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Seasonal dynamics in a near-shore isotopic niche and
spatial subsidies from multi-trophic aquaculture

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25 **Abstract**

26 A poorly understood food web dynamic concerns possible seasonal variation in spatial
27 subsidies associated with multi-trophic aquaculture and their effects on extractive and
28 naturally occurring organisms. We used the stable isotopes, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and circular
29 statistics to investigate niche overlap across a year-long period at an experimental multi-
30 trophic aquaculture facility in British Columbia, Canada. A two-source mixing model
31 revealed that particulate organic matter was the most important food source for all sample
32 invertebrates (mean range 40-98%) compared to farm effluent (mean range 3-35%). There
33 were significant month-to-month changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for all species except for the
34 brooding transparent tunicate. We did not detect any directionality for the entire community,
35 but did identify variable directional shifts for each species, suggesting resource partitioning
36 driven by competition and/or morphology-based differences in feeding strategies. This was
37 further supported by seasonal variation in inter- and intraspecific isotopic niche widths.
38 Isotopic niche overlap among co-occurring invertebrates appeared to be stronger during
39 winter and summer than autumn months. Our study provides valuable insights on the role of
40 multi-trophic derived effluent on a near-shore marine community comprised of both natural
41 and cultured species within the same feeding guild.

42

Introduction

Some 40% of the world's population live within 100 kilometres of a maritime coast (Sale et al. 2014). Coastal regions also support a large marine aquaculture industry (FAO 2016). The combination of proximity to the coast and high population densities renders coastal ecosystems susceptible to manifold alteration and a wide range of negative environmental impacts including those associated with aquaculture (Halpern et al. 2008). The suite of negative environmental impacts related to aquaculture includes, but is not limited to, the release of large amounts of organic effluent, including fish feed waste, faeces, and other metabolites (Mazzola and Sarà 2001; Yokoyama 2002; Herbeck et al. 2013). The waste stream is known to cause eutrophication, environmental toxicity, and anoxia in the surrounding water column, thus adversely affecting pelagic and benthic communities (Holmer et al. 2005; Sarà 2007; MacDonald et al. 2011). Among the management options to mitigate the effects of waste output from aquaculture is multi-trophic aquaculture, an ecological approach to seafood production whereby extractive species (e.g., mussels, sea urchins, and macroalgae) are co-cultured alongside finfish as a means of recycling waste (Sarà et al. 2009; Cranford et al. 2013; Weldrick and Jelinski 2016). Effectively, the farmed extractive recipient community effectively receives a "spatial subsidy" (*sensu* Polis et al. 1997) in the form of energy and nutrients from the donor finfish. The subsidized extractive species, such as suspension-feeders, are of major commercial interest, and thus are a value-added economic benefit to the farm.

Notwithstanding the promise of IMTA in mitigating the effects of the waste stream in finfish aquaculture, there is little understanding of the annual variation in food web structures in and near IMTA farms in relation to resource partitioning of effluent as a food resource.

The concept of resource partitioning, or differences in how similar species use resources (Schoener 1974), is fundamental to understanding niche dynamics and competition. Examples of resource partitioning are wide ranging and include, for example, partitioning of time (Kronfeld-Schor and Dayan 2003), foraging sites (Field et al. 2005), prey size (Michaletz et al. 1987), and food (Grossman 1986). The term “niche width” refers to the variety of resources a population exploits and how they may be partitioned in regards to food (Roughgarden 1974). Accordingly, for trophic niches, niche width is often expressed as dietary diversity (Bearhop et al. 2004).

Stable isotopes have been widely used to characterize the trophic niche, particularly in regard to the relative position of individuals in $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ niche space (DeNiro and Epstein 1978; Newsome et al. 2007). Isotopic signatures are expressed as δ , the ratio of heavy to light isotopes, and reported in parts per thousand as per mil. Their use derives from the fact that stable isotopes in the proteins of consumers closely parallel those of the proteins in their diet (Hobson and Clark 1992; Newsome et al. 2007). Conventionally, nitrogen ratios of ^{15}N to ^{14}N exhibit stepwise enrichment (i.e., increase in the value of $\delta^{15}\text{N}$) at each trophic level (DeNiro and Epstein 1981). The $\delta^{15}\text{N}$ in the tissues of consumers tend to be between 2.5 and 5 times greater than those of their diets (e.g., DeNiro and Epstein 1981). The ratio of $\delta^{13}\text{C}$ also increases with trophic level, but to a much lesser degree than $\delta^{15}\text{N}$, in the order of 1. Thus, $\delta^{13}\text{C}$ is used mainly to reflect primary carbon sources (DeNiro and Epstein 1978).

Populations living in seasonal environments are exposed to regular or systematic changes in environmental conditions, such as variation in temperature, food availability, and vulnerability to predation (Fretwell 1972; Holt 1977). The isotopic values of an animal’s tissue reflect change over time when seasonality affects food resources for predators and prey (Goering et al. 1990). For example, seasonality may cause a switch to a new diet that is isotopically distinct from the

previous diet, or a change in the proportional contributions of prey items to consumers (Hobson and Clark 1992). Often these proportions are calculated using a stable isotopic mixing model (e.g., Parnell et al. 2013b; Phillips et al. 2014). Accordingly, a dual element (x, y), three-source, mass-balance, linear mixing model of three equations yields three unknown proportional source contributions (f_1, f_2, f_3) for a mixture (m) when ^{TM}X and ^{TM}Y values are known for mixtures and sources (the latter adjusted to account for isotopic discrimination).

A key unknown insofar as the ecological effects of multi-trophic aquaculture relates to how variability in space and time of a larger food web structure and trophic interactions in and adjacent to caged fish can improve our understanding of the functional response to seasonal variation in effluent inputs. The aim of this study was to assess the role of finfish effluent on the trophic niche of a range of consumer species, including wild and cultured species, and importantly over a near year-long period. We used a stable isotopes approach, a Bayesian isotope mixing model, and novel quantitative approach using circular (directional) statistics to compare differences in food web structure across eight periods of time spread over a one year time period. A particular advantage of circular statistics is that they quantify temporal changes of each food web component occurring in a $\delta^{13}C$ – $\delta^{15}N$ bi-plot (i.e., niche space).

Materials and Methods

Study Site

The study was conducted on a 5 km² IMTA farm located within Kyuquot Sound on Vancouver Island, British Columbia, Canada (50°3'10"N, 127°18'45"W). The farm site is characterized by circular tidal flow with a photic depth of approximately 30 metres. Low-current flow rate runs laterally through a series of seven sablefish (*Anoplopoma fimbria*) cages (15.2 x

15.2 m², and 18.3 m deep) that hold approximately 4,500 to 10,000 sablefish per cage. The ocean current flows through to a series of 250 shellfish droplines spaced 1 metre apart and deployed in a raft-type system (14 m across x 75 m long). Each dropline has 12 tiers, the top of which is approximately 5 metres deep, with each tier housing approximately 25 to 50 Pacific scallops (*Patinopectin yessoensis*). The current then passes through a number of parallel sugar kelp (*Saccharina latissima*) lines approximately 50 metres in length, spaced 1 metre apart. The seafloor is mainly fine-grained silt.

Sample Collection and Preparation

Samples were collected monthly between June 2009 and April 2010. Sablefish samples were obtained from the on-site Pacific SEA Lab. Pacific scallops (*P. yessoensis*), blue mussels (*Mytilus edulis*), sea urchins (*Strongylocentrotus franciscanus*), broadbase tunicates (*Cnemidocarpa finmarkeinsis*), brooding transparent tunicates (*Corella inflata*), hairy tunicates (*Boltenia villosa*), California sea cucumber (*Parastichopus californicus*), red algae (*Rhotophyte* sp.), and wild sugar-wrack kelp (*S. latissima*) were manually retrieved from within and adjacent to shellfish droplines. Farmed sugar-wrack kelp samples were collected along the kelp lines. Samples were dissected and processed on site. Prior to dissection, samples were rinsed with distilled water. Using a scalpel or surgical blade (Dubois et al. 2007), only tissues known to have high turnover rates and low variability were sampled, namely the liver of the sablefish (Perga and Gerdeaux 2005; Michener and Lajtha 2007), whole soft bodies (dissected from the shell and operculae of bivalves) of the blue mussels, tunicates, and anemones, gonads for sea urchins (*S. franciscanus*) (Berger and Jelinski 2008), as well as sections of the body wall in sea cucumbers (Iken et al. 2001). Sablefish faeces within dissected large intestines were removed by pipette.

Holdfasts were removed from sugar-wrack kelp samples, and the remaining whole fronds were used in the isotopic analysis.

Monthly triplicate samples of each species were analyzed. Tissues were soaked in 5% HCl in order to remove inorganic carbonates potentially highly enriched in ^{13}C (Simenstad and Wissmar 1985; Pinnegar and Polunin 1999). Dissected tissue samples, fish faeces, and samples of commercial fish feed (Taplow Feeds, Vancouver, BC) were oven-dried at 60°C for a minimum of 24 hours, then ground to a fine powder using a mortar and pestle (Sarà et al. 2004). One milligram samples of all tissues were weighed in tin containers (6 x 4 mm and 5 x 8 mm for oily samples) in preparation for sample continuous flow isotope ratio spectrometry (CF-IRMS) analysis.

Isotopic Analysis

Isotope analysis was carried out at the University of California–Davis Stable Isotope Facility. CF-IRMS was performed using a Europa Hydra 20/20 mass spectrometer. Generally, isotopic data are reported in the conventional δ notation;

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \cdot 1000(\text{‰})$$

where R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively. The resulting analytical reproducibility from the triplicate samples measured 0.3‰ for carbon and 0.4‰ for nitrogen, based on the international standards: Pee Dee Belemnite (PDB) for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$.

Data Analysis

We sought to determine the percent contribution of IMTA-derived waste effluent along with marine POM values within filter-feeding consumers using the multi-source system isotope mixing models Stable Isotope Mixing Models in R (simmr) developed by Parnell et al. (2013a). We used the isotopic signatures of marine POM measured by Markel (2011) sampled within the same period and approximately 5.3 nautical miles from our site in Kyuquot Sound, which had values for $\delta^{13}\text{C}$ of $-20.3 \pm 0.9\text{‰}$ and $\delta^{15}\text{N}$ of $6.9 \pm 0.9\text{‰}$. Use of this data was necessitated by an oversight on our part in failing to collect POM samples at the farm. The proportional contribution of putative food sources to invertebrate consumer diets was assessed using average isotopic ratios of each consumer and source, as well as trophic enrichment factors (TEFs), which is the difference in isotopic composition between consumers and diet source (Post 2002). The mixing model Stable Isotope Analysis in R (SIAR-R package ‘simmr’ version 0.2) uses a Bayesian approach to analyse all possible combinations of potential food source contributions, taking into account natural variation and uncertainty (Parnell et al. 2010; Phillips et al. 2014). We used TEFs of 2.2‰ for $\delta^{13}\text{C}$ and 3.6‰ for $\delta^{15}\text{N}$, based on experimentally determined values of Dubois et al. (2007) for similar suspension feeding species.

Isotopic signatures were tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variance using Levene’s test (Zar 1999). A one-way MANOVA was used to compare organic matter sources and sampling dates. The independent variables were “date” and “group,” and dependent variables were the carbon or nitrogen isotopic ratios. *F*-tests were generated from the estimated marginal means with Šidák’s correction owing to multiple comparisons. Most statistical analyses were performed using software SPSS version 17.0 for Windows (SPSS 17.0, Chicago, IL).

To evaluate the nature of month-to-month ‘shifting’ in isotopic niche width on a community scale, we employed a circular (i.e., directional) statistical approach (Batschelet 1981; Dekar et al. 2009; Schmidt et al. 2007; 2009). Circular statistics involves the analysis of angular data, which ranges from 0 to 360° or 0 to 2 π radians (Batschelet 1981; Zar 1999). They are based on the von Mises distribution (also known as the circular normal distribution), which is a continuous probability distribution of a circle. Directional change in niche width is calculated by measuring the difference between two values, or points, in trophic niche space for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Schmidt et al. 2007). The points represent both direction (angle of change, θ) and length (magnitude of change). The angles of change can also be averaged representing a mean vector of change, each with a direction (mean angle, μ) and a length (r). A parameter r equalling, or closely approximating θ represents a uniform distribution, whereas the closer r is given a value of 1, the more concentrated of the data angles. As with Gaussian distributions, descriptive statistics such as standard deviation and variance can be calculated for circular data (Zar 1999).

Isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were converted into degrees or angles within a circular distribution for data representing one year. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were corrected for circular analysis. Rayleigh’s test (Zar 1999) was employed in order to test the null hypothesis that the isotopic signatures among samples do not vary in time (i.e., have no mean direction). This method graphically arrays isotopic values along the circumference of a circle with arrows representing time (Batschelet 1981). Each arrow is a sample group: the direction of the arrow refers to the direction of change within trophic niche space, and the length of the arrow represents how far the sample group has moved within trophic niche space (Schmidt et al. 2007; 2009). Thus for example, in the event of community-wide directionality, all arrows would be pointing in a similar direction, thereby signifying influence of a particular ecological driver (e.g.,

autochthonous vs. allochthonous inputs). Arrow diagrams were created using the Oriana 3.0 software package (Rockware, Inc., Golden, Colorado, USA), which was also used to perform Rayleigh's Z and P tests (for uniformity of angular distributions) and Watson-Williams multi-sample F test (for differences between mean angles of direction for different monthly sampling transitions). Significance levels were set at $\alpha = 0.05$.

For each co-occurring invertebrate species, we determined isotopic niche width and compared isotopic niche overlap across each season using Bayesian and sample size-corrected standard ellipse areas (SEA_b and SEA_c , respectively) within SIBER (Stable Isotope Bayesian Ellipses in R; statistical package version 2.0.2, R Core Development Team, 2016). For more details on SEA_c calculations, see Jackson et al. (2011). Isotopic diversity across seasons and species was compared through the Bayesian posterior approach calculating 100,000 iterations to generate a range of average SEA_b metric values and 95% confidence interval.

Results

Food Web Reconstruction and Food Source Partitioning

The mean stable isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) compositions of all samples are in Table 1. They differed significantly as a function of organic matter (OM) source (Wilks' $\lambda = 0.035$, $F_{26,124} = 20.87$, $p < 0.05$) and OM type (Wilks' $\lambda = 0.269$, $F_{6,144} = 22.30$, $p < 0.05$) (Table 2). When comparing isotopic ratios of all organic source types in Table 1 (fish, invertebrates, primary producers, and aquaculture-derived effluent), mean $\delta^{15}\text{N}$ ratios of *A. fimbria* were not statistically distinct from the effluent ($p = 0.128$), while all invertebrates and primary producers were different ($p < 0.05$). No $\delta^{13}\text{C}$ ratios were significantly different among organic source types ($p > 0.05$).

Isotopic values for sablefish were only slightly more enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than its source fish feed ($p = 1.00$ and $p = 0.261$, respectively). Fish feed and sablefish excretion had similar values for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($p > 0.05$). *Rhodophyte* sp. and sugar-wrack kelp samples (wild and IMTA) had the most depleted levels of $\delta^{15}\text{N}$ (5.46‰, 3.72‰, and 4.93‰, respectively), and thus represent the lowest trophic level within the community.

Dietary Mixing Model

We found that IMTA waste effluent was not the main food source found within the diets of invertebrates grown adjacent to the project site (Fig. 2). Fish feed and faeces were combined as a food source as they were not sufficiently distinct in isospace (Fig. 1); combining them also helped with precision of model estimates following Phillips et al. (2005). POM featured most prominently in the diets of all invertebrates, including the intended extractive Pacific scallop (blue mussel: 43-98%; sea urchin: 17-98%; Pacific scallop: 9-95%; broadbase tunicate: 4-79%; brooding transparent tunicate: 29-97%; hairy tunicate: 37-95%; and sea cucumber: 4-82%). Bayesian isotope mixing model (simmr) results are presented in Appendix A, Table A1.

Temporal Variability of IMTA Food Web Structure

When we tested for month-to-month variability, we found significant differences in all isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios as a function of sampling date (Wilks' $\lambda = 0.042$, $F(16, 348) = 4.415$, $p < 0.05$) (Table 2). Brooding transparent tunicates and *Rhodophyte* sp. had consistent isotopic ratios throughout sampling dates ($p > 0.05$). With the exception of transparent tunicates, *C. inflata*, and *Rhodophyte* sp., all other species and aquaculture waste effluent exhibited enrichment and depletion spikes between June 2009 and April 2010 ($p > 0.05$). All monthly time steps showed significant directionality in isotopic signatures of organic matter ($p < 0.05$; Table 3), except for the January to February 2010 time step, the only time that showed no significant

directionality ($p = 0.06$; Table 3). All sampling date transitions, except for August to October 2009, were characterized by increasingly enriched $\delta^{13}\text{C}$ values (Table 3). For the sampling time step of August to October 2009, the mean vector was calculated to be 343.4° , which is indicative of a shift to more negative $\delta^{13}\text{C}$ values (Table 3; see also Appendix B, Fig. A1c). Finally, comparing the mean angles of direction on all eight sampling transition periods revealed that all eight calculated angles of change are from populations with different mean angles ($F_{7, 51} = 1.243, p = 0.297$).

The isotopic niche of brooding transparent tunicates was distinct from other co-occurring epibionts over seasons, in that they exhibit relatively wide vertical niche width (along y-axis) with the least overlap with other species (Fig. 3). Pacific scallop demonstrated among the narrowest of the isotopic niches calculated over all seasons. Niche overlap was greatest among summer and winter months (69.1% and 45.4%, respectively); negligible overlap was measured during autumn (1.8%) (Fig 3).

Discussion

Coastal ecosystems are among the most productive yet highly threatened in the world. Human demand from this region for farmed fish and shellfish is significant and growing as the world's population is increasing. Global annual per capita consumption of seafood averages 20 kilograms, and half of that supply currently comes from aquaculture (FAO 2016). Marine aquaculture production exceeds 26 million metric tons (t) per year, and global food fish production has outpaced human population growth between 1961 and 2013 at an annual rate of 3.2% (FAO 2016). A major challenge relates to sustainability of coastal ecosystems and aquatic resources. Integrated Multi-Trophic Aquaculture is an engineered ecological farming method that involves cultivating several species from one trophic level in

such a way that the waste stream (i.e., uneaten feed and/or by-products) is used by another species at one or more other trophic levels in the system. A broad range of studies have documented various aspects of multi-trophic aquaculture as it relates to food webs (e.g., Sarà et al. 2009; Cranford et al. 2013; Brager et al. 2015; Weldrick and Jelinski 2016). Jelinski (2015) explained how one can apply principles of landscape ecology as well-developed for terrestrial systems to near-shore systems, to better understand near-shore marine dynamics, including food web structures. These principles apply to this study particularly as it relates to the notion of spatial subsidies as articulated by Polis and colleagues (e.g., Polis et al 1997). A spatial subsidy, in the context of landscape ecology, is the transfer of a donor-mediated resource (nutrients, energy, detritus) that is passed from one habitat to a recipient (consumer) in a second habitat.

Among the outstanding questions related to the efficacy of multi-trophic aquaculture insofar as their ability to mitigate environmental effects relates to the waste stream and the implications for seasonal dynamics in food web structures in and near the facility. We used stable isotopes to reconstruct a marine food web within and adjacent to an IMTA farm, and applied a two-source Bayesian-based mixing model to examine food partitioning in the diets of wild and harvested marine organisms over a 9 month period. Using a circular statistics we did not detect any directionality for the entire community of study. We did, however, identify variable (seasonal) directional shifts for each species, suggesting that resource partitioning strategies may play a role, possibly driven by competition for the same range of resources and/or different feeding mechanisms characteristic of the species in the study. This was further supported by seasonal variation in inter- and intraspecific isotopic niche widths. Studies that employ circular statistics to measure community-wide food web

dynamics in marine systems are few but growing in use (Schmidt et al. 2007; 2009; Dekar et al. 2009; Bergamino et al. 2016). A particular advantage of circular statistics is that they quantify changes of each food web component occurring in a $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plot (i.e., niche space).

Reconstructing Food Webs and Measuring Dietary Proportions

Aside from brooding transparent tunicates, all the sampled invertebrate species that clustered together in niche space were retrieved adjacent to the shellfish nets (specifically, blue mussel, sea urchin, sea cucumber, broadbase tunicate, hairy tunicate, and Pacific scallop). Not surprisingly, sablefish were the most enriched species overall, exhibiting isotopic signatures similar to fish feed, indicating that fish feed comprises the primary diet of sablefish. Insofar as the next lower trophic level (i.e., the primary consumers), all but brooding transparent tunicates had isotopic signatures similar to sugar-wrack kelp. Results from the two-source mixing model confirmed this general pattern. Given its wide niche width, it is possible that this species of solitary ascidian is capable of consuming a high diversity of particles that include kelp, possibly resuspended from sediment to a size within their preferred diet range (Stuart and Klumpp 1984). The diet of primary consumers revealed a variable food source mixture, but primarily comprised of POM along with aquaculture-derived waste. This finding is in accord with similar studies that showed the importance of POM in anthropogenically altered coastal food webs (e.g., Gao et al. 2006; Bongiorni et al. 2015). For example, sea cucumber measured the highest proportion of aquaculture effluent (mean proportions 27% fish feed and 26% fish faeces) within their diets. Our results lend support to a growing literature demonstrating sea cucumbers as being

good candidates for IMTA harvestable extractives (Paltzat et al. 2008; Nelson et al. 2012; Hannah et al. 2013).

Seasonal Scale Isotopic Variation in Marine Food Webs Adjacent to IMTA

In contrast to other studies that examined temporal isotopic changes in near-shore food webs that found distinct patterns (e.g., Simenstad and Wissmar 1985; Goering et al. 1990; Kang et al. 1999; Vizzini and Mazzola 2002; 2003), there was no clear detection of isotopic enrichment in summer months, nor significant depletion in winter months. The lack of agreement may be a reflection of the fact that coastal ecosystems are subject to significant seasonal variability in environmental drivers including temperature (Johannessen and Macdonald 2009), freshwater and terrestrial inputs (Mulkins et al. 2002; Berger et al. 2010), upwelling (Harris 2001), carbon and nitrogen availability (Forbes and Denman 1991), and irradiance levels (Keller et al. 1997). For instance, during winter, nitrates are typically driven to surface waters by increased storm activity and hydrodynamic mixing (Rochford 1975; Wu et al. 1999). Primary producers are recipients of a more enriched form of nitrogen available at this time (Wada et al. 1975; Michener and Schell 1994). The contribution phytoplankton make to the diets of higher trophic consumers typically decreases due to their low abundance. By summer, nitrate largely becomes less available and primary producers shift to recycled ammonia, which has more depleted $\delta^{15}\text{N}$ (Michener and Schell 1994; Vizzini and Mazzola 2003). For consumers such as suspension-feeders, low signatures during this time generally reflect the integration of the phytoplankton spring bloom.

While there was no community-wide directionality throughout the eight time steps, our study highlights the utility of circular metrics as an effective, vector-based approach to assessing seasonal variability in food web responses to a range of environmental change (Layman et al.

2012). For instance, a red tide event was observed at the farm under study in late August 2009. Following this event we found significant depletion of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in sugar-wrack kelp and $\delta^{13}\text{C}$ enrichment in *Rhodophyte* sp. These observations were corroborated by longer arrow lengths found along the arrow diagrams, indicating significant change in isotopic niche space from one time step to the next (see Appendix B, Fig. A1). By comparison, Minagawa and Wada (1984) found lower than predicted $\delta^{15}\text{N}$ values for zooplankton and fish sampled during a red tide event. It remains unclear, for example, whether depletion in $\delta^{15}\text{N}$ values of primary producers in our study is related to the red tide event considering no similar isotopic depletion occurred with our primary consumers. However, the $\delta^{15}\text{N}$ depletion exhibited by the primary producers is more likely with a result of a shift in food source such as nitrate to recycled ammonia (Minagawa and Wada 1984; Vizzini and Mazzola 2003), possibly caused by the same environmental factors (e.g., increased irradiance levels) that caused the red tide event.

IMTA-generated fish faeces and uneaten fish feed were not found to be disproportionately high within the diets of sampled species, including the harvested Pacific scallop. It is possible that particles tend to undergo a downward, vertical flux. At the same farm as our study, Brager et al. (2015) used high-resolution 3-D spatial surveys to measure the effect on the surrounding particle field of effluent derived from sablefish aquaculture. They found low-level release of waste feed near the surface of the water column within and between the sablefish pens, and negligible amounts detected between 5 and 500 m outside of the farm boundaries.

The differences in isotopic signatures and niche widths for each filter-feeding species at each time step and by season may also be a reflection of morphological differences in feeding mechanisms relative to resource partitioning or trophic plasticity (Riera 2009). For example, on the basis of stable isotope variability, Cresson et al. (2016) found that nine species of co-

occurring suspension feeders in the French Mediterranean exhibited different patterns of resource partitioning. They attributed these differences to a variation in food diversity and availability between summer and winter, and post-capture sorting strategies between food items therein. The same explanation may hold true in our study. All three tunicate species demonstrated generalist-feeding traits as revealed by their large isotopic niche widths on both spatial and temporal scales, as compared to the relatively narrow niche widths of the intended farmed Pacific scallop. By comparison, Rodriguez and Ibarra-Obando (2008) found instances where tunicate species (both native and introduced) outcompeted intended harvestable species apparently due to their general ability to retain a wide range of particle sizes of food.. Ribes et al. (1998) found that the tunicate, *Halocynthia papillosa*, fed on a diet ranging in size from 0.6 μm (heterotrophic bacteria) to 70 μm (pennate diatoms). By comparison, Lesser et al. (1992) found that *M. edulis* selected phytoplankton in the 3-5 μm size, whereas solitary ascidians selected for larger phytoplankton size classes (>16 μm). Bivalves commonly demonstrate large intraspecific variability in labial palp-to-gill ratio as a morphological adaptation to variation in the turbidity of the water column (Dutertre et al. 2009). Thus, morphologically-based feeding strategies as it relates to interspecific competition and foraging strategies may account for variability in isotopic niche widths, coupled with the effects of interspecific competition.

To conclude, we hypothesized that a marine food web situated within and adjacent to an integrated multi-trophic aquaculture farm would be subject to seasonal directionality as regards isotopic variability. While we did not detect community-wide directionality, we did find that IMTA provides food subsidies to naturally occurring epibionts and primary producers. Whether using neighboring natural communities to aid in dealing with effluents from nearby aquaculture is a good management strategy or acceptable to society should be open to debate. Our findings

also lend support to recommendations for rearing California sea cucumber (Hannah et al. 2013) and green sea urchin (Orr et al. 2014) alongside finfish in a multi-trophic setting. Last, our analysis represents, to our knowledge, the first use of circular statistics to explore and characterize seasonal dynamics of food webs in relation to multi-trophic aquaculture, as well as other studies on spatial subsidies in near-shore ecosystems.

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Tables

Table 1. Stable isotope ratios (‰) for all organic matter sources sampled between June and August 2009. Data include sample size (n), mean δ values, and standard error of the means.

Organic matter source	n	$\delta^{13}\text{C}$	$\pm\text{SE}$	$\delta^{15}\text{N}$	$\pm\text{SE}$
<u>Fish</u>					
<i>Anoplopoma fimbria</i>	6	-21.55	0.50	12.82	0.32
<u>Aquaculture-derived effluent</u>					
Fish faeces	6	-21.84	0.29	11.16	0.46
Fish feed	3	-21.62	0.27	10.48	0.26
<u>Invertebrates</u>					
<i>Patinoplectin yessoensis</i>	9	-21.91	0.32	8.27	0.07
<i>Mytilus edulis</i>	8	-18.53	0.31	8.68	0.31
<i>Strongylocentrotus franciscanus</i>	5	-19.31	0.65	9.86	0.30
<i>Cnemidocarpa finmarkeinsis</i>	4	-18.76	0.42	9.04	0.13
<i>Corella inflata</i>	7	-18.10	0.53	5.11	0.53
<i>Boltenia villosa</i>	8	-18.67	0.27	8.61	0.14
<i>Parastichopus californicus</i>	2	-17.99	0.25	8.61	0.50
<u>Primary producers</u>					
<i>Saccharina latissima</i> (wild)	3	-19.27	0.12	3.72	0.71
<i>Saccharina latissima</i> (IMTA)	8	-17.42	0.83	4.93	0.72
<i>Rhodophyte</i> sp.	7	-26.91	1.64	5.46	0.51
POM (Markell 2011)	44	-20.27	0.91	6.93	0.93

Table 2. One-way MANOVA results of organic matter sources and consumer isotopes by sampling date. Fish feed and POM were not included due to insufficient data across sampling dates. Significance set at $\alpha = 0.05$.

Organic matter source x sample date	Wilks' λ	(df1, df2) F	p
<i>Anoplopoma fimbria</i>	0.071	(10, 24) 6.624	0.000
Fish faeces	0.152	(12, 28) 3.653	0.002
<i>Strongylocentrotus franciscanus</i>	0.219	(14, 38) 3.092	0.003
<i>Patinoplectin yessoensis</i>	0.009	(16, 46) 27.074	0.000
<i>Mytilus edulis</i>	0.059	(16, 42) 8.213	0.000
<i>Cnemidocarpa finmarkeinsis</i>	0.128	(14, 40) 5.130	0.000
<i>Corella inflata</i>	0.363	(16, 42) 1.733	0.078
<i>Boltenia villosa</i>	0.099	(16, 44) 6.008	0.000
<i>Saccharina latissima</i>	0.221	(8, 20) 2.817	0.029
<i>Rhodophyte</i> sp.	0.142	(4, 6) 2.485	0.153
All organic matter	0.042	(16, 348) 4.415	0.000

Table 3. Community-level circular statistical values for variables describing sample groups within the aquaculture farm over time. Rayleigh's test values are significant at $\alpha = 0.05$, meaning the distribution of angular variance departs from uniformity.

Sampling Date	No. obs	Mean vector		Circular SD	Rayleigh's test	
		μ	r		Z	P
June-July 2009	6	22.9	0.89	28.0	4.73	0.004
July-August 2009	10	9.3	0.83	35.5	6.81	0.000
August-October 2009	8	343.4	0.73	45.0	4.31	0.009
October-November 2009	8	2.7	0.79	39.5	4.97	0.003
November-January 2010	7	44.8	0.84	33.4	4.99	0.003
January-February 2010	6	25.3	0.67	50.9	2.72	0.060
February-March 2010	6	21.3	0.82	35.8	4.06	0.010
March-April 2010	8	12.9	0.63	20.8	3.21	0.035

Figures

Fig. 1 Isotope structure of the food web within and adjacent the aquaculture farm in June to August 2009. Each data point depicts means and standard error (error bars) of each organic matter source. Each shape represents trophically different organic matter.

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Fig. 2 Box-and-whisker plots of fractional dietary contribution of each potential food source farm effluent and POM biomass to each consumer species estimated from simmr model outputs using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The box boundaries represent the 25th and 75th percentile, the horizontal line is the median, and the whiskers mark the 2.5th and 97.5th percentiles.

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Fig. 3 (A-D) Seasonal sample size-corrected standard ellipse areas (SEA_c) and convex hulls of isotopic data from each invertebrate species; (E-F) Bayesian estimates of standard ellipse areas (SEA_b) and modes (black dots) and size-corrected standard ellipse area modes (magenta squares) overlaying grey-shaded density plots representing 50, 75, and 95% credible intervals.

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Appendices

Appendix A: Bayesian Mixing Model Output (simmr)

Table A1 Bayesian isotope mixing model (simmr) results (mode of the percentage contribution followed by 95% credibility intervals) for the contribution of the different potential food sources for the seven different consumers. SD $\delta^{13}\text{C}$ and SD $\delta^{15}\text{N}$ refer to the residual error, or the variability in $\delta^{13}\text{C}/\delta^{15}\text{N}$ (in %) that cannot be explained by diet alone.

	IMTA-waste effluent	POM	SD $\delta^{13}\text{C}$	SD $\delta^{15}\text{N}$
Blue mussel	3 (0-41)	80 (43-98)	0.5(0.0-1.4)	3.0(1.5-6.7)
Sea urchin	3 (0-54)	44 (17-98)	1.3(0.5-3.9)	2.7(0.1-8.0)
Pacific scallop	4 (0-57)	42 (9-95)	3.1(1.9-6.1)	4.3(2.0-8.8)
Broadbase tunicate	35 (0-60)	40 (4-79)	0.8(0.0-4.1)	3.8(0.9-20)
Brooding transparent tunicate	4 (0-48)	66 (29-97)	1.3(0.3-2.7)	6.7(3.8-13.8)
Hairy tunicate	4 (0-45)	63 (37-95)	0.4(0.0-1.2)	3.3(1.6-7.1)
Sea cucumber	33 (0-58)	43 (4-82)	0.9(0.0-16.5)	4.8(0.0-58)

12 **Appendix B:** Arrow diagrams from circular statistical analysis. Directional food web changes
13 are depicted in each diagram within $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ space between each month (Fig. A1a-h). An
14 arrow represents a sample group; the direction of the arrow represents the direction of change
15 within trophic niche space; and the length of the arrow represents how far the sample group has
16 moved within trophic niche space (Schmidt et al. 2009). Arrow diagrams were created using the
17 Oriana 3 software package.

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Fig. A1(a-h) Arrow diagrams for angle (θ) and magnitude (arrow length) of change for each IMTA sample group between time periods. For data label abbreviations, see Table 1. Each arrow represents a different sample group, arrow direction represents isotopic change with which the sample group moved from one month to another, and arrow length represents magnitude of change (‰) in trophic niche space. Concentric lines depict magnitude of change increasing outwards corresponding to increasing trophic position from one sample session to the next. The bold line dissecting the circles represents mean vector of change (μ) and the bold outer arc segment represents the 95% confidence interval around the mean vector of change

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