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Canthaxanthin as a potential tracer of salmon feed in mussels (*Mytilus* spp.) and sea urchins (*Strongylocentrotus droebachiensis*)

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ABSTRACT

The large amount of fish feed involved in salmon aquaculture results in high nutrient loading to the surrounding environment. The near-field effects of this loading are well documented but the full zone of influence, or far-field effect, remains poorly understood. We investigated the potential of the carotenoid canthaxanthin, a common additive in farmed Atlantic salmon diets, as a biochemical tracer to identify this zone of influence in two benthic invertebrate species that are locally abundant around salmon farms: the blue mussel *Mytilus edulis* (or *Mytilus trossulus*), a suspension feeder, and the green sea urchin *Strongylocentrotus droebachiensis*, an omnivorous grazer. To measure persistence of canthaxanthin in the digestive gland of mussels or the gonad of sea urchins, both species were individually fed three levels of salmon feed (6, 13, 26 mg d^{-1} for mussels; 70, 150, 300 mg d^{-1} for sea urchins) for 13 d to measure pigment uptake rates, followed by a non-pigmented diet for 7 d to measure pigment loss rates. Mussels exhibited a relatively rapid uptake of canthaxanthin (1 to 3 d), which subsequently declined to zero within 3 d following cessation of the pigmented diets. The sea urchins exhibited slower initial uptake (4 to 10 d) and the pigment signal lasted up to 46 d, suggesting retention of canthaxanthin. To examine the scale of dispersion of feed-derived particulate material in nature, canthaxanthin uptake by sea urchins was measured at fixed intervals along a transect extending 1 km from a salmon cage in Passamaquoddy Bay, New Brunswick, Canada. Pigment concentration in the gonad dropped from ~5 to 0.5 μg g^{-1} within the first 100 m from the cage and remained at this low level along the remainder of the transect, likely reflecting low background levels of salmon farm-derived particles within the bay. Our study demonstrates the potential of canthaxanthin as an organic tracer for salmon aquaculture, and the use of blue mussel digestive gland for short-term detection and sea urchin gonad for long-term detection, in accordance with the rapid ingestion and nutrient storage functions of the respective tissues.

1. Introduction

The large amount of fish feed used in salmon aquaculture, combined with the normal physiological losses associated with fish feeding, result in increased nutrient loading to the surrounding environment. Near-field effects of this loading from caged aquaculture sites have been well documented and include changes in sediment geochemistry, increased soluble nutrients in the surrounding water column, and altered benthic community structure (Beveridge, 1984; Brown et al., 1987; Buschmann et al., 1996; Frid and Mercer, 1989; Giles, 2008; Handy and Poxton, 1993; Hargrave, 2010; Wu et al., 1994). The succession of benthic species richness, abundance and biomass radiating from an organic enrichment site has been modeled (Pearson and Rosenberg, 1978), but the full zone of influence of salmon farms, or range of far-field effects, is not well known. The variable nature of aquaculture sites (including variation in currents, water temperature, substratum type, depth, and local species composition) complicates the monitoring and assessment of far-field effects. Consequently, the development of a far-field aquaculture-specific tracer would provide an effective means of quantifying aquaculture feed exposure in wild marine populations surrounding aquaculture sites.

Previous studies have investigated benthic (redox potential, sediment oxygen consumption, dissolved nutrient fluxes) and water-column (seston, water quality indices) indicators to identify the impact zone of finfish aquaculture (Brown et al., 1987; Christensen et al., 2000; Giles, 2008; Karakassis et al., 2000; Lander, 2006; Neofitou et al., 2010; Wu et al., 1994). Most studies have concluded that the range of influence of caged salmon sites does not extend beyond 300 m (Brown et al., 1987; Christensen et al., 2000; Giles, 2008; Karakassis et al., 2000; Lander, 2006; Neofitou et al., 2010). In contrast, Wu et al, (1994) suggested that environmental impacts can extend 1 to 1.5 km in sub-tropical aquaculture sites.

Although it is widely understood that fish farm effluents can negatively impact the local environment, they have also been shown to

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increase growth rates and biomass of certain species. For example, mussels (*Mytilus* spp.), seaweeds (*Porphyra* spp.) and sea urchins (*Paracentrotus* sp.) have been shown to benefit from salmon feed or salmon feces in their diets (Chopin et al., 1999; Cook and Kelly, 2007; Lander, 2006; MacDonald et al., 2011), indicating the potential for biological filtration of salmon farm effluents through polyculture (Barrington et al., 2009; Chopin et al., 2001; Lander, 2006; Neori et al., 2004; Reid et al., 2010; Troell et al., 2009; Vandermeulen and Gordin, 1990).

One advantage of the uptake of aquaculture-derived material by other organisms is that it presents an opportunity to apply an aquaculture-specific dietary tracer to determine the range of influence of aquaculture effects. In this study we explore the use of the carotenoid pigment canthaxanthin, a commonly used additive in farmed Atlantic salmon diets, as a tracer. Canthaxanthin used in salmon feed is produced synthetically and antioxidants (ethoxyquin) are added as stabilizers. To detect the pigment in the environment, we used two benthic invertebrates, known to ingest salmon feed, as biological indicators: the blue mussel *Mytilus edulis* and possibly *Mytilus trossulus* – both species and their hybrids co-occur in the northeastern Gulf of Maine and are difficult to distinguish morphologically (Mallet and Carver, 1995; Rawson et al., 2001) – and the green sea urchin *Strongylocentrotus droebachiensis*. Canthaxanthin is not a naturally occurring pigment in blue mussels or green sea urchins (Campbell, 1970; Griffiths and Perrot, 1976; Tsushima, 2007). The objectives of our study were to (1) measure the uptake and excretion of canthaxanthin in the digestive gland of mussels and the gonad of sea urchins fed different levels of salmon feed in the laboratory, and (2) measure the uptake of canthaxanthin in the gonad of sea urchins at increasing distances from a salmon cage and at two heights above bottom.

2. Methods

2.1. Laboratory feeding experiments

2.1.1. Mussels

Blue mussels, *M. edulis* or *M. trossulus*, 4–5 cm in shell length were collected from an intertidal mudflat in Passamaquoddy Bay, near St. Andrews, New Brunswick. Mussels were fed 3 levels of a standardized diet of salmon feed at daily intervals: 6, 13 or 26 mg mussel^{-1} d^{-1} (C1, C2, C3), corresponding to canthaxanthin levels of 0.22, 0.43 and 0.87 μg mussel^{-1} d^{-1}, respectively. Diet treatments were applied to closed system tanks containing filtered seawater and a submerged titanium coil to maintain water temperature at ~10 °C. An airstone was used to keep the water oxygenated and the feed particles in suspension. Groups of 90 mussels were placed in each of 2 replicate tanks per diet treatment. The treatment for each tank was prepared daily by weighing the required quantity of salmon feed (between 0.2 and 2 g) and grinding it into fine particles (using a mortar and pestle) that were sprinkled into the tanks. The weight of feed used in each diet treatment at each feeding time was standardized to the number of individuals in a tank. Canthaxanthin levels in the salmon feed were measured by liquid–liquid extraction and high performance liquid chromatography (HPLC) (see Section 2.3).

The experiment was split into two stages. The first stage monitored uptake rates of canthaxanthin in the digestive gland of mussels for 13 d. The digestive gland was selected to develop a short-term pigment detection method. Mussels exhibit high filtration and ingestion rates and, consequently, turnover rates of material in the digestive gland are also expected to be high (Bayne et al., 1989; Schulte, 1975; Winter 1973). The second stage monitored excretion rates, as loss of pigment from the digestive gland, for 7 d following the cessation of a salmon feed diet. During the uptake stage, 9 mussels were randomly sampled at days 1, 4, 7, 10 and 13. The excised digestive glands of 3 individuals were pooled for analysis (to obtain enough tissue for the extraction process) and maintained in labeled plastic scintillation vials at −80 °C until extraction. After the uptake stage, the tanks were cleaned and the titanium coils and airstones were removed to convert to a flow-through system of filtered seawater for the excretion stage. Sampling continued every 3 d as per the uptake stage.

Mussels were maintained without food in a flow-through control tank throughout the experiment to ensure that the pigment absorbed by the mussels was a result of the salmon feed diets they were fed. Six replicates were removed for analysis at day 1 and again at day 20 (Fig. 1).

2.1.2. Sea urchins

Green sea urchins, *S. droebachiensis*, 3–5 cm in test diameter were collected using SCUBA from Tongue Shoal (Fig. 2), Passamaquoddy Bay. The design of this experiment was similar to that of the mussel experiment, with 2 replicate tanks for each of three diet treatments, as well as a control tank (Fig. 1). Each tank contained 30 urchins individually contained within an upright plastic cylinder (8 cm diameter) with a wire mesh bottom, an open top, and perforated (1-cm diameter holes) around the circumference to permit water flow. The plastic cylinders rested on metal grids elevated 5 cm off bottom to permit water flow under the array. Sea urchins were fed 3 levels of salmon feed at daily intervals: 70, 150, 300 mg urchin^{-1} d^{-1} (C1, C2, C3), corresponding to canthaxanthin levels of 2.5, 5 and 10 μg^{-1}urchin^{-1} d^{-1}, respectively. The diet treatments were prepared by weighing out the appropriate amounts of salmon feed and dropping the feed pellets directly into each cylinder containing a sea urchin.

The sea urchin experiment was also split into uptake and excretion stages. During the uptake stage, sea urchins were haphazardly sampled every 3 d for 13 d. Gonads were sampled to assess long-term pigment detection because of their nutrient storage function (Andrew, 1986; Lawrence and Lane, 1982; Walker, 1982). Excised tissues were maintained at −80 °C until extraction. Upon completion of the uptake stage the tanks were cleaned and the sea urchins were returned to their respective cylinders in a flow-through system. During the excretion stage, designed to monitor pigment loss, the sea urchins were fed ~2 g of fresh kelp daily and sampled every 3 d for the remaining 8 d. Five remaining urchins were removed from the cylinders and maintained in their tanks and sampled at day 46, almost 4 weeks after the main experiment.

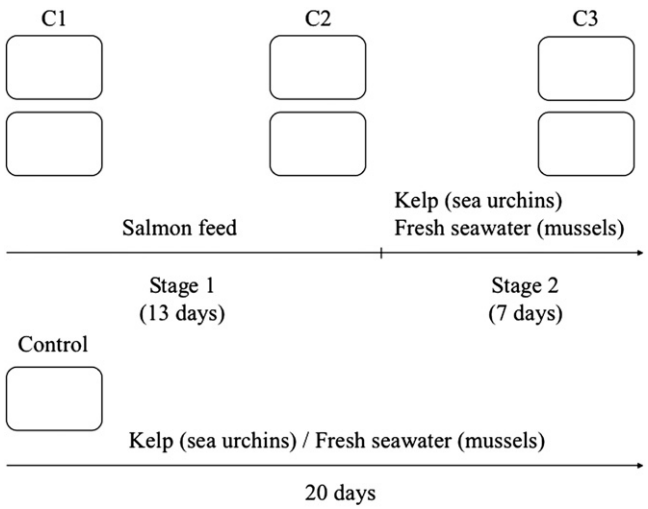

Fig. 1. Design of laboratory feeding experiments for mussels (*Mytilus edulis*, *Mytilus trossulus*) and sea urchins (*Strongylocentrotus droebachiensis*).