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Field assessment of Pacific oyster (*Crassostrea gigas*) growth and ingestion of planktonic salmon louse (*Lepeophtheirus salmonis*) larvae at an Atlantic salmon (*Salmo salar*) farm in British Columbia, Canada

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ABSTRACT

Integrated culture of bivalves at marine finfish farms may help lessen nutrient loading while producing a commercially-valuable crop and increasing the farms' social license to operate. Integrated culture of filter-feeding bivalves at salmon farms may also provide natural mitigation of the occurrence of planktonic sea lice larvae. This study assessed the growth of Pacific oysters (*Crassostrea gigas*) cultured at a commercial Atlantic salmon (*Salmo salar*) farm in British Columbia (BC) and the extent to which the cultured oysters contributed to mitigation of the occurrence of the salmon louse (*Lepeophtheirus salmonis*). Oysters were deployed in trays at 1-, 3-, and 6-m depths around one end of the farm and at a reference site approximately 150 m away. The height, length, and width of the shell and weight [whole wet, soft-tissue wet, dry, and ash-free dry (organic) weights] of oysters were measured at intervals following deployment. Salmon louse reduction was assessed monthly by comparing the water-borne density of larval sea lice among three bivalve cages and three controls (non-bivalve cages), and by examining oyster digestive tracts for *L. salmonis* DNA using PCR. All seven oyster-size variables increased significantly over time with significant effects of depth and position around the farm. In general, oysters at 1 and 3 m were significantly larger than those at 6 m. Side of the fish cage was used as a blocking factor in the experimental design and had a significant effect on final oyster size; at the end of the study, oysters at the farm were either significantly larger or not significantly different than oysters at the reference site, depending on the side of deployment. There was no significant variation in mean larval density due to time or treatment (bivalve versus non-bivalve). Larval lice densities were highest in January 2014. However, at that time there was no evidence of *L. salmonis* DNA in oyster digestive tissues.

1. Introduction

1.1. Integrated multi-trophic aquaculture

Integrated multi-trophic aquaculture (IMTA) can be defined as the farming of aquaculture species from different trophic levels, whereby portions of the waste nutrients of one species are captured and converted into energy by others, mimicking synergistic relationships found in natural ecosystems (Chopin et al., 2012). Most solid organic waste nutrients from salmon farms accumulate on the ocean floor at the site (Brooks, 2001; Sutherland et al., 2001), though smaller organic particulates held in suspension may also be observed directly beside fish pens (Brager et al., 2015; Sutherland et al., 2001). Organic extractive species cultured beneath or in close proximity to fish cages may help capture a portion of those waste nutrients, diverting them towards the growth of commercially-valuable products.

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1.2. Finfish–bivalve integrated multi-trophic aquaculture

Several studies have demonstrated positive impacts of finfish farms on the growth of filter-feeding bivalves such as mussels (MacDonald et al., 2011; Stirling and Okumuş, 1995) and oysters (Jiang et al., 2012; Jones and Iwama, 1991) cultured nearby. However, other investigations have reported no significant increase in bivalve growth near finfish cages compared to their growth in control locations (Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2014). Conflicting results are not entirely surprising since the suspended organic particulate "plume" around finfish cages will be site dependent and may occur only intermittently (Brager et al., 2015) if at all (Taylor et al., 1992). Those studies also varied with respect to fish species and the number and distance of reference sites. For example, Taylor et al. (1992) examined mussel growth near Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum 1792) farms in British Columbia (BC), Canada compared to reference sites 600 and 800 m away, and did not detect nutrient enrichment (seston, chlorophyll *a*) or find any evidence of increased mussel growth closer to the farms (measured at 3, 15, and 75 m) than at the control sites (600 and 800 m). However, the salmon farms described in Taylor et al. (1992) produced only 3700 and 2500 kg of fish (consuming approximately 9250 and 6200 kg of feed, respectively, over one year) during the study. As a comparison, a commercial Atlantic salmon (*Salmo salar*) farm in Canada contains three orders of magnitude more fish biomass (DFO, 2015) and would presumably create a greater suspended organic nutrient plume available to cultured bivalves. Jones and Iwama (1991) concluded that the growth rates of Pacific oysters (*Crassostrea gigas*) were significantly higher within commercial-scale Chinook salmon cages, compared to control animals outside the cages and from distant (4 or 6 km) reference sites.

1.3. Sea lice at salmon farms in BC

Sea lice are ectoparasitic copepods of the family Caligidae. Infestations are more likely at high host densities, such as those found at salmon farms, and can result in economic losses from reduced fish growth, feed conversion, and market value as well as chemotherapeutic treatment costs and direct or indirect mortality (Torrissen et al., 2013). In BC, two species of sea lice commonly parasitise farmed and wild salmonids: the salmon louse (*Lepeophtheirus salmonis*) and the herring louse (*Caligus clemensi*). It has been suggested that sea lice from Atlantic salmon farms in BC contribute to increased salmon louse infections on wild salmonids (Bateman et al., 2016), leading to higher mortality rates than would occur naturally (Krkošek et al., 2007, 2006). Controlled laboratory studies have confirmed and provided context on the magnitude of those effects on fish physiology, particularly in smaller species such as pink salmon (Brauner et al., 2012; Jones et al., 2008).

The salmon louse life cycle begins with two free-swimming naupliar stages followed by one free-swimming infective copepodid stage. The number of larvae released from a salmon farm depends on the parasite abundance, which is a function of the number of fish and mean level of infection per fish (Orr, 2007; Tully and Whelan, 1993). On a large scale, salmon louse larval movement is dictated by hydrological processes (Stucchi et al., 2011; Tully and Nolan, 2002). Field studies suggest that planktonic sea lice accumulate in shallow water (Costelloe et al., 1996; McKibben and Hay, 2004; Norði et al., 2015; Penston et al., 2011, 2004), and farmed salmon held at shallower depths (0–4 m) develop significantly greater sea lice infections than those held at greater depths (4–12 m) (Hevrøy et al., 2003). Nauplii and copepodids are also capable of swimming and respond to gradients of light (Heuch et al., 1995), salinity (Heuch, 1995), pressure, and temperature (Bron et al., 1993; Heuch et al., 1995; Norði et al., 2015), which aid host location. Once attached to a suitable host, copepodids progress through the remaining chalimus, pre-adult, and adult life stages.

1.4. IMTA bivalves and salmon lice

In addition to potentially recycling small organic particulate wastes at salmon farms, filter-feeding bivalves have been proposed as a potential biomitigation tool for fish diseases (Skår and Mortensen, 2007) and harmful phytoplankton (Delegrange et al., 2015). The ability of bivalves to ingest and digest salmon louse larvae in the laboratory has been well documented. Molloy et al. (2011) verified copepodid presence in the buccal cavity and stomach contents of a laboratory population of blue mussels (*Mytilus edulis*) following 30 and 60 min exposures to larval lice. In another laboratory study, all species of shellfish tested (mussels (*Mytilus* spp., Pacific oysters (*C. gigas*), Pacific scallops (unconfirmed hybrid: *Mizuhopecten yessoensis* × *Patinopecten caurinus*), and basket cockles (*Clinocardium nuttallii*)) ingested and digested salmon louse larvae (Webb et al., 2013). That ingestion/digestion occurred with and without algae present, with no significant effect of temperature (5, 10, and 15 °C) (Webb et al., 2013). In a third series of laboratory experiments, the ingestion of larval *L. salmonis* by bivalves was increased by the provision of light to concentrate the larvae (Bartsch et al., 2013). Evidence for mitigation of sea louse infestation by cultured bivalves in the field would expand the potential environmental and social benefits of IMTA and encourage further development of alternative, non-chemical strategies to mitigate salmon louse problems. The present study examined two important aspects of IMTA: growth augmentation of Pacific oysters in the vicinity of an active commercial Atlantic salmon farm in BC, and the extent to which the oysters served to mitigate the abundance of salmon lice larvae within the farm.

2. Materials and methods

2.1. Study site

The study was undertaken at a Grieg Seafood BC Ltd. commercial Atlantic salmon farm at Turnour Island, BC (Fig. 1) between September 2013 and August 2014. The site was a 2 × 7 floating, square-cage array (Fig. 2) stocked with salmon in April 2012 and fully harvested by June 2014.

2.2. Oyster deployment

Large seed Pacific oysters (mean shell height ± SE = 81.1 ± 0.9 mm, $n = 90$) were purchased in July 2013 from Mac's Oysters Ltd. (Fanny Bay, BC) in Dark Sea trays [Dark Sea Enterprises, West Vancouver, BC (L × W × H: 60 × 60 × 8 cm, mesh size: 12 × 12 mm)]. During the 10-hour transport to the study site the oysters were maintained at 10 °C and covered with seawater-soaked blankets to prevent desiccation. One stack of oyster trays consisted of three occupied trays with a lid on the top tray. One stack was deployed at 1-, 3-, and 6-m depths every 1 m around five outer walkways of the fish cage array designated as sides A–E for a total of 125 stacks in total at the farm (Fig. 2). A further 15 stacks (five at each depth) were placed at a reference site approximately 150 m distant from the farm, designated as side F (Fig. 2). In total, each tray held on average 67 oysters and each stack 201 oysters. Stack depths were positioned systematically, *i.e.* non-randomly, around the pens to prevent entanglement of adjacent 6-m trays in the high-flow farm environment (Fig. 2). The shallower depths of 1, 3, and 6 m were chosen to maximize interactions between the oysters and sea lice larvae (Costelloe et al., 1996; McKibben and Hay, 2004; Norði et al., 2015; Penston et al., 2011, 2004) and natural food sources.

2.3. Oyster growth measurements

Oysters were sampled approximately every four months, starting in late July 2013, when they were deployed, and then in November 2013, March 2014, and August 2014 before they were removed from the site. Ten oysters were haphazardly sampled out of three randomly selected