

**Evidence for asymmetric assimilation of an anthropogenic resource subsidy in
a freshwater food web**

by

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ABSTRACT

EVIDENCE FOR ASYMMETRIC ASSIMILATION OF AN ANTHROPOGENIC RESOURCE SUBSIDY IN A FRESHWATER FOODWEB

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Anthropogenic subsidies occur readily, especially with growing human development neighboring natural systems. Considering the immense quantity of anthropogenic resources contributed to natural systems, determining how these subsidies permeate into native food webs is not well understood. Using a combination of stable isotope and fatty acid bio-tracers, my research attempts to estimate the pathway(s) of assimilation of an anthropogenic resource provided by freshwater cage aquaculture. My results point to an asymmetric assimilation of feed by native organisms into and through the structured food web. Specifically, two pelagic fish species (one an intermediate consumer and the other a top predator) show elevated trophic position and higher levels of essential fatty acids due to consumption of the feed, with no signs of consumption of feed through the littoral pathway. Further, I find that the assimilation of feed has an increasing effect on the proportions of n-3 LC PUFAs in the tissue of fish indicating that the feed subsidy has implications for fish health. My findings provide new insight into an emerging research area that asks how anthropogenic resource subsidies effect properties of food webs. Since many habitats experience both natural and anthropogenic subsidy inputs, the multiple tracer approach employed here shows promise for studying how subsidies may affect species health and behavior and food web structure and stability.

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OVERVIEW

General Introduction

Ecosystems are open systems exchanging energy flows with surrounding habitats. Polis et al., (1997) made an argument for the powerful role of natural subsidies crossing ecological boundaries and allochthonous resource inputs have since been stressed repeatedly as major drivers of food web dynamics and biodiversity (Polis et al., 1997; Polis & Hurd 1996; Polis & Winemiller 1996; Nakano et al., 1999; Hall et al., 2000; Takimoto et al., 2002). Increasing human development globally has had drastic effects on nutrients and organic material within natural systems. Just as resources from neighboring habitats affect food web dynamics, so do anthropogenic sources from nearby human activities. In large ecosystems with many subsidy inputs (both natural and anthropogenic), it is likely that there are both resource and consumer subsidies occurring simultaneously (Allen et al., 2016). In order to be able to study their effects concurrently, we must first be able to trace the pathway of a single resource subsidy through a food web. I argue that delineating the consumptive pathway of an anthropogenic resource will help to expand our knowledge of the implications of resource subsidies on food web dynamics and stability. In this study I trace the fate of an anthropogenic resource provided by cage aquaculture feed pellets in a freshwater food web and give insight on how this will have implications for dynamics and stability.

The present overview addresses current knowledge and need for both research in the subsidy and aquaculture literature and techniques used to identify diet origin, and gives insight into how the use of stable isotopes and fatty acids may be a promising tool for the identification of the pathway of consumption of an anthropogenic resource within a freshwater food web.

Subsidy Overview

Subsidies are sources of energy that cross habitat boundaries (e.g., terrestrial to aquatic) and provide resources to the receiving system. These energy sources may be in the form of nutrients, detritus, prey, or consumers, causing drastically different outcomes to the recipient food web depending on the quality and the quantity of the resource that is received. Resource subsidies have significant impacts on species interactions and food web dynamics. Inputs of nutrients and detritus generally increase the abundance of producers and prey organisms in the recipient

habitat (Polis et al., 1997). Increased growth at the producer level can result in bottom-up effects, whereby increases in the abundances of populations at lower trophic levels support higher abundances of consumer populations than would otherwise be possible without the subsidy (Polis et al., 1997). If the subsidy is a consumer rather than a resource, top-down effects are likely to occur. With the addition of more consumers in a habitat, the consumers exploit prey populations to lower levels than would be expected by only the action of resident consumers (Polis et al., 1997; Schmidt & Ostfeld, 2008). Subsidizing top trophic level organisms can cause predators to suppress their prey, thereby releasing the next lower trophic level from predation. Thus, subsidies may alter predator-prey relationships and result in a disproportionate impact on the communities of the recipient habitat. In each case, the outcome on community structure, food web dynamics and stability is truly dependent on the nature of the subsidy (i.e., nutrients, prey, and consumers), the duration of the subsidy (i.e., pulsed or continuous) and what trophic level is being subsidized (Marczak et al., 2007; Richardson et al., 2010; Marcarelli et al., 2011; Allen et al., 2016).

In addition to natural resource subsidies, human derived (anthropogenic) subsidies are increasingly common due to human development in close proximity to natural environments. Anthropogenic resource inputs from wastewater, agriculture, aquaculture, etc., can provide a novel source of food for native populations (Newsome et al., 2014; Newsome et al., 2015). In cases of anthropogenic resource use, the organism's flexible, opportunistic feeding behaviors allow them to respond by including these novel resources in their diet. As with natural subsidies, the impact of anthropogenically-driven subsidies depends on where the subsidy enters the food web, as well as the fate of this novel resource in the food web (i.e. the pathway that the resource is incorporated into within the food web).

Given the importance of the fate of a subsidy in the food web for determining the impacts on food web dynamics, surprisingly few studies have documented the pathway of a resource subsidy once it has entered the food web. The subsidy is often traced into the food web to one species, but the pathway in which the subsidy is assimilated further is not actually followed; rather, it is simply implied (Anderson & Polis 1998, Carpenter et al., 2005; Cole et al., 2006). Whether a resource subsidy is equally distributed across the food web or preferentially utilized by one compartment of the food web may effectively re-wire, or re-route, whole system carbon flows, and thus the nature of resources subsidies needs to be studied further.

Aquaculture Overview

Freshwater aquaculture is a rapidly growing agricultural food production practice that often involves rearing fish in cages suspended within a lake. Each year in Ontario alone, the freshwater cage aquaculture industry produces 4,510 tonnes of rainbow trout, which has an estimated economic contribution of \$80 million (Moccia & Bevan, 2016). Increased demand for fish protein to meet the needs of an ever-growing population highlights the need for aquaculture policy that effectively protects the environment but enables industry growth. However, a lack of functional government regulations and guidelines prevent the cage culture industry from expanding existing sites and accessing new sites (Fisheries and Oceans Canada, 2006; Moccia & Bevan, 2016). Therefore, a better understanding of how freshwater cage aquaculture facilities affect lake ecosystems is required.

The environmental impact of waste from the fish culture industry, notably from cage culture operations, is increasingly a matter of close scrutiny. There are many environmental implications associated with cage aquaculture as waste feed and fecal matter are directly released into the natural system. These environmental issues are categorized into nutrient loading, habitat alteration and effects on native fish populations (Fisheries and Oceans Canada, 2006; Kullman et al., 2009) and have been primarily studied in marine systems. Research has been conducted on water quality changes due to high levels of dissolved nitrogen and phosphorus causing eutrophication, algae blooms, and decreased oxygen (Folke et al., 1994; Fernandez-Jover et al., 2007b; Sara, 2007, Guo et al., 2009). The effects of eutrophication and oxygen depletion have been mediated by developing high density, low phosphorus feed and by establishing farm sites that have improved water circulation (Cho and Bureau 1997). Cage aquaculture has also been shown to alter the habitat in which it is situated by increasing sedimentation and organic matter concentrations, in sediment close to the cages (Cho & Bureau, 2001; Carroll et al., 2003). When waste settles under the cages, it is degraded by bacteria who consume oxygen in the process. This degradation process by bacteria can lead to significant reduction in dissolved oxygen and create anoxic conditions (Cho & Bureau, 2001). Sediment changes are often found in shallow lake areas that are likely not suitable for long term sustainable aquaculture sites due to their already high nutrient levels and low flushing abilities. The technique of moving the cages (fallowing) is a mechanism used to mitigate sedimentation because the area where the cages once

were, is able to recover when the cage location is changed (Carroll et al., 2003, Cho & Bureau, 2001, Guo et al, 2009). Although decreased water quality and habitat alteration have associated implications for native organisms, there is also the potential for the particulate organic matter to be assimilated by native organisms, but this possibility is much less studied.

Cage aquaculture operations provide a continuous point source of organic matter through the release of excess feed and fecal matter that can be utilized either directly or indirectly by native fish populations (Ackefors & Enell, 1990, Kullman et al., 2009). Excreted waste and uneaten food are released directly into the surrounding environment and thereby introduce an allochthonous source of limiting nutrients (P and N) and organic solids that alter resource abundances within the system (Yan, 2005; Johnston et al., 2010; Oksanen, 2013). This organic material can act as a food source for invertebrate and fish species, and studies have shown that natural fish populations surrounding cage culture operations exhibit a shift in diet towards that of the released cage culture feed and waste (Fernandez-Jover et al., 2007a; Fernandez-Jover et al., 2011). Although this research comes mainly from marine environments, Kullman et al. (2009) set up an experimental study in a freshwater lake where they established a cage aquaculture operation and monitored the effects of aquaculture for several years. They found the effects of waste from cage aquaculture on native organisms included, increased abundances of benthic organisms, increased growth, reproduction and densities of invertebrates and small fish. While informative, the results of this study are limited; Kullman et al.'s study was conducted in a small lake where effects would arguably be more pronounced than in the larger lakes typically chosen by the aquaculture industry. There is a clear need for a natural experiment done at a spatial scale that is more consistent with the lakes commonly chosen for aquaculture. However, this research faces the significant challenge of designing such natural experiments in a way that the energy and nutrient flux in open systems can be quantified.

Bio-tracers as Diet Indicators

Stable isotopes and fatty acids are common bio-tracers used to measure important food web parameters including an organism's diet (Vander Zanden et al., 1999; Fernandez-Jover et al., 2011; McMeans et al., 2012). A single piece of tissue used for bio-tracer analysis represents a time integrated diet estimate that reflects many snapshots of stomach contents. In freshwater ecosystems, stable isotope ratios of carbon ($\delta^{13}\text{C}$) change very little from prey to predator

(enriched from prey to predator at 0-1‰) and can differentiate dietary sources between the pelagic and littoral energy pathways. The stable isotope ratio of nitrogen ($\delta^{15}\text{N}$) of an organism is typically enriched by 3.4‰ relative to its diet, and can be used to determine the trophic position of an organism (Vander Zanden et al., 1999; Post et al., 2002). Analysis of fatty acid composition can determine dietary components because the fatty acid composition of a consumer is influenced by that of its prey (Ackman et al., 1980). Many studies in both freshwater and marine ecosystems have shown that as levels of certain fatty acids increase in the diet items, there is a concomitant increase in these fatty acid levels in consumers (Castell et al., 1972; Boggio et al., 1985; Bergstrom, 1989; Gomes et al., 1993; Bell et al., 2001; Heissenberger et al., 2010; McMeans et al., 2016). Thus, stable isotopes and fatty acids provide an excellent tool to quantify the flow of carbon-based energy and nutrients among habitats.

Researchers have taken advantage of the fact that aquaculture waste is enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Therefore isotopically enriched feed corresponds to an isotopic enrichment in native organism tissue (Salazar-Hermoso, 2007; Kullman et al., 2009). When no differences in isotopic signatures of organisms between reference and affected sites, it is often due to failure to use reference sites far enough away from the aquaculture to use as a distant control. In one study conducted in Lake Huron, reference sites were only 1-2 km away from their affected sites (Johnston et al., 2010). Many fish have a greater home range than 1-2 km and could very easily swim from their control sites to farm sites thus explaining why they didn't find differences in fish isotopic signatures between sites.

Resources subsidies flow into neighboring habitats, often having distinct stable isotope signatures compared to resources in the receiving habitat, allowing the ability to identify organisms that have consumed the resource. Stable isotopes have been used to indicate marine subsidies entering terrestrial habitats by examining the isotope signatures of consumer tissue within the receiving habitat (Anderson and Polis, 1998; Barrett et al., 2005). Anderson and Polis (1998) used isotopes of carbon and nitrogen to show that coastal consumers received a significant part of their diet directly and indirectly from marine sources. Coastal spiders and scorpions were significantly more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than inland spiders and scorpions. Even less studied, is the identification of anthropogenic resource subsidy consumption by native organisms. DeBruyen et al., (2003) evaluated the role of sewage as a resource in the St. Lawrence River however, they were unable to quantify the diet composition of primary

consumers at the sewage-exposed sites because they could not physically separate sewage derived particulate organic matter from autochthonous production. Subsidy studies that have identified subsidy resource use by native organisms using stable isotopes, make assumptions about the pathway of assimilation to the focal species of interest. In order to move forward in studying dynamics and stability implications from anthropogenic subsidy input, we need to be able to trace a subsidy through a food web to determine what species and trophic groups are taking advantage of the novel resource.

Fatty acid composition analysis is commonly used for determining dietary sources in field and experimental studies of trophic interactions in freshwater systems (Fernandez-Jover et al., 2011; McMeans et al., 2012). For example, a study conducted by Olsen et al. (2015) fed Atlantic cod 3 different diets: a control, a herring fillet diet that had significantly higher levels of the marine long-chained mono-unsaturated fatty acids (MUFA) 20:1n-11, 20:1n-9 and 22:1n-11, and a salmon feed diet was rich in 18:1n-9 and had significantly higher of the poly-unsaturated fatty acids (PUFA) 18:2n-6 and 18:3n-3. The study showed that cod fed the herring fillet diet had significant increases in 20:1n-9, 22:1n-11 and 20:1n-11, which correlated to the high level of these FA in the feed, and rainbow trout fed the salmon feed had significant increases in 18:2n-6, 18:3n-3, and 18:1n-9, which also correlated to the high levels of these FA in the salmon feed. Studies in marine cage culture systems have also shown that the fatty acid composition of fish surrounding the cage culture is similar to that of the cage culture feed and significantly different than that of control fish (Fernandez-Jover et al., 2007a; Fernandez-Jover et al., 2011). Therefore, using fatty acids to determine dietary components of natural fish populations near freshwater cage culture operations may be able to detect if and where released excess feed and waste enters a food web and the pathway of assimilation that the excess feed and waste follows after it has entered. Although some studies find that aquaculture waste is being assimilated by native organisms, such studies often only look at one species or one trophic category. In the case of Fernandez-Jover et al. (2011), they only include a top-predator fish species, which does not allow for any conclusions based on whole food webs. By including base level organisms, intermediate consumers, and higher level top predators, a clearer picture of the food web consequences of waste provided by aquaculture can be captured.

Although, several studies have used single bio-tracers to determine if aquaculture waste is entering the food web, they are often inconclusive but may have come to a more robust

conclusion about the fate of waste in the surrounding ecosystem if they had used a combination of bio-tracers.

Linking Aquaculture to Subsidy Research

Recent food web theory shows that changes in resource abundance and shifts in consumer diets as a result of allochthonous energy sources can influence food web structure (Vander Zanden & Vadeboncoeur, 2002; McCann & Rooney, 2009). When energetic resources from a neighboring system flow into a receiving system, they increase productivity (Polis 1997, Marczak et al., 2007), thus increasing growth and abundance of the recipient species (Allen et al., 2016). These energetic resources or flows of energy, are commonly referred to as resource subsidies (Polis et al., 1997). Although cage aquaculture waste uptake by native organisms has been studied, the ecological implications to the food web linking ideas to subsidy theory has never been done. It is recognized that the food web impacts of subsidies depend on both where the subsidy enters the food web and how it is then distributed through the food web, and surprisingly few studies have emerged that have documented the actual subsidy pathway(s). Most research has documented the "entry" of the subsidy into the recipient food web (e.g., Anderson & Polis 1998; Spiller et al., 2010) and the same is true for aquaculture waste assimilation. The impact of released cage culture feed and waste on lake food webs remains largely unknown.

Here, I have highlighted a need for research on the effects of freshwater cage culture waste on native organisms since freshwater systems may respond differently than marine systems as a result of differences in physical processes, water chemistry, and biotic communities (Johnston et al., 2010). Linking aquaculture research with ecological implications will allow us to grasp a more holistic view of the effects of aquaculture and the subsidy provided by waste. I also indicate that studies combining multiple common bio-tracers may come to more robust conclusions about the fate of aquaculture waste within the surrounding system than those only using one bio-tracer. Finally, studies that incorporate more than one species will be able to determine structural changes in the food web associated with aquaculture waste assimilation and how the consumption of this feed subsidy may have physiological consequences to the fish population. In the study that follows, I evaluate the use of a combination of bio-tracers (i.e., stable isotopes and fatty acids) to identify the pathways of assimilation of an anthropogenic resource provided by cage aquaculture feed in a freshwater environment and give insight on the

effects of aquaculture originated organic matter on native organisms and the associated potential ecological implications.

INTRODUCTION

Historically, community ecologists investigated phenomena at pre-defined spatial scales that ignored the flow of nutrients and materials across boundaries (Forbes, 1887; Levin & Paine 1974). In the 1990's, a series of influential papers made cogent arguments that a greater understanding of food web structure and dynamics required ecologists to shift their perspective away from considering habitats as single, isolated entities. Instead, they argued it is essential to embrace the fact that populations, communities, and ecosystems are intimately connected by cross-boundary flows, commonly referred to as resource subsidies, on the landscape (Likens & Bormann 1974; Polis et al., 1997; Polis & Hurd 1996; Polis & Winemiller 1996).

A body of research on resource subsidies has emerged that not only argues for the ubiquitous existence of subsidies in nature, but also importantly suggests that subsidies should fundamentally alter the structure and dynamics of recipient ecosystems (see an excellent summary by Polis and Strong 1997). When resource subsidies from a neighboring system flow into a receiving system, they necessarily increase productivity, thus increasing the growth and abundance of the recipient species (Polis & Hurd 1996; Barrett et al., 2005). Richardson (1991) showed experimentally that stream detritivore invertebrate species rely on seasonal terrestrial subsidies during resource deficits to stimulate productivity, supporting the findings of many previous studies (Nakano et al., 1999; Hall et al., 2000; Takimoto et al., 2002; Yang et al., 2008). Additionally, theory has argued that food web stability in recipient ecosystems is increased at low to moderate levels of allochthonous (i.e., organic material formed outside of the receiving ecosystem) resource inputs, although very strong subsidies can also drive top-down cascading suppression in consumers by predator species that can be destabilizing (Polis and Strong 1997, Huxel & McCann 1998; Takimoto et al., 2002). Resource subsidies often ignite trophic cascades and alter competitive relationships, thus impacting the maintenance of diversity within the recipient community (Anderson & Polis, 1998; Nakano et al., 1999; Baxter et al., 2005, Schmidt & Ostfeld 2008; Spiller et al., 2010). Depending on the nature of the subsidy (i.e., nutrients, prey, and consumers), the duration of the subsidy (i.e., pulsed or continuous), and what trophic level is being subsidized, ecologists are finding that subsidies can play out a myriad of different outcomes on food web dynamics, stability and functioning (Marczak et al., 2007; Richardson et al., 2010; Marcarelli et al., 2011; Allen et al., 2016).

While natural resource subsidies occur readily (e.g., Polis and Hurd 1996; Spiller et al., 2010), anthropogenic activities also frequently result in resource inputs into natural habitats (e.g., aquaculture, wastewater, agriculture). These human-driven resource inputs often act as a novel source of food for native populations. Areas of human development are highly productive, allowing organisms the opportunity to take advantage of these allochthonous energy inputs. Dingoes in the outback of Australia, for example, utilize human derived waste in garbage facilities from mining companies (Newsome et al., 2014). Likewise, nutrient enrichment in the St. Lawrence River from sewage attracts fish that in turn exploit this enrichment, driving increases in biomass densities of consumers (DeBruyn et al., 2004). In cases of anthropogenic resource use, organisms' flexible, opportunistic feeding behaviors allow them to respond by including these novel resources in their diets. Eveleigh et al. (2007) describe the phenomenon of mobile organisms being drawn to areas of high resource density as the "birdfeeder effect". As with natural subsidies, the impact of anthropogenically-driven subsidies depends on where the subsidy enters the food web, as well as the fate of this novel resource in the food web (i.e., the pathway that the resource is incorporated into within the food web).

Although it is recognized that the food web impacts of subsidies depends on both where the subsidy enters the food web and how it is then distributed through the food web, surprisingly few studies have emerged that have quantified subsidy pathway(s). Most research has documented the "entry" of the subsidy into the recipient food web (e.g., Anderson & Polis 1998; Spiller et al., 2010). The subsidy is traced into the food web but the pathway in which the subsidy is assimilated further is not actually followed. Instead, the pathway is simply implied (Anderson & Polis 1998, Carpenter et al., 2005; Cole et al., 2006). Food web architecture can frequently cover large spatial scales, with more mobile generalist species at higher trophic levels coupling across different ecosystems, relatively distinct macro-habitat divisions, or compartments (Krause et al., 2003; McCann et al., 2005; Rooney et al., 2008). Thus, whether a resource subsidy is equally distributed across the food web (hereafter, called **diffuse** assimilation of a subsidy into the food web), or preferentially utilized by one compartment of the food web (hereafter, referred to as an **asymmetric** assimilation of a subsidy), may effectively re-wire, or re-route, whole system carbon flows.

Compartmentalized food web structure suggests that resource subsidies may enter food webs and alter different pathways (energy channels) asymmetrically. For example, a subsidy

may enter a freshwater food web in the pelagic (offshore) zone, subsidize consumers occupying that area, and move through that pathway to a mobile higher order predator that consumes organisms in both the pelagic and littoral (nearshore) energy channels. If generalist top predators derive excess energy from one pathway (e.g., pelagic energy channel), this can indirectly allow them to suppress a completely different pathway or compartment (littoral energy channel). Therefore, asymmetric assimilation of subsidies can restructure the pathway of carbon through webs in a manner that may fundamentally alter community composition, trophic structure and stability. In comparison, if a resource subsidy was diffusely assimilated, the food web may become more top heavy and more likely to promote trophic cascades (Okansen et al., 1981; Nakano et al., 1999). The ability to detect the major pathways impacted by a given subsidy promises to allow us to fully understand how subsidies may directly and indirectly alter community biomass in ecological communities.

In this study, I seek to define the pathway of a resource subsidy that emanates from a freshwater cage aquaculture facility in Lake Huron. Cage aquaculture operations provide a continuous point source of organic matter through the release of excess feed and fecal matter that can be utilized directly or indirectly by native fish populations (Ackefors & Enell, 1990). Excreted waste and uneaten food are released directly into the surrounding environment and thereby introduce an allochthonous source of limiting nutrients (P and N) and organic solids that alter resource abundances within the system (Yan, 2005; Johnston et al., 2010). Cage aquaculture operations are positioned within an existing aquatic ecosystem (marine or freshwater) and exist in a range of settings (e.g., cold, deep water offshore or warm, shallow water near shore) that may drive differential uptake of waste material (i.e., feed, fish feces) within the food web. In the marine environment, coastal sea-cage fish farms are known to drive wild fish aggregations in the vicinity of the cage culture (i.e., the birdfeeder effect) producing higher biomass of fish (Dempster et al., 2002; Dempster et al., 2009; Fernandez-Jover et al., 2008 and Tuya et al., 2006). Wild fish often benefit from this allochthonous feed resource (Fernandez-Jover et al., 2007a). Much less is known about freshwater cage aquaculture, and less still about the fate of this waste within the food web itself (i.e., whether it enters diffusely or asymmetrically in the aquatic food web). Here, I aim to address both gaps in knowledge by investigating the fate of an anthropogenic resource provided by cage aquaculture in a freshwater system.

The recent development of bio-tracers (e.g., stable isotopes, fatty acids) that provide time integrated diet estimates have placed ecologists in a position to empirically follow the fate of cage aquaculture feed in receiving food webs. Stable isotopes of nitrogen ($\delta^{15}\text{N}$) increase at each trophic link in the food web and can be used to determine trophic position. Additionally, because aquaculture feed is of marine and terrestrial origin, it is often enriched in $\delta^{15}\text{N}$ compared to available freshwater resources in Lake Huron and allows for tracing the consumption of a possible feed subsidy. Fatty acids are physiologically and structurally important lipids that are largely obtained from the diet (although biosynthesis is possible for some fatty acids, Tocher 2003), and studies in both freshwater and marine ecosystems have shown that as levels of fatty acids increased in the diet there was a concomitant increase in these fatty acids levels in the consumer (Castell et al., 1972; Bell et al., 1986; Boggio et al., 1985; Bergstrom, 1989; Gomes et al., 1993; Bell et al., 2001; Heissenberger et al., 2010; McMeans et al., 2016). Because feed has been shown to contain higher levels of certain fatty acids (e.g., the essential fatty acids 18:2n-6 and 18:3n3 that cannot be biosynthesized by fish and must be obtained from the diet; Tocher 2003) compared to freshwater resources (Tocher 2003), a multiple bio tracer approach combining fatty acids and stable isotopes provides an avenue to rigorously define the fate of feed in a freshwater ecosystem.

In what follows, I explore whether, and how, a commercial cage aquaculture operation potentially subsidizes a recipient adjacent freshwater food web. I use Lake Huron—where most of Ontario’s cage culture industry is located—as a model system to depict the fate of an anthropogenic resource subsidy provided by feed from a cage aquaculture facility. This is an appropriate system to test for the presence of an anthropogenic resource subsidy given the ecological flexibility of many freshwater fish species that are likely able to exploit such a resource subsidy. Further, I considered that insights would be best achieved in an oligotrophic system where nutrients and productivity are low and where human-provided resources (cage aquaculture feed) were available. I used a simplified food web with both a pelagic and littoral energy pathway, consisting of primary consumers, prey fish, and a generalist top predator that obtains resources from both energy pathways. I hypothesize that feed from the rainbow trout aquaculture operation had three possible fates within the food web (Figure 1). 1) The **No Uptake Hypothesis**: Cage aquaculture feed is not assimilated into the food web because none of the species in the food web consume it (Figure 1a), 2) the **Asymmetric Enrichment Hypothesis**:

Cage aquaculture feed is asymmetrically assimilated into the food web, such that it enters the food web through one compartment (either pelagic or littoral, Figure 1b), or 3) the **Diffuse**

Enrichment Hypothesis: Cage aquaculture feed is symmetrically assimilated into the food web, such that species from both the littoral and pelagic zones as well as the top predator derive energy from the feed (Figure 1c). I predicted that organisms consuming feed would have a higher $\delta^{15}\text{N}$ -based trophic position compared to organisms not consuming the feed because feed is known to be enriched in $\delta^{15}\text{N}$ (Kullman et al., 2009).

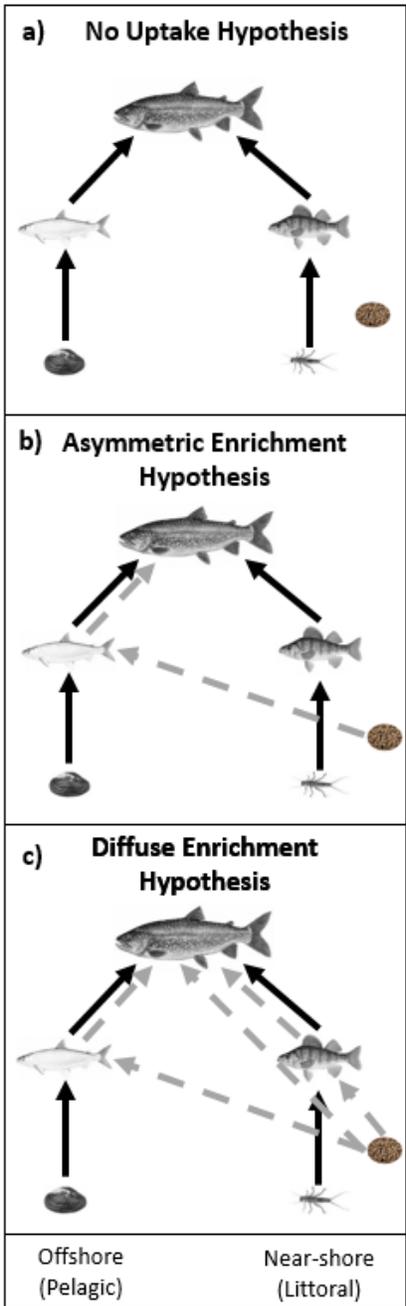


Figure 1: Three **potential pathways of assimilation of cage aquaculture feed into the freshwater food web**. Solid black arrows represent the classical freshwater food web feeding pathways. Pelagic and littoral resources chains, each containing fish and invertebrate prey, are coupled by lake trout. Grey dashed arrows represent potential pathways of feeding on feed from the cage aquaculture facility based on three hypotheses: (a) Feed is not consumed by any native organisms, (b) feed is asymmetrically assimilated by one energy channel (pelagic pathway in this example), or (c) feed is symmetrically assimilated into the food web, such that species from both the pelagic and littoral energy channels consume it.

METHODS

Sample Collection

Samples were collected from the cage aquaculture area and from 3 other control areas within Lake Huron that are not associated with the aquaculture facility (hereafter unassociated sites, UA). Aquaculture-associated (hereafter A_{assoc}) samples are defined as those collected from sites in Big Sound Bay, Georgian Bay, Lake Huron where the cage aquaculture facility is located. The cage aquaculture facility was established in 1981 and produces 1,000-1,300 tonnes of rainbow trout annually (Gord Cole, personal communication, August 2015). Farmed rainbow trout are reared in cages suspended within the lake where water is free to move into the cages and waste (feed and feces) is released into the lake. The feed pellets are comprised of 20% marine fish meal, 12% marine fish oil, 6% canola oil and 25% to 30% terrestrial animal by products (Gord Cole, personal communication, August 2015). Sample collection in Parry Sound occurred in June and July over the course of two years (2014 and 2015). UA samples were collected >30km away from the cage aquaculture facility on the western side of Georgian Bay (Colpoys Bay, Hope Bay, and Stokes Bay), Lake Huron. UA samples were collected from these areas as controls to compare to A_{assoc} samples and were chosen as control sites because of their lack of aquaculture facilities, similar habitats to the A_{assoc} area and the presence of a common top-predator (lake trout). Samples were collected from UA areas in July and August 2015.

I collected tissue samples from a representative set of organisms in the lake food web for stable isotope and fatty acid analysis. Organisms representing the pelagic energy pathway consisted of mussels (*Unionidae spp.*), zebra mussels (*Dreissena polymorpha*), and cisco (*Coregonus artedii*), while the littoral energy pathway was characterized by aquatic insect larvae (*Plecoptera spp.*, *Ephemeroptera spp.*, *Trichoptera spp.*), snails (*Gastropoda spp.*), and yellow perch (*Perca flavescens*). The common piscivore lake trout (*Salvelinus namaycush*) was chosen to represent the top-predator in the lake food web.

Fish and invertebrate samples were collected from both A_{assoc} sites and UA sites. Fish samples were collected using Ontario multi-mesh gill nets following a modified broad-scale fish community monitoring (BSM) protocol (Sandstrom et al., 2013). Both overnight and daytime sets were employed in the sampling protocol. All gill net sets were for a duration of 12 hours before retrieving fish. Weight (g), length (mm), and a muscle tissue plug from behind the dorsal

fin were collected from each fish caught. Non-predatory insect larvae, snails and mussels were collected from shorelines by hand and using kick-nets to act as baselines for stable isotope analysis. These primary consuming invertebrates are commonly used to account for spatial variation in stable isotopes at the base of the food web (Post, 2002) and are needed in the estimation of consumer trophic positions (see 'Data Analysis' section below). Invertebrate samples were collected in the A_{assoc} area from shorelines both proximate to the cage aquaculture and at a distant site from the cage culture. The purpose of collecting baselines from both proximate and distant locations within the A_{assoc} area was to see if there are localized effects of cage culture feed on invertebrate diet due to minimal feed dispersal around the cages. Muscle tissue plugs and invertebrate samples for stable isotope analysis were stored in vials at -20°C until analysis. Muscle tissue plugs and invertebrate samples for fatty acid analysis were stored in vials at -80°C until analysis.

In addition to the samples collected at A_{assoc} and UA sites, dry food pellets (feed) and farmed rainbow trout were collected directly from the aquaculture facility in 2014 and 2015. Samples from the cage aquaculture facility were frozen at -80°C for future stable isotope and fatty acid analysis. Feed and farmed rainbow trout tissue is as a reference material to which I compare the A_{assoc} samples to when determining if native biota are deriving energy from cage culture waste.

Stable isotope analysis

Stable isotopes are described as a ratio of the sample compared to a standard. The standards for carbon and nitrogen are PeeDee Belemnite river limestone and atmospheric N_2 respectively. The standard for carbon has a relatively high ^{13}C content such that most organism samples are less enriched (more negative) than the standard and the standard for nitrogen has a relatively low ^{15}N content such that most organic samples are more enriched (more positive) than the standard. This isotopic ratio, reported as δX , is calculated by:

$$\delta\text{X} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 100$$

where X is the heavy stable isotope, and R is the ratio of the heavy isotope to the common isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) (Peterson and Fry 1987; Vander Zanden et al., 2000).

Stable carbon isotope ratios identify diet origins within a food web because they discriminate among different basal resources and exhibit little ($<1\%$) $\delta^{13}\text{C}$ enrichment with

transfer along the food web (DeNiro & Epstein 1978; Fry 2007). Typically, carbon can be used to delineate the pathway of feeding for a consumer back to basal resources because baselines in the littoral and pelagic zones of the lake differentiate based on their carbon isotope signatures. However, the $\delta^{13}\text{C}$ of feed did not differentiate from littoral baselines in my study (data not shown) and $\delta^{13}\text{C}$ was therefore not used as a bio tracer of the feed subsidy. However, $\delta^{13}\text{C}$ values were used in the calculations of consumer trophic position (see ‘Data Analysis’ section below) to account for different $\delta^{15}\text{N}$ values between the littoral and pelagic baselines (Post 2002). Values of $\delta^{15}\text{N}$ can be used to estimate an organism’s vertical position in the food web, which reflects the number of trophic transfers in the pathway from a basal resource to a top predator (hereafter trophic position), because $\delta^{15}\text{N}$ exhibit a predictable 3-4‰ fractionation between predators and prey (DeNiro & Epstein 1981; Fry 2007; Vander Zanden et al., 2000). Because feed had a significantly higher $\delta^{15}\text{N}$ values than natural baselines (see ‘Results’), I also used $\delta^{15}\text{N}$ to trace the consumption of feed by fish

Frozen fish and invertebrate muscle tissue samples as well as cage aquaculture feed samples were thawed in the lab and dried at 60°C for 48 hours. Once dried, they were individually ground using a mortar and pestle and scooped into a labelled centrifuge tube. The samples were sent to the University of Windsor GLIER (Windsor, ON, Canada) laboratories for isotopic analysis. $\delta^{13}\text{C}$ isotopic values were corrected for fat content using the equation

$$\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C} + (-3.32 + 0.99 \times \text{C:N})$$

(Post et al., 2007; Vander Zanden et al., 2001). Stable isotopes of $\delta^{13}\text{C}$ were used to acquire measures of percent littoral carbon in the diet for each individual prey fish and lake trout, which was then used in combination with $\delta^{15}\text{N}$ values in the trophic position calculations (Tunney et al., 2012).

Fatty acid analysis

I use fatty acids to determine if the dietary components indicative of feed are elevated in fish from A_{assoc} sites compared to a UA site (Colpoys Bay). Fatty acid analysis was conducted on lake trout, cisco, and yellow perch from the A_{assoc} area and UA area as well as cage aquaculture feed samples and farmed rainbow trout. Frozen tissue was sent to Ryerson University where the fatty acid analysis was conducted using a modified Folch method (Folch et al., 1957, as described in detail by McMeans et al., 2012). The % lipid content and fatty acid composition

expressed as both μg fatty acid/g dry tissue (abbreviated as $\mu\text{g/g}$) and relative proportions (individual fatty acid/sum of all fatty acids, expressed as a %) of all samples were obtained.

Data analysis

All statistical analyses were performed using R version 3.2.3, and all tests were considered significant at $\alpha = 0.05$. Data was tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test.

I used ANOVA with Tukey's post-hoc tests to determine if feed was significantly higher in $\delta^{15}\text{N}$ in than the natural pelagic and littoral baseline organisms within the A_{assoc} area and to explore spatial variation in the A_{assoc} area baselines. Specifically I compared $\delta^{15}\text{N}$ of baselines within the A_{assoc} area from sites proximate to and distant from the cage aquaculture facility to explore whether proximate baseline invertebrates were consuming feed (distant invertebrates were assumed not to consume feed).

To test the hypothesis that fish consuming feed would have higher $\delta^{15}\text{N}$ -based trophic positions than fish not consuming feed, I first calculated the trophic positions of A_{assoc} and UA fish. As per established methodology, I used a two source mixing model to calculate contribution of littoral carbon to the diet of lake trout to then calculate trophic position (Post 2002). The calculation for proportion of littoral carbon for an individual is

$$\text{Prop. Littoral Carbon} = (\delta^{13}\text{C}_{\text{predator}} - \delta^{13}\text{C}_{\text{pelagic inverts}}) / (\delta^{13}\text{C}_{\text{littoral inverts}} - \delta^{13}\text{C}_{\text{pelagic inverts}})$$

where $\delta^{13}\text{C}_{\text{predator}}$, $\delta^{13}\text{C}_{\text{pelagic inverts}}$ and $\delta^{13}\text{C}_{\text{littoral inverts}}$ are the carbon signatures of lake trout, pelagic baselines and littoral baselines respectively (Tunney et al. 2012). The scale of this equation ranges from 0 – 1, where values closer to 0 indicate greater use of pelagic carbon sources and values approaching 1 indicate greater use of littoral carbon sources (Vander Zanden et al. 1999). Trophic position of lake trout was calculated as

$$\text{Trophic Position} = 2 + (\delta^{15}\text{N}_{\text{lake trout}} - (\delta^{15}\text{N}_{\text{pelagic inverts}} \times (1-\alpha) + \delta^{15}\text{N}_{\text{littoral inverts}} \times \alpha)) / 3.4$$

(Vander Zanden et al. 1999; Vander Zanden et al., 2000), where 3.4 is the assumed, per trophic level increase due to fractionation in ^{15}N , 2 is the trophic position of primary consumers used as the baseline values, α is the proportion of littoral carbon and weighs the trophic position estimate according to baseline contributions. Only lake trout >250mm were used for analysis to control for ontogenetic shifts in diet. For intermediate consumers, I did not use proportion of littoral

carbon in the trophic position calculation for cisco or yellow perch because these species do not couple into both habitats to the same degree as a top predator does. Thus, the calculation for trophic position of intermediate consumers is

$$\text{Trophic Position (Eqn 2)} = 2 + (\delta^{15}\text{N}_{\text{prey fish}} - (\delta^{15}\text{N}_{\text{invert average}})) / 3.4$$

(Vander Zanden et al., 2001) where all parameters are the same as the previous equation with the exception of $\delta^{15}\text{N}_{\text{invert average}}$. $\delta^{15}\text{N}_{\text{invert average}}$ is the $\delta^{15}\text{N}$ average for invertebrates in the littoral zone (when calculating trophic position for littoral prey fish) or $\delta^{15}\text{N}$ average for pelagic invertebrates (when calculating trophic position of pelagic prey fish).

In order to compare the trophic position of A_{assoc} fish to the trophic position of fish from a wider range of lakes, I obtained fish and invertebrate data from lakes across Ontario that were gathered in conjunction with ongoing Ministry of Natural Resources and Forestry (MNR) lake biodiversity surveys to use as controls for my study. My prediction was that A_{assoc} fish would have higher $\delta^{15}\text{N}$ -based trophic positions due to their consumption of ^{15}N enriched feed compared to these control fish from across Ontario. Lakes were sampled from June-August in 2008-2011. Full details for the fish community netting protocol and isotope collection can be found elsewhere (Sandstrom et al., 2013). Previous work has shown that trophic position of lake trout starts to level off in lakes $>1,000$ ha, with the maximum lake trout trophic position occurring in lakes $>10,000$ (Tunney et al. 2012). I calculated trophic position for lake trout, cisco and yellow perch from lakes $>1,000$ ha (60 lakes in total) using the methods described in the previous paragraph. I also collected data on trophic position of lake trout and prey fish from lakes $>10,000$ ha (17 lakes in total) from published literature (Tunney et al., 2012; Vander Zanden et al., 2000). All of the Ontario lakes sampled did not have aquaculture facilities, thus making them suitable as control sites.

To test the hypotheses of feed uptake and thus determine if A_{assoc} fish had significantly higher trophic positions due to feed consumption, I compared mean trophic position of lake trout, cisco, and yellow perch from A_{assoc} sites to controls. I conducted one sample t-tests, where the mean trophic positions of a species from each lake within a control lake category (i.e., $>10,000$ ha lakes, $>1,000$ ha and UA sites in Lake Huron) were compared to the mean trophic position in the A_{assoc} for that species.

Principle component analysis (PCA) was used to investigate patterns in fatty acid composition across all samples and locations. Fatty acid data were standardized to a mean of zero and unit variance prior to their inclusion in further analyses. All fatty acids that composed an average of >1% across all samples were included in the PCA to eliminate fatty acids that are very rare in the system and to satisfy the PCA requirements (McGarigal et al., 2000). Principle component scores for each of the fatty acids were used to determine the fatty acids that are most important for differentiating feed from natural biota in the system (these fatty acids are subsequently referred to as ‘feed indicator fatty acids’, FIFA). I then conducted a PCA for each species using FIFA profiles. Each point on the plot represents a FIFA fatty acid profile for one individual of the particular species in that plot. This provided a visual representation of the correlations of FIFA from different areas and species groups.

To further test our hypotheses regarding feed consumption, a dissimilarity statistic using Euclidean distances was used to quantify the compositional dissimilarity between samples based on their FIFA profiles. This measure delivers robust and reliable dissimilarity results and is one of the most commonly applied measurements to express relationships in ecology, environmental sciences, and related fields (Clarke & Warwick, 1994; Hixson et al., 2016). The purpose of the distance matrix is to construct the data points in a multi-dimensional space, which configures the data in a similarity/ dissimilarity matrix. Analysis of similarities (ANOSIM) was used to test differences based on the dissimilarity matrix among fish from A_{assoc} and UA sites. ANOSIM is a multivariate analysis that uses a resemblance matrix and carries out an approximate analogue of ANOVA. ANOSIM generates a value of R that ranges between 0 and 1: a value of 0 represents no difference among a set of samples (the null hypothesis) and a value of 1 represents complete dissimilarity among set of samples (Hixson et al., 2016). Finally, I used MANOVA with a Tukey HSD post-hoc test to test whether the proportions of FIFA and omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) 20:5n-3 and 22:6n-3 proportions were significantly different in A_{assoc} fish compared to UA fish.

RESULTS

Both littoral and pelagic baseline $\delta^{15}\text{N}$ values within the A_{assoc} area were significantly lower than the $\delta^{15}\text{N}$ values for feed ($F_{2,51} = 23.11$, $p < 0.001$). Proximate baseline $\delta^{15}\text{N}$ values did not differ significantly from distant baseline $\delta^{15}\text{N}$ values for both littoral ($F_{3,38} = 6.57$, $p = 0.88$) and pelagic baseline organisms ($F_{3,38} = 6.57$, $p = 0.99$).

A_{assoc} lake trout and cisco had significantly higher trophic positions than conspecifics from all control lake categories (Figure 2, Table 1). Within Lake Huron, all UA lake trout average trophic positions were significantly lower than the A_{assoc} average lake trout trophic position (Table 1). I was only able to obtain cisco trophic position data for one of the UA sites within Lake Huron and was therefore not able to test for significance. However, the average trophic position of cisco from the A_{assoc} area was 4.17 and the average trophic position for cisco in the UA area was 3.62, following the trend of higher trophic positions in A_{assoc} fish (Table 1).

Of all control lake categories, I only had enough data to test for significance in the comparison of mean yellow perch trophic position from the A_{assoc} area and lakes $>1,000\text{ha}$. I found no significant difference (Table 1). In all other cases, there is no significant difference in the A_{assoc} area mean yellow perch trophic position compared to controls.

The first two principle components (PC) extracted by the PCA of fatty acid profiles of all samples, explained 70.2% of the variance in the data (Table 2). The cage aquaculture feed and farmed rainbow trout separated from the native biota as a result of higher 18:1n-9, 18:2n-6, and 18:3n-3 (Fig.3). These 3 fatty acids were subsequently named as FIFA (feed indicator fatty acids) as they were most correlated with the feed.

A second PCA performed on FIFA indicate A_{assoc} individuals separate on PC1 from UA individuals due to higher 18:2n-6 and 18:3n-3 in A_{assoc} individuals and higher 18:1n-9 in UA individuals (Fig. 4). A_{assoc} and UA yellow perch did not separate on either PC1 or PC2 of the PCA indicating similar fatty acid profiles between these two groups. Based on the ANOSIM performed on FIFA, lake trout (R statistic = 0.5445; $p = 0.003$) and cisco (R statistic = 0.8545; $p = 0.048$) differed significantly between A_{assoc} and UA. Yellow perch from A_{assoc} area were not significantly different from UA area (R statistic = 0.001; $p = 0.841$), supporting the results of the PCA.

Based on the MANOVA performed on FIFA proportions A_{assoc} lake trout have significantly higher proportions of the FIFA 18:2n-6 and 18:3n-3 compared to UA lake trout. A_{assoc} lake trout also have significantly higher proportions of n-3 LC PUFA (Tables 4 & 5). A_{assoc} cisco have significantly higher levels of the FIFA 18:3n-3, as well as n-3 LC PUFA compared to UA cisco (Tables 4 & 5). There was no significant difference in yellow perch proportions the FIFA, or n-3 LC PUFA between A_{assoc} and UA individuals.

Table 1: Examining differences in trophic position between A_{assoc} fish and control fish using one sample t-tests.

Site	Lake Trout					Cisco					Yellow Perch				
	n	TP	df	t	p	n	TP	df	t	p	n	TP	df	t	p
A_{assoc}	1	4.86	-	-	-	1	4.17	-	-	-	1	3.59	-	-	-
UA	4	4.61	2	29.65	<0.001	1	3.62	-	-	-	1	3.84	-	-	-
Lakes >1,000ha	60	4.44	59	17.19	<0.001	23	3.49	22	10.61	<0.001	27	3.63	26	0.94	0.82
Lakes >10,000ha	17	4.46	16	16.55	<0.001	4	3.44	3	2.66	0.038	3	3.65	-	-	-

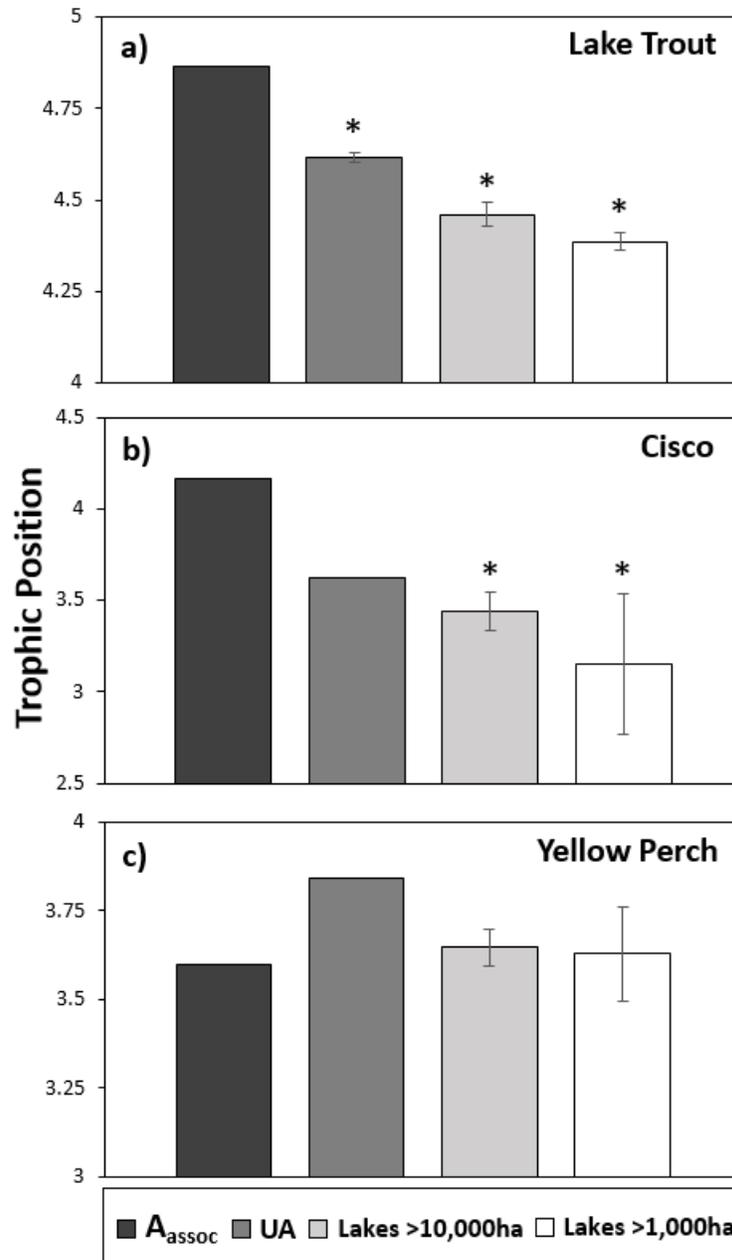


Figure 2: Mean a) lake trout, b) cisco, and c) yellow perch trophic position and standard error from A_{assoc} , UA, control lakes >10,000 ha and control lakes >1,000ha. * indicates significant differences between A_{assoc} fish and controls based on one sample t-tests.

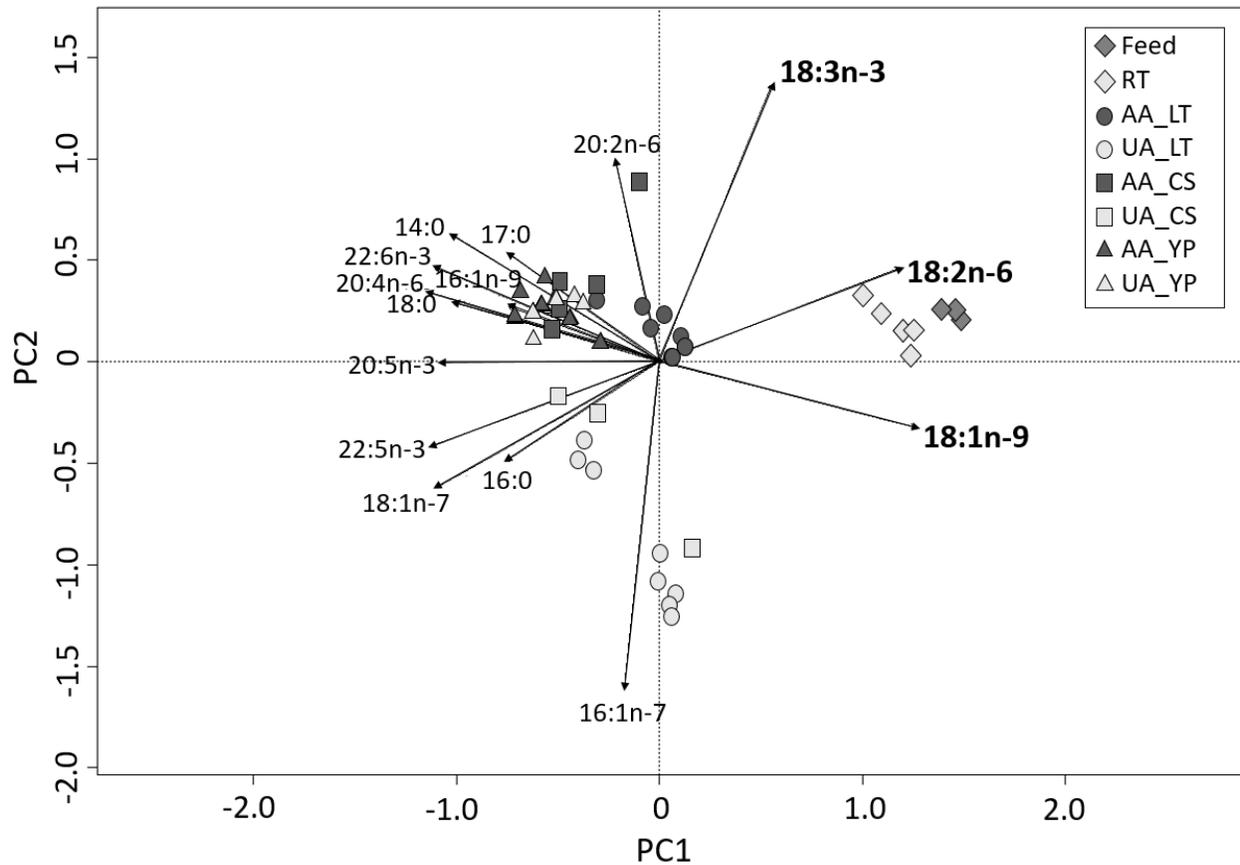


Figure 3: Bi plot of the component scores (symbols) and fatty acid variable loadings (vectors) on the first two principal components from a PCA performed on standardized fatty acid proportions in on lake trout (LT), cisco (CS), yellow perch (YP), farmed rainbow trout (RT) and farm feed pellets (Feed) from aquaculture associated (AA) and unassociated (UA) sites. Both scores and variables are scaled proportional to eigenvalues.

Table 2: Eigenvalues, proportion explained and unscaled weights of each fatty acid variable on the first three principal components (PC) of a PCA performed on lake trout, cisco, yellow perch, farmed rainbow trout and farm feed pellets fatty acid proportions.

Principal Components	PC1	PC2	PC3
Eigenvalue	7.8843	2.6521	1.17417
Cumulative Proportion	0.5256	0.7024	0.78071
Fatty Acid			
14:0	0.28162	0.225846	0.13962
16:0	0.20762	-0.1766	-0.25368
16:1n-9	0.20349	0.097671	-0.21528
16:1n-7	0.04718	-0.5792	-0.06924
17:0	0.20348	0.192944	0.236583
18:0	0.27868	0.105102	0.43833
18:1n-9	0.34449	-0.11563	-0.0159
18:1n-7	0.30032	-0.22247	0.21864
18:2n-6	0.32255	0.166473	0.224286
18:3n-3	0.15138	0.490837	0.110383
20:2n-6	0.05956	0.356175	-0.65662
20:4n-6	0.31919	0.126134	0.117719
20:5n-3	0.29394	0.001307	-0.23709
22:5n-3	0.30775	-0.14898	0.003426
22:6n-3	0.30417	0.167468	-0.07163

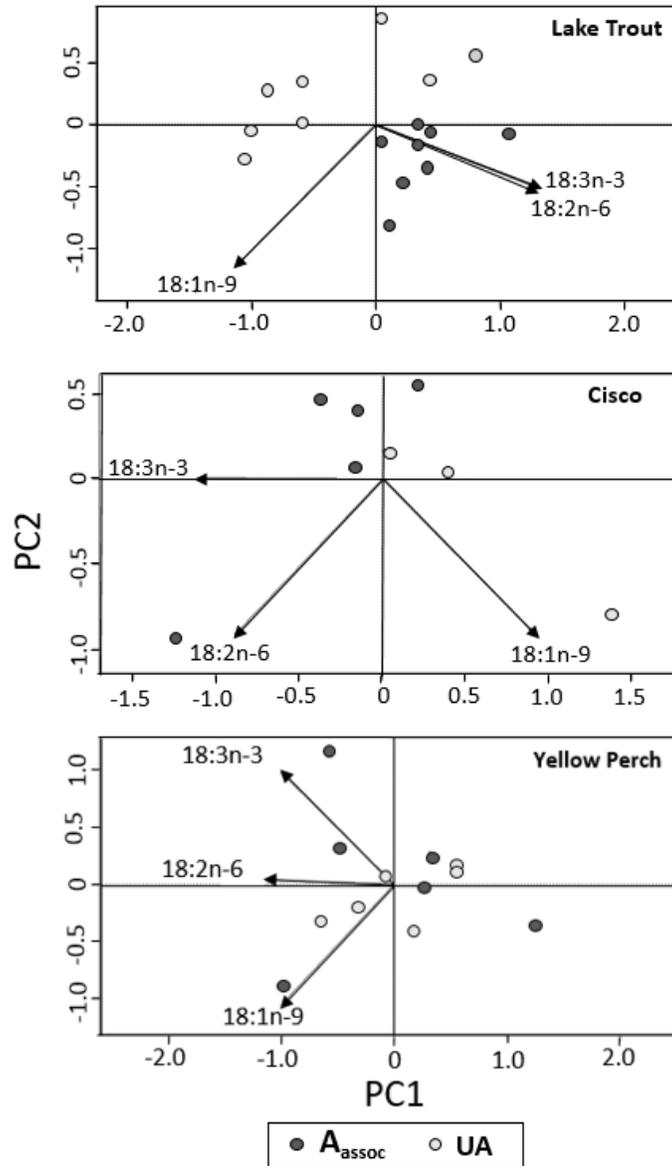


Figure 4: Feed indicator fatty acid principle component analysis bi-plots illustrate the difference in fatty acid profiles among species from both aquaculture associated (A_{assoc}) and unassociated (UA) areas. Each point on the plot represents a FIFA fatty acid profile for one individual of the particular species in that plot. Fatty acid vectors are directionally oriented in these plots, indicating an association between the vector and the fatty acid profile of individuals in the vicinity of the vector. The standardized fatty acid proportion data used to run the PCA was then used to configure the data in a dissimilarity matrix (Euclidian distances) to run ANOSIM. Lake trout (R statistic = 0.5445; $p = 0.003$) and cisco (R statistic = 0.8545; $p = 0.048$) differed significantly between A_{assoc} and UA. Yellow perch from A_{assoc} area were not significantly different from UA area (R statistic = 0.001; $p = 0.841$).

Table 3: Eigen values, proportion explained and unscaled weights of each FIFA on the first two principal components of the PCA used to illustrate differences in individuals from A_{assoc} and UA areas for each species

Principal Components	Lake Trout		Cisco		Yellow Perch	
	PC1	PC2	PC1	PC2	PC1	PC2
Eigenvalue	2.5580	0.4011	2.1213	0.7228	1.8377	0.7157
Cumulative Proportion	0.8527	0.1337	0.7071	0.2409	0.6126	0.8511
Fatty Acid						
18:1n-9	-1.311	-1.331	1.056	-1.046	-1.153	-1.218
18:2n-6	1.494	-0.593	-1.041	-1.075	-1.337	0.0401
18:3n-3	1.497	-0.574	-1.286	0.012	-1.174	1.1497

Table 4: Fatty acid table showing differences in proportions of FIFA and n-3 LC PUFAs in A_{assoc} sites compared to UA sites.

Species	Feed	Rainbow Trout	Lake Trout		Cisco		Yellow Perch	
Site	Aassoc	Aassoc	Aassoc	UA	Aassoc	UA	Aassoc	UA
n	3	5	8	8	5	3	6	6
Fatty Acids								
18:1n-9	39.54 ± 0.48	39.72 ± 3.21	18.76 ± 3.25	20.40 ± 5.01	10.54 ± 1.26	15.34 ± 6.90	7.16 ± 0.98	7.22 ± 0.76
18:2n-6 (LIN)	17.17 ± 0.12	14.68 ± 0.85	3.73 ± 0.16	3.08 ± 0.74	3.97 ± 0.60	3.77 ± 0.19	3.88 ± 0.68	3.90 ± 0.22
18:3n-3 (ALA)	4.68 ± 0.01	4.96 ± 0.47	3.53 ± 0.27	2.04 ± 1.13	4.10 ± 0.51	2.78 ± 0.44	3.49 ± 0.44	3.36 ± 0.21
∑n-3 LCPUFA	3.16 ± 0.05	6.23 ± 0.82	23.21 ± 2.12	17.73 ± 2.03	24.89 ± 1.53	20.84 ± 3.12	25.90 ± 2.5	26.79 ± 1.71

Table 5: Post hoc MANOVA results of FIFA and n-3 LC PUFA proportions in fish from A_{assoc} sites compared to UA sites.

Species	Lake trout			Cisco			Yellow Perch		
	F	Df	P(>F)	F	Df	P(>F)	F	Df	P(>F)
18:1n-9	0.5972	1,14	0.4525	2.6477	1,6	0.1548	0.0124	1,10	0.9134
18:2n-6 (LIN)	5.9551	1,14	0.0029	0.2904	1,6	0.6094	0.4086	1,10	0.537
18:3n-3 (ALA)	13.312	1,14	0.0026	13.714	1,6	0.010	0.008	1,10	0.9305
∑n-3 LCPUFA	8.3944	1,14	0.0117	2.6548	1,6	0.0154	0.3307	1,10	0.578

DISSCUSSION

Anthropogenic subsidies to ecosystems are widespread and will likely only grow with increased human populations and development. It is therefore critical to determine how anthropogenic subsidies permeate into native food webs and effect food web dynamics. Recent empirical and theoretical results have argued that natural food webs are constructed from separate, or compartmentalized, energy channels (e.g., warm nearshore versus cold offshore energy channels) coupled by mobile generalist top predators capable of taking advantage of the resources from these different compartments. Because of this, I hypothesized that there are three fates of an anthropogenic subsidy in this system: (1) No assimilation of the novel resource; (2) diffuse assimilation whereby organisms of both nearshore and offshore zones consume the feed, and (3) asymmetric assimilation whereby one compartment of the food web consumes the feed. Given the placement of the cage aquaculture facility in the offshore deep water zone, I predicted that the anthropogenic subsidy would be assimilated asymmetrically into the offshore cold water pelagic food compartment. My results support this prediction because lake trout and cisco, but not yellow perch or baseline organisms, had higher $\delta^{15}\text{N}$ -based trophic positions and higher proportions of feed indicator fatty acids (FIFA) in A_{assoc} compared to both control sites within Lake Huron and a range of Ontario lakes, suggesting that aquaculture feed enters the food web through the pelagic pathway.

My results, which are based on a novel mixed bio-tracer method, suggest that the assimilation of feed by native organisms occurs asymmetrically through a pelagic intermediate consumer and a top predator species. Feed had higher in $\delta^{15}\text{N}$ than the natural pelagic and littoral baselines in the A_{assoc} area, which allowed us to use $\delta^{15}\text{N}$ to trace the pathways by which feed entered the food web. I found no enrichment in $\delta^{15}\text{N}$ in baselines proximate to the cage aquaculture compared to distant baselines, indicating that the cage aquaculture feed was not being consumed by either pelagic or littoral baselines. Trophic position of cisco and lake trout from the A_{assoc} area were, however, significantly higher compared to trophic position of cisco and lake trout from control lakes >1,000ha and control lakes >10,000ha, strongly indicating the consumption of feed by cisco and lake trout in the A_{assoc} area. In contrast the trophic position of littoral yellow perch from the A_{assoc} area was not higher than the controls, indicating that consumption of aquaculture feed did not enter the littoral prey food web.

Solid waste produced by cage aquaculture reared fish is in two forms; uneaten feed and fecal waste. It is important to note that I did not test the isotopic signature of fecal waste from farmed rainbow trout which, in addition to feed, is a major output from cage aquaculture. It is estimated that 15-30% of applied feed is released as fecal waste and uneaten feed can range from 2-5% (Bureau et al., 2003; Osuchowski, 2013). Previous studies have found that fecal waste is enriched in $\delta^{15}\text{N}$ in a similar fashion as the feed (Fernandez-Jover et al., 2011; Kullman et al., 2009); therefore, similar results would be expected whether cisco or lake trout were consuming rainbow trout feces or feed. I also observed feed in the stomachs of pelagic prey fish while sampling (L. Johnson, personal observation), supporting the direct consumption of feed by these species.

I found that feed was distinguished by high levels of 18:1n-9, 18:2n-6 and 18:3n-3, which coincides with the findings of others (McMeans et al., 2016; Fernandez-Jover et al., 2011). The proportions of the FIFA 18:2n-6 and 18:3n-3 were significantly higher in cisco and lake trout, but not yellow perch, from the A_{assoc} sites compared to UA sites, indicating asymmetric assimilation of feed through the pelagic pathway. Higher 18:1n-9 in the UA compared to A_{assoc} fish may be attributed to the fact that fish can readily synthesize 18:1n-9, meaning this FIFA may not be as robust of a diet indicator as 18:2n-6 and 18:3n-3 that can only originate in a fish's tissues from the diet. Alternatively, the fish from the UA area could have higher proportions of 18:1n-9 and the A_{assoc} fish high 18:2n-6 and 18:3n-3 due to high levels of these fatty acid in baseline organisms. Although I lacked baseline samples to test this idea, I found no evidence in the literature indicating that invertebrates in freshwater systems have high levels of essential fatty acids 18:2n-6 and 18:3n-3. Therefore, it is unlikely that the higher levels of 18:2n-6 and 18:3n-3 in A_{assoc} cisco and lake trout are attributed to higher proportions in baseline organisms. Further, the stable isotopes supported the fatty acids in suggesting that feed enters the food web through the pelagic pathway. Thus, the combined use of stable isotopes and fatty acids in this study proved to be a powerful tool for tracing the pathway of an anthropogenic subsidy.

Here, I have harnessed a mix of bio-tracers (i.e., stable isotopes and fatty acids) to directly follow the fate of a cage aquaculture feed subsidy in an aquatic food web. This mix of bio-tracers allows us a more rigorous, empirical approach for following the fate of this resource subsidy through the food web as they are integrated into the tissue of the organism over time. Much of the current literature revolves around the impacts of terrestrial carbon on lake

ecosystems, using bio-tracers to determine the entry point in the food web. There has been much less effort employed to follow the pathway of subsidized energy through the web. My work highlights that the further development and deployment of multiple tracers (e.g., DNA barcoding) promises to aid our ability to follow human-driven subsidies through ecosystems and allow us to begin to understand the dynamical implications of subsidies.

Both empirical and theoretical studies have demonstrated how cross-habitat subsidies may impact local food web dynamics, stability, and ultimately biodiversity (Polis, 1997; Holt, 2002, Sears et al., 2004). In a broad sense, constant subsidies like the one studied here may be expected to impact the stability of ecosystems in two ways that are dependent on the quantity of the subsidy. Firstly, small amounts of resources subsidized to a system may allow the existence of a species, or set of species, that would not be able to persist without this novel energy source (Huxel et al., 2002). Such a weak subsidy is unlikely to allow these now persisting species to drive suppression of other species through predation or competition. Alternatively, larger amount of subsidies can play a powerful role in muting or inhibiting consumer-resource cycles in resident food webs (Huxel et al., 2002; Takimoto et al., 2002). However, while stabilizing in a dynamic sense, this same large subsidy can translate into strong indirect effects of suppression through competition or predation on other species (Polis et al., 1997; Huxel et al., 2002). While I have documented asymmetric assimilation of a feed subsidy it remains to determine how this anthropogenic resource will impact the resident food web's dynamics. Notably, the A_{assoc} area (Parry Sound, Lake Huron) is one of only two known cases of a sustainably reproducing lake trout in all of Lake Huron suggesting the potential that feed may be allowing their persistence (i.e., case one above).

One of the major ecological concerns surrounding anthropogenic subsidies and freshwater cage aquaculture is their possible effects on native organisms and the environment. In other studies, increased size, growth and abundance of fish have been attributed to food resources provided by cage aquaculture (Kilambi et al., 1978). Kullman et al., 2009 conducted an experimental study in a freshwater lake where they established a cage aquaculture operation and monitored the effects for several years. They found the effects of waste from cage aquaculture on native organisms included, increased abundance of benthic organisms, increased growth, reproduction and densities of invertebrates and small fish. While I did not measure growth or abundance, I do provide evidence that fish from the A_{assoc} habitat have higher proportions of fatty

acids associated with fish health in their tissue than in unsubsidized areas. Specifically, A_{assoc} fish had higher proportions of the n-3 LC PUFA 20:5n-3 and 22:6n-3, as well as their precursors 18:2n-6 and 18:3n3. Thus, the feed-enhanced pelagic pathway, may have unintentionally provided a health benefit to fish by increasing levels of fatty acids that are known to play a role in immune function, growth, reproduction and survival (Tocher, 2003; Sargent, 1999; McMeans et al., 2016).

There is a need for aquaculture policy that effectively protects the environment however, a better understanding of how freshwater cage aquaculture affects lake ecosystems is required to produce a more complete view of the environmental implications of this practice. Cage aquaculture operations located in deep water with strong currents accumulate significantly less organic waste under the cages and the waste that does settle beneath the cages is further dispersed over a larger area by means of bottom currents (Fisheries and Oceans Canada, 2006; Fernandez-Jover et al., 2007b; Cho & Bureau, 1997). The cage aquaculture facility in this study is located in very deep water and thus, may have a higher assimilative capacity for organic waste compared to shallow locations where waste accumulates in high quantities below the cages. The widespread enrichment in trophic position and increased proportions in FIFA in pelagic fish observed in my study suggests that waste from the cage aquaculture is rapidly dispersed and assimilated by native biota. The ability to rapidly assimilate feed may actually reduce the environmental effects of cage culture by eliminating sedimentation of waste. With the aim of reducing environmental impact, new diet formulations with high digestibility and quality ingredients have also increased nutrient retention, thus reducing the amount of waste output from aquaculture facilities (Fisheries and Oceans Canada, 2006; Cho & Bureau, 1997; Cho & Bureau, 2001). A combination of appropriate site choice, newly developed feed, and the assimilative capacity of native organisms should be considered when choosing new sites for this industry in order to reduce environmental impacts of this practise on the freshwater ecosystem.

Our results broadly inform the consumptive pathway of feed subsidies through aquatic food webs, however the reduced mobility of particulate waste in the lake environment may contribute to our results. Particulate waste falls directly below the cages, which are located in the pelagic zone of the lake. Therefore, it is directly available to organisms within that zone of the lake and they are more likely to consume it. In this case, mobile cold water adapted lake trout consume the feed directly or consume cisco that have garnered energy from the feed deposited

directly into the deep cold water habitat. That the feed enters asymmetrically through the cold pathway may argue that different placements of cage aquacultures could alter these results (e.g., a nearshore cage culture may drive feed consumed by the nearshore warm pathway). Many predatory fish are mobile tend to be more generalist foragers since their mobility exposes them to a greater number of habitats and prey types (Pyke et al., 1977). If predatory fish are mobile and can take advantage of these resources, then even though the cage culture is located in the cool, deep water zone, predators from other zones of the lake may be taking advantage of this subsidy as well. It remains to see if other top predators flock to this like a birdfeeder effect (Eveleigh et al., 2007) or whether the thermal differences between nearshore and offshore limit the abilities of other mobile predators that may be more thermally constrained to the warm nearshore zone. In order to get a more complete picture of the food web effects of this anthropogenic resource, future studies should be done on other top predators within this system.

In summary, the unique isotope and fatty acid signature of feed from the cage aquaculture facility allowed us to trace the pathway of this anthropogenic resource through a freshwater food web. This study illustrates that the combination of fatty acid and stable isotope biomarkers could further our ability to not only detect the entry of an anthropogenic resource subsidy, but also trace its fate through the food web. The result of an asymmetric assimilation through the pelagic pathway has implications for dynamic and stability of the food web. I suggest that this subsidy may contribute persistence of a top predator species that may be resource limited in this nutrient poor oligotrophic system. This study has also allowed for a better understanding of the environmental impacts of both subsidies and aquaculture by showing the direct consumption of waste. Finally I have also showed higher levels of healthy fatty acids in fish consuming feed which may have physiological consequences such as increased survival and reproductive success. Now that the fate of the feed has been revealed and some of its consequences to the native fish population discussed, there is a clear need to unpack the many implications of this research, such as the development of sustainable aquaculture, the effects of aquaculture on adjacent ecosystems, and the maintenance of biodiversity in lake ecosystems, all of which could benefit or hinder the food web.

CONCLUSIONS

A major breakthrough in the study of ecological systems was the shift in thinking of habitats as single, isolated entities, to the idea that all ecosystems are connected by flows of energetic resources (Polis & Winemiller et al., 1996; Polis et al., 1997). These energetic resources or flows of energy, are commonly referred to as resource subsidies (Polis et al., 1997) and few studies have emerged that document patterns of assimilation of subsidies within a food web.

Recent empirical and theoretical results have argued for food webs to be constructed from separated, or compartmentalized, energy channels (e.g., warm near shore versus cold offshore pelagic) coupled by mobile, generalist top predators capable of taking advantage of the resources from multiple compartments. Because of this, I hypothesized that there are three fates of an anthropogenic subsidy in this system: No assimilation of the novel resource, diffuse assimilation whereby organisms of both nearshore and offshore zones consume the feed and asymmetric assimilation whereby one zone of the food web consumes the feed. Given the placement of the cage aquaculture facility in the offshore zone and the compartmentalization of food web structure, I predicted that the anthropogenic subsidy would be assimilated into the food web via an asymmetric pathway as opposed to diffusely throughout the food web.

Here, I have harnessed a mix of bio-tracers (i.e., stable isotopes and fatty acids) to directly follow the fate of a cage aquaculture feed subsidy in an aquatic food web. This mix of bio-tracers allows us a more rigorous empirical approach at following the fate of this resource subsidy through the food web. I have used a simplified food web comprised of two food chains coupled by a top predator to develop intuition about how an anthropogenic subsidy provided by cage aquaculture is assimilated into the native food web. Data repeatedly show an asymmetric pathway of assimilation of aquaculture feed. This pattern of uptake within the food web is consistent with predictions based on mobile consumer accessibility and food web compartmentalization.

Linking aquaculture research with ecological implications will allow us to grasp a more holistic view of the effects of aquaculture and the subsidy provided by waste. We also indicate that studies combining multiple common bio-tracers may come to more robust conclusions about the fate of aquaculture waste within the surrounding system, than those only using one bio tracer. Finally, studies that incorporate more than one species will be able to determine structural

changes in the food web associated with aquaculture waste assimilation and how the consumption of this feed subsidy may have physiological consequences to the fish population. In the study that follows, we evaluate the use of a combination of bio-tracers (i.e. stable isotopes and fatty acids) to identify the pathways of assimilation of an anthropogenic resource provided by cage aquaculture feed in a freshwater environment and give insight on the effects of aquaculture originated organic matter on native organisms and the associated potential ecological implications.

The results of this study show that stable isotopes and fatty acids are a useful tool in tracing the pathway of an anthropogenic resource through a freshwater food web. We are able to trace the asymmetric uptake of an anthropogenic resource within a freshwater system by incorporating more than one species and more than one trophic group. By linking aquaculture research with ecological implications future research will grasp a more holistic view of the effects of aquaculture and the subsidy provided by waste. This asymmetric uptake may have implications for food web dynamics and stability. Furthermore the assimilation of this anthropogenic resource may help to address environmental concerns of anthropogenic subsidies and effects on organism health.

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