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Resource subsidies from multi-trophic aquaculture affect isotopic niche width in wild blue mussels (*Mytilus edulis*)☆

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ABSTRACT

Blue mussels (*Mytilus edulis*) are boreo-temperate, filter-feeding bivalves common to intertidal areas. As filter-feeders they have been employed in open-water, multi-tropic aquaculture systems to reduce organic benthic loading though the exploitation of suspended particulate organic materials. We compared $\delta^{13}C$ and $\delta^{15}N$ signatures and the isotopic niches of mussels growing in, and adjacent to, an integrated multi-trophic aquaculture (IMTA) farm in British Columbia, Canada, and using this information evaluated the contribution of aquaculture-derived effluent to their diet. Farm-sampled mussels had the least intraspecific isotopic variation compared to mussels sampled at the reference site. The interaction between time (i.e. sampling dates) and site did not significantly affect the isotopic composition of mussels; however significant variation was detected in $\delta^{15}N$ values as a function of sampling date and particulate organic matter. A two-source isotopic mixing model indicated that marine particulate organic matter and IMTA farm effluent were approximately equal in importance (~46 % and ~54 %, respectively) to the diet of IMTA-retrieved mussels. Uptake of IMTA farm waste by *M. edulis* supports their use as economic extractives while also mitigating farmed sablefish (*Anoplopoma fimbria*) nutrient loading to the aquatic environment.

1. Introduction

Caged fish-farming releases large amounts of solid organic effluent including fish feed waste, faeces, and other metabolites (Mazzola and Sarà, 2001; Yokoyama et al. 2002; Herbeck et al. 2013). This waste stream can cause eutrophication, environmental toxicity and anoxia in the surrounding water column, thus adversely affecting the pelagic and benthic environment though organic enrichment (Holmer et al., 2005; Sarà, 2007; MacDonald et al., 2011). Integrated multi-trophic aquaculture (IMTA) is an ecological approach to seafood production whereby extractive species (e.g., mussels, sea cucumbers, sea urchins and macroalgae) are co-cultured alongside finfish as a means of recycling their waste output (Sarà et al., 2009, Reid et al., 2010; Cranford et al. 2013; Reid et al., 2013). In IMTA-type facilities the effluents move from a donor system (e.g., penned finfish) through to an adjacent farmed extractive recipient community (e.g., filter feeders), thus acting as a "spatial subsidy" (*sensu* Polis et al., 1997). Such subsidies may also affect the abundance and diversity of nearby infaunal communities, directly and indirectly, through successional responses to organic and inorganic enrichment (Karakasis et al., 1999; Naylor et al., 2000; Chopin et al., 2012). While IMTA has promise for ecologically efficient, low environmental impact, profitable seafood production that is societally acceptable, major questions remain. Among these are the nature of trophic linkages and nutrient transfers of suspended organic materials between cultivated fed species and extractive species, and the effects of the waste stream on nearby ecosystems.

Mussels within the *Mytilus edulis* complex are boreo-temperate, filter-feeding bivalves common in intertidal rocky ecosystems. As filter-feeders they have been employed in some open-water, multi-tropic aquaculture systems to reduce organic benthic loading though the exploitation of suspended particulate organic materials. The overall objective of this study was examine the proportion of sablefish (*Anoplopoma fimbria*) culture solids (i.e. faeces and waste feed) in the diet of *M. edulis*, a harvestable biofilter, at an IMTA farm on Vancouver Island, Canada. We used a stable isotope in a "who eats whom" framework (Peterson and Fry, 1987) to directly quantify trophic niche width in regards to the relative position of individuals in $\delta^{13}C$–$\delta^{15}N$ niche space (DeNiro and Epstein, 1978; Frederiksen, 2003). Metabolic fractionation of $\delta^{13}C$ signatures within organic tissues of consumers averages 1-1.5‰ for marine invertebrates (DeNiro and Epstein, 1978; Peterson and Fry, 1987; France and Peters, 1997). Isotopic signatures of $\delta^{15}N$ within tissues tend to fractionate at rates approximately 3‰ relative to food sources, and hence are useful for the identification of trophic position within a food web (DeNiro and Epstein, 1978; Frederiksen, 2003). Specifically, we hypothesized that IMTA-derived effluent of fish feed, faeces and associated metabolites from farmed sablefish subsidize the diets of *M. edulis* naturally recruited on aquaculture structures, and that this will be reflected in their isotopic

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signatures and, more specifically, trophic niche width. We focused on months where consumer signatures are less affected by non-farm particulate organic matter sources, which are generally high in summer months principally as a function of enhanced phytoplankton (Dauby et al. 1990).

2. Materials and methods

2.1. Study site

The study took place in a 5 km^2 IMTA farm situated in Kyuquot Sound (N 50°2′43.836″ W 127°17′57.2172″) on northwest Vancouver Island, British Columbia, Canada (Fig. 1). The farm is characterized by a slow circular tidal flow with a photic depth of about 28 m and a 3 m average semi-diurnal tidal range. The current flow runs laterally through a series of seven cages (15.2 × 15.2 m, and 18.3 m deep) holding 4500 to 10,000 sablefish per cage. The current continued through a series of 250 shellfish droplines spaced one metre apart from a raft 14 m × 75 m. Each dropline has 12 tiers, the top tier is about 5 m deep, with each tier colonized by 25–50 Pacific scallops (*Patinopectin yessoensis*). The current then passes through a number of sugar kelp (*Saccharina latissima*) lines. The sea floor is mainly fine-grained silt. Sampling for wild blue mussels was also undertaken at a reference site, 500 m northeast and upstream of the IMTA farm, where five similarly sized shellfish lantern nets were set up 2 months in advance of sampling to facilitate recruitment. We selected this distance to avoid potential waste plumes from the IMTA farm based on the findings of Ye et al. (1991), Wu et al. (1994), and Lander et al. (2013).

2.2. Sample collection

We conducted the study during the winter and spring months of October 2009 to March 2010 when the diets of suspension feeders are less affected by algal blooms (Dauby et al., 1990). Samples within sea cages and shellfish pens were collected monthly from October 2009 to March 2010. Wild blue mussels were retrieved from shellfish nets that were temporarily lifted out of the water. Soft bodies of mussels were dissected by scalpel and surgical blade. Sablefish were obtained from the onsite Pacific SEA Lab and faecal contents within dissected large intestines were removed by pipette.

We sampled mussel tissues and sablefish faeces on a monthly basis. Tissues were placed in 5% HCl and rinsed in distilled water to remove inorganic carbonates prior to drying (Bosley and Wainright, 1999; Kaehler and Pakhomov, 2001). Mussel tissues, sablefish faeces, and commercial fish feed (Taplow Aquafeed©) were oven-dried at 60 °C to constant weight, then ground by pestle on mortar (Sarà et al., 2004). Dry samples (1 mg) were placed in tin combustion containers (6 × 4 mm) in preparation for continuous flow isotope ratio mass spectrometry (CF-IRMS).

2.3. $\delta^{13}C$ and $\delta^{15}N$ analysis

The CF-IRMS analyses were performed at the University of California Davis Stable Isotope Facility on a Europa Hydra 20/20 mass spectrometer. Isotopic results are expressed in the δ notation as $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is either carbon (C) or nitrogen (N) and R is the ratio of heavy to light stable isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Triplicate samples resulted in an analytical reproducibility of 0.3‰ for $\delta^{13}C$ and 0.4‰ for $\delta^{15}N$ based on the standards: Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

2.4. Dietary and isotopic niche width analyses

Isotopic mixing models are often used to evaluate the proportion of a particular food source within the diet of consumers (e.g., Bustamante and Branch, 1996; Kaehler et al., 2000; Nadon and Himmelman, 2006). Using this approach, we evaluated effluent from the IMTA farm and marine particulate organic matter (POM) as potential food sources

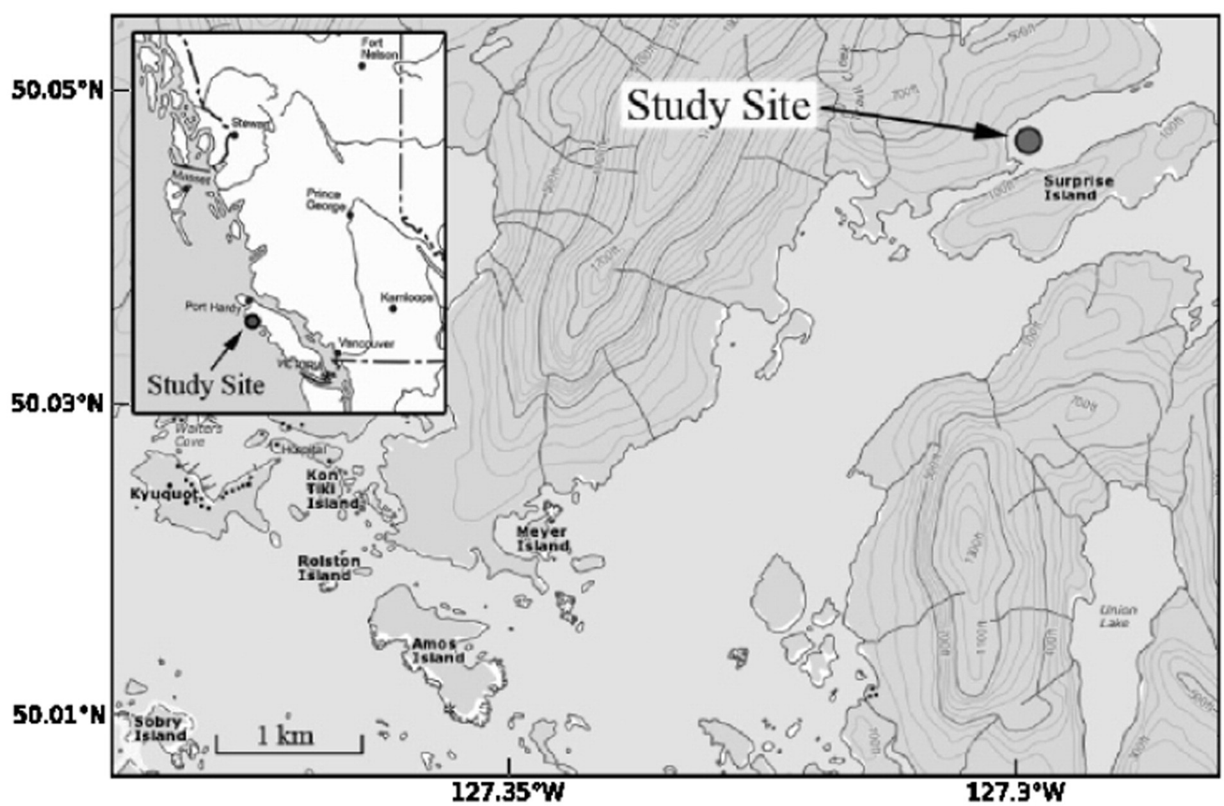

Fig. 1. Kyuquot Sound, British Columbia, Canada—location of study site.