variability in (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ values of different size fractions of surface POM. When a range of sizes is given (shown below), the largest size is chosen as representative of the sample. Symbols represent different data sources: \circ ($<5\ \mu\text{m}$, $5\text{--}64\ \mu\text{m}$)—Trull *et al.* (2008), \times ($0\text{--}10\ \mu\text{m}$, $10\text{--}90\ \mu\text{m}$, $90\text{--}200\ \mu\text{m}$)—Tiselius and Fransson (2016), $+$ ($0.2\text{--}2\ \mu\text{m}$, $2\text{--}20\ \mu\text{m}$)—Hunt *et al.* (2017), ∇ ($0.2\text{--}2\ \mu\text{m}$, $2\text{--}20\ \mu\text{m}$)—Im and Suh (2016), $*$ ($<3\ \mu\text{m}$, $3\text{--}8\ \mu\text{m}$, $8\text{--}20\ \mu\text{m}$, $20\text{--}150\ \mu\text{m}$)—Rau *et al.* (1990), \triangleright ($0.2\text{--}2\ \mu\text{m}$)—Waite *et al.* (2007a). Dotted lines represent lines of best fit ($r = 0.57$, $P = 0.03$; $r = 0.6$, $P = 0.02$).

CHALLENGES IN STABLE ISOTOPE VALUES: TURNOVER RATES FOR PRODUCERS VERSUS CONSUMERS

Another major consideration with stable isotope analysis is reconciling the difference in the turnover time of the elements in tissues of organisms at different trophic positions (O'Reilly *et al.*, 2002). Phytoplankton primary producers have hourly or daily turnover rates and represent

a near instantaneous measure, while their consumers have tissue turnover rates of weeks to months and therefore average prey isotope values over prolonged, often poorly constrained time intervals. This creates a challenge of reconciling the stable isotope values of high turnover primary producers (e.g. POM) with slower turnover primary consumers (e.g. zooplankton). The trophic positions for primary consumers can be calculated using the formula:

$$\text{Trophic Position}_{\text{Consumer}} = \frac{\delta^{15}\text{N}_{\text{Consumer}} - \delta^{15}\text{N}_{\text{POM}}}{2.5} + 1$$

assuming that POM is trophic position 1 and 2.5 is the mean fractionation for herbivores (Vander Zanden and Rasmussen, 2001). When considering all studies that have corresponding tunicate, copepod (non-carnivorous) and POM samples available, the mean trophic position (TP) of pelagic tunicates ($\text{TP} = 0.93 \pm 0.82$) was significantly lower than that of copepods (ANOVA; $\text{TP} = 1.92 \pm 1.36$; $F_{1,24} = 5.23$, $P = 0.03$) and not significantly different than that of POM (assuming POM $\text{TP} = 1$, $P = 0.75$; Table II). Since pelagic tunicates and non-carnivorous copepods are both primary consumers, they should have trophic position ≥ 2 . It is possible that the difference in trophic position for pelagic tunicates and copepods reflects their different feeding strategies, with most copepods feeding (*sensu* assimilating) on larger prey than pelagic tunicates (Kleppel, 1993). However, this highlights the difficulties in using POM as a baseline, in this case not providing a representative baseline of the primary diet items of pelagic tunicates. A detailed analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation across species showed that variation in the $\delta^{15}\text{N}$ baseline and variation in the trophic fractionation value are important additional sources of error when estimating trophic position (Vander Zanden and Rasmussen, 2001).

UTILITY OF PELAGIC TUNICATES AS A TROPHIC BASELINE

By now there are at least five studies where pelagic tunicates have been used as a primary consumer baseline in food web interpretations (Richoux and Froneman, 2009; Cherel *et al.*, 2008, 2010; Stowasser *et al.*, 2012; Menard *et al.*, 2014). Pelagic tunicates are filter feeders and all suspended particles present in the water, from detritus through phytoplankton, have been found in their stomachs (e.g. Hopkins, 1985; Vargas and Madin, 2004; Lombard *et al.*, 2010; von Harbou *et al.*, 2011; Lawrence *et al.*, 2018) making them potentially attractive

Table I: Sampling dates and locations for $\delta^{13}C$ and $\delta^{15}N$ values of POM and pelagic tunicates

Cruise	Sampling date (mm/yyyy)	Location	POM n	Pelagic tunicates	
				n	Species
Agulhas110	04/2003	Southern Ocean	44	19	<i>Salpa thompsoni</i>
PS-ANT21-4	04/2004	Southern Ocean	3	10 [^]	<i>S. thompsoni</i> , <i>Ihleia racovitzai</i>
MIE_1	10/2004	Hawaii	6	7	<i>Salpa</i> sp.
<i>La Curieuse</i>	01/2005	Southern Ocean Kerguelen Islands	7*	10	<i>S. thompsoni</i>
PS-ANT23-3	12/2005	Southern Ocean	10	4	<i>S. thompsoni</i> , <i>I. racovitzai</i>
PS-ANT23-6	07/2006	Southern Ocean	15	39 [^]	<i>S. thompsoni</i> , <i>I. racovitzai</i>
JR161	11/2006	Southern Ocean	4	15	<i>S. thompsoni</i>
JR177	02/2008	Southern Ocean	10	25	<i>S. thompsoni</i>
TAN08-06	05/2008	Chatham Rise	42	89 [^]	<i>S. fusiformis</i> , <i>Thetys vagina</i> , <i>lasis zonaria</i>
JR200	03/2009	Southern Ocean	3	44	<i>S. thompsoni</i>
TAN11-16	11/2011	Chatham Rise	31	109	<i>S. fusiformis</i> , <i>T. vagina</i> , <i>I. zonaria</i> , <i>Pyrosoma</i> sp.
ANT28-3	01/2012	Southern Ocean	76	124	<i>S. thompsoni</i> , <i>Pegea confoederata</i>

n—number of replicates. *Samples taken from consecutive cruise in Trull *et al.* (2008). [^]Samples used to test the effect of lipid removal and tissue type.

Table II: Mean (\pm SD) $\delta^{13}C$ and $\delta^{15}N$ values for POM, pelagic tunicates and copepods (non-carnivorous). TP – trophic position

POM		Pelagic tunicates			Copepods			Source
$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	TP	$\delta^{13}C$	$\delta^{15}N$	TP	
-26.74 \pm 1.36	-1.17 \pm 1.6	-27.74 \pm 0.73	1.82 \pm 0.59	2.20	-29.52 \pm 1.71	4.17 \pm 1.62	3.14	This study (PS-ANT23-6 data)
-24.8 \pm 3.3	1.8 \pm 0.8	-24.5 \pm 1.8	2.7 \pm 0.8	1.36	-22.92 \pm 0.94	5.22 \pm 0.56	2.37	Stowasser <i>et al.</i> (2012)
-22.7 \pm 0.48	4 \pm 1.5	-19.3 \pm 0.87	3.46 \pm 2.33	0.78	-18.81 \pm 0.83	4.59 \pm 2.3	1.24	Richoux and Froneman (2009)
-28.21 \pm 2.2	0.38 \pm 0.6	-28.96 \pm 0.6	3.86 \pm 0.5	2.39	-28.6 \pm 0.14	4.05 \pm 0.07	2.47	Pinkerton <i>et al.</i> (2013)
-19.81 \pm 0.002	3.63 \pm 0.27	-19 \pm 1.28	2.75 \pm 1.98	0.65	-19.35 \pm 0.35	5.35 \pm 2.33	1.69	McClain-Counts <i>et al.</i> (2017)
-22.63 \pm 0.86	5.6 \pm 1.35	-21.93 \pm 0.35	6.7 \pm 0.2	1.44	-21.48 \pm 0.76	6.78 \pm 1.06	1.47	Henschke <i>et al.</i> (2015)
-25.11 \pm 1.37	2.20 \pm 2.27	-22.76 \pm 1.29	0.35 \pm 1.46	0.26	-21.45 \pm 0.58	3.82 \pm 0.57	1.65	Banaru <i>et al.</i> (2013)
-23.39 \pm 0.15	7.67 \pm 0.4	-23.53 \pm 0.54	8.24 \pm 0.58	1.23				Ishak <i>et al.</i> (2017)
	5.91 \pm 1.8		5.58 \pm 1.61	0.87				Wu <i>et al.</i> (1997)
	7.15 \pm 0.98		5.28 \pm 1.34	0.25				Annasawmy <i>et al.</i> (2018)
-27.4 \pm	0.53	-28.1	1.8	1.51	-28.7	9.6	4.63	Wada <i>et al.</i> (1987)
-23.30 \pm 0.15	7.00 \pm 1.23	-21.33 \pm 0.65	3.75 \pm 0.57	-0.30				Waite <i>et al.</i> (2007a)
	7.35 \pm 0.35		3.95 \pm 0.35	-0.36		3.7	-0.46	Montoya <i>et al.</i> (1992)
	4.5		5.5	1.40				Hunt <i>et al.</i> (2015)
-21.5 \pm 0.2	6.1 \pm 2.5	-22 \pm 2.2	4.3 \pm 2.6	0.28	-21.5 \pm 1	6.2 \pm 1.6	1.04	Davenport and Bax (2002)

first consumer surrogates. They are efficient feeders, deploying a very fine mucous net to capture particles from a size range <1 μ m to 1 mm (Vargas and Madin, 2004; Sutherland *et al.*, 2010; Lawrence *et al.*, 2018). However, studies have demonstrated that pelagic tunicates have varying retention and assimilation rates of different particles (Vargas and Madin, 2004; von Harbou *et al.*, 2011; Metfies *et al.*, 2014; Conley *et al.*, 2017; Dadon-Pilosof *et al.*, 2017; Walters *et al.*, 2018). Although these filter feeders can be used as a baseline in food web studies, they may only reflect a particular food chain because of their selective prey assimilation. This issue was seldom explored in current stable isotope literature. Here we combined our data with a literature review of studies where pelagic tunicates and POM were sampled consecutively in the same area and

produced 67 $\delta^{15}N$ estimates and 58 $\delta^{13}C$ estimates from 12 species (see Tables I and II). The trophic enrichment in both carbon and nitrogen stable isotope values for tunicates relative to POM was highly variable, ranging from -3.7 to 4.5%, and did not show any clear pattern (Fig. 3a). Considering 48% of all nitrogen and 43% of all carbon enrichment values were negative, this indicates how difficult it can be to reconcile the diets of primary consumers using bulk POM. Tunicate $\delta^{13}C$ enrichment relative to POM significantly declined with increasing chlorophyll *a* concentration ($r = -0.5$, $P < 0.01$), whereas there was no significant trend between $\delta^{15}N$ enrichment and chlorophyll *a* concentration (Fig. 3b and c). Generally, low chlorophyll *a* concentrations suggest communities with higher proportions of heterotrophic carbon (Hewes *et al.*, 1990) and members

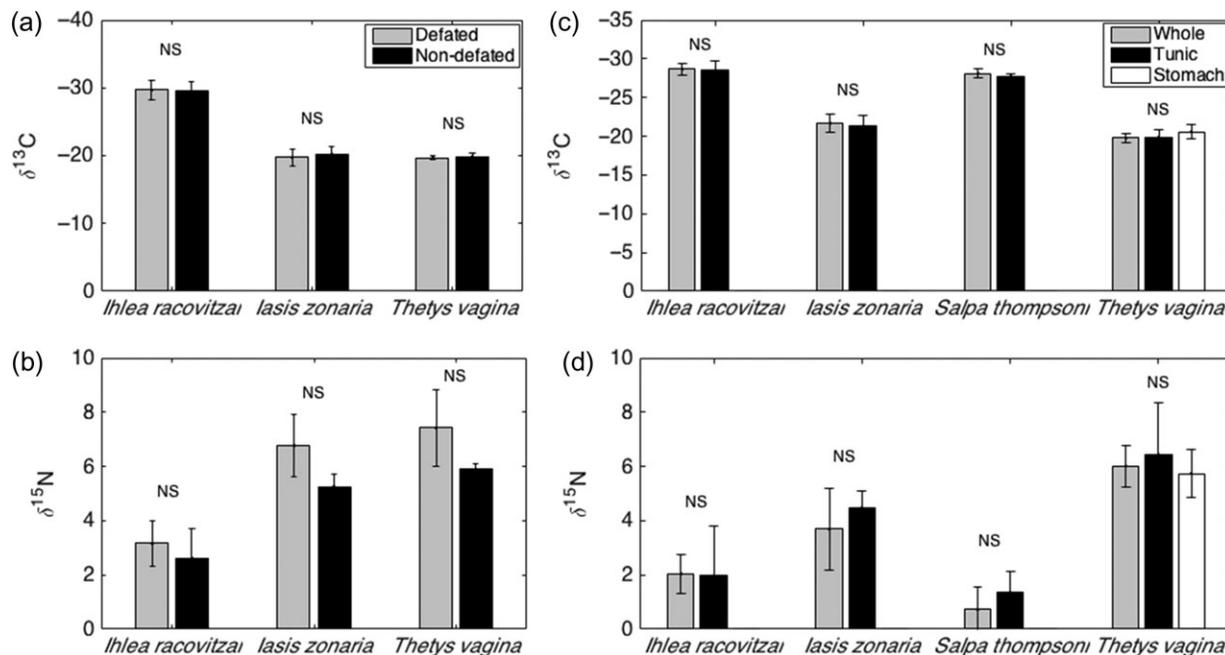


Fig. 2. (a and b) The effect of lipid removal on mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of three salp species: *Ihlea racovitzai* ($n = 30$), *Iasis zonaria* ($n = 6$) and *Thetys vagina* ($n = 8$) and (c and d) the variability of mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for different body sections (the whole animal, tunic and stomach) for four salp species: *I. racovitzai* ($n = 32$), *I. zonaria* ($n = 31$), *T. vagina* ($n = 46$) and *Salpa thompsoni* ($n = 8$). NS—not significant ($P > 0.05$).

comprised of smaller cell sizes (e.g. Waite *et al.*, 2007b). The fact that tunicates were enriched relative to POM under low chlorophyll *a* conditions suggests they were preferentially assimilating a smaller, more heterotrophic size fraction of the plankton community, such as flagellates or microzooplankton. Salps have been found to be direct consumers of bacteria, ciliates and autotrophic and heterotrophic dinoflagellates (Vargas and Madin, 2004; Sutherland *et al.*, 2010), and may have their energy demands met by bacterial biomass (Sutherland *et al.*, 2010). Appendicularians have been shown to consume ciliates and viruses (Lombard *et al.*, 2010; Lawrence *et al.*, 2018). Furthermore, there is mounting tracer (fatty acid composition) and genetic evidence pointing to selective feeding by salps and other pelagic tunicates irrespective of the natural prey assemblage (von Harbou *et al.*, 2011; Metfies *et al.*, 2014; Conley *et al.*, 2017; Dadon-Pilosof *et al.*, 2017; Walters *et al.*, 2018). This is understandable because, as continuous feeders, food items consumed generally have a short stomach residence time (a matter of hours) and they have no means of breaking hard parts of protected prey items, such as frustules of diatoms (Pakhomov *et al.*, 2006; von Harbou *et al.*, 2011). For example, in *Salpa thompsoni* and *Ihlea racovitzai* tissue fatty acid signatures and metagenomic analysis of stomach contents suggests high assimilation of small flagellates and not of diatoms,

the latter often passing through the salp stomach undigested (von Harbou *et al.*, 2011; Metfies *et al.*, 2014). The disconnect between POM stable isotope values and salp stable isotope values under high chlorophyll *a* conditions reflects this non-consumption of diatoms by salps, as diatoms generally dominate during high chlorophyll *a* conditions (e.g. Hewes *et al.*, 1990). Despite their large feeding range, salps may preferentially feed on pico- and nanoplankton constituents of the microbial loop, including heterotrophic organisms.

RE-INTERPRETATION OF SALP BASELINE STUDIES

It is difficult to re-interpret the results of all the previous five studies that used pelagic tunicates as a primary consumer baseline because corresponding chlorophyll *a* concentrations and/or POM stable isotopes were not always sampled and in order to assess the appropriateness of salps as a baseline proxy, knowledge of the phytoplankton community is needed. These data are available for only two of the studies: Richoux and Froneman (2009) and Stowasser *et al.* (2012). During sampling, Richoux and Froneman (2009) found low chlorophyll *a* concentrations and a picoplankton dominated phytoplankton community. This suggests that in

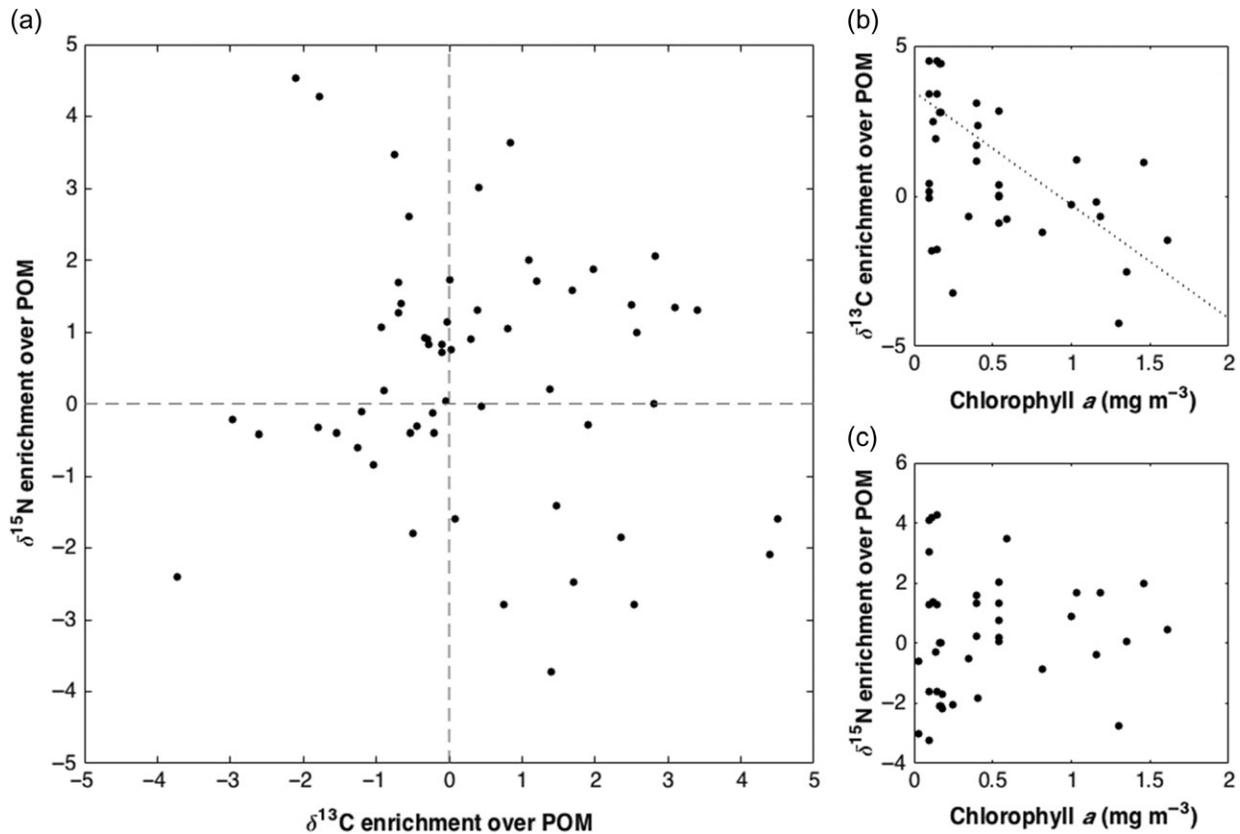


Fig. 3. (a) Bi-plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment for tunicates with respect to POM (dashed lines). (b) $\delta^{13}\text{C}$ and (c) $\delta^{15}\text{N}$ enrichment for tunicates with respect to POM across varying chlorophyll *a* concentrations. Dotted line represents line of best fit ($r = -0.5$, $P < 0.01$). These data include all tunicate and POM data from Tables I and II.

this case, *Salpa thompsoni* was an appropriate baseline primary consumer, and correspondingly salp $\delta^{13}\text{C}$ values were enriched compared to POM (akin to Fig. 3b). Alternatively, Stowasser *et al.* (2012) sampled high chlorophyll *a* water (mean \pm SD: 2.52 ± 1.63) and POM $\delta^{13}\text{C}$ values were high, potentially suggesting an increased proportion of larger cells. Here, large herbivorous copepods consuming a larger size range than *S. thompsoni* may be a more appropriate baseline. The use of herbivorous copepods as a trophic position 2 baseline as opposed to *S. thompsoni* would result in a negative discrepancy of ~ 0.74 trophic positions (using data from Table II) compared to those calculated in Stowasser *et al.* (2012). Yet, without knowledge of the phytoplankton community composition and feeding selectivity of first consumer metazoans, it is difficult in hindsight to confidently recalculate these trophic positions and salps could theoretically be an appropriate baseline group here. However, apart from identifying an appropriate baseline, when calculating trophic positions in classical food webs it is important to consider the contribution of the baseline primary consumer to secondary consumers and higher trophic positions. Pelagic tunicates have

few predators in comparison to copepods and other crustacean zooplankton (Henschke *et al.*, 2016). Thus, even if pelagic tunicates represent primary consumers, a better baseline proxy should be the primary consumers, such as copepods, that are the prey of secondary consumers, otherwise food web calculations will be based on an organism that has limited energy transfer to higher trophic positions.

CONCLUDING REMARKS

A recent study using compound specific stable isotope analysis of essential and non-essential amino acids found that salps were representative first level consumers (trophic position 2) in both low and high chlorophyll *a* areas of the Antarctic Polar Frontal Zone (Kruse *et al.*, 2015). While this should hardly be disputed, in light of dietary findings, the usage of pelagic tunicates as a baseline organism representative of the classical food web should be critically re-evaluated. Pelagic tunicates could be a promising baseline surrogate in the food webs when they are part of it (e.g. consumed) but will be

“erroneous” surrogates in any other (e.g. crustacean dominated) food webs. Furthermore, although salps represent a first level consumer, they are both competitors and consumers of the microbial food web constituents, thus reflecting a unique food chain. Using salps as a first consumer surrogate, despite obvious convenience, may result in increased propagation of error in the trophic positions of higher order consumers. Currently, we do not have a good understanding of the stable isotope values of microzooplankton, so it is difficult to accurately determine the true trophic position of pelagic tunicates. However, as the fractionation to microzooplankton may be minimal but still uncertain (Montagnes *et al.*, 2010; Gutiérrez-Rodríguez *et al.*, 2014; Landry and Decima, 2017), applying average trophic fractionation values may underestimate the number of trophic steps within food webs that are dominated by the microbial loop. Until this can be resolved, when using primary consumers as a baseline proxy in food web studies their feeding preferences need to be considered. Depending on the organism, they are likely to be indicative of different trophic pathways; pelagic tunicates represent pico- and nano- producers, whereas primary consuming copepods would generally represent nano- and micro- producers and the choice of which primary consumer to use as a baseline proxy should vary depending on the phytoplankton community. While they may be a convenient group to use as a baseline proxy, pelagic tunicates are not a perfect primary consumer surrogate for food web studies considering the uncertainties associated with their diet assimilation efficiency, trophic fractionation, relevance as a baseline proxy for the classical food web, and their limited contribution to higher trophic positions given their restricted predation. While they have their own set of limitations, we instead recommend the use of herbivorous copepods, size fractionated if required, as surrogates for primary consumers.

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