TRACKING SEA LICE, LEPEOPHTHEIRUS SALMONIS, BETWEEN HOST FISH POPULATIONS USING STABLE CARBON AND NITROGEN ISOTOPES

by

SUSAN DEAN

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ABSTRACT

This study was undertaken to assess the use of carbon and nitrogen stable isotope analyses as methods to identify source host fish populations of sea lice, *Lepeophtheirus salmonis*. The delta carbon signatures of parasitic copepodid sea lice used to infect cultured Atlantic salmon in the laboratory were found to be statistically indistinguishable from the delta carbon signatures of blood (ANOVA, $p = 1.000$) and mucus (ANOVA, $p = 0.430$) sampled from the wild pink salmon population from which the sea lice originated. As a result, delta carbon signatures show potential as tools to track the movement of sea lice between fish populations. In contrast, delta nitrogen analysis did not show such promise as the natal host fish, wild pink salmon, and novel host fish, cultured Atlantic salmon, between which sea lice were transferred during the study, did not display distinct isotope signatures and thus could not be differentiated. This study found that when applying stable isotope analysis as a method to studying the dispersal of sea lice (1) the blood and mucus of potential source host fish populations should be sampled when assessing their possible relationship to sampled sea lice, (2) sea lice in no later than the parasitic copepodid stage should be analysed, (3) sea lice should be given time to clear their guts of any host materials before analysis takes place, (4) sea lice sampled from the gills should not be not grouped with sea lice sampled from the body surface or fins, and (5) replication unit for sampled parasitic copepodids should be set at the individual and not host fish level. The application of stable carbon isotopes to tracking the movement of sea lice between host fish is a promising method for directly identifying sources of sea lice epizootics and of quantifying the exchange of sea lice between host fish populations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER 1: GENERAL INTRODUCTION

The sea louse, or salmon louse, *Lepeophtheirus salmonis*, is currently garnering substantial attention in both the public and scientific arenas. This ectoparasitic copepod of wild and farmed fish (Kabata, 1979, Wootten et al., 1982) has historically been present on wild fish in numbers too scant to cause severe pathological effects (White, 1940, Boxshall, 1974, Wootten et al., 1982, Nagasawa, 1987, Berland, 1993). However, in almost all countries with major sea farming operations, heavy wild sea lice epizootics have now taken place (Anon, 1997). Consequently, it has been suggested that farm operations serve as amplification reservoirs for indigenous sea lice (McVicar, 1997). Although correlations in the location and timing of farmed and wild epizootics have been identified (Bjorn et al., 2001, Morton and Williams, 2003), no direct relationship has been established displaying a transfer of sea lice between cultured and wild fish (Bjorn et al., 2001, Tully and Nolan, 2002, Butterworth et al., 2004). Development of a technique to track sea lice movement between hosts would allow for the identification of sea lice epizootic sources and facilitate the development of strategies to mitigate the effects of sea lice infection. Stable isotope analysis presents such a technique.

This literature review will discuss (1) the life history of *L. salmonis*, (2) factors influencing the infection of host fish, (3) the physiological and behavioural impacts of *L. salmonis* on host fish, (4) susceptibility of fish farms to epizootics, (5) current examples of suspected transmission of sea lice from farmed to wild stocks, (6) methods employed for tracking larvae in situ, (7) principles behind carbon and nitrogen stable isotope analyses, and (8) the application of stable isotope analysis to tracking sea lice between hosts.

The Sea Louse, Lepeophtheirus salmonis

The sea louse, *L. salmonis*, is an ectoparasitic, caligid copepod commonly found on both farmed and wild salmon in the northern hemisphere (Kabata, 1979, Wootten et al., 1982). Host fish include Atlantic salmon (*Salmo salar*), Chum salmon (*Oncorhynchus keta*), pink salmon (*Oncorhynchus gorbuscha*), Coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), Sockeye salmon (*Oncorhynchus nerka*), Chinook salmon (*Oncorhynchus tshawytscha*), coastal cutthroat trout (*Oncorhynchus clarki*), and brook trout (*Salvelinus fontinalis*) (Kabata, 1979, Wootten et al., 1982). *L. salmonis* have also been found on non-salmonid hosts, including sand lance (*Ammodytes hexapterus*), white sturgeon (*Acipenser transmontanus*), and saithe (*Pollachius virens*) (Kabata, 1973, Bruno and Stone, 1990). More recently, three-spine sticklebacks (*Gasterosteus aculeatus*) have also been recognized as important, and potentially only temporary, hosts (Jones et al., 2006).

The life history of sea lice is not solely comprised of a parasitic phase. During Nauplius I, the first free-swimming life stage, nauplii emerge from eggs produced by female sea lice and subsequently mature through the nauplius II and free-swimming copepodid stages, all of which comprise a planktonic phase (Johnson and Albright, 1991a). During this time, the sea lice are lecithotrophic and obtain their energy for growth and development from internal energy reserves, in the form of lipids, formed during embryogenesis (Kunz, 1985, Tucker et al., 2000). The planktonic phase can last between four days and two weeks (Johnson and Albright, 1991b). Following the free-swimming copepodid stage, sea lice become infective and attach to host fish, thereby entering the parasitic phase (Johnson and Albright, 1991a). Sea lice then mature through four chalimus stages, during which time they are affixed to their host fish by a frontal filament and cannot move (Grimnes and Jakobsen,

1996). This is followed by two pre-adult stages and one adult stage in which the sea lice are mobile on their host (Johnson and Albright, 1991a). These mobile lice are capable of moving between fish but most often remain on their initial host (Bruno and Stone, 1990, Todd et al., 1997, Murray, 2002, Jones et al., 2006). During attachment, both mobile and immobile parasitic lice consume host mucus, skin, and/or blood (Kabata, 1979, 1988). The development time for lice is heavily dependent on temperature and can last between three to seventeen weeks (Tully, 1989, 1992). At 10°C, development from egg to adult takes approximately 40 days and 52 days for males and females, respectively (Johnson and Albright, 1991b).

Infection of Host Fish

A number of factors have been found to increase the susceptibility of fish to infection by sea lice. These include proximity to lice sources, including the amount of time spent in coastal waters (Johnson et al., 1996, Tully et al., 1999), stress induced by fresh to saltwater transition (Dawson et al., 1999), unfavourable environmental conditions, such as low dissolved oxygen levels, high water temperatures, or toxic plankton blooms (Johnson et al., 1996), current infection with parasites (Wootten et al., 1982), and sexual maturation (Johnson et al., 1996). Host species also plays an important role in sea lice infection dynamics as both the probability and persistence of infection are influenced by different skin structures and general behaviours, such as swimming speed and depth distribution, of host species (Nagasawa et al., 1991). In particular, Atlantic salmon have been found to be more susceptible to infection with *L. salmonis* while coho salmon have been observed with relatively lower numbers of attached lice (Johnson and Albright, 1992). Factors influencing sea lice settlement behaviour also influence infection rates. Salinity, light, and host surface

area have all been found to impact attachment of the parasites to host fish (Genna et al., 2005).

Host Response to Lice Infection

Newly parasitic sea lice typically attach to the fins and gills of host fish. They often have a negligible effect on fish health during these early stages of development due to their small size and scattered distribution (Pike, 1989, Bron et al., 1991). In contrast, detrimental impacts resulting from sea lice infection frequently take place following maturation to the mobile pre-adult and adult stages (Grimnes and Jakobsen, 1996, Johnson et al., 1996, Bjorn and Finstad, 1997, Finstad et al., 2000). During these stages, sea lice often move to the head, the area between the dorsal and adipose fins, the peri-anal region, or the external operculum as these areas are characterized by thin skin and minimal scale cover, thus allowing easy access to blood and flesh for consumption (White, 1940, Wootten et al., 1982, Bron et al., 1991, Jaworski and Holm, 1992, Jónsdóttir et al., 1992). This movement, the resulting concentrated distribution of the sea lice, and the larger size of the pre-adult and adult stages have been found to induce the greatest impact on host fish.

This impact can be separated into two distinct categories, the first being direct damage to epithelial surfaces from sea lice attachment and feeding and the second being indirect damage associated with a prolonged stress response (Nolen et al., 1999). The former is characterized by skin irritation, skin erosion, and haemorrhaging, the consequences of which include increased host susceptibility to ultraviolet damage as well as secondary infection, osmoregulatory failure, and anaemia due to the dilution and leakage of blood components (Brandal et al., 1976, Brandal and Egidius, 1979, Wootten et al., 1982, McArdle and Bullock, 1987, Grimnes and Jakobsen, 1996, Wagner and McKinely, 2004). The latter

category involves a chronic stress response that no longer aids fish in overcoming the stress associated with sea lice infection and that may, instead, further compromise fish health (Wendelaar Bonga, 1997). Nolan et al. (1999) found that infection of post-smolt Atlantic salmon with a relatively low level of sea lice (n=10/fish) induced a chronic stress response in which the integrity of fish skin and gill epithelia were compromised thereby disturbing osmotic regulation and increasing host susceptibility to secondary infection.

Behavioural changes caused by the combined effect of both the direct and indirect impacts of sea lice infection can bring about additional adverse effects. Wagner et al. (2003) found swimming ability of infected Atlantic salmon was impaired due to compromised osmotic functioning and hypothesised that reduced swimming ability could lower survival rates by disabling the capacity of fish to forage for food and escape from predators. Dawson et al. (1999) found infection with sea lice disrupted the dominance hierarchy of Atlantic salmon in the laboratory as dominant fish no longer consumed the greatest proportion of daily meal. This shift in feeding dominance was interpreted as development of an instable social structure within the group. Birkeland and Jakobsen (1997) observed post-smolt sea trout with heavy loads of sea lice prematurely return to streams in an effort to remove freshwater-intolerant lice. This behaviour is of particular concern because fish then spend less time developing energy stores at sea, thus lowering health and, potentially, reproductive success (Berg et al., 1986, Tully et al., 1993, Whelan, 1993, Johnson et al., 1996, Bjorn et al., 2001).

Ultimately, mortality in host fish can be caused by the direct effect of sea louse attachment and feeding and the indirect effect of a chronic whole-body stress response (Wootten et al., 1982, Grimnes and Jakobsen, 1996, Morton and Williams, 2003, Bjorn et al.,

2001). Yet, it is important to note sea lice are a natural component of functioning ecosystems and do not always reduce fish health or contribute to population decline (Kabata, 1979, 1988, McVicar, 1997). Wagner et al. (2003) demonstrated that low infection with sea lice (0.2 sea lice/gram of host fish) did not affect the overall health of fish and Dawson et al. (1999) found that infected Atlantic salmon were able to recover from damage caused by sea lice, even with parasites still attached. Indeed, the severity of a stressor, such as sea lice infection, is dependent on a number of factors and no uniform reaction to parasite infection exists (Murray, 2002). Such factors include, but are not limited to, fish gender, water temperature and quality, season, fish physiological condition, fish species and strain, fish age, and inherited or acquired individual characteristics of host fish (Wendelaar Bonga, 1997). As a result, defining when innocuous, everyday events escalate to the level of potentially fatal stressors is difficult, if not impossible.

Sea Lice in Aquaculture Production

Although there is no threshold rate of infection that defines when mortality in hosts will certainly occur, relatively high infection levels of 30 to 350 sea lice per host fish can increase the likelihood of host mortality (Grimnes and Jakobsen, 1996). If not carefully controlled, the conditions under which farmed fish are housed and handled may facilitate the establishment of sea lice epizootics within aquaculture operations. Firstly, the practices of grading, transport, and routine maintenance can further strain the health of penned fish already stressed by their intensive farming environment (Barton and Iwama, 1991). As discussed earlier, stress within fish can lead to immunosupression thereby resulting in an increased susceptibility to disease, including a lowered resistance to sea lice (Nolan et al., 1999). Secondly, it is suggested the time of seawater residence is positively correlated with

sea lice infection levels (Johnson et al., 1996, Morton and Williams, 2003). Sea farms represent an over-wintering site for sea lice in coastal waters (Morton and Williams, 2003) by providing ample, year round hosts on which sea lice may grow and reproduce. This is in contrast to wild fish, which are most often present in less dense aggregations and which return to freshwater to spawn, thereby, providing only temporary and less intense habitats for parasitic sea lice. Lastly, as noted earlier, swimming speed affects the ability of sea lice to infect hosts. It is suggested the slower swimming speeds of cultured fish facilitate lice attachment and infection (Dawson et al., 1999). All of these factors contribute to the belief that fish farms are capable of producing sea lice at levels sufficient to impact fish health.

Transfer of Sea Lice between Farmed and Wild Populations

Relative to background levels, greater numbers of planktonic lice have been found proximate to farming operations in Ireland (Costelloe et al., 1996), Scotland (Penston et al., 2004), and Canada (Morton et al., 2004), raising concern that these infection levels are then transferred from farmed to wild host populations in numbers sufficient to cause disease and mortality. Wild sea lice epidemics have been found to occur around the same time as those on nearby farmed sites (MacKinnon, 1997, Pike and Wadsworth, 1999) and fish near farming operations have been observed with significantly higher loads of lice than those in areas with little farming activity in Scotland (Mackenzie et al., 1998), Norway (Bjorn et al., 2001), Ireland (Birkeland and Jakobsen, 1997, Tully et al., 1999), and Canada (Morton and Williams, 2003, Morton et al., 2004). Yet, to date no direct relationship has been established displaying a transfer of sea lice between farmed and wild hosts. This may be attributed to the nature of the free-swimming phase in the life history of sea lice. It is believed sea lice are most commonly transferred between hosts through the release of free-swimming progeny and

not through the movement of adults. Tracking the transfer of lice between fish, therefore, necessitates following individuals in the plankton through the nauplius I, nauplius II, and copepodid stages. This presents a considerable methodological challenge as during these stages sea lice can disperse over large distances, dilute rapidly following release into coastal waters, and suffer high rates of mortality. In addition, during the free-swimming stages sea lice are nearly microscopic and are difficult to morphologically classify by species.

Tracking of Larvae

Several tagging methods have been employed to track larval dispersal in situ, including vital or fluorescent tissue stains, calcium carbonate markers, radioactive labels, and genetic markers (reviewed by Levin, 1990). Staining can be advantageous because there are a variety of stains to choose from, costs are relatively low, and the marking of large numbers of individuals can be relatively easy. However, stains can be toxic, not retained in recovered animals due to fading, and too obvious to potential predators thus increasing predation risk (Levin, 1990). In addition, the detection of stained individuals can be labour intensive in the laboratory and subject to error (Levin, 1990). Calcium carbonate markers, which influence calcium carbonate deposition and involve chelation to or replacement of consumer calcium (Levin, 1990), are not applicable to tracking sea lice larvae since these larvae do not have calcium carbonate structures that persist throughout their life history. Radioactive elements may be incorporated into the tissues of animals and are easily detected, but may not be retained indefinitely, represent environmental contaminants, and are often dangerous to use (Levin, 1990).

Several promising approaches to tracking sea lice involve the use of genetic markers (Hobson, 1999), including allozyme analysis, random amplification of polymorphic DNA

(RAPD) analysis, and microsatellite polymerase chain reaction (PCR) typing (Todd et al., 1997, Nolen et al., 2000). These techniques conveniently negate the need for both artificial tags and the recapture of individuals as all sea lice are innately marked with genetic information. Furthermore, the use of genetic markers is well suited to the small size of sea lice as most of these methods only require minimal amounts of genetic material (Nolen et al., 2000). However, a number of drawbacks are associated with the application of genetic markers. A lack of genetic differentiation between geographically connected populations can hinder identification of specific sea lice epizootic sources. Such genetic homogeneity is often attributed to the dispersal of sea lice during the planktonic phase, the mobility of migratory host fish, and the ability of adult sea lice to move between hosts (Todd et al., 1997, Nolen et al., 2000).

Each of the aforementioned genetic techniques also has their own unique drawbacks. Microsatellite typing requires initial research on the development of microsatellite-PCR assays and template DNA preparation methods suitable to the individual parasites under study. Identification of useful PCR primer sets in sea lice has proven difficult and has therefore limited the effective application of this technique (Nolen et al., 2000). Allozyme analysis does not display a random sample of the genome and is frequently hindered by a lack of polymorphism during quantitative analyses (Todd et al., 1997). RAPD analysis does provide a random sample of the genome and is more effective in genetically differentiating between populations; however, it is currently incapable of providing direct evidence regarding epizootic sources because sea lice originating from wild fish have been found to exhibit low background frequencies of RAPD markers associated with the identification of farmed sea lice. This similarity in wild and farmed genetic markers is primarily attributed to

the fact that farmed and wild sea lice in the same geographic areas often have the same genetic origins because all farm sites are initially infected with sea lice originating from wild populations. As a result, it is impossible to definitively state whether RAPD markers are indicators of the transfer of sea lice from farmed to wild populations or whether they are, instead, reflective of alleles associated with the wild sea lice populations originally transferred to the farmed sites. Therefore, only probabilistic statements regarding the likelihood of the origins of sea lice can often be made using RAPD analysis.

In summary, it is very difficult to recapture and identify artificially marked individuals in plankton samples, thus, the application of mark-recapture techniques to studying the movements of artificially tagged larvae in the plankton is discouraged (Levin, 1990). The use of genetic markers in tracking sea lice between host populations is promising yet no method currently exists to efficiently and directly identify sources of sea lice epizootics.

Stable Carbon and Nitrogen Isotope Analysis

Stable isotope analysis has been used to overcome many of the issues associated with tracking individuals using markers and has been used to study small animals (Dauby, 1995) and to track animals where directly following them is not possible (Primavera, 1996). While previously a tool of the earth sciences, the production of advanced, user-friendly analytical equipment has facilitated the application of stable isotope analysis to a broader range of research areas, including the atmospheric sciences, physiology, and ecology (Ehleringer and Rundel, 1989, Hobson and Wassenaar, 1999). This approach involves measuring the relative abundances of isotopes within samples, which are then expressed with the differential notation:

$$\delta X_{std} = \left(\frac{R_{sample} - R_{std}}{R_{std}} \right) \times 1000$$

where X is the stable isotope of interest, such as carbon or nitrogen, and δX_{std} is the ratio of heavy to light isotopes in delta units relative to a standard. R_{sample} and R_{std} are the absolute isotope ratios of the sample and the standard, respectively (Ehleringer and Rundel, 1989). Values in delta notation are expressed in parts per thousand (‰) to amplify relatively minor differences between stable isotope signatures. The use of standards overcomes the problem of day to day electronic fluctuations within equipment thus facilitating high precision and repeatability over the short and long terms and allowing values from different isotope facilities to be compared (Fry and Sherr, 1984, Ehleringer and Rundel, 1989). The standards for nitrogen and carbon stable isotope analyses are atmospheric air and carbonate or organic materials referenced to PeeDee Belemnite (PDB), respectively (Fry and Sherr, 1989).

Application of stable isotope analysis is based on the premise the isotopic composition of a consumer closely resembles the mean isotopic composition of its diet (Focken and Becker, 1998). Therefore, if an organism has a single food source, its isotopic composition should reflect the value of that food source. Isotopes of carbon and nitrogen have commonly been used because they are naturally occurring and are assimilated into consumers with comparatively predictable changes to their relative abundances (Peterson and Fry, 1987). This change between consumer and diet is termed "trophic shift" and is the process by which animals typically become enriched in heavier isotopes (e.g. ^{13}C and ^{15}N) relative to their diet because the lighter isotopes (e.g. ^{12}C and ^{14}N) are preferred in metabolic process such as excretion and respiration (Hobson and Clark, 1992, Focken and Becker, 1998). Trophic shift for stable carbon isotopes is typically 0-1‰ per trophic level, which is relatively small (DeNiro and Epstein, 1978). As a result, delta carbon signatures are often

used to identify primary food sources of animals (Vander Zanden et al., 1997, March and Pringle, 2003). In contrast, nitrogen trophic transfer typically results in a 2-5‰ increase, or enrichment, in the delta nitrogen signatures of consumers relative to their diets (Minagawa and Wada, 1984). Consequently, the $^{15}N/^{14}N$ ratio is most frequently used to determine trophic position of consumers because organisms with higher $^{15}N/^{14}N$ ratios are assumed to be relatively higher in the food chain (Vander Zanden et al., 1997).

Whole organisms on the same diet and tissues within a single organism often display significantly different isotope signatures as lipid content influences stable carbon signature (Focken and Becker, 1998) and non-essential amino acid content influences stable nitrogen signature (Gaebler et al., 1966, DeNiro and Epstein, 1978, 1981). Lipids are reduced in the heavier ^{13}C isotope relative to other components, such as proteins and carbohydrates, due to the preferential uptake of ^{12}C during lipid synthesis from glucose (DeNiro and Epstein, 1977). As a result, tissues and organisms with relatively greater lipid contents display lighter, more negative delta carbon signatures. Lipid content has no effect on the stable nitrogen signatures of tissues which are, instead, influenced by ratios of essential to non-essential amino acids (Pinnegar and Polunin, 1999). Essential amino acids are not synthesized by animals and are incorporated directly from the diet without isotopic fractionation (DeNiro and Epstein, 1978). In contrast, synthesis or partial modification of non-essential amino acids results in the removal of ^{14}N enriched amine groups and the preferential retention of ^{15}N (Gaebler et al., 1966, Macko et al., 1986). Consequently, animals and individual tissues enriched in non-essential amino acids tend to have relatively higher delta nitrogen signatures (Pinnegar and Polunin, 1999). Another factor which may be considered in the creation of isotopically distinct tissues is the process of isotopic routing (Schwarcz, 1991). Diets of free-range and

wild animals are often heterogeneous, therefore, tissues synthesized at different times may be made from materials with different isotope signatures. As a result, both the composition of tissues and the food ingested and assimilated during their creation determine their final isotopic signature.

Several factors influence the host-parasite isotopic relationship. The balance between feeding and excretion rates, in association with absorption efficiency, plays a large role in mediating trophic step fractionation because it is largely during these processes that fractionation occurs (Gannes et al., 1998, Ponsard and Averbuch, 1999). In addition, the host-parasite isotopic relationship is affected by preferential assimilation of more labile compounds within ingested host materials, consumption of host tissues enriched in lipids such as blood and muscle, changes in metabolism such as that experienced during maturation, reproduction, and starvation, and/or changes in host habitat, life history stage, and feeding (Pinnegar et al., 2001, Deudero et al., 2002, Olive et al., 2003). Although parasites have often been assumed to comply with the predator-prey paradigm in which consumers are consistently enriched in the heavier stable isotopes of ^{15}N and ^{13}C (Power and Klein, 2004), this is not always the case. Parasites can be depleted in the heavier isotopes relative to their hosts (Boag et al., 1998, Pinnegar et al., 2001) and even parasites of the same species can be either isotopically enriched or depleted relative to their hosts due to the consumption of different tissues during separate stages in their life history, such as with the copepod *L. branchialis* (Deudero et al., 2002). However, it must be noted that endoparasites, and not ectoparasites, typically break from expected enrichment patterns (Pinnegar et al., 2001, Olive et al., 2003, Langellotto et al., 2005). This is because endoparasites are often able to absorb amino acids directly through their body surface, thereby avoiding metabolic fractionation and

alteration of the $^{15}N/^{14}N$ ratio, and reuse their own excreted nitrogen, thus lowering their net delta nitrogen signature as excreted nitrogen is characteristically depleted in ^{15}N (Howell, 1976, Pinnegar et al., 2001).

The combined application of both carbon and nitrogen stable isotope analysis reduces the uncertainty associated with using a single isotope tracer (Petersen et al., 1985). Although stable carbon isotope ratios are not greatly influenced by trophic transfer, they can, as noted above, be influenced by the lipid content of an organism or tissue. As a result, stable carbon isotope ratios can vary within a single organism that has body parts with different lipid contents or between individuals on the same diet that have different lipid levels. Stable nitrogen ratios are less variable with regards to the biochemical makeup of tissues (DeNiro and Epstein, 1981, Minagawa and Wada, 1984), however, as noted earlier, they may be influenced by the ratio of essential to nonessential amino acids (Pinnegar and Polunin, 1999). It is possible to analyse single samples for both carbon and nitrogen stable isotopes due to advances in analytical techniques (Preston and Owens, 1983). As a result, nitrogen and carbon stable isotope ratios have been used in concert to investigate the isotopic properties of many aquatic ecosystems (Marcogliese and Cone, 1997).

Tracking Sea Lice Using Stable Isotope Analysis

The trophic relationship between sea lice and their hosts is an ideal case in which to apply stable isotope analysis (Doucett et al., 1999). As sea lice typically parasitize one host fish in their lifetime, the application of complex mixing models in order to differentiate between several food sources is not necessary (Langellotto et al., 2005). It may be noted three-spine sticklebacks have recently been identified as potential temporary hosts for *L. salmonis* (Jones et al., 2006). This finding does not compromise the potential use of stable

isotope analysis in tracking sea lice as, although capable of detaching from host fish in several stages, the parasites most often remain attached to host fish once settled. Furthermore, sea lice in the chalimus stages are non-motile and cannot physically detach from their hosts (Johnson and Albright, 1991a). Butterworth et al. (2004) found that adult lice collected from various farmed populations could be differentiated from one another as well as from lice collected from wild populations using nitrogen isotope ratios alone, demonstrating the potential application of stable isotope analysis for tracking sea lice between host populations. Essentially, if the unique signatures of host fish imparted onto adult sea lice are then passed on to sea lice offspring then all lice progeny would be inherently tagged with natal isotope signatures reflecting their source population. All sea lice progeny would then effectively act as indicators of their source population for as long as their natal isotope signature was maintained. Given this strict criteria for the application of this approach, the objectives of this study were to examine the longevity of natal isotope signatures in sea lice offspring and to assess the applicability of using stable isotope analysis in distinguishing origins of parasite infestations.

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CHAPTER 2: TRACKING SEA LICE, LEPEOPHTHEIRUS SALMONIS, BETWEEN HOST FISH POPULATIONS USING STABLE CARBON AND NITROGEN ISOTOPES

A version of this chapter will be submitted for publication.

INTRODUCTION

The sea louse, *Lepeophtheirus salmonis*, is an ectoparasitic, caligid copepod commonly found on both farmed and wild salmon in the northern hemisphere (Kabata, 1979, 1988). The life history of this species includes a free-swimming phase, comprised of the nauplius I, nauplius II and copepodid stages, followed by a parasitic phase, comprised of four chalimus stages, two pre-adult stages, and one adult stage (Johnson and Albright, 1991a). Development from egg to adult takes about 40 days and 52 days at 10°C for males and females, respectively (Johnson and Albright, 1991b).

Throughout the planktonic phase sea lice do not consume external food sources and obtain energy for growth and development from internal energy reserves formed during embryogenesis (Kunz, 1985, Tucker et al., 2000). During the parasitic phase, however, sea lice consume host fish mucus, skin, and/or blood, at which time they can cause considerable disease when abundant on host fish (Kabata, 1979, 1988, Grimnes and Jakobsen, 1996, Morton and Williams, 2003). Such epizootic levels are not unusual in salmon farming operations (Wooten et al., 1982) but few cases were noted on wild fish until recently (Johnson and Albright, 1992). High levels of infection on wild populations have now been recorded in almost all countries with established large-scale salmon aquaculture industries (Anon, 1997). This has raised questions regarding the origins of sea lice parasitizing farmed stocks, whether farms act as amplification reservoirs for indigenous sea lice, and especially whether sea lice are transferred from farm operations to wild stocks at levels sufficient to cause disease and mortality.

Wild sea lice epidemics have been observed to occur about the same time as those on nearby farmed sites (MacKinnon, 1997, Pike and Wadsworth, 1999) and fish raised in

experimental study sites exposed to farming operations have been found to display significantly higher loads of lice than those in areas with little farming activity (Bjorn et al., 2001). Yet, there is no direct evidence for the transfer of sea lice between farmed and wild hosts, which is usually attributed to the nature of the free-swimming developmental stages. It is believed sea lice are most commonly transferred between hosts through the release of free-swimming progeny and not through the movement of adults (Murray, 2002, Jones et al., 2006). Tracking the transfer of lice between fish, therefore, necessitates following individuals in the plankton through the nauplius I, nauplius II, and free-swimming copepodid stages. This presents a considerable methodological challenge since these sea lice stages are small, can potentially disperse large distances, are present in high numbers, and suffer high rates of mortality. Under these circumstances, directly tracking or recapturing tagged individuals is extremely challenging.

Stable isotope analysis using delta carbon and delta nitrogen signatures presents an effective and efficient approach to tracking sea lice between hosts. These elemental signatures are imparted onto the parasites during consumption of host mucus, skin and/or blood and can be likened to unique fingerprints that reflect each parasite's host population. Butterworth et al. (2004) found adult lice collected from farmed and wild populations could be differentiated from one another using delta nitrogen signatures. If these unique signatures are imparted to and retained by free-swimming offspring, all sea lice progeny could be inherently marked with natal stable isotopic signatures and could in turn act as indicators of their source populations. The objectives of this study were to examine the longevity of natal isotope signatures in second generation sea lice and their

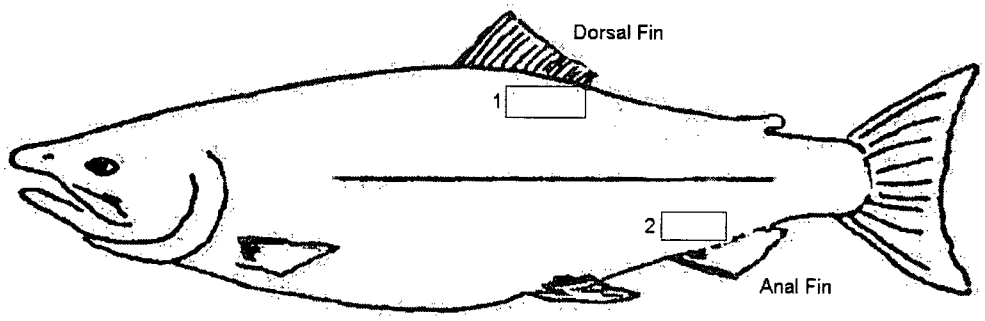
applicability in distinguishing the origins of sea lice infestations in populations of cultured and wild salmon.

METHODS

Sample Collection

In September of 2005, seven wild adult pink salmon parasitized by the sea louse *L. salmonis* were sampled in Fife Sound (n=1), Tribune Channel (n=1), Knight Inlet (n=2), Sutlej Channel (n=1), and Georgia Strait (n=2) on the west coast of British Columbia, Canada. Mucus, blood, skin, muscle, fin, and gill samples were sampled from each fish for stable carbon and nitrogen isotope analysis. Blood was taken posterior to the anus and mucus was sampled from both sides of the body. Muscle tissue was removed from near the anal fin and separate skin samples were taken from near the dorsal (skin1) and anal (skin2) fins, respectively (Fig. 2.1a). The dorsal, caudal, and pectoral fins, as well as the gills from the left side of the body, were removed for analysis using scissors. Gravid female sea lice were removed from each of the seven host fish using forceps and their location was noted according to six general areas (Fig. 2.1b).

a.



b.

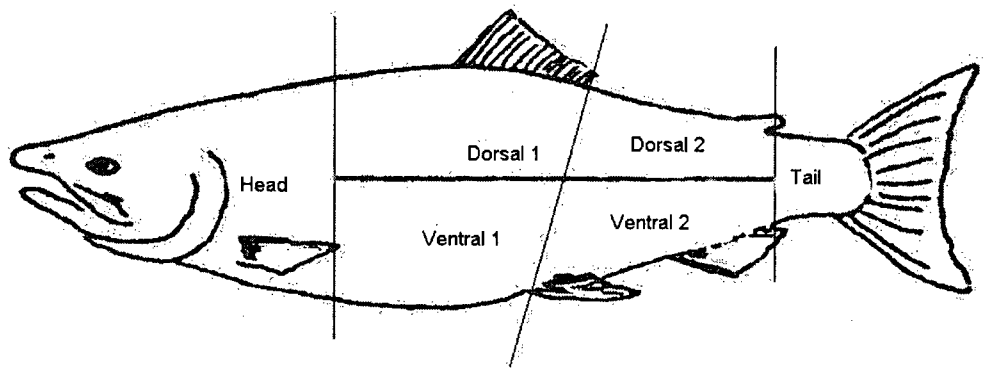


Fig. 2.1 (a) Sampling locations of skin samples from Atlantic and pink salmon from near the dorsal fin and skin and muscle samples from near the anal fin (b) Five divisions of pink salmon examined for adult sea lice.

The distal portions of egg strands from gravid sea lice were removed using scissors and placed in jars of sea water, leaving the proximate portions of the egg strands still attached to the adult lice. All host fish samples and adult sea lice with proximate egg strands still attached were stored on ice for the duration of the four day trip and during transport back to the laboratory. On arrival to the laboratory, host fish tissues and adult lice samples, with partial egg strands still attached, were stored at -80°C. The detached egg strands were haphazardly separated into three groups and placed in individual aerated tanks with flow-through, filtered, ambient seawater. One week was allotted for growth from hatching to the parasitic copepodid stage to maximize the number of lice maturing to the parasitic copepodid stage while minimizing loss of infectivity of the copepodids as they aged (Tucker et al., 2000). The free-swimming lice were sampled daily for the first two days and every second day for the remaining four days to allow assessment of potential changes in the stable carbon and nitrogen isotope signatures of the planktonic sea lice over time. During this period, water oxygen content and temperature were monitored with an Oxyguard Handy Gamma Oxygen meter and salinity was monitored

with a Vee Gee STX-3 refractometer (temperature 12.6°C, SE ± 0.1; oxygen 8.5 ppm, SE ± 0.0; salinity 31.0, SE ± 0.0).

Subsequently, approximately 7,000 of the newly infective copepodids were introduced to cultured juvenile Atlantic salmon (average weight 107.8 g, SE ± 1.7); average length 22.4 cm, SE ± 0.3). This was done to assess whether parasitic sea lice adopted the isotopic signatures of their new hosts and, if so, on what time scale. The sea lice were transferred from wild pink salmon to pellet-fed Atlantic salmon to emulate the transfer of lice between farmed and wild fish in the field. The infection level was roughly 2 lice per gram of host fish (230 lice per L or 10 lice per cm length of host fish). Infection took place over 8 hours in 30 L of salt water at 12.2°C (SE ± 0.1), 31.0 (SE ± 0.0) salinity, and 9.5 ppm O^2 (SE ± 1.0). Pure oxygen was periodically bubbled into the water as it was previously observed turbulence from a continuous flow of air inhibited settlement of copepodid sea lice. Oxygen levels were not allowed to fall below 7.0 ppm O^2. In addition, no water was exchanged during infection in order to prevent loss of infective copepodids. Low water temperatures were, instead, maintained by placing the infection bath within a larger tank of flowing, ambient seawater.

Host fish were haphazardly moved using a dipnet from the infection bath into one of three replicate tanks following the infection treatment. Sampling of host fish tissues and attached sea lice took place immediately following infection (i.e. Time = 0 hours), 12 hours post-infection (pi), 36 hours pi, and 2, 3, 4, 6, 8, 10, and 12 days pi. Host fish tissues were sampled for two purposes, the first was to test that isotopic change within the parasitic sea lice was due to processes at the lice level and not due to fluctuations in the isotopic composition of the salmon feed. The second purpose was to investigate the

stability of host fish tissues over time to assess whether natal isotope signatures retained by sea lice can be effectively compared to the isotopic signatures of their natal host fish population. During each sampling, a total of three fish, one from each tank, were removed using a dipnet and euthanized. Blood, mucus, skin from near the dorsal and anal fins, muscle, fin, and gill tissues were sampled from cultured Atlantic salmon as described above for the wild pink salmon, with exception that both sets of gills were removed for analysis. These samples were pooled due to their small size. Attached sea lice were removed using forceps and their position on the body, gills, or fins was noted. Subsequently, all lice were viewed under 4X magnification to confirm that they were living at the time of sampling, thus ensuring they had the opportunity to consume host material until their removal. Movement of appendages and/or body contractions within a 2 minute period were used to indicate live sea lice. Those failing to demonstrate movement were discarded. Sea lice from the gills, fins, and body were then refrigerated at 4°C to allow for the digestion and excretion of any host material in the gut. Following refrigeration, the sea lice were frozen at -80°C until preparation for stable isotope analysis.

In addition, samples of sea lice with full guts were collected from the gills. These samples were frozen immediately following their removal from host fish at -80°C. Sea lice with empty and full guts were sampled to examine the effect of gut content on the isotopic signatures of parasitic sea lice. In summary, parasitic sea lice were collected from a total of 30 fish during 10 sampling periods over 12 days. Water temperature (11.3°C, SE ± 0.0), oxygen content (7.5 ppm, SE ± 0.0), and salinity (32.0, SE ± 0.0) were monitored as described for planktonic sea lice rearing.

Sample Preparation

Host fish muscle, gill, skin, and fin samples were rinsed with distilled water to remove host mucus. In addition, skin and fin samples were rasped with a scalpel and scrubbed with a small plastic brush, respectively, to remove scales. Fin tissues were then cut from attached cartilaginous material using a dissecting scope and scalpel. All tissue samples were placed in individual scintillation vials and dried at 60°C (Power and Klein, 2004) for 60 hours. Following drying, muscle, gill, and blood samples were ground using a mortar and pestle. Fin and skin samples, due to their small size, were crushed using the tip of a stainless steel probe. All tissues except mucus were then placed in 8X5-mm tin capsules for analysis. Mucus samples were rehydrated using distilled water. Scale fragments remained attached to the scintillation vials during this process thus allowing pure mucus to be separated from the scales. The pure mucus was then pipetted into 8X5-mm tin capsules and dried at 60°C for 60 hours. Dry weights of tissue samples from the wild pink salmon and cultured Atlantic salmon were recorded (Table 2.1).

Table 2.1 Dry weights of body samples from pink salmon (N=7) and Atlantic salmon (N=30) (SE values in brackets).

Sample Type	Pink Salmon Dry Weights (mg)	Atlantic Salmon Dry Weights (mg)
Gills	1.1 (± 0.1)	0.9 (± 0.0)
Blood	1.0 (± 0.0)	1.1 (± 0.0)
Skin 1 (from near dorsal fin)	1.0 (± 0.0)	1.1 (± 0.0)
Skin 2 (From near anal fin)	1.0 (± 0.0)	1.1 (± 0.0)
Muscle	1.0 (± 0.0)	1.1 (± 0.0)
Fin	0.7 (± 0.1)	0.4 (± 0.0)
Mucus	1.3 (± 0.1)	1.6 (± 0.1)

Planktonic and second generation sea lice were rinsed in distilled water and staged according to Johnson and Albright (1991a) under 4X magnification. The basal plates at the tips of sea lice frontal filaments were removed as these structures are not

sheathed and replaced during growth and may, therefore, mask changes in the isotopic signatures of sea lice over time (Gonzalez-Alanis et al., 2001). Sea lice were then placed in tin capsules according to their stage, position on host fish, and gut content. Gravid female lice, with proximate egg strands still attached, were rinsed in distilled water to remove host mucus. Remaining eggs were then detached using scissors, placed in tin capsules, and dried at 60°C for 60 hours, resulting in an average dry weight of 0.5 mg (SE ± 0.1). Adult lice were placed in 20-ml scintillation vials and dried at 60°C (Power and Klein, 2004) for 60 hours. Each adult louse was then ground individually using a mortar and pestle and placed in a tin capsule (average dry weight 0.9 mg, SE ± 0.0). Samples of the feed consumed by the cultured Atlantic salmon were ground using a mortar and pestle and dried at 60°C for 60 hours.

Sample Analyses

All host fish and lice samples were analysed at the UC Davis Stable isotope Facility and all feed samples were analysed at the Pacific Centre for Isotope and Geochemical Research (PCIGR) at the University of British Columbia using methods described in Butterworth et al. (2004).

Statistical Analysis

Statistical analyses were performed using SPSS (version 14.0, SPSS Inc.). Values greater than 2 standard deviations from the mean were considered extreme and were removed from both the delta carbon and delta nitrogen data sets (Langellotto et al., 2005). Alpha was set at 0.05 for all analyses and non-parametric tests (i.e. Kruskal-Wallis) were applied in lieu of parametric tests when normality assumptions could not be met. Carbon

and nitrogen data were tested separately and were never statically compared to one another.

Isotopic differences among tissues from within the wild adult pink salmon were assessed using a one-way analysis of variance (ANOVA). Tukey's *post-hoc* test was applied following significant ANOVA results. Isotopic differences between tissues from within the cultured Atlantic salmon and between the tissues and the Atlantic salmon feed were assessed using a one-way ANOVA. Both the delta nitrogen and delta carbon data sets were inverse transformed during statistical analyses to attain a normal distribution. Due to uneven sample sizes and heterogeneous variances between sample groups, Games-Howell's *post-hoc* test was applied following significant ANOVA results (Field, 2005).

Isotopic signatures of tissues within the wild and cultured salmon were compared using one-way ANOVA. These were followed by Games-Howell's *post-hoc* tests, again, due to uneven sample size and heterogeneous variances between tissue groups. The natural logarithms of the delta nitrogen values were used to attain normal distribution (Field, 2005).

Potential changes over time in the stable carbon isotope signatures of tissues within the Atlantic salmon were assessed using one-way ANOVA for blood, skin, and gill tissues and, because statistical assumptions of normality could not be met with any transformations, non-parametric Kruskal-Wallis tests for mucus, muscle, and fin tissues. The effect of time on the stable nitrogen isotope signatures of tissues within the Atlantic salmon was assessed using one-way ANOVA for all tissues.

The relevance of host fish as replication units for adult sea lice was assessed using a one-way ANOVA for the delta carbon data and a non-parametric Kruskal-Wallis test for the delta nitrogen data. The delta carbon values were inverse transformed to meet the normality assumption of the ANOVA (Field, 2005). Replication unit for egg strand delta carbon and delta nitrogen signatures was assessed using non-parametric Kruskal-Wallis tests. The relevance of host fish as replication units for the second generation parasitic lice was assessed using three way ANOVA for both delta carbon and delta nitrogen. The main factors were host fish, sea lice position on host fish, and sea lice stage. The delta nitrogen data were square transformed to meet the normality assumption of the ANOVA (Field, 2005).

The effect of gut content (i.e. empty or full) on the delta carbon signatures of parasitic copepodid and chalimus III sea lice was investigated using independent two-tailed T-tests. A non-parametric Mann-Whitney test was used to analyse the delta carbon signatures of early chalimus sea lice as the data residuals were not normally distributed. The effect of gut content on the delta nitrogen signatures of parasitic copepodid, early chalimus, and chalimus III sea lice was assessed using two-tailed, independent T-tests.

The effect of sea lice attachment site (i.e. fins, body, or gills) on the delta carbon and delta nitrogen signatures of sea lice was assessed, when possible, in the parasitic copepodid, early chalimus, and chalimus III stages. In the case of delta carbon analysis, insufficient samples were obtained from the body surface in the parasitic copepodid stage so an independent, two-tailed T-test was used to compare sea lice from the fins and gills only. In the case of delta nitrogen analysis, the effect of host fish attachment site on the isotopic signatures of parasitic copepodids could not be assessed because too few values

were available for all attachment sites in this stage. As a result, the effect of sea lice attachment site on delta nitrogen signature was only assessed in the early chalimus and chalimus III stages. Furthermore, too few samples were available from the fins in the early chalimus stages to allow analysis so a two-tailed independent T-test was used to compare sea lice from the body and gills only. Sufficient sample sizes were collected from each attachment site (i.e. fins, body, and gills) in the chalimus III stage so a one-way ANOVA was used to assess the effect of position on delta nitrogen signature in the chalimus III stage. Tukey's *post-hoc* test was employed when significant ANOVA results for delta carbon and delta nitrogen analyses were observed.

The isotopic signatures of sea lice from the adult stage to the chalimus III stage of offspring were compared to those of the wild pink salmon and cultured Atlantic salmon host tissues using one-way ANOVA. Significant ANOVA results were followed by Games-Howell's *post-hoc* tests as sample sizes and variances were different between samples (Field, 2005).

RESULTS

Stable Isotope Analyses of Wild and Cultured Host Fish Tissues

Delta carbon signatures of tissues within wild adult pink salmon are displayed in Figure 2.2. Significant differences were found between tissues (ANOVA, p = 0.000) (Table 2.2a). Skin1 and skin2 had significantly higher delta carbon values than all other

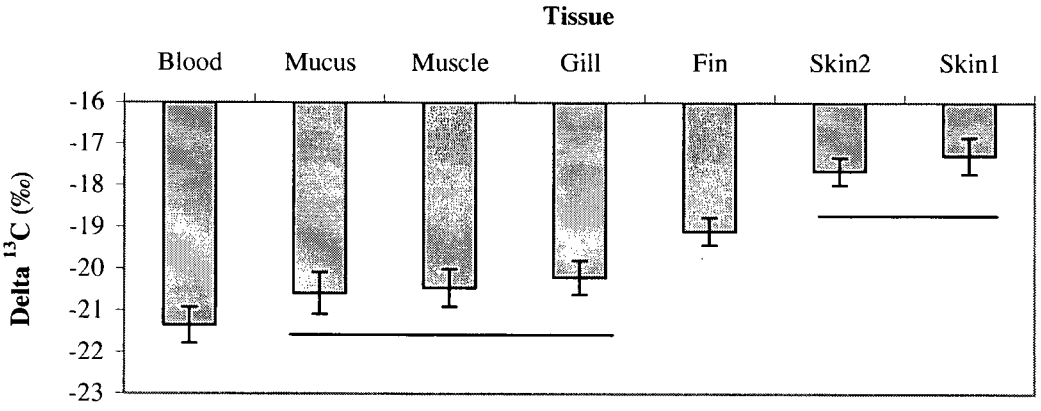


Fig. 2.2 Average delta carbon signatures of tissues within wild adult pink salmon. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

Table 2.2a P-values from statistical analyses (α = 0.05) of delta carbon signatures of tissues within wild pink salmon (Tukey's *post hoc* results in grey, top right) and cultured Atlantic salmon (Games-Howell's *post hoc* results in black, bottom left) (statistically significant differences are bolded).

	BLOOD	MUCUS	SKIN1	SKIN2	MUSCLE	GILLS	FINS	FEED
BLOOD		0.037	0.000	0.000	0.009	0.001	0.000	-
MUCUS	0.000		0.000	0.000	**0.998**	**0.739**	0.000	-
SKIN1	0.000	0.000		**0.701**	0.000	0.000	0.000	-
SKIN2	0.000	0.000	0.000		0.000	0.000	0.000	-
MUSCLE	0.000	**0.315**	0.000	0.000		**0.949**	0.000	-
GILLS	0.008	0.004	0.000	0.000	0.000		0.001	-
FINS	0.000	**1.000**	0.000	0.000	**0.097**	0.000		-
FEED	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table 2.2b P-values from statistical analyses ($\alpha = 0.05$) of delta nitrogen signatures of tissues within wild pink salmon (Tukey's *post hoc* results in grey, top right) and cultured Atlantic salmon (Games-Howell's *post hoc* results in black, bottom left) (statistically significant differences are bolded).

	BLOOD	MUCUS	SKIN1	SKIN2	MUSCLE	GILLS	FINS	FEED
BLOOD		1.000	1.000	0.999	0.970	0.999	0.048	-
MUCUS	0.006		0.999	1.000	0.900	1.000	0.090	-
SKIN1	0.000	0.003		0.993	0.989	0.997	0.032	-
SKIN2	0.000	0.000	**0.999**		0.790	1.000	0.152	-
MUSCLE	0.000	0.002	**1.000**	**1.000**		0.845	0.004	-
GILLS	**1.000**	0.004	0.000	0.000	0.000		0.167	-
FINS	0.001	**0.980**	**0.135**	0.024	**0.108**	0.001		-
FEED	0.001	0.000	0.010	0.002	0.000	0.001	0.000	

tissues tested but did not differ from one another (Tukey, $p = 0.701$). Values for blood and fins were statistically significantly different from all other groups. Mucus was significantly different from all tissues but muscle (Tukey, $p = 0.998$) and the gills (Tukey, $p = 0.739$), which were also statistically indistinguishable from one another (Tukey, $p = 0.949$). Overall, blood had the lowest delta carbon signatures and skin1 and skin2 had the highest.

Delta nitrogen signatures of tissues within wild adult pink salmon are displayed in Figure 2.3. A significant difference was found between the tissues (ANOVA, $p = 0.012$), however, in general, *post-hoc* comparisons between tissues did not reveal many significant differences (Table 2.2b). Specifically, blood, skin1, and muscle were significantly different from the fins (Tukey, $p = 0.048$, $p = 0.032$, $p = 0.004$, respectively) but no other differences between the delta nitrogen signatures of tissues in wild adult pink salmon were found.

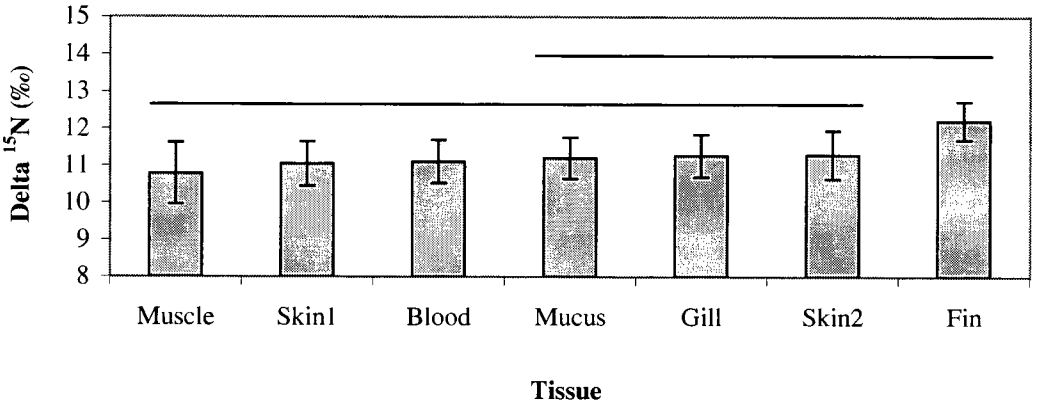


Fig. 2.3 Average delta nitrogen signatures of tissues within wild adult pink salmon. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

Delta carbon signatures of tissues within cultured juvenile Atlantic salmon, the second set of hosts, are displayed in Figure 2.4. Once again, tissues were found to have significantly different delta carbon signatures (ANOVA, p = 0.000) (Table 2.2a, above). The only tissues that did not differ significantly were the fins and mucus (Games-Howell, p = 1.000), fins and muscle (Games-Howell, p = 0.097), and muscle and mucus (Games-Howell, p = 0.315). All other tissue comparisons yielded significant results. As with the pink salmon, blood had the lowest average delta carbon signature and skin1and skin2 had

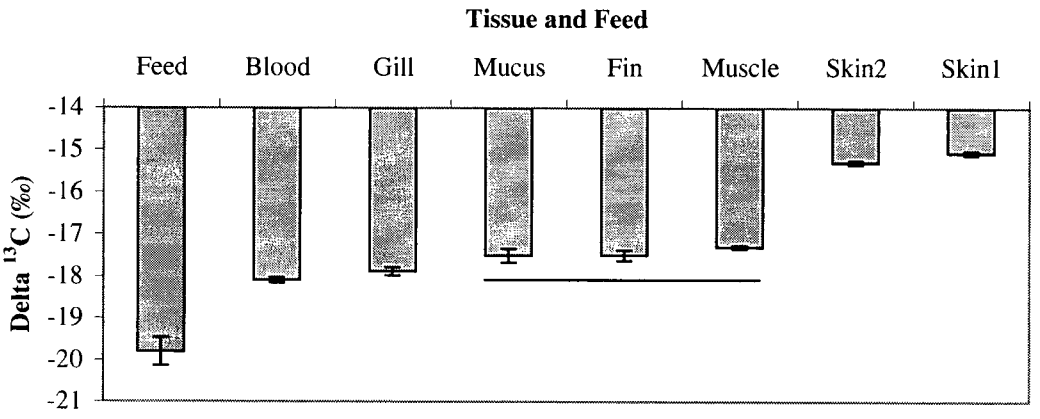


Fig. 2.4 Average delta carbon signatures of tissues within cultured juvenile Atlantic salmon and feed. Error bars represent 95% CI. Connected horizontal line denotes values that are not statistically significantly different.

the highest. The confidence intervals were considerably smaller for the cultured as opposed to the wild fish. All cultured Atlantic salmon tissues were enriched in the heavier ^{13}C isotope relative to the feed and the trophic shift between the tissues and the feed was larger than the 0-1‰ enrichment reported by DeNiro and Epstein (1978). It may be noted that differences between delta carbon signatures of tissues were not the same for the cultured and wild fish (see Figs. 2.2 and 2.4).

Delta nitrogen signatures of tissues within cultured juvenile Atlantic salmon are displayed in Figure 2.5. Once again, confidence intervals for the cultured fish were noticeably smaller than those of the wild fish. Like all previous within-fish analyses, significant differences were found between the delta nitrogen signatures of tissues (ANOVA, p= 0.000) (Table 2.2b). The gills and blood were not significantly different

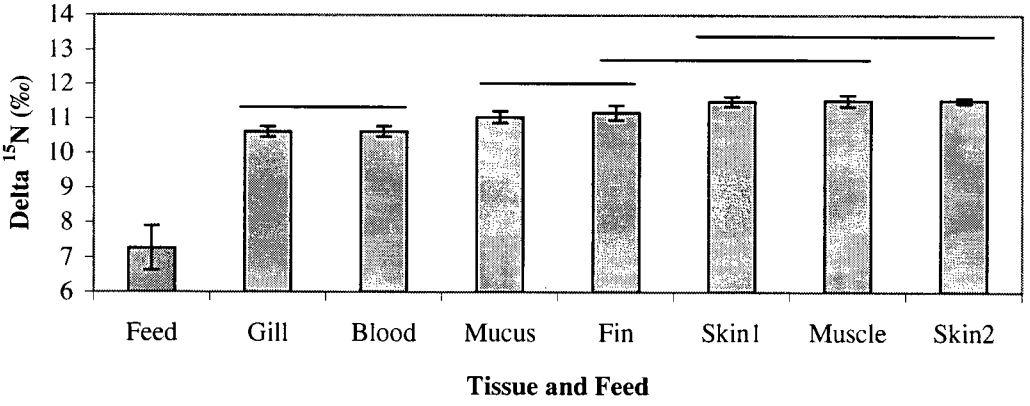


Fig. 2.5 Average delta nitrogen signatures of tissues within cultured juvenile Atlantic salmon and feed. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

from each other but were significantly different from all other tissues (Games-Howell, p = 1.000) and mucus was significantly different from all tissues but the fins (Games-Howell, p = 0.980). The fins were also not significantly different from skin1 and muscle (Games-Howell, p = 0.135, p = 0.108, respectively) and skin1 and skin2 could not be

distinguished from one another (Games-Howell, $p = 0.999$). In addition, neither skin1 nor skin2 significantly differed from muscle (Games-Howell, $p = 1.000$, $p = 1.000$). All other tissue comparisons were significant. This is unlike the delta nitrogen results for the wild fish in which significant differences between tissues were less prevalent.

The delta carbon and delta nitrogen signatures of tissues within wild adult pink salmon and cultured juvenile Atlantic salmon were compared to assess whether transfer from the wild to the cultured hosts would provide newly settled parasitic sea lice with novel stable isotope signatures. Introduction to food with new stable isotope ratios facilitates the study of tissue turnover in newly settled lice because the rate at which sea lice body tissues convert from the initial to the new isotope signatures can then be measured. The investigation of tissue turnover in sea lice provides an understanding of the longevity of sea lice natal isotope signatures and allows assessment of the practicality of using stable isotopes to track sea lice between host salmon populations. Significant differences were found between delta carbon signatures of tissues within the two host fish populations (ANOVA, $p = 0.000$) (see Figs. 2.2 and 2.4). The delta carbon signatures of the wild host gills, blood, mucus, muscle, and fins were significantly different from all tissues within the cultured Atlantic salmon (Games-Howell, $p < 0.006$). In contrast, the delta carbon signatures of skin1 and skin2 from the wild hosts were significantly different from only the delta carbon signatures of skin1 and skin2 from the cultured Atlantic salmon (Games-Howell, $p < 0.002$). Unlike with the delta carbon signatures, the delta nitrogen signatures of tissues within the host populations rarely differed from one another (see Figs. 2.3 and 2.5). Only the delta nitrogen signature of the wild pink salmon fins

varied significantly from any cultured tissues (Games-Howell, cultured blood p = 0.002, cultured mucus p = 0.019, cultured gill p = 0.003, cultured fin = 0.038).

No significant differences were found over time for the delta carbon signatures of blood (ANOVA, p = 0.052), mucus (non-parametric Kruskal-Wallis, p = 0.895), skin1 (ANOVA, p = 0.855), skin2 (ANOVA, p = 0.166), and muscle (non-parametric Kruskal-Wallis, p = 0.198) (Figure 2.6). ANOVA results for the gills did display significant

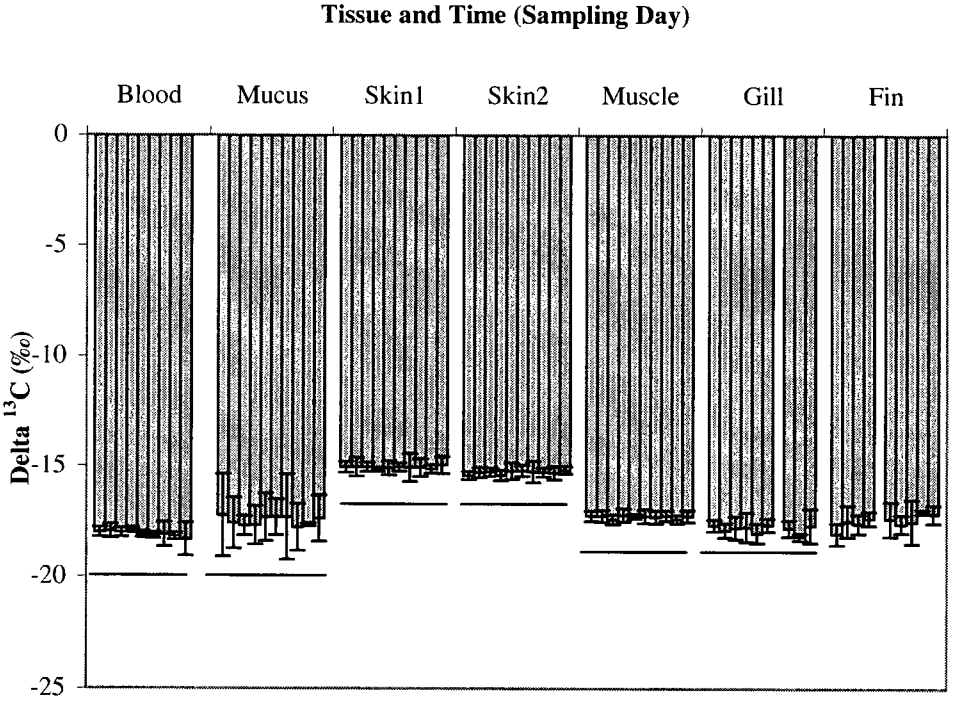


Fig. 2.6 Delta carbon signatures of tissues within cultured juvenile Atlantic salmon over a period of 12 days. Error bars represent 95% CI. Tissues were sampled on 10 separate days, as represented by the 10 columns for each tissue. From the left to right, the columns display sampling on day 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and day 12. No fin samples were available on day 3. Connected horizontal lines denote values that are not statistically significantly different.

statistical results (ANOVA, p = 0.035), however, Tukey's *post-hoc* test displayed no significant differences over time. The delta carbon signatures of the fins (non-parametric Kruskal-Wallis, p = 0.022) were found to change over time. This was likely due to small

sample size and sampling error associated with the difficulty of separating fin tissues from attached cartilaginous material. It may be noted the confidence intervals for mucus, fin, and gill tissues were generally larger than those for the other tissues, perhaps further illustrating the sampling difficulty associated with these tissues.

Delta nitrogen signatures of tissues within the cultured juvenile Atlantic salmon over the same 12 day period are shown in Figure 2.7. No statistical differences over time were found for mucus (ANOVA, $p = 0.176$), skin1 (ANOVA, $p = 0.253$), skin2 (ANOVA, $p = 0.113$), and the gills (ANOVA, $p = 0.061$). Significant differences over time were found for the fins (ANOVA, $p = 0.009$), blood (ANOVA, $p = 0.004$), and muscle (ANOVA, $p = 0.002$).

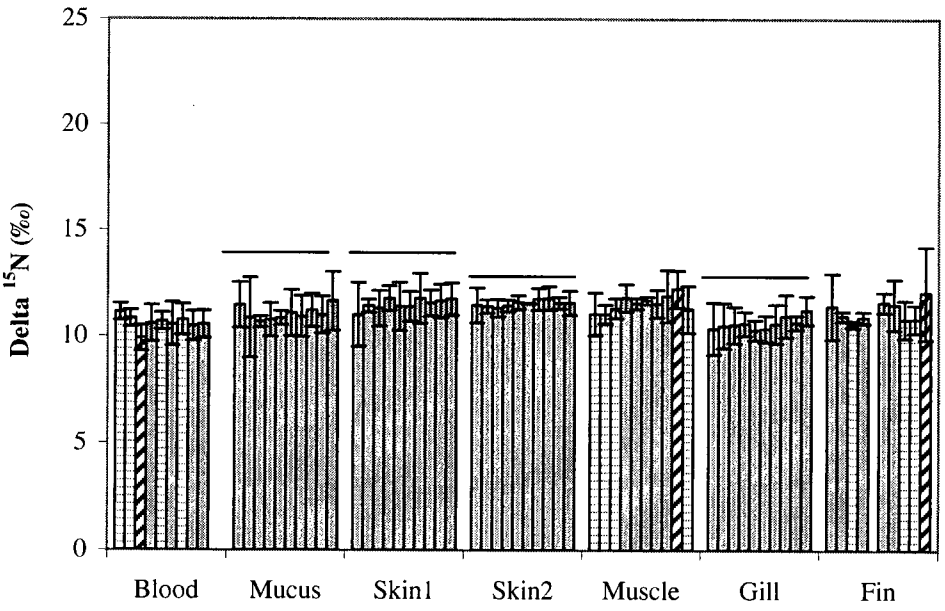


Fig. 2.7 Delta nitrogen signatures of tissues within cultured juvenile Atlantic salmon over a period of 12 days. Error bars represent 95% CI. Tissues were sampled on 10 separate days, as represented by the 10 columns for each tissue. From the left to right, the columns display sampling on day 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and day 12. The days marked by the light horizontal bars were significantly different from the days marked by the dark diagonal bars within each tissue group. No blood samples were available on sample day 12 and no fin samples were available on day 3. Connected horizontal lines denote values that are not statistically significantly different.

Stable Isotope Analyses of Sea Lice

The relevance of host fish as replication units for adult sea lice, egg strands, and second generation parasitic sea lice was investigated to assess whether delta carbon and delta nitrogen signatures of sea lice and egg strands are determined at the host or individual level. As displayed in Figures 2.8 and 2.9, it was found host fish had a

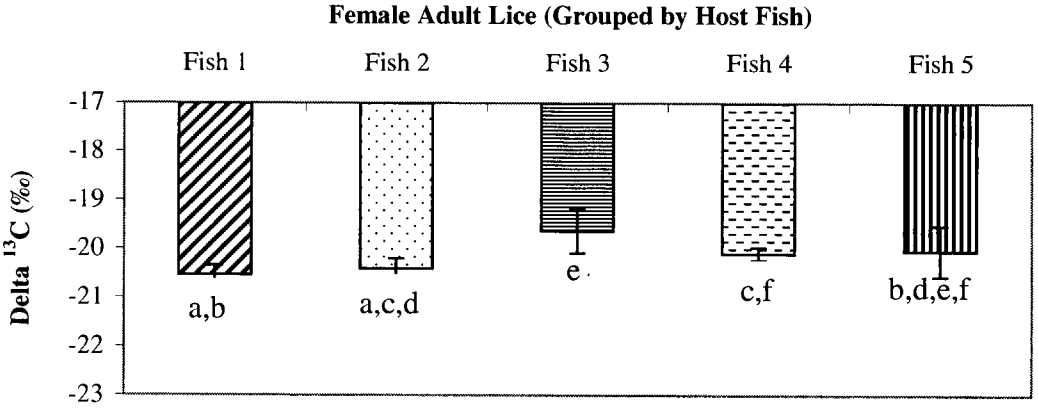


Fig. 2.8 Effect of wild host fish on the delta carbon signatures of female adult lice sampled from the ventral 2 position. Error bars represent 95% CI. Letters denote values that are not statistically significantly different.

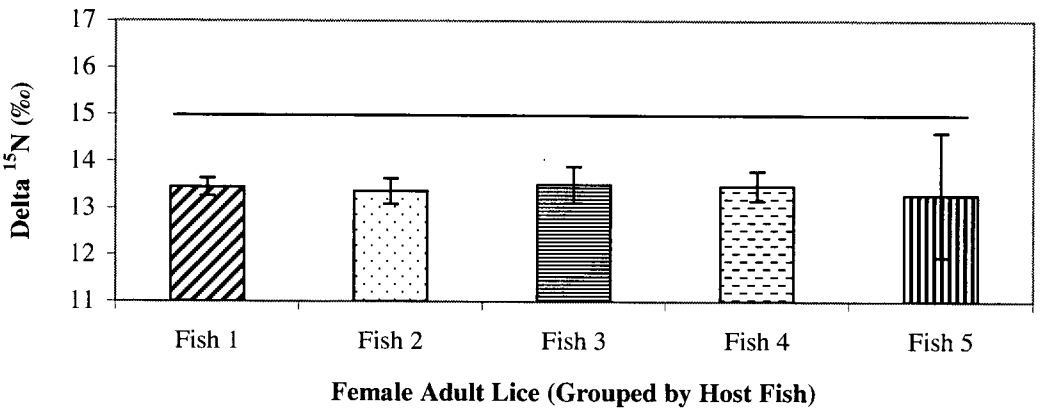


Fig. 2.9 Effect of wild host fish on the delta nitrogen signatures of female adult lice sampled from the ventral 2 position. Error bars represent 95% CI. Connected horizontal line denotes values that are not statistically significantly different.

significant effect on the delta carbon (ANOVA, $p < 0.000$) but not the delta nitrogen (non-parametric Kruskal-Wallis, $p = 0.956$) signatures of gravid female sea lice, respectively. It was found wild host fish did not have a significant effect on the delta carbon and delta nitrogen signatures of egg strands (non-parametric Kruskal-Wallis, $p = 0.103$; ANOVA, $p = 0.177$, respectively), as shown in Figures 2.10 and 2.11. In addition,

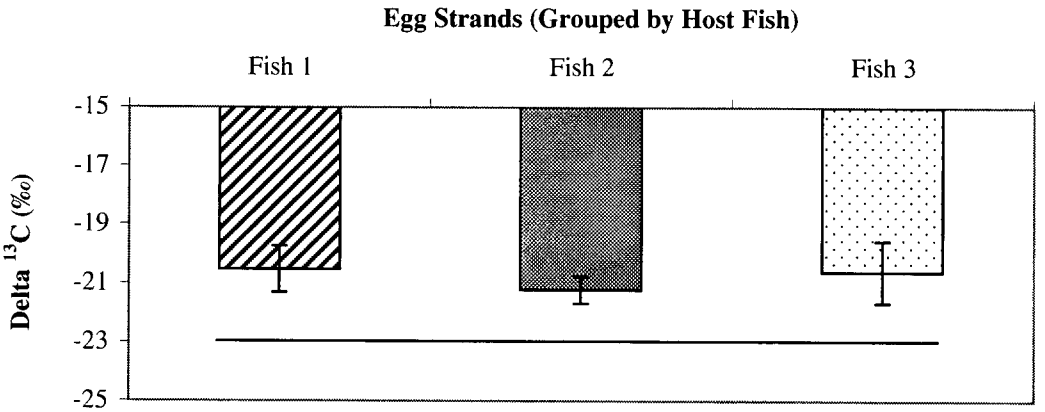


Fig. 2.10 Effect of wild host fish on the delta carbon signatures of egg strands. Error bars represent 95% CI. Connected horizontal line denotes values that are not statistically significantly different.

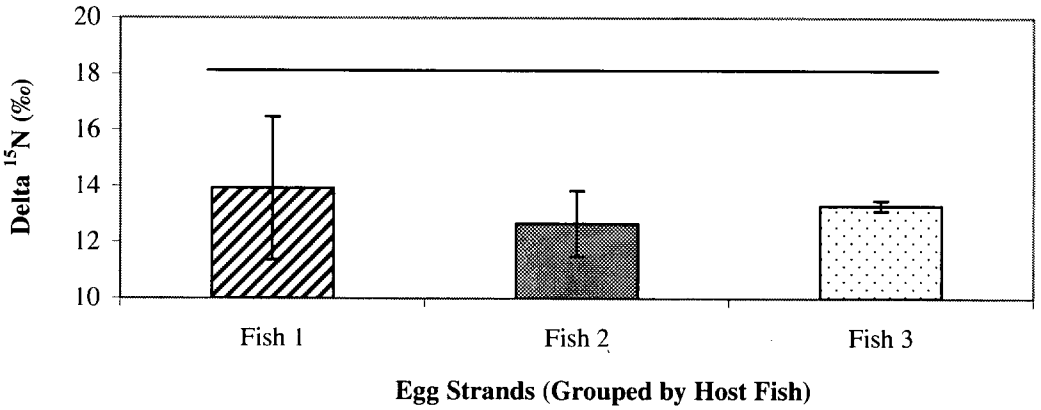


Fig. 2.11 Effect of wild host fish on the delta nitrogen signatures of egg strands. Error bars represent 95% CI. Connected horizontal line denotes values that are not statistically significantly different.

there was a non-significant main effect of cultured host fish on the delta carbon and delta nitrogen signatures of second generation parasitic sea lice (Univariate GLM, p = 0.301, p = 0.612, respectively) (Figs. 2.12 and 2.13). No significant interaction was found between host and position nor host and stage in the delta carbon (Univariate GLM, host vs position interaction, p = 0.694, host vs stage interaction, p = 0.575) and delta nitrogen (Univariate GLM, host vs position interaction p = 0.736, host vs stage interaction p = 0.518) three-way ANOVA analyses.

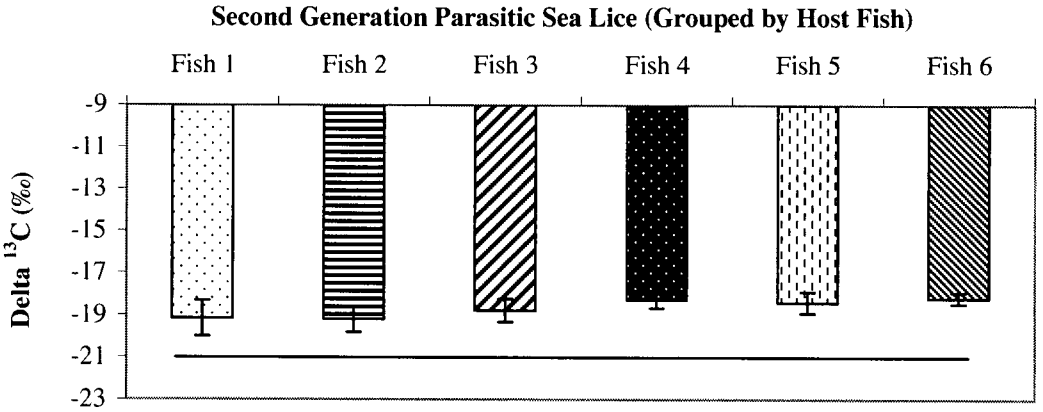


Fig. 2.12 Effect of host fish on the delta carbon signatures of second generation parasitic sea lice. Error bars represent 95% CI. Connected horizontal line denotes values that are not statistically significantly different.

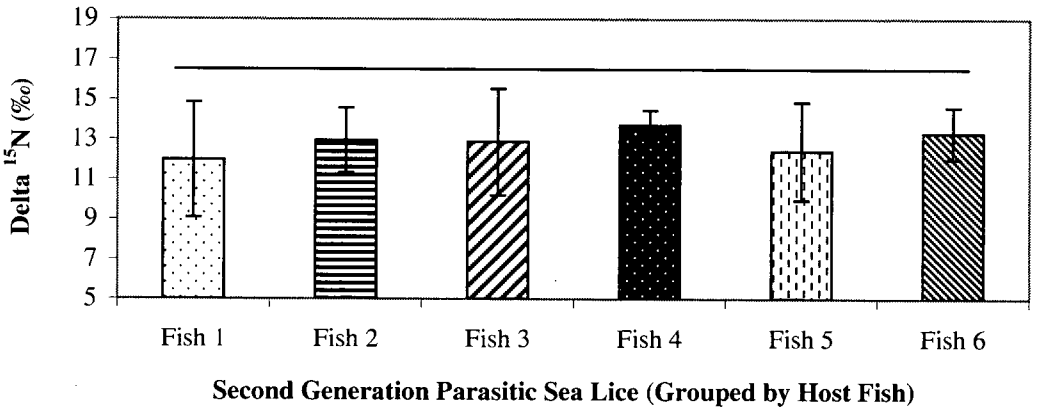


Fig. 2.13 Effect of host fish on the delta nitrogen signatures of second generation parasitic sea lice. Error bars represent 95% CI. Data were square transformed for statistical analysis. Connected horizontal line denotes values that are not statistically significantly different.

Gut content was found to have a significant effect on the delta carbon signatures of parasitic copepodids (Independent two-tailed T-test, $p < 0.000$) but not on the delta carbon signatures of sea lice in the early chalimus (Mann-Whitney, $p = 0.223$) or chalimus III (Independent two-tailed T-test, $p = 0.152$) stages (Fig. 2.14). As shown in Figure 2.15, gut content was not found to have a significant effect on the delta nitrogen signatures of sea lice in the parasitic copepodid and early chalimus stages (Independent two-tailed T-test, $p = 0.270$, $p = 0.068$, respectively) but was found to have a significant effect on the chalimus III stage (Independent two-tailed T-test, $p = 0.039$).

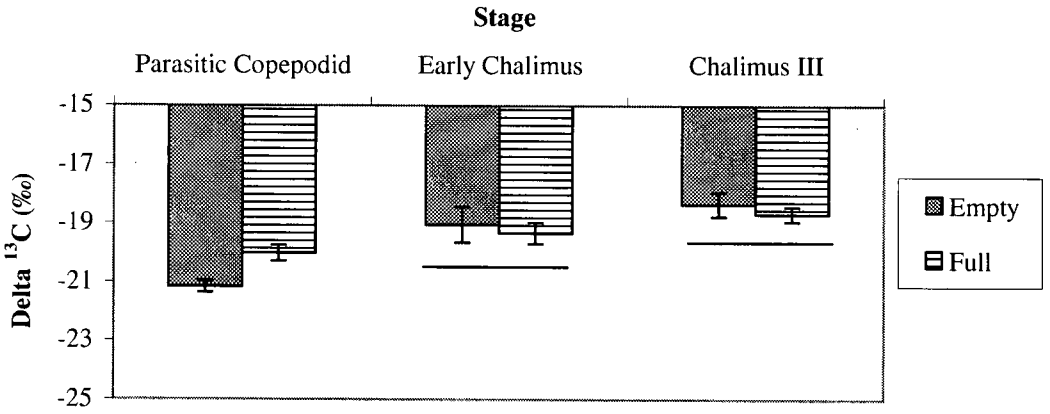


Fig. 2.14 Effect of gut content on the delta carbon signatures of gill lice in the parasitic copepodid, early chalimus, and chalimus III stages. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

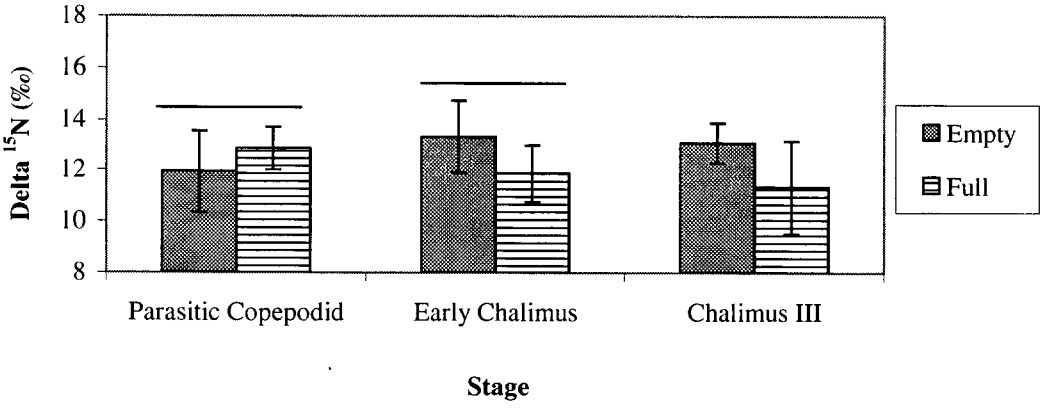


Fig. 2.15 Effect of gut content on the delta nitrogen signatures of gill lice in the parasitic copepodid, early chalimus, and chalimus III stages. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

The effect of sea lice position on host fish was studied to assess the validity of grouping sea lice from the body, fins, and gills during sample collection and analysis. It was found location on host fish had no significant effect on the delta carbon signatures of lice in the parasitic copepodid, early chalimus, and chalimus III stages (Independent two-tailed T-test, $p = 0.698$, ANOVA, $p = 0.714$, $p = 0.658$, respectively) (Fig. 2.16). Likewise, position on host fish had no effect on the delta nitrogen signatures of early chalimus sea lice (Independent two-tailed T-test, $p = 0.0910$ (Fig. 2.17). However, body location was found to have a significant effect on the delta nitrogen signatures of chalimus III sea lice (ANOVA, $p = 0.008$); delta nitrogen signatures of sea lice from the gills were found to be significantly different from those of sea lice from the body surface and fins (Tukey, $p = 0.009$, $p = 0.042$, respectively).

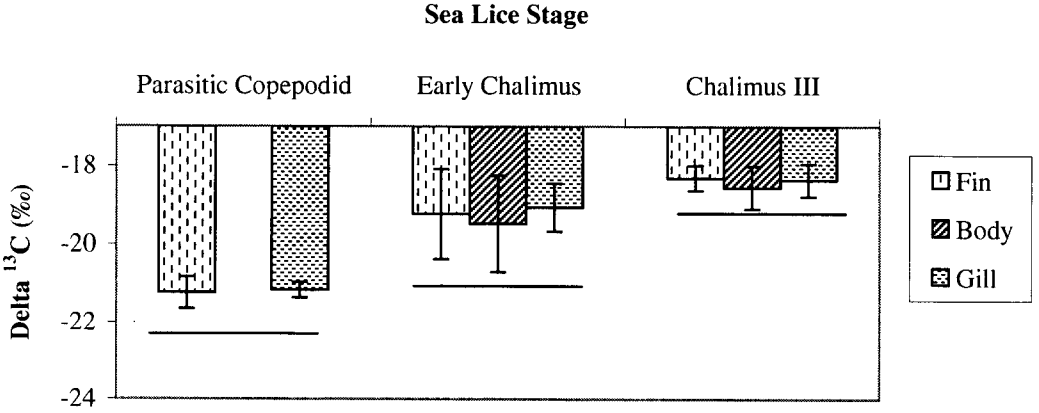


Fig. 2.16 Effect of position on the delta carbon signatures of sea lice in the parasitic copepodid, early chalimus, and chalimus III stages. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

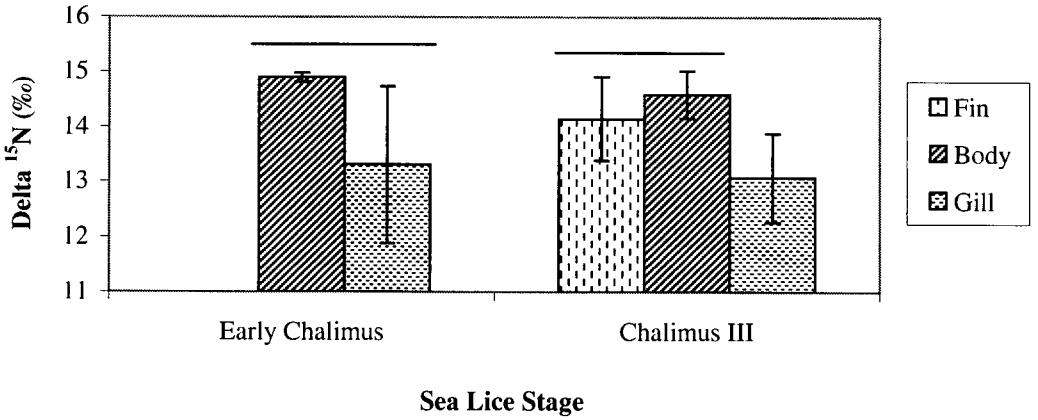


Fig. 2.17 Effect of position on the delta nitrogen signatures of sea lice in the early chalimus and chalimus III stages. Error bars represent 95% CI. Connected horizontal lines denote values that are not statistically significantly different.

Changes in the isotopic composition of adult sea lice to second generation chalimus III sea lice were observed relative to the host fish populations to assess the longevity of natal isotope signatures in sea lice progeny. Refer to Table 2.3 for the p-values from statistical comparisons between the delta carbon signatures of sea lice and the two host populations. A steady decline in sea lice delta carbon signatures was

Table 2.3 Test results where p > 0.000 from comparisons between the delta carbon signatures of sea lice stages and those of tissues of wild and cultured host fish (Games-Howell, 95% CI). P-values for each comparison are displayed in the cell where the respective row and column of given tissues intersect. Bolded p-values indicate significant results.

↓ Increasing Delta Carbon Signature	Host Fish Tissue/ Sea Lice Stage	Adult	Egg Strand	Nauplius	Planktonic Copepodid	Parasitic Copepodid	Early Chalimus	Chalimus III
	Wild Blood	0.009	**0.860**	**1.000**	**0.992**	**1.000**	0.004	0.000
	Wild Mucus	**0.854**	**1.000**	**0.160**	**0.071**	**0.430**	**0.182**	0.000
	Cultured Fish Feed	**0.299**	**0.976**	0.002	0.000	0.029	**0.115**	0.000
	Wild Muscle	**0.966**	**0.994**	0.047	0.020	0.162	**0.261**	0.000
	Wild Gill	**1.000**	**0.599**	0.007	0.004	0.023	**0.568**	0.000
	Wild Fin	0.013	0.000	0.000	0.000	0.000	**1.00**	**0.267**
	Cultured Blood	0.000	0.000	0.000	0.000	0.000	**0.080**	**0.698**
	Cultured Gill	0.000	0.000	0.000	0.000	0.000	0.032	**0.113**
	Wild Skin2	0.000	0.000	0.000	0.000	0.000	0.012	**0.069**
	Cultured Mucus	0.000	0.000	0.000	0.000	0.000	0.006	0.001
	Cultured Fin	0.000	0.000	0.000	0.000	0.000	0.006	0.001
	Cultured Muscle	0.000	0.000	0.000	0.000	0.000	0.003	0.000
	Wild Skin1	0.000	0.000	0.000	0.000	0.000	0.003	0.015
	Cultured Skin2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Cultured Skin1	0.000	0.000	0.000	0.000	0.000	0.000	0.000

observed from the adult to the free-swimming copepodid stage, indicating a relative depletion in ^{13}C over these stages (Fig. 2.18). In contrast, delta carbon signatures became progressively less negative during maturation from the parasitic copepodid to the chalimus III stage, indicating a relative enrichment in ^{13}C over time. Adult to early chalimus sea lice were statistically indistinguishable from wild pink salmon mucus. Egg strand to parasitic copepodid sea lice were statistically indistinguishable from wild pink salmon blood. The earliest non-significant result relative to the cultured Atlantic salmon occurred between the delta carbon signatures of the early chalimus lice and the blood of the cultured hosts. Chalimus III sea lice were indistinguishable from both the blood and the gills of the cultured Atlantic salmon.

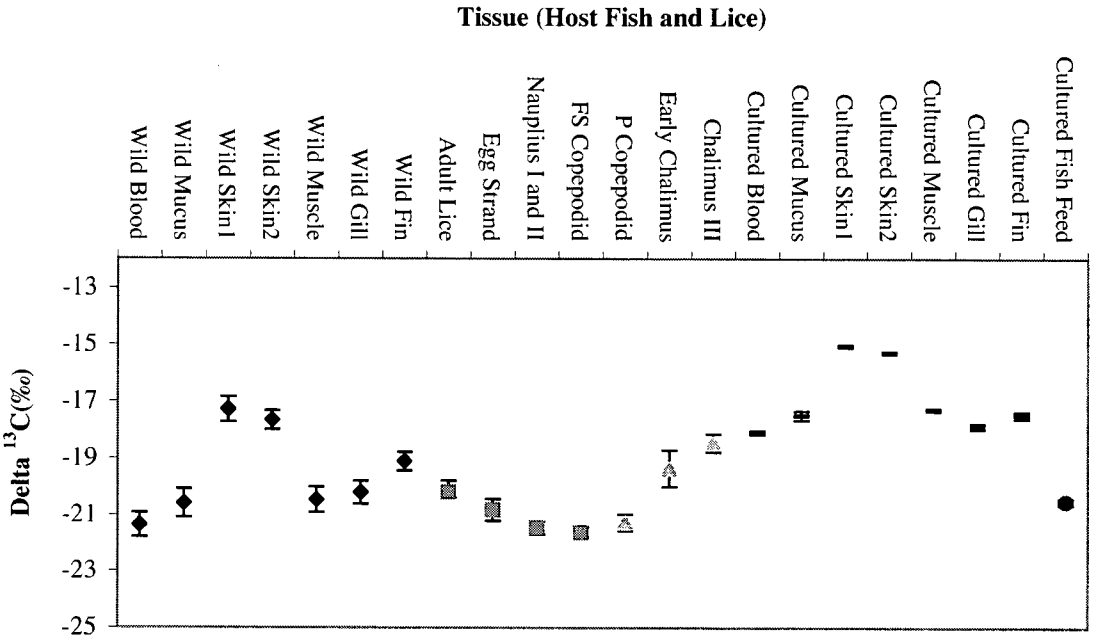


Fig. 2.18 Delta carbon signatures of sea lice from the adult to the second generation chalimus III stage relative to wild pink salmon and cultured Atlantic salmon host fish tissues. Error bars represent 95% CI. On the x-axis, "FS copepodid" represents free-swimming copepodids and "P copepodid" represents parasitic copepodids.

In contrast to the delta carbon analysis, delta nitrogen signatures of adult lice, egg strands, and planktonic sea lice became progressively enriched in the heavier isotope over time (Fig. 2.19). The signatures of the second generation parasitic sea lice became progressively depleted in the heavier isotope and only returned to approximately the same delta nitrogen value as the adult lice as opposed to surpassing it as in the delta carbon analysis. Furthermore, the only comparisons to yield non-significant statistical results were between the egg strands and the fin tissues of the wild pink salmon (Games-Howell, $p = 0.624$) and the early chalimus stages and all of the wild and cultured salmon tissues. The latter non-significant results were most likely the product of sampling or analysis error as manifested in the large confidence interval associated with the delta nitrogen signature of the early chalimus stages.

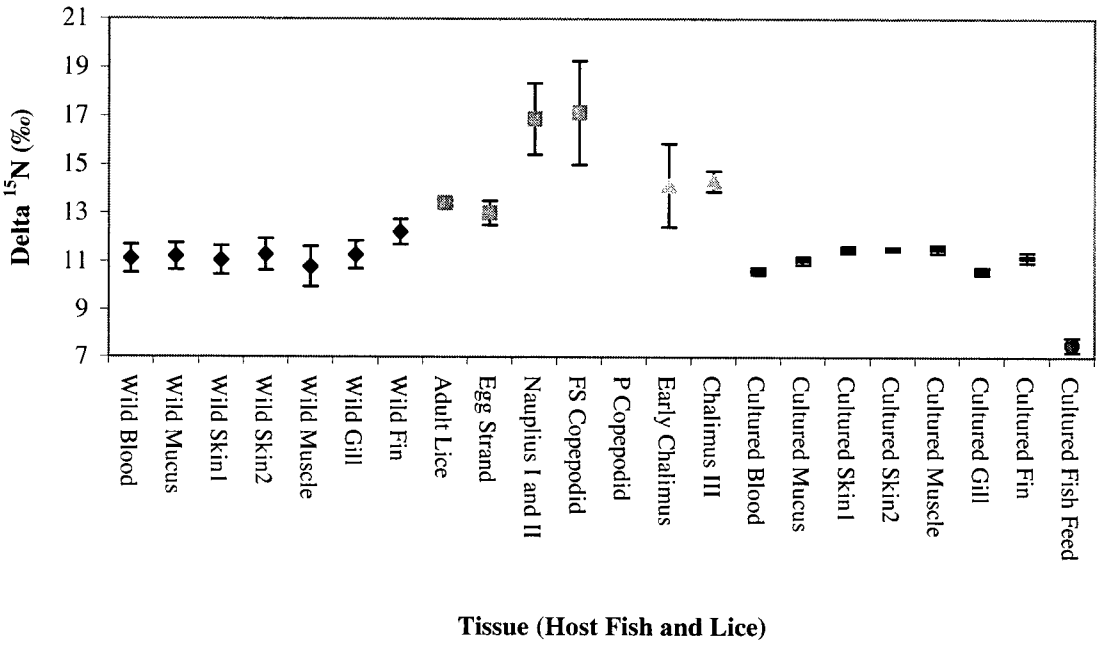


Fig. 2.19 Delta nitrogen signatures of sea lice from the adult to the second generation chalimus III stage relative to wild pink salmon and cultured Atlantic salmon host fish tissues. Error bars represent 95% CI.

DISCUSSION

Sea lice, *L. salmonis*, parasitizing novel host fish were found to maintain their natal stable carbon isotope signature. Stable carbon isotope ratios, therefore, show potential as tools to successfully distinguish between sea lice, *L. salmonis*, originating from either wild or cultured salmon. This is the first study to report a method capable of providing direct evidence regarding sources of sea lice epizootics. Application of this technique is based on the concept that all sea lice progeny are inherently tagged with natal isotope signatures. These signatures are the product of the isotopic composition of host fish tissues consumed by maternal sea lice during egg strand synthesis. As host fish populations exhibit unique stable carbon isotope ratios, natal sea lice signatures can be likened to distinct 'fingerprints' that provide innate links between the sea lice and their source host fish populations. The retention of these 'fingerprints' over time allows identification of sea lice origins following the release of sea lice into the plankton and even following attachment of sea lice to new host fish. As a result, stable carbon isotope signatures can be used to quantify the transfer of sea lice between farmed and wild populations and can, therefore, serve as a tool to assess the impact of such transfer. In contrast, delta nitrogen signatures were found to be ineffective tools in identifying source populations of sea lice progeny as host populations were not isotopically distinct and could not be differentiated.

Adult female sea lice likely consumed blood or mucus, or a combination of the two, as they displayed typical delta carbon consumer-diet enrichment of +0-1‰ relative to these tissues (DeNiro and Epstein, 1978). Muscle and gill tissues in this study were within this 0-1‰ range, however, adult sea lice were sampled from near the anal fin on

the body surface and did not have access to gill or muscle tissues. Although it is possible adult sea lice do not follow typical delta carbon enrichment patterns, the finding that sea lice clearly followed typical delta nitrogen enrichment patterns of 2-5‰ (Minagawa and Wada, 1984) relative to their diet implies conventional delta carbon enrichment patterns are probable. As female sea lice most likely consume host fish blood and mucus, it would be expected the natal isotope signatures of offspring reflect these tissues as well. This was found to be the case. It is, therefore, recommended that blood and mucus be sampled when assessing potential host fish populations in the field as these tissues appear to determine the natal isotopic signature of sea lice progeny.

It is not recommended other tissues be sampled in lieu of blood and mucus as tissues within fish were found to display different isotopic signatures and the relationships between tissues were inconsistent between fish populations. In the case of delta carbon analysis, isotopic differences between tissues were likely due to variable lipid concentrations within the tissues as higher amounts of lipids are often associated with lighter, more negative delta carbon signatures (Focken and Becker, 1998). This is consistent with the finding that fish blood displayed the lowest ^{13}C content as blood is considered a relatively fattier tissue within fish (McDonald and Milligan, 1992). Delta nitrogen signatures of tissues are influenced by ratios of essential to non-essential amino acids (Pinnegar and Polunin, 1999). Essential amino acids are not synthesized by animals and are incorporated directly from the diet without alteration of their isotopic signature (DeNiro and Epstein, 1978). Alternatively, non-essential amino acids are synthesized or partially modified within animals, resulting in isotopic fractionation through the removal of ^{14}N enriched amine groups (Gaebler et al., 1966, Macko et al., 1986). Consequently,

tissues enriched in non-essential amino acids may have relatively higher delta nitrogen signatures (Pinnegar and Polunin, 1999). Once again, it is not recommended alternative tissues be sampled in place of blood or mucus due to the observed differences in isotopic signature between tissues.

The delta carbon signatures of fish blood and mucus were not found to change significantly over time. This is essential for the successful application of stable isotope analysis as a method for tracking sea lice as the free-swimming phase in the life history of sea lice results in a time lag between the hatching of nauplii I and the attachment of parasitic copepodids to new host fish. If the isotopic signatures of tissues within the original host population change during this lag, there is no way of identifying the natal populations of sea lice progeny. Essentially, natal isotope signatures of sea lice retained in the free-swimming and parasitic phases would be relicts of host tissue signatures no longer in existence. The approximate duration of the time lag between hatching and attachment to new hosts was estimated from growth and survival studies on *L. salmonis* by Johnson and Albright (1991b) and was found to be approximately 6-12 days at 10°C. As noted above, the delta carbon signatures of fish blood and mucus were found to be stable over this period. Consequently, the natal signatures retained by sea lice progeny can be compared to the isotopic signatures of the host fish tissues from which they were potentially synthesized, regardless of the time lag between hatching and maturation to the parasitic copepodid stage.

In contrast, the delta nitrogen signatures of fish blood, muscle, and fin tissue were unstable over time. The variation observed in fish blood may be attributed to blood's role as a moderator for tissue specific ^{15}N uptake and release within tissues as this role allows

both ingested food and fractionation patterns to influence fish blood amino acid composition (Miller, 2000). For example, Karlsson et al. (2006) found free amino acid concentration in plasma increased rapidly within hours of feeding and often returned to, or even exceeded baseline levels, within 48 hours. The observed changes in blood delta nitrogen signature in this study may, therefore, be due to tissue sampling occurring at different times relative to feeding.

The delta nitrogen signature of fish muscle tissue may have varied over time because, although the intention was to sample red muscle, red and white muscle are closely related and it is difficult to sample one without the other (Robinson and Mead, 1973). Red and white muscle are dissimilar in their isotope signatures (Pinnegar and Polunin, 1999), hence, the apparent variation in delta nitrogen signature of muscle over time may have been due to the collection of samples with different combinations of red and white muscle. The change in fin tissues over time is most likely due to sampling error as a result of the difficulty associated with separating fin tissue from attached cartilage. It is recommended that the variability associated with blood samples and that the sampling difficulties associated with muscle and fin tissues be considered when selecting tissues for delta nitrogen analysis.

Factors that influence isotope signatures must be accounted for in any statistical analyses employed to determine lice origins. Several factors need be considered when sampling sea lice in the field, including sea lice stage, gut content, and attachment site on host fish. It was found the delta carbon signatures of sea lice egg strands, nauplii, free-swimming copepodids, and parasitic copepodids were indistinguishable from the blood of their natal host population and that sea lice from the egg strand to the early chalimus

stage were indistinguishable from the mucus. Thus, in order to optimize the chances of identifying the origins of newly attached sea lice, it is recommended that sea lice in no later than the parasitic copepodid stage be sampled since sea lice in this stage have had a limited amount of time to feed on new host tissues with distinct isotope signatures.

Gut content was found to influence the isotopic signature of sea lice, therefore, it is recommended that sampled sea lice are held in filtered seawater for several hours until they have had time to clear their guts. Relative to parasitic copepodid sea lice with empty guts, parasitic copepodid sea lice with full guts were found to display stable carbon isotope signatures more similar to the signatures of their new host fish. Essentially, undigested host fish tissues within the guts of newly parasitic sea lice may shift the whole-body isotopic signature of sea lice towards that of their new host population, while individuals with empty guts have isotopic signatures that reflect trophic shifts associated with the assimilation of host fish tissues.

Sea lice samples from the fins and body surface should not be grouped with sea lice sampled from the gills. Chalimus III sea lice parasitizing host fish gills were found to have significantly lower delta nitrogen signatures relative to chalimus III sea lice parasitizing the fins and body surface. This may be due to the preferential consumption of blood by sea lice on the gills and the infrequent consumption of blood by body surface and fin lice (Johnson and Albright, 1992) as blood is characteristically depleted in ^{15}N isotopes. Position on host fish was not found to have a significant effect on the delta carbon signatures of newly settled sea lice, yet, as a precaution due to observed differences in delta nitrogen signatures, it is recommended that sea lice collected from fin and body surfaces not be grouped with those sampled from gills.

Processes at the host fish level, such as feeding, assimilation of diet, respiration, and excretion, did not significantly influence the stable carbon or stable nitrogen isotope signatures of sea lice progeny in the egg strand and early parasitic stages (parasitic copepodid to chalimus III stages). As a result, egg strand tissues and second generation parasitic sea lice should be treated as individual samples and not be grouped by host fish. This practice will prevent unnecessary averaging of individual stable isotope signatures of sea lice from the same host fish and will minimize sampling effort for sea lice as fewer fish need be captured to obtain a given number of sea lice samples. In the case of adult sea lice, host fish did influence the delta carbon but not the delta nitrogen signatures. As a result, adult sea lice should be grouped at the host fish level for all stable isotope analyses as a precautionary approach.

Egg strands and planktonic sea lice did not display the progressive enrichment in ^{13}C that is characteristic of non-feeding animals (Hobsen et al., 1993). This is likely due to the process of ecdysis, in which the ^{13}C-rich chitinous exoskeletons of sea lice are shed during growth (DeNiro and Epstein, 1978). When feeding, input of isotopic material in the form of food is balanced by relatively greater assimilation of heavier isotopes into tissues and preferential removal of lighter isotopes through respiration and excretion, resulting in the typical consumer-diet isotopic enrichment patterns of + 0-1‰ for carbon and + 2-5‰ for nitrogen (DeNiro and Epstein, 1977, 1978, Minegawa and Wada, 1984). During non-feeding periods, ^{12}C-rich lipid stores are preferentially utilized for energy, thus decreasing the relative amount of ^{12}C in the body, and, at the same time, ^{12}C continues to be preferentially removed through respiration and excretion while not being replaced through the consumption of external food sources (Chevel et al., 1988, Castellini

and Rea, 1992). The end result is a net loss of ^{12}C from the body tissues of organisms and subsequent enrichment of the delta carbon signature. In the case of sea lice, however, it appears removal of ^{12}C through metabolic processes and the breakdown of lipids is outweighed by the loss of ^{13}C during ecdysis, resulting in a lower delta carbon signature due to a greater loss of ^{13}C relative to ^{12}C over time. This is supported by the finding that delta nitrogen signatures of planktonic sea lice followed typical enrichment patterns during the non-feeding stages because chitinous exoskeletons are depleted in the heavier nitrogen isotope. Instead of opposing one another, the processes of ecdysis, respiration, and excretion act in concert to progressively raise the delta nitrogen signatures of planktonic sea lice over time (DeNiro and Epstein, 1981). This is the first report of the effect of ecdysis on the stable isotopic signatures of moulting animals during fasting.

Wild fish tissues displayed greater isotopic variation between fish than did cultured fish tissues. This is most likely due to the homogeneous pellet diet of the cultured Atlantic salmon verses the heterogeneous wild diet of the pink salmon. Essentially, new tissues within the cultured fish would have always been constructed with the same isotopic signature due to the homogeneity of pellet feed. In contrast, the process of isotopic routing, in which tissues synthesized at different times are made from materials with different isotope signatures, likely resulted in greater isotopic variation between the same tissues within individual wild fish (Schwarcz, 1991).

Lastly, all tissues sampled from the cultured Atlantic salmon had delta nitrogen signatures within +2-5‰ of their feed and, therefore, displayed typical delta nitrogen consumer-diet relationships (Minagawa and Wada, 1984). In contrast, these same tissue samples did not show typical delta carbon enrichment of +0-1‰ relative to the feed

(DeNiro and Epstein, 1978), instead displaying greater enrichment than was expected. Typical enrichment patterns are associated with whole body analyses and not with individual analysis of particular tissues. The delta carbon enrichment patterns observed may be attributed to the sampling of tissues with lower lipid concentrations in comparison to other tissues within the sampled fish. It is possible typical enrichment patterns may have been observed had entire fish been sampled. Delta nitrogen analyses likely yielded results within the expected +2-5‰ enrichment range because little variation was found between the delta nitrogen signatures of tissues. Fundamentally, the lack of variation between tissue-specific delta nitrogen signatures resulted in the net nitrogen isotopic signature of the whole fish being represented in individual tissues.

In summary, analysis of stable carbon isotopes appears to be an acceptable tool to identify the origins of sea lice from either wild or farmed fish. The same was not true for using stable nitrogen isotopes. Recommendations for the application of stable isotopes in tracking sea lice in the field are as follows:

- Sea lice should be sampled in the parasitic copepodid stage in order to minimize alteration of the natal signature due to the consumption and assimilation of tissues of new host populations with novel isotopic compositions;
- Blood and mucus should be sampled when assessing potential natal host populations because the delta carbon signatures of these tissues are likely imparted onto sea lice progeny as natal isotope signatures;
- Following removal from host fish, sea lice should digest and expel any host fish material within their guts to avoid masking their true isotopic signature;

- Sea lice sampled from the gills should not be grouped with sea lice from the fins or body surface as gill sea lice display isotopically low signatures due to their preferential consumption of blood;
- Delta carbon values of adult sea lice should be grouped at the host fish level to avoid pseudo-replication, whereas replication unit for egg strands and newly parasitic sea lice (parasitic copepodid to chalimus III stages) should be at the individual level.

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CHAPTER 3: CONCLUDING DISCUSSION

CONCLUSIONS

Sea lice, *L. salmonis*, are currently the most controversial topic regarding possible disease interactions between wild and cultured fish (McVicar, 1997). Although it is probable that there are instances in which sea lice are transferred between farm operations and wild stocks, the extent of this has yet to be quantified (McVicar, 1997). Currently, the literature provides only correlative data displaying potential interactions between aquaculture operations and the surrounding marine environment. For example, fish proximate to salmonid sea farms have been found to display greater levels of infection relative to fish in areas distant from farm operations in Canada (Morton and Williams, 2003, Morton et al., 2004), Norway (Bjorn et al., 2001, Bjorn and Finstad, 2002), Ireland (Birkeland and Jakobsen, 1997), and Scotland (Mackenzie et al., 1998). Likewise, higher concentrations of planktonic sea lice have been observed near aquaculture operations relative to sites distant from farming activities in Scotland (Penston et al., 2004), Ireland (Costelloe et al., 1996), and Canada (Morton et al., 2004). This indirect evidence can be interpreted in numerous ways and as such has not abated discussion regarding the role of aquaculture in wild sea lice epizootics. Quite the opposite, such data have acted to polarize opinions in both the scientific and non-scientific communities (McVicar, 1997, Morton and Williams, 2003). As a result, many have not addressed issues surrounding sea lice with total impartiality (McVicar, 1997). The use of stable isotopes to directly track sea lice dispersal and to determine sea lice origins presents the first method that may allow the origins of sea lice sampled on wild fish to be determined directly. Stable isotopes may, therefore, provide evidence to help settle the

sea lice debate and, more importantly, to help establish effective management programs that target sources of sea lice epizootics.

The current study is the first to demonstrate that delta carbon signatures of second generation parasitic copepodid sea lice reflect host fish delta carbon signatures and that stable isotope analysis may serve as an effective tool to identify sea lice natal host populations in situ. It was found that delta nitrogen signatures cannot be used to track sea lice as host populations are not isotopically distinct and cannot be differentiated. This is in contrast to the findings of Butterworth et al. (2004) in which delta nitrogen signatures, and not delta carbon signatures, were demonstrated to be effective tools in differentiating between sea lice from farmed and wild fish and between sea lice from different farms. The apparent incongruence between this study and that of Butterworth et al. (2004) may be attributed to the diets consumed by host fish in each of the respective studies. By chance, fish in this study consumed diets similar in delta nitrogen signature and fish in Butterworth et al.'s (2004) study consumed diets similar in delta carbon signature. It is, therefore, advisable that dual nitrogen and carbon stable isotope analysis be used when applying this technique to account for potential carbon or nitrogen isotopic homogeneity between the populations under study.

In addition, methodological issues were addressed to facilitate the application of this technique in the field. In particular, it was recommended that (1) blood and mucus be used to assess potential source populations, (2) sea lice be sampled in the parasitic copepodid stage, (3) sea lice be given time to clear host material from their guts before analysis, (4) sea lice from the gills not be grouped with sea lice from the fins and body surface, and (5) newly settled parasitic sea lice be treated as individual samples and not

be grouped by host fish. Despite the successful identification of delta carbon as an effective tracer, small sample sizes hampered sea lice analyses and prevented addressing several methodological questions thoroughly, such as the effect that the location of sea lice on host fish bodies has on sea lice's isotopic signatures. Finally, this research was conducted in the lab and this novel approach should ideally be applied in the field. As a result, there are a number of opportunities for future research.

The most pressing topic for further study is the application of stable carbon isotopes to identifying natal host populations of sea lice in the field. For the first time ever, a direct link between host populations could then be established, thus facilitating development of sea lice management strategies directed towards source populations of epizootics. Within the laboratory, however, it would be interesting to assess how the isotopic signatures of second generation sea lice change when introduced to hosts with not only higher but also lower isotopic signatures relative to their natal host population. Such research would aid in interpreting sea lice data from the field.

In addition, closer consideration of tissues consumed by adult sea lice would provide a greater understanding of the isotopic connection between progeny and natal host fish. In the current study, the typical consumer-diet delta nitrogen enrichment of 2-5‰ (Minegawa and Wada, 1984) was evident because the delta nitrogen signatures of the host tissues were very similar. In contrast, the relationship between the delta carbon signatures of adult sea lice and their diet was ambiguous. Host tissues exhibited different delta carbon signatures and sea lice were both enriched and depleted relative to potential food sources. Fundamentally, it was not clear whether sea lice had, indeed, fed on mucus and blood, as would be expected due to the 0-1‰ enrichment (DeNiro and Epstein, 1978),

or whether sea lice had, instead, consumed other host tissues and were not displaying typical enrichment patterns. A study involving adult sea lice fed a diet of known isotopic content would facilitate assessment of sea lice-host fish enrichment patterns. Such work could then be used to infer what tissues adult sea lice generally consume in the field, thereby providing valuable information on the tissues used for the synthesis of progeny. Similarly, it would be interesting to investigate the effect of manufactured feeds composed of land-based plant meals, as opposed to marine-based fish meals, on the isotopic composition of cultured fish and their associated sea lice. Marine and terrestrial organic matter exhibit unique delta carbon signatures as a result of the different processes associated with primary production in each environment (Fry and Sherr, 1984). It is likely that isotopic differences between cultured and wild populations would be enhanced by the incorporation of land-based materials into feeds, thereby enhancing our ability to differentiate between farmed and wild epizootic sources. Lastly, as noted earlier, small sea lice sample sizes hampered study of the effects of gut content and position on second generation sea lice. It would be beneficial to address these topics again but with larger sample sizes and, therefore, greater statistical power.

In conclusion, sea lice may be tracked between host fish populations using stable carbon isotope signatures. This is the first report of a method capable of providing direct evidence regarding the natal sources of sea lice and of ending debate surrounding the role of aquaculture operations in wild epizootics.

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