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Lipid and fatty acid composition of parasitic caligid copepods belonging to the genus *Lepeophtheirus*

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ABSTRACT

Sea lice are copepod ectoparasites that constitute a major barrier to the sustainability and economic viability of marine finfish aquaculture operations worldwide. In particular, the salmon louse, *Lepeophtheirus salmonis*, poses a considerable problem for salmoniculture in the northern hemisphere. The free-swimming nauplii and infective copepodids of *L. salmonis* are lecithotrophic, subsisting principally on maternally-derived lipid reserves. However, the lipids and fatty acids of sea lice have been sparsely studied and therefore the present project aimed to investigate the lipid and fatty acid composition of sea lice of the genus *Lepeophtheirus* obtained from a variety of fish hosts. Total lipid was extracted from eggs and adult female *L. salmonis* obtained from both wild and farmed Atlantic salmon (*Salmo salar* L.) sampled at two time points, in the mid 1990s and in 2009. In addition, *L. salmonis* from wild sea trout (*Salmo trutta* L.) and *L. hippoglossi* from wild Atlantic halibut (*Hippoglossus hippoglossus* L.) were sampled and analyzed. The lipids of both females and egg strings of *Lepeophtheirus* were characterized by triacylglycerol (TAG) as the major neutral (storage) lipid with phosphatidylcholine and phosphatidylethanolamine as the major polar (membrane) lipids. The major fatty acids were 22:6*n*−3 (DHA), 18:1*n*−9 and 16:0, with lesser amounts of 20:5*n*−3, 22:5*n*−3 and 18:0. *L. salmonis* sourced from farmed salmon was characterized by higher levels of 18:2*n*−6 and 18:3*n*−3 than lice from wild salmon. Egg strings had higher levels of TAG and lower DHA compared to females, whereas *L. hippoglossi* had lower levels of TAG and higher DHA than *L. salmonis*. The results demonstrate that the fatty acid compositions of lice obtained from wild and farmed salmon differ and that changes to the lipid and fatty acid composition of feeds for farmed salmon influence the louse compositions.

1. Introduction

Copepods are a group of small crustaceans found in most marine and freshwater habitats of the world. More than 14,000 species have thus far been described, with sizes typically being in the range of <1 to 6 mm. Many copepods are herbivorous, often feeding on phytoplankton, whilst others may be detritivores, predators or commensals, some of which are fully parasitic. Copepods are considered to be the most numerous metazoans on the planet, exceeding the numbers of the other two hyperabundant groups: insects and nematodes. As such, they are generally assumed to constitute the predominant biomass of zooplankton and are the major food for many fish, larger crustaceans, sea mammals and seabirds (Skjoldal et al., 2004). Many copepods, particularly those in cold or deep waters, build up large lipid energy reserves through feeding on phytoplankton. These lipids are stored in oils sacs and/or as oil droplets. In some species lipid may accumulate to between 50 and 70% of body dry weight (Kattner and Krause, 1989; Lee et al., 2006; Falk-Petersen et al., 2009), making copepods the principal source of dietary lipid for many plankton-feeding fish species.

Parasitic copepods of the genus *Lepeophtheirus* constitute one of the most serious pathogens of marine farmed salmonids around the world (Johnson et al., 2004). It is estimated that sea lice infection by species belonging to the genera *Lepeophtheirus* and *Caligus*, cost the world's eight major salmon-producing countries, a combined total of over €300 million (Costello, 2009). Sea lice can pose a considerable risk to fish health and can inhibit growth, cause external damage and, in extreme cases, lead to mortality (Pike and Wadsworth, 1999). Sea lice are therefore a major constraint to farm production in coldwater salmoniculture and have also been suggested to be a threat to wild salmonid populations such as sea trout (Ford and Myers, 2008).

The species of copepod parasite of prime concern to mariculture and wild fisheries in Scotland is the salmon louse, *Lepeophtheirus salmonis*, which is the most pathogenic marine ectoparasite of Atlantic salmon (*Salmo salar* L.). The life cycle of this species is well characterized, comprising 10 stages separated by moults and five developmental phases (Kabata, 1979). After hatching from paired egg strings carried by host-attached adult females, the lice progress through two free-swimming planktonic nauplius stages before developing into copepodids, which infect a new fish host (Schram, 1993). After attachment,

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development proceeds through four chalimus and two sexually differentiated preadult stages before sexual maturity is reached at the adult stage. During development on the fish host, lice survive by feeding exclusively on host material including mucus, skin and blood (Brandal et al., 1976; Jonsdottir et al., 1992). Off the host, free-living stages of *L. salmonis,* nauplii and copepodids, are sustained by body reserves until the infective copepodid larva attaches to a new host (Boxaspen, 2006).

Despite considerable research into the biology, genetics and control of sea lice (Pike and Wadsworth, 1999; Boxaspen, 2006), very little is known about lipids and lipid metabolism in *L. salmonis* and in parasitic copepods in general. The period of survival of the free-swimming copepodid stage is constrained by its endogenous energy supplies (Boxaspen, 2006). The available energy reserves of *L. salmonis* copepodids were estimated at 7800 cal g^{-1} dry weight by bomb calorimetry, this being similar to reserves reported for copepodid stages of other parasitic and free-living copepods during winter (Tucker et al., 2000). This figure declined sharply between 1–2-day-old and 7-day-old copepodids with those at 7 days having substantially depleted reserves. In addition to their role as energy reserves, lipids are also key to the parasite's ability to immuno-modulate the host. In this respect, *L. salmonis* has been demonstrated to secrete prostaglandin E_2, an arachidonic acid (ARA; 20:4*n*−6) metabolite (Fast et al., 2004).

In contrast to the parasitic copepods, there has been considerable research into lipid storage and lipid metabolism in free-living copepods (see Lee et al., 2006; Kattner et al., 2007). An initial comparison of lipids in free-living and parasitic species (Lee, 1975) reported that, unlike the lipids of many free-living marine copepods that use wax esters (WE) as their primary energy store, *L. salmonis* from Pacific coho (*Oncorhynchus kisutch*) and pink *(O. gorbuscha*) salmon and other parasitic caligid copepods used triacylglycerol (TAG) as their main energy store. This was confirmed for *L. salmonis* from Atlantic salmon by Tucker et al. (2000). Given the paucity of information concerning lipids in caligid copepods, the present study therefore investigated lipid class and fatty acid compositions of total lipids of sea lice of the genus *Lepeophtheirus* obtained from a variety of fish hosts.

2. Materials and methods

2.1. Samples and sampling

Individual lice of the genus *Lepeophtheirus* were collected from infected fish obtained from various sites in Scottish waters (Table 1). Collected sea lice were maintained in fresh seawater (minimum 33 ppt) at 10 °C with aeration for approximately 24 h prior to processing. This study used samples of *L. salmonis,* collected from wild and farmed Atlantic salmon (*Salmo salar* L.) at two time points (early summer 1995 and summer 2009), and also those collected from wild sea trout (*Salmo trutta* L.) in summer 1996. Samples obtained in 2009 were used fresh whilst older samples were stored at −80 °C prior to use. The Atlantic salmon were all sampled in sea water mostly from various sea lochs whereas the sea trout was sampled in freshwater. Samples of L. *hippoglossi* collected from wild Atlantic halibut (*Hippoglossus hippoglossus* L.) in summer 1998 were also examined. All lice samples were adult females without egg strings. For three samples from farmed salmon, egg strings were carefully removed and also used for analysis.

Individual fresh or frozen lice were processed by macerating them in 30 μL of homogenization buffer (1 Mm Tris–HCl, pH 7.0, 0.1 mM EDTA, 0.1 mM 2-mercaptoethanol) using a pellet pestle (Anachem, Luton UK). Samples were then flash frozen in liquid nitrogen and stored at −70 °C until required. Salmon muscle samples were skinned and boned white muscle fillets that were flash frozen in liquid nitrogen and stored at −70 °C. The muscle samples were thawed and homogenized into a paté prior to lipid extraction.

2.2. Lipid extraction

Total lipid was prepared according to the method of Folch et al. (1957). Sea lice samples or 0.5 g samples of salmon muscle paté were added to 5 mL ice cold chloroform/methanol (2:1, by volume) containing 0.01% butylated hydroxytoluene as an antioxidant, and were homogenized using an IKA Ultra-Turrax T8. Tubes of homogenate were left on ice for 1 h. A further 1 mL of chloroform/methanol (2:1, v/v) was then added along with 1.5 mL aqueous KCl (0.88%). Samples were left on ice for a further 5 min and were then centrifuged at 600 g_{ave} for 5 min to separate the mixture. The lower organic layer was filtered through Whatman No. 1 filter paper into clean test tubes, the solvent evaporated under a stream of oxygen-free nitrogen (OFN) and the dry lipid extract resuspended in chloroform/methanol (2:1, v/v).

2.3. Lipid class composition analysis

Lipid class separation was performed by high-performance thin-layer chromatography (HPTLC). The concentration of the lipid extracts was adjusted to 10 mg/mL in chloroform/methanol (2:1, v/v) and two μL of each sample loaded as 2 mm streaks, 1 cm up on HPTLC plates (10 cm × 10 cm × 0.15 mm), precoated with silica gel 60 (Merck, Darmstadt, Germany). The plate was developed to approximately 5 cm with methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) then, after drying in air for 30 min, developed fully with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The lipid classes were visualized and quantified by charring at 160 °C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by densitometry using a Camag 3 TLC Scanner (Camag, Muttenz, Switzerland) and winCATS software (Henderson and Tocher, 1992). The identities of individual lipid classes were confirmed by comparison with reference to the Rf values of authentic standards run alongside samples on HPTLC plates and developed in the above solvent systems.

Table 1
Details of the *Lepeophtheirus* von Nordmann, 1832 samples collected in Scottish waters and used for the current study.

Species	Host	Locality (date)	Latitude/longitude	Samples collected
Lepeophtheirus hippoglossi (Krøyer, 1837)	*Hippoglossus hippoglossus* L. (W)	North Atlantic (06/98)	58° 52′ 56.87″N/7° 27′ 04.63″W	Adult female lice (S)
Lepeophtheirus salmonis (Krøyer, 1837)	*Salmo trutta* L. (W)	River Ewe (07/96)	57° 50′ 23.43″N/5° 34 56.21W	Adult female lice (S)
	Salmo salar L. (W)	Loch Duich (06/95)	57° 13′ 48.41″N/5° 28′ 02.04″W	Adult female lice (S)
		Armadale, Skye (06/09)	57° 03′ 30.90″N/5° 53′ 09.19″W	Adult female lice (NS)
	Salmo salar L. (F)	Loch Duich (06/95)	57° 14′ 49.70″N/5° 29′ 00.19″W	Adult female lice (S)
		Loch Fyne (site 2; 04/95)	56° 13′ 40.35″N/5 02′ 30.38″W	Adult female lice (S)
		Loch Fyne (site 1; 05/95)	56° 04′ 02.78″N/5 17′ 25.78″W	Egg strings
		Loch na Keal, Mull (06/95)	56° 26′ 02.05″N/6° 12′ 13.94″W	Adult female lice (S)
		Loch Linnhe (04/95)	56° 31′ 38.61″N/5° 32′ 42.41″W	Adult female lice (S)
		Lumlash Bay, Arran (05/95)	55° 31′ 48.38″N/5° 06′ 15.2″W	Adult female lice (S)
		Lumlash Bay, Arran (05/95)	55° 31′ 48.38″N/5° 06′ 15.2″W	Egg strings
		Shuna (04/95)	56° 13′ 49.68″N/5° 35′ 20.15″W	Egg strings
		Machrihanish (07/09)	55° 25′ 24.45″N/5° 44′ 54.40″W	Adult female lice (S)

F, farmed; NS, not starved; S, starved for 24 h prior to processing; W, wild.