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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 *Lepeophtheirus salmonis* obtained from both wild and farmed Atlantic salmon (*Salmo salar* L.) sampled at two time points, in the mid 1990's 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 22:6n-3 (DHA), 18:1n-9 and 16:0, with lesser amounts of 20:5n-3, 22:5n-3 and 18:0. *L. salmonis* sourced from farmed salmon were

characterized by higher levels of 18:2n-6 and 18:3n-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 50-70 % 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 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, 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; 20:4n-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 ^{o}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 -80ºC 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, 0.1mM 2-mercaptoethanol) using a pellet pestle (Anachem, Luton UK). Samples were then flash frozen in liquid nitrogen and stored at -70 ^{o}C until required. Salmon muscle samples were skinned and boned white muscle fillets that were flash frozen in liquid nitrogen and stored at -70 ^{o}C until required. 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 one hour. 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 re-suspended 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 x 10 cm x 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 ºC 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.

2.4. Fatty acid composition analysis

Fatty acid composition was determined by gas chromatography analysis of fatty acid methyl esters (FAME) prepared by acid-catalyzed transmethylation (Christie, 1993). Approximately 0.5 mg of total lipid was taken into a clean test tube and the solvent evaporated under a stream of OFN before 1 ml toluene and 2.5 ml of 1 % H_2SO_4 in methanol were added and the sample incubated overnight at 50^{o}C (Christie, 1993). After cooling, FAME were extracted by addition of 2 ml of 2 % $KHCO_3$ and 5 ml of isohexane/diethyl ether (1:1, v/v) containing 0.01% BHT, followed by thorough mixing and centrifugation at 600 g_{ave} for 2 min. The upper layer was removed into a clean test tube and the lower layer further extracted with a further portion of 5 ml of isohexane/diethyl ether (1:1, v/v) without BHT. After centrifugation at 600 g_{ave} for 2 min, the upper layer was removed and added to the previous upper layer, and the solvent evaporated under a stream of OFN, before the crude FAME extract was resuspended in 100 μl isohexane. The FAME were purified by thin-layer chromatography (TLC) prior to GC analysis. Samples were applied as 2 cm streaks to TLC plates (20 cm x 20 cm x 0.25 mm) and FAME separated from non-derivatized lipid classes using hexane/diethyl ether/acetic acid (90:10:1, by vol.) as developing solvent. The FAME were located on the plate by staining standards run at the side of the plate using an Iodine spray. The areas of silica containing FAME were scraped into clean test tubes and FAME eluted using isohexane/diethyl ether (1:1). After centrifugation the supernatant solvent was removed into a clean test tube, the solvent evaporated under OFN and the FAME resuspended in 200 μL of isohexane. The FAME were separated and quantified by gas-liquid capillary chromatography using a Fisons GC8600 gas chromatograph (Fisons Ltd., Crawley, U.K) equipped with a 30 m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak Ltd., London, U.K.) and on-column injection. Hydrogen was used as carrier gas and temperature programming was from 50 ^{o}C to 150 ^{o}C at 40 ^{o}C min^{-1} and then to 230 ^{o}C at 2.0 ^{o}C min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher and Harvie, 1988). Data were collected and processed using the Chromcard for Windows (Version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy).

2. 5. Materials

BHT was obtained from Sigma Chemical Co. (Poole, U.K.). HPTLC (10 cm x 10 cm x 0.15 mm) and TLC (20 cm x 20 cm x 0.25 mm) plates, precoated with silica gel 60 (without fluorescent

indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK (Loughborough, England).

2.6. Statistical analysis

All data are presented as means ± SD (n = 3). The significance of differences between samples were determined by one-way analysis of variance (ANOVA) followed, where appropriate, by Tukey's comparison test (Zar, 1999). Percentage data and data that were identified as non-homogeneous (Bartlett's test) were subjected to arcsine transformation before analysis. Differences were regarded as significant when $P < 0.05$.

3. Results

3.1. Effect of location on lipid class and fatty acid compositions of sea lice from farmed salmon

Triacylglycerol (TAG) was the major lipid class in salmon lice, constituting between 35 – 45 % of total lipid with 9-13 % cholesterol. Similar amounts of the major polar lipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were seen, with lesser amounts of other phosphoglycerides such as phosphatidylserine (PS) and phosphatidylinositol (PI), and the major sphingolipid, sphingomyelin (Table 2). There were few major differences in lipid class composition between samples and, although TAG levels varied, no significant differences in TAG or the proportions of total polar and neutral lipids were apparent. Free fatty acids (FFA) varied between 8 and 11 %, but these values were possibly partly artifactual resulting from *post-mortem* lipolytic action during homogenization and storage.

The fatty acid composition of the lice was characterized by about 25 % saturated fatty acids, predominantly 16:0, around 35 % monounsaturated fatty acids, predominantly 18:1n-9, 2-3 % n-6 polyunsaturated fatty acids (PUFA) including 20:4n-6 (ARA, arachidonic acid), and 27-32 % n-3 PUFA (Table 3). No major differences between the fatty acid compositions of lice obtained from farmed salmon at different sites were observed. Saturated fatty acids (mainly16:0) showed little variation (23-25 %), whereas monounsaturated fatty acids (largely 18:1n-9) showed slightly more (30-40 %), although none of the differences were significant (Table 3). There was more variability in the levels of certain PUFA, particularly 18:2n-6 and the main n-3 long-chain polyunsaturated fatty acid (LC-PUFA), 22:6n-3 (DHA, docosahexaenoic acid) (Table 3). There were no significant differences in the levels of the other important LC-PUFA, ARA and 20:5n-3 (EPA, eicosapentaenoic acid) between lice from the different populations of farmed salmon (Table 3).

3.2. Lipid class and fatty acid compositions of female sea lice and their egg strings

Very clear differences between the relative lipid class compositions of females and the egg strings were observed, with eggs containing significantly higher proportions of TAG and neutral lipids than females (Table 4). Thus, the lipid in egg strings comprised 80 % neutral lipid, predominantly TAG at around 70 % and around 7 % cholesterol. Consequently the proportions of all polar lipid classes were significantly lower in egg strings compared to females although the relative proportions were similar with PC and PE predominating (Table 4).

Compared to the female lice, the fatty acid compositions of the egg strings were characterized by higher proportions of monounsaturated fatty acids, particularly 18:1n-9 but also 20:1, and lower levels of total PUFA, specifically n-3 PUFA, particularly DHA (Table 5). Few differences in fatty acid composition of the egg strings obtained from the different locations could be discerned.

3.3. Effect of year of sampling on lipid class and fatty acid compositions of sea lice from wild and farmed salmon

Irrespective of sampling time point, the lipid class compositions of lice obtained from farmed or wild salmon showed few differences (Table 6). Clear trends were apparent for higher cholesterol levels in lice obtained from farmed fish and also for lower levels of FFA in the 2009 samples (Table 6). Relatively few major effects of louse source (wild / farmed) or year were apparent in the fatty acid compositions of the sea lice. However, there was a clear trend for higher 18:2n-6 and 18:3n-3 in the lice from the farmed fish compared to lice from wild fish (Table 7). The farmed fish also tended to have lower 16:0, 16:1n-7 and EPA, and higher 22:1 and 20:4n-3 compared to the lice from wild salmon. The levels of 18:2n-6, 18:3n-3, 20:4n-3 and DHA were higher in lice from farmed fish in 2009 compared to 1995 (Table 7). In contrast, there was essentially no difference in the fatty acid composition of lice obtained from wild salmon in 1995 and those samples obtained in 2009.

The host-parasite transfer of fatty acids can be observed by comparing the compositions of female lice, their egg strings and the muscle of host salmon (Table 8). The higher level of 18:2n-6 in the lice from farmed fish in 2009 is a reflection of the relatively high level of this fatty acid (> 5 %) in the salmon, and this fatty acid is also present in eggs. However, there are differences in the fatty acid compositions of the three samples. For instance the lice and eggs have generally higher saturated fatty acids and LC-PUFA (especially ARA and DHA), and lower monounsaturated fatty acids and C18 PUFA (18:2n-6 and 18:3n-3) compared to the salmon muscle (Table 8). Total phospholipids were characterized by higher percentages of n-3 PUFA, total PUFA and saturated fatty acids compared to TAG, whereas TAG showed higher proportions of monounsaturated fatty

acids and n-6 PUFA (Table 9). This was the pattern in female lice, egg strings and salmon muscle, although it was most pronounced in the fish muscle.

3.4. Lipid class and fatty acid compositions of sea lice from salmon, sea trout and halibut

Essentially no differences in lipid class composition between *L. salmonis* obtained from wild salmon and wild sea trout were significant with the difference in lyso-PC (a lipolytic product of PC) probably being due to differences in sampling. In contrast, *L. hippoglossi* was characterized by significantly lower levels of TAG and total neutral lipids compared to *L. salmonis* samples (Table 10). This was also reflected in higher proportions of PC, PE and sphingomyelin in halibut lice (Table 10).

The halibut lice had generally lower levels of saturated fatty acids than lice obtained from salmon (Table 11). However, there was a very clear variation in the levels of 18:1n-9 and total monoenes between the louse species, with levels decreasing significantly from salmon to trout to halibut (Table 11). Other than 24:1n-9, all the monoenes were significantly lower in halibut lice compared to the salmonid lice. In contrast, the levels of n-6, n-3 and total PUFA were significantly higher in halibut lice compared to the salmonid lice (Table 11). Interestingly, the higher n-3 PUFA in halibut was entirely due to higher DHA as levels of EPA and 22:5n-3 were significantly lower in halibut lice compared to the salmonid lice.

4. Discussion

In many free-living copepods, periods of dietary surplus and extensive feeding, such as occurs during plankton blooms, may foster phases of rapid growth. In some habitats, however, such as those of polar regions, phytoplankton blooms have only short durations and so many animals build up significant stored lipid reserves to carry them through periods of low food availability. These stored reserves function to allow survival through dark winters with low primary production, and are also used for reproductive purposes. Such lipid storage has been particularly noted for many zooplankton species (Lee et al., 2006). TAG is by far the most common form of energy store in krill species (Lee et al., 2006) and, in addition, is used as the main lipid store in most fish and marine mammals such as seals. However, a peculiarity in many marine organisms, such as calanoid copepods, is the occurrence of wax esters (WEs) as the main lipid store (Lee et al., 2006). Wax esters comprise long-chain fatty acids esterified to long-chain alcohols and there has been considerable discussion as to their advantages as storage products. It is suggested that WEs are suited to use as long-term energy reserves while TAG can be used for short-term energy supplies, or that WEs may be used for buoyancy regulation (Lee et al., 2006). However, few animals have only WEs as the only storage lipid. In many cases, there will also be various levels of TAG present

277 depending on developmental stage. The present study has confirmed sparse earlier data (Lee, 1975)
278 suggesting that parasitic caligid copepods of the genus *Lepeophtheirus* sp. store their lipid
279 essentially as TAG with only small, trace amounts of WE. This perhaps supports the hypothesis that
280 WEs are only required for long-term storage as the utilization of stored lipid in the parasitic
281 copepods will only be required over a short time period between fish hosts.

282 The major phospholipid classes (membrane lipids) in the sea lice were PC and PE as in fish
283 and most animals in general, and there were no major differences in the relative percentages of
284 these membrane lipids. The major difference in lipid class composition observed between different
285 samples was in the proportion of neutral (storage) lipid, TAG (Tocher, 2003). The major fatty acids
286 observed in sea lice and their egg strings were 16:0, 18:1n-9, EPA and DHA, with lesser amounts of
287 18:0, 16:1n-7, 20:1, ARA and 22:5n-3, which is a pattern characteristic of fish (Tocher, 2003).
288 Therefore, the general fatty acid composition of lice is suggested to be a reflection of the fatty acid
289 composition of the fish host.

290 Some of the differences in fatty acid composition observed between the samples were also
291 reflective of the differences in lipid class composition discussed above. Neutral lipids like TAG
292 generally have higher levels of monounsaturated fatty acids (monoenes) and lower PUFA as they
293 are used primarily as an energy store, whereas phospholipids have higher PUFA as they are
294 membrane lipids and PUFA are essential for membrane function, possibly in terms of membrane
295 fluidity but more so for enzyme, receptor and carrier protein activities (Tocher, 2003). Lee (1975)
296 reported the fatty acid composition of TAG and phospholipids from *L. salmonis* and showed that
297 TAG had higher monoenes and lower PUFA than phospholipids. In the present study, this pattern
298 was also clearly observed in the salmon muscle, female lice and egg string samples. Indeed all the
299 samples in the present study that contained higher proportions of TAG were characterized by higher
300 proportions of monoenes and lower proportions of PUFA and, especially, LC-PUFA. This was
301 observed in the salmon louse versus egg string comparisons and also in the halibut versus salmonid
302 lice comparisons.

303 It is probable, however, that the sea lice can modify the fatty acids obtained from the host.
304 The adult female lice and the salmon muscle had similar levels of TAG (Table 8) but the sea lice
305 showed lower levels of C18 PUFA, 18:2n-6 and 18:3n-3 and higher levels of the LC-PUFA ARA
306 and DHA. This may indicate that they were able to convert the shorter chain fatty acids to the LC-
307 PUFA by fatty acid desaturation and elongation, or, alternatively, differences in PUFA composition
308 could be generated by selective oxidation of C18 PUFA and selective retention of LC-PUFA in lice
309 (Tocher, 2003). Previously, Lee (1975) reported that the DHA:EPA ratio in *L. salmonis* lipids were
310 2:1 in TAG and 6:1 in phospholipid. In the present study, the DHA:EPA ratios in *L. salmonis* and

311 their egg strings were around 8:1 and 3:1 in phospholipids and TAG, respectively, and only 4:1 and
312 2:1 in salmon muscle phospholipids and TAG.
313 The fatty acid composition of fish, and animals in general, is most strongly influenced by
314 diet (Tocher, 2003). As salmon lice are considered to obtain all their nutrition from the fish host,
315 differences in the fatty acid composition of the diet of the fish host could similarly be reflected in
316 the composition of the lice, as observed in the present study. Thus, the fatty acid composition of lice
317 from farmed salmon in 2009 showed a difference compared to lice from farmed fish in 1995,
318 specifically in terms of increased levels of 18:2n-6 and 18:3n-3. This is because feeds for salmon
319 farming are being changed to more sustainable formulations, in particular replacing the marine
320 resources, fishmeal and fish oil, global supplies of which are limiting, with more sustainable plant
321 meals and vegetable oils (Tacon and Metian, 2008). Currently, around 25 % of fish meal and 50 %
322 of fish oil is now generally substituted with plant alternatives in salmon feeds. Lipids in fishmeal
323 and fish oil have high EPA and DHA and low C18 PUFA whereas plant meals and oils contain no
324 LC-PUFA whatsoever, and high levels of C18 fatty acids, especially18:1n-9 and 18:2n-6 but also
325 18:3n-3 (Gunstone and Harwood, 2007). Therefore, the increased levels of C18 PUFA in the
326 farmed samples from 2009 were expected but it was surprising that levels of EPA and DHA were
327 maintained. This may be due to the specific fish oil included in the diets, as fish oils with higher
328 LC-PUFA sourced from the southern hemisphere, may act to compensate for reduced levels of fish
329 oil in the feed (Pratoomyot et al., 2008). In contrast, no differences in fatty acid compositions of lice
330 from farmed and wild salmon were observed in 1995 samples due to fact that salmon feeds at that
331 time comprised almost exclusively fishmeal and fish oil and, therefore, farmed fish would be
332 getting essentially the same diet (fish) as the wild fish. Similarly, no difference was observed in the
333 fatty acid composition of lice from wild fish sampled in 1995 and 2009. Differences in sea louse
334 composition resulting from changes in salmon feed components may not be simply of academic
335 interest. It is recognised, for instance, that *L. salmonis* secretes immuno-modulatory products
336 including the prostaglandin E_2 (Fast et al., 2004), a derivative of the omega-6 fatty acid ARA,
337 which may function to protect the parasite from the host's immune response. Changes in the fatty
338 acid profile of the parasite could therefore potentially affect, in a positive or negative fashion, the
339 interaction between parasite and host, leading to changes in infection intensity and / or host injury.
340 In conclusion, the present study has confirmed that parasitic caligid copepods of the genus
341 *Lepeophtheirus* store their lipid essentially as TAG. Egg strings had higher lipid contents than adult
342 female lice and this was reflected in higher TAG levels. Differences in fatty acid composition
343 observed between the samples partly reflected the differences in lipid content and TAG levels but it
344 is also likely that the endogenous metabolism of the lice modifies their fatty acid composition.
345 Changes in the fatty acid composition of salmon feeds is reflected in the fatty acid composition of

346 the lice and their egg strings and this may have consequences for the ability of lice to suppress the
347 host's immune response. The fact that changes to the lipid and fatty acid composition of feeds for
348 farmed salmon influence the composition of sea lice indicates that further investigation of lipid and
349 fatty acid metabolism of *L. salmonis* is warranted.
350
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353
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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 analysed
Lepeophtheirus hippoglossi (Krøyer, 1837)	*Hippoglossus hippoglossus* L. (W)	North Atlantic (06/98)	58º 52' 56.87"N / 7º 27' 04.63"W	Adult female lice (S)
Lepeophtheirus salmonis (Krøyer, 1837)	*Salmo trutta* L. (W)	River Ewe (07/96)	57º 50' 23.43"N / 5º 34 56.21W	Adult female lice (S)
	Salmo salar L. (W)	Loch Duich (06/95)	57º 13' 48.41"N / 5º 28' 02.04"W	Adult female lice (S)
		Armadale, Skye (06/09)	57º 03' 30.90"N / 5º 53' 09.19"W	Adult female lice (NS)
	Salmo salar L. (F)	Loch Duich (06/95)	57º 14' 49.70"N / 5º 29' 00.19"W	Adult female lice (S)
		Loch Fyne (site 2; 04/95)	56º 13' 40.35"N / 5 02' 30.38"W	Adult female lice (S)
		Loch Fyne (site 1; 05/95)	56º 04' 02.78"N / 5 17' 25.78"W	Eggstrings
		Loch na Keal, Mull (06/95)	56º 26' 02.05"N / 6º 12' 13.94"W	Adult female lice (S)
		Loch Linnhe (04/95)	56º 31' 38.61"N / 5º 32' 42.41"W	Adult female lice (S)
		Lumlash Bay, Arran (05/95)	55º 31' 48.38"N / 5º 06' 15.2"W	Adult female lice (S)
		Lumlash Bay, Arran (05/95)	55º 31' 48.38"N / 5º 06' 15.2"W	Eggstrings
		Shuna (04/95)	56º 13' 49.68"N / 5º 35' 20.15"W	Eggstrings
		Machrihanish (07/09)	55º 25' 24.45"N / 5º 44' 54.40"W	Adult female lice (S)

F, Farmed; NS, not starved; S, starved for 24 hrs prior to processing; W, wild.

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Table 2. Lipid class composition (percentage of total lipid) of *L. salmonis* obtained from Atlantic salmon farmed at various locations.

Lipid class	Duich	Linnhe	Mull	Arran	Shuna	Fyne
PC	13.5 ± 2.9	14.3 ± 1.5	14.6 ± 2.6	12.7 ± 0.3	12.6 ± 1.3	15.9 ± 0.9
PE	14.2 ± 1.8	12.2 ± 3.8	13.3 ± 0.9	10.8 ± 2.0	12.6 ± 1.9	14.6 ± 0.9
PS/PI/PA/CL	9.9 ± 1.9	7.8 ± 2.8	10.1 ± 3.8	7.7 ± 1.6	7.4 ± 2.7	8.0 ± 1.6
Sphingomyelin	3.5 ± 1.2	4.4 ± 0.9	3.5 ± 1.9	3.2 ± 0.3	2.9 ± 0.8	4.2 ± 0.2
LPC	0.5 ± 0.3 ^{b}	0.2 ± 0.2 ^{b}	0.8 ± 0.3 ^{ab}	1.2 ± 0.1 ^{a}	0.7 ± 0.2 ^{ab}	0.7 ± 0.2 ^{ab}
Total polar	41.5 ± 7.4	38.9 ± 5.0	42.2 ± 8.5	35.6 ± 3.5	36.2 ± 6.7	43.4 ± 2.5
Total neutral	58.5 ± 7.4	61.1 ± 5.0	57.8 ± 8.5	64.4 ± 3.5	63.8 ± 6.7	56.6 ± 2.5
Cholesterol	13.3 ± 0.8 ^{a}	10.8 ± 1.1 ^{ab}	12.7 ± 1.6 ^{a}	8.7 ± 0.5 ^{b}	11.7 ± 2.0 ^{ab}	10.9 ± 0.6 ^{ab}
Triacylglycerol	34.7 ± 8.4	40.8 ± 5.7	36.0 ± 10.6	45.4 ± 5.3	44.4 ± 6.5	35.0 ± 3.2
Free fatty acid	10.4 ± 1.8	9.4 ± 0.6	9.1 ± 3.4	10.2 ± 1.4	7.7 ± 1.4	10.7 ± 2.7
Steryl/wax ester	Trace	Trace	Trace	Trace	Trace	Trace

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).
CL, cardiolipin; LPC, lyso-PC; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

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Table 3. Fatty acid composition (percentage of total fatty acids) of *L. salmonis* obtained from Atlantic salmon farmed at various locations.

Fatty acid	Duich	Linnhe	Mull	Arran	Shuna	Fyne
14:0	2.1 ± 0.0 ^{b}	2.5 ± 0.2 ^{b}	2.2 ± 0.3 ^{b}	2.4 ± 0.2 ^{b}	2.4 ± 0.2 ^{b}	3.1 ± 0.1 ^{a}
16:0	17.9 ± 0.5	17.1 ± 2.8	16.8 ± 0.7	17.1 ± 0.9	16.6 ± 0.9	16.9 ± 1.9
18:0	3.9 ± 0.5	3.7 ± 0.8	3.7 ± 0.2	3.4 ± 0.3	2.9 ± 0.3	3.9 ± 0.9
Total saturated[1]	25.4 ± 0.9	24.1 ± 3.6	23.5 ± 1.0	23.5 ± 0.5	22.6 ± 0.6	25.0 ± 3.5
16:1n-9	1.7 ± 0.4	1.8 ± 1.2	2.1 ± 0.3	2.0 ± 0.2	2.2 ± 0.5	3.0 ± 0.1
16:1n-7	2.3 ± 0.2 ^{b}	3.1 ± 1.1 ^{ab}	2.7 ± 0.3 ^{ab}	3.1 ± 0.2 ^{ab}	3.7 ± 0.4 ^{ab}	3.6 ± 0.1 ^{a}
18:1n-9	22.5 ± 2.5 ^{ab}	24.8 ± 3.2 ^{ab}	23.2 ± 3.7 ^{ab}	28.2 ± 1.9 ^{a}	25.4 ± 2.7 ^{ab}	19.0 ± 3.7 ^{b}
18:1n-7	1.4 ± 0.2	1.4 ± 0.0	1.3 ± 0.3	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.3
20:1[2]	2.8 ± 0.5	2.9 ± 0.3	2.2 ± 0.2	2.7 ± 0.2	2.4 ± 0.1	2.6 ± 0.1
22:1[3]	2.1 ± 0.4 ^{a}	1.0 ± 0.1 ^{b}	1.1 ± 0.3 ^{b}	1.0 ± 0.1 ^{b}	1.3 ± 0.1 ^{b}	1.4 ± 0.5 ^{ab}
24:1n-9	2.5 ± 1.2 ^{a}	1.7 ± 0.1 ^{ab}	1.2 ± 0.0 ^{b}	0.9 ± 0.2 ^{b}	0.9 ± 0.1 ^{b}	0.7 ± 0.3 ^{b}
Total monoenes	35.2 ± 3.2	36.6 ± 4.8	33.8 ± 4.0	39.6 ± 1.6	37.3 ± 2.2	30.0 ± 5.3
18:2n-6	1.0 ± 0.1 ^{ab}	0.4 ± 0.0 ^{d}	1.2 ± 0.1 ^{a}	0.8 ± 0.0 ^{bc}	0.6 ± 0.0 ^{cd}	1.4 ± 0.4 ^{a}
20:4n-6	1.2 ± 0.4	1.0 ± 0.1	1.0 ± 0.4	1.3 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
Total n-6PUFA[4]	2.9 ± 0.5 ^{a}	2.0 ± 0.1 ^{b}	2.6 ± 0.5 ^{ab}	2.6 ± 0.3 ^{ab}	1.8 ± 0.1 ^{b}	2.8 ± 0.3 ^{a}
18:3n-3	0.3 ± 0.0 ^{bc}	0.3 ± 0.0 ^{bc}	0.5 ± 0.0 ^{c}	0.3 ± 0.0 ^{bc}	0.4 ± 0.0 ^{b}	0.7 ± 0.2 ^{a}
20:4n-3	0.6 ± 0.1 ^{ab}	0.4 ± 0.2 ^{b}	0.7 ± 0.1 ^{b}	0.7 ± 0.1 ^{ab}	0.8 ± 0.1 ^{a}	0.6 ± 0.1 ^{ab}
20:5n-3	4.3 ± 0.8	4.6 ± 1.1	4.8 ± 0.6	4.7 ± 0.1	5.2 ± 0.2	4.0 ± 0.4
22:5n-3	3.0 ± 0.5 ^{ab}	2.5 ± 0.9 ^{b}	3.5 ± 0.4 ^{a}	3.3 ± 0.6 ^{ab}	3.0 ± 0.3 ^{ab}	2.3 ± 0.7 ^{b}
22:6n-3	18.4 ± 3.1 ^{b}	20.8 ± 4.6 ^{ab}	25.3 ± 3.4 ^{ab}	20.6 ± 0.4 ^{ab}	23.0 ± 0.8 ^{ab}	25.8 ± 2.0 ^{a}
Total n-3PUFA[5]	26.9 ± 4.5	28.8 ± 6.0	35.1 ± 3.7	29.9 ± 0.8	32.7 ± 0.2	33.6 ± 3.0
Total PUFA	29.7 ± 4.5	30.8 ± 6.0	37.7 ± 3.7	32.5 ± 1.1	34.5 ± 0.1	36.4 ± 2.8

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).
[1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.7 %; [2], Predominantly n-9 isomer;
[3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6,20:3n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.3 %; [5], Totals include 18:3n-3 and 20:3n-3 present in some samples at up to 0.2 %; PUFA, polyunsaturated fatty acids.

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Table 4. Lipid class compositions (percentage of total lipid) of *L. salmonis* females and their egg strings

	Females			Egg strings		
Lipid class	Arran	Shuna	Fyne	Arran	Shuna	Fyne
PC	12.7 ± 0.3 ^{a}	12.6 ± 1.3 ^{a}	15.9 ± 0.9 ^{a}	8.7 ± 0.8 ^{b}	9.3 ± 0.9 ^{b}	9.6 ± 0.8 ^{b}
PE	10.8 ± 2.0 ^{a}	12.6 ± 1.9 ^{a}	14.6 ± 0.9 ^{a}	6.4 ± 0.1 ^{b}	5.9 ± 0.6 ^{b}	6.4 ± 1.6 ^{b}
PS/PI/PA/CL	7.7 ± 1.6 ^{a}	7.4 ± 2.7 ^{a}	8.0 ± 1.6 ^{a}	4.5 ± 0.7 ^{ab}	2.8 ± 1.7 ^{b}	2.5 ± 0.7 ^{b}
Sphingomyelin	3.2 ± 0.3 ^{a}	2.9 ± 0.8 ^{a}	4.2 ± 0.2 ^{a}	1.4 ± 0.3 ^{b}	1.6 ± 0.2 ^{b}	1.5 ± 0.2 ^{b}
LPC	1.2 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.3 ± 0.2	0.2 ± 0.3	0.5 ± 0.8
Total polar	35.6 ± 3.5 ^{a}	36.2 ± 6.7 ^{a}	43.4 ± 2.5 ^{a}	21.2 ± 0.2 ^{b}	19.7 ± 2.1 ^{b}	20.5 ± 2.5 ^{b}
Total neutral	64.4 ± 3.5 ^{b}	63.8 ± 6.7 ^{b}	56.6 ± 2.5 ^{b}	78.8 ± 0.2 ^{a}	80.3 ± 2.1 ^{a}	79.5 ± 2.5 ^{a}
Cholesterol	8.7 ± 0.5 ^{b}	11.7 ± 2.0 ^{a}	10.9 ± 0.6 ^{a}	7.0 ± 0.4 ^{b}	7.1 ± 0.2 ^{b}	6.6 ± 0.1 ^{b}
Triacylglycerol	45.4 ± 5.3 ^{b}	44.4 ± 6.5 ^{b}	35.0 ± 3.2 ^{b}	67.1 ± 2.3 ^{a}	70.2 ± 3.8 ^{a}	69.3 ± 3.0 ^{a}
Free fatty acid	10.2 ± 1.4 ^{a}	7.7 ± 1.4 ^{ab}	10.7 ± 2.7 ^{a}	4.7 ± 2.0 ^{bc}	3.1 ± 1.5 ^{c}	3.5 ± 0.7 ^{c}
Steryl/wax ester	Trace	Trace	Trace	Trace	Trace	Trace

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).

CL, cardiolipin; LPC, lyso-PC; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine;

PI, phosphatidylinositol; PS, phosphatidylserine.

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Table 5. Fatty acid compositions (percentage of total fatty acids) of *L. salmonis* females and their egg strings

	Females			Egg strings		
Fatty acid	Arran	Shuna	Fyne	Arran	Shuna	Ardcastle
14:0	2.4 ± 0.2 ^{b}	2.4 ± 0.2 ^{b}	3.1 ± 0.1 ^{a}	2.4 ± 0.2 ^{b}	2.5 ± 0.2 ^{b}	1.5 ± 0.1 ^{c}
16:0	17.1 ± 0.9 ^{ab}	16.6 ± 0.9 ^{ab}	16.9 ± 1.9 ^{ab}	17.4 ± 1.4 ^{a}	16.9 ± 0.7 ^{ab}	14.5 ± 0.8 ^{b}
18:0	3.4 ± 0.3	2.9 ± 0.3	3.9 ± 0.9	3.2 ± 0.3	2.9 ± 0.1	3.4 ± 0.3
Total saturated[1]	23.5 ± 0.5 ^{ab}	22.6 ± 0.6 ^{ab}	25.0 ± 3.5 ^{a}	24.3 ± 2.6 ^{a}	23.2 ± 1.0 ^{ab}	19.9 ± 1.3 ^{b}
16:1n-9	2.0 ± 0.2 ^{b}	2.2 ± 0.5 ^{b}	3.0 ± 0.1 ^{a}	1.7 ± 0.1 ^{b}	2.0 ± 0.4 ^{b}	1.8 ± 0.1 ^{b}
16:1n-7	3.1 ± 0.2 ^{b}	3.7 ± 0.4 ^{ab}	3.6 ± 0.1 ^{ab}	3.5 ± 0.1 ^{ab}	3.9 ± 0.2 ^{a}	3.6 ± 0.3 ^{ab}
18:1n-9	28.2 ± 1.9 ^{ab}	25.4 ± 2.7 ^{b}	19.0 ± 3.7 ^{b}	30.5 ± 0.7 ^{a}	30.9 ± 1.9 ^{a}	30.7 ± 0.4 ^{a}
18:1n-7	1.6 ± 0.1 ^{b}	1.4 ± 0.2 ^{b}	1.5 ± 0.3 ^{b}	1.7 ± 0.1 ^{b}	1.5 ± 0.2 ^{b}	2.1 ± 0.0 ^{a}
20:1[2]	2.7 ± 0.2 ^{bc}	2.4 ± 0.1 ^{c}	2.6 ± 0.1 ^{bc}	3.2 ± 0.1 ^{ab}	3.2 ± 0.2 ^{ab}	3.5 ± 0.3 ^{a}
22:1[3]	1.0 ± 0.1	1.3 ± 0.1	1.4 ± 0.5	1.1 ± 0.5	1.4 ± 0.2	1.1 ± 0.1
24:1n-9	0.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.3	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.3
Total monoenes	39.6 ± 1.6 ^{bc}	37.3 ± 2.2 ^{c}	30.0 ± 5.3 ^{c}	42.6 ± 0.4 ^{ab}	43.9 ± 2.3 ^{ab}	44.0 ± 0.6 ^{a}
18:2n-6	0.8 ± 0.0 ^{b}	0.6 ± 0.0 ^{b}	1.4 ± 0.4 ^{a}	0.7 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	0.7 ± 0.0 ^{b}
20:4n-6	1.3 ± 0.2 ^{ab}	0.9 ± 0.1 ^{c}	0.9 ± 0.1 ^{c}	1.4 ± 0.1 ^{a}	1.0 ± 0.1 ^{bc}	1.5 ± 0.2 ^{a}
Total n-6PUFA[4]	2.6 ± 0.3 ^{b}	1.8 ± 0.1 ^{c}	2.8 ± 0.3 ^{ab}	2.6 ± 0.2 ^{b}	1.8 ± 0.1 ^{c}	3.1 ± 0.1 ^{a}
18:3n-3	0.3 ± 0.0 ^{b}	0.4 ± 0.0 ^{b}	0.7 ± 0.2 ^{a}	0.4 ± 0.0 ^{b}	0.3 ± 0.0 ^{b}	0.3 ± 0.0 ^{b}
20:4n-3	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
20:5n-3	4.7 ± 0.1	5.2 ± 0.2	4.0 ± 0.4	4.8 ± 0.3	5.1 ± 0.5	5.2 ± 0.8
22:5n-3	3.3 ± 0.6	3.0 ± 0.3	2.3 ± 0.7	2.5 ± 0.6	2.4 ± 0.1	2.7 ± 0.5
22:6n-3	20.6 ± 0.4 ^{ab}	23.0 ± 0.8 ^{a}	25.8 ± 2.0 ^{a}	17.3 ± 1.9 ^{b}	17.6 ± 0.4 ^{b}	18.8 ± 3.1 ^{ab}
Total n-3PUFA[5]	29.9 ± 0.8 ^{ab}	32.7 ± 0.2 ^{a}	33.6 ± 3.0 ^{a}	25.9 ± 2.9 ^{b}	26.4 ± 1.0 ^{ab}	27.8 ± 4.4 ^{ab}
Total PUFA	32.5 ± 1.1	34.5 ± 0.1	36.4 ± 2.8	28.5 ± 3.1	28.2 ± 1.1	30.9 ± 4.4

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).
[1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.7 %; [2], Predominantly n-9 isomer; [3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.3 %; [5], Totals include 18:3n-3 and 20:3n-3 present in some samples at up to 0.2 %; PUFA, polyunsaturated fatty acids.

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Table 6. Lipid class composition (percentage of total lipid) of *L. salmonis* obtained from wild and farmed Atlantic salmon in 1995 and 2009

	Wild		Farmed	
Lipid class	1995	2009	1995	2009
PC	13.9 ± 1.5	12.5 ± 0.1	13.5 ± 2.9	13.2 ± 0.5
PE	14.1 ± 2.6	12.6 ± 1.7	14.2 ± 1.8	13.3 ± 1.6
PS/PI/PA/CL	5.2 ± 3.0	6.3 ± 4.5	9.9 ± 1.9	6.8 ± 0.6
Sphingomyelin	3.7 ± 0.8	3.3 ± 0.3	3.5 ± 1.2	3.1 ± 0.2
LPC	0.3 ± 0.2 ^{ab}	0.5 ± 0.1 ^{a}	0.5 ± 0.3 ^{a}	0.0 ± 0.0 ^{b}
Total polar	37.2 ± 5.3	35.2 ± 3.1	41.5 ± 7.4	36.5 ± 2.0
Total neutral	62.8 ± 5.3	64.8 ± 3.1	58.5 ± 7.4	63.5 ± 2.0
Cholesterol	10.5 ± 0.7 ^{b}	8.3 ± 0.5 ^{c}	13.3 ± 0.8 ^{a}	10.6 ± 0.3 ^{b}
Triacylglycerol	42.2 ± 7.5	49.1 ± 4.5	34.7 ± 8.4	47.1 ± 2.8
Free fatty acid	10.1 ± 1.5 ^{a}	7.4 ± 1.3 ^{ab}	10.4 ± 1.8 ^{a}	5.8 ± 0.5 ^{b}
Steryl/wax ester	Trace	Trace	Trace	Trace

Both wild and farmed samples were obtained from Loch Duich in 1995, and from Armadale (wild) and Machrihanish (farmed) in 2009. Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).
CL, cardiolipin; LPC, lyso-PC; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

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Table 7. Fatty acid composition (percentage of total fatty acids) of *L. salmonis* obtained from wild and farmed Atlantic salmon in 1995 and 2009.

	Wild		Farmed	
Fatty acid	1995	2009	1995	2009
14:0	2.0 ± 0.1 ^{ab}	2.0 ± 0.1 ^{ab}	2.1 ± 0.0 ^{a}	1.8 ± 0.1 ^{b}
16:0	18.7 ± 0.7 ^{a}	18.3 ± 1.4 ^{ab}	17.9 ± 0.5 ^{ab}	16.2 ± 0.3 ^{b}
18:0	3.8 ± 0.1	3.7 ± 0.4	3.9 ± 0.5	3.6 ± 0.1
Total saturated[1]	25.1 ± 1.1	24.7 ± 2.1	25.4 ± 0.9	22.6 ± 0.3
16:1n-9	1.7 ± 0.3	1.8 ± 0.1	1.7 ± 0.4	1.5 ± 0.1
16:1n-7	2.7 ± 0.1 ^{b}	3.2 ± 0.1 ^{a}	2.3 ± 0.2 ^{c}	2.1 ± 0.0 ^{c}
18:1n-9	25.0 ± 1.2	27.0 ± 2.7	22.5 ± 2.5	21.8 ± 1.0
18:1n-7	1.5 ± 0.1	1.7 ± 0.1	1.4 ± 0.2	1.4 ± 0.3
20:1[2]	2.2 ± 0.2	2.4 ± 0.1	2.8 ± 0.5	2.2 ± 0.1
22:1[3]	1.0 ± 0.0 ^{b}	0.6 ± 0.1 ^{c}	2.1 ± 0.4 ^{a}	1.0 ± 0.0 ^{b}
24:1n-9	1.1 ± 0.1	1.1 ± 0.1	2.5 ± 1.2	1.2 ± 0.1
Total monoenes	35.3 ± 1.2	37.6 ± 2.9	35.2 ± 3.2	31.2 ± 1.0
18:2n-6	0.4 ± 0.1 ^{c}	0.3 ± 0.0 ^{c}	1.0 ± 0.1 ^{b}	2.2 ± 0.1 ^{a}
20:4n-6	1.2 ± 0.3	1.4 ± 0.1	1.2 ± 0.4	1.5 ± 0.1
Total n-6PUFA[4]	2.3 ± 0.1	2.0 ± 0.2	2.9 ± 0.5	4.4 ± 0.2
18:3n-3	0.2 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}	0.3 ± 0.0 ^{b}	0.5 ± 0.0 ^{a}
20:4n-3	0.4 ± 0.1 ^{b}	0.4 ± 0.1 ^{b}	0.6 ± 0.1 ^{b}	1.0 ± 0.0 ^{a}
20:5n-3	5.1 ± 0.2 ^{ab}	6.0 ± 0.5 ^{a}	4.3 ± 0.8 ^{b}	5.5 ± 0.1 ^{ab}
22:5n-3	4.3 ± 0.3 ^{a}	3.5 ± 0.3 ^{ab}	3.0 ± 0.5 ^{b}	3.5 ± 0.2 ^{ab}
22:6n-3	22.1 ± 1.2 ^{ab}	20.9 ± 2.7 ^{ab}	18.4 ± 3.1 ^{b}	25.7 ± 1.2 ^{a}
Total n-3PUFA[5]	32.2 ± 1.6 ^{ab}	31.1 ± 3.5 ^{ab}	26.9 ± 4.5 ^{b}	36.8 ± 1.3 ^{a}
Total PUFA	34.5 ± 1.6 ^{ab}	33.1 ± 3.8 ^{ab}	29.7 ± 4.5 ^{b}	41.1 ± 1.4 ^{a}

Both wild and farmed samples were obtained from Loch Duich in 1995, and from Armadale (wild) and Machrihanish (farmed) in 2009. Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$).

[1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.7 %;

[2], Predominantly n-9 isomer; [3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6 20:3n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.3 %; [5], Totals include 18:3n-3 and 20:3n-3 present in some samples at up to 0.3 %; PUFA, polyunsaturated fatty acids.

Table 8. Lipid content (percentage of wet weight), triacylglycerol (TAG) content (percentage of total lipid), and fatty acid composition (percentage of total fatty acids) of *L. salmonis* and their egg strings along with the muscle of the Atlantic salmon from which they were collected.

Fatty acid	Salmon muscle	Female lice	Egg strings
Lipid content	3.5 ± 0.1 [b]	1.6 ± 0.2 [c]	6.1 ± 1.2 [a]
TAG	54.4 ± 4.4 [b]	47.1 ± 2.8 [b]	72.0 ± 1.4 [a]
14:0	3.2 ± 0.5 [a]	1.8 ± 0.1 [b]	1.9 ± 0.1 [b]
16:0	11.6 ± 0.2 [b]	16.2 ± 0.3 [a]	16.4 ± 0.4 [a]
18:0	3.0 ± 0.0 [c]	3.6 ± 0.1 [b]	3.9 ± 0.1 [a]
Total saturated[1]	18.4 ± 0.8 [b]	22.6 ± 0.3 [a]	22.7 ± 0.4 [a]
16:1n-9	0.0 ± 0.0 [c]	1.5 ± 0.1 [a]	1.3 ± 0.0 [b]
16:1n-7	3.6 ± 0.0 [a]	2.1 ± 0.0 [b]	2.1 ± 0.1 [b]
18:1n-9	16.1 ± 0.4 [c]	21.8 ± 1.0 [b]	23.6 ± 0.4 [a]
18:1n-7	2.1 ± 0.1 [a]	1.4 ± 0.3 [b]	1.6 ± 0.1 [b]
20:1[2]	8.7 ± 0.3 [a]	2.2 ± 0.1 [c]	2.9 ± 0.0 [b]
22:1[3]	9.7 ± 0.5 [a]	1.0 ± 0.0 [b]	1.2 ± 0.0 [b]
24:1n-9	2.0 ± 0.9	1.2 ± 0.1	0.9 ± 0.1
Total monoenes	42.3 ± 0.4 [a]	31.2 ± 1.0 [c]	33.6 ± 0.7 [b]
18:2n-6	5.3 ± 0.1 [a]	2.2 ± 0.1 [b]	2.2 ± 0.1 [b]
20:4n-6	0.7 ± 0.0 [b]	1.5 ± 0.1 [a]	1.7 ± 0.1 [a]
Total n-6PUFA[4]	7.0 ± 0.2 [a]	4.4 ± 0.2 [b]	4.9 ± 0.2 [b]
18:3n-3	1.2 ± 0.0 [a]	0.5 ± 0.0 [b]	0.6 ± 0.0 [b]
20:4n-3	1.5 ± 0.1 [a]	1.0 ± 0.0 [b]	1.0 ± 0.1 [b]
20:5n-3	5.6 ± 0.3 [ab]	5.5 ± 0.1 [b]	6.3 ± 0.4 [a]
22:5n-3	1.3 ± 1.5 [b]	3.5 ± 0.2 [a]	3.0 ± 0.1 [ab]
22:6n-3	14.4 ± 0.7 [c]	25.7 ± 1.2 [a]	21.6 ± 0.5 [b]
Total n-3PUFA[5]	25.9 ± 2.7 [b]	36.8 ± 1.3 [a]	32.8 ± 0.8 [a]
Total PUFA	32.9 ± 2.9 [b]	41.1 ± 1.4 [a]	37.8 ± 0.9 [a]

Samples were obtained from Machrihanish in 2009. Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$). [1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.7 %; [2], Predominantly n-9 isomer; [3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6 20:3n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.3 %; [5], Totals include 18:4n-3 and 20:3n-3 present in some samples at up to 0.3 %; PUFA, polyunsaturated fatty acids.

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Table 9. Fatty acid compositions (percentage of total fatty acids) of phospholipids and triacylglycerols of *L. salmonis* and their egg strings along with the muscle of the Atlantic salmon from which they were collected

	Phospholipids			Triacylglycerols		
	Salmon muscle	Female lice	Egg strings	Salmon muscle	Female lice	Egg strings
14:0	1.3 ± 0.1	2.9 ± 0.2	4.6 ± 0.0	4.4 ± 0.0	1.5 ± 0.0	1.7 ± 0.0
16:0	19.6 ± 0.1	18.5 ± 0.5	18.2 ± 0.4	11.6 ± 0.0	17.0 ± 1.1	18.5 ± 0.3
18:0	5.9 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	2.8 ± 0.0	4.3 ± 0.2	4.6 ± 0.1
Total saturated[1]	27.3 ± 0.1	25.3 ± 0.6	26.2 ± 0.5	19.4 ± 0.1	23.3 ± 1.3	25.3 ± 0.2
16:1n-9	0.0 ± 0.0	1.7 ± 0.1	1.7 ± 0.0	0.0 ± 0.0	1.5 ± 0.0	1.3 ± 0.0
16:1n-7	1.2 ± 0.0	1.9 ± 0.0	1.5 ± 0.1	4.5 ± 0.0	2.4 ± 0.0	2.3 ± 0.1
18:1n-9	5.7 ± 0.0	16.1 ± 0.7	16.6 ± 1.4	19.9 ± 0.0	28.3 ± 0.9	27.7 ± 0.0
18:1n-7	1.7 ± 0.0	1.3 ± 0.4	0.9 ± 0.0	2.3 ± 0.1	1.9 ± 0.2	1.8 ± 0.2
20:1[2]	1.4 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	10.9 ± 0.0	3.2 ± 0.0	3.6 ± 0.0
22:1[3]	0.6 ± 0.0	0.6 ± 0.0	0.9 ± 0.9	12.3 ± 0.2	1.4 ± 0.0	1.4 ± 0.0
24:1n-9	1.1 ± 0.0	0.9 ± 0.1	2.4 ± 2.1	1.0 ± 0.0	0.9 ± 0.1	0.7 ± 0.0
Total monoenes	11.7 ± 0.2	23.7 ± 0.5	25.5 ± 1.8	51.0 ± 0.1	39.6 ± 0.6	39.0 ± 0.2
18:2n-6	1.6 ± 0.0	1.6 ± 0.0	1.1 ± 0.0	6.1 ± 0.0	2.8 ± 0.0	2.4 ± 0.1
20:3n-6	0.9 ± 0.3	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:4n-6	1.7 ± 0.0	1.0 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	1.8 ± 0.2	2.0 ± 0.0
Total n-6 PUFA	5.1 ± 0.4	3.4 ± 0.2	2.8 ± 0.6	7.9 ± 0.0	5.6 ± 0.3	5.4 ± 0.1
18:3n-3	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
18:4n-3	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.9 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
20:4n-3	1.0 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	1.5 ± 0.0	1.2 ± 0.0	1.1 ± 0.0
20:5n-3	11.1 ± 0.0	4.8 ± 0.0	4.1 ± 0.1	4.4 ± 0.0	6.1 ± 0.3	6.5 ± 0.2
22:5n-3	2.0 ± 0.0	4.1 ± 0.0	3.6 ± 0.2	2.3 ± 0.0	3.3 ± 0.3	2.8 ± 0.0
22:6n-3	40.1 ± 0.3	36.8 ± 0.9	36.1 ± 2.0	9.6 ± 0.0	18.7 ± 1.1	18.2 ± 0.5
Total n-3 PUFA	55.3 ± 0.3	47.1 ± 0.9	45.0 ± 1.9	21.2 ± 0.0	30.6 ± 1.7	29.7 ± 0.2
Total PUFA[6]	61.0 ± 0.1	51.0 ± 1.1	48.3 ± 1.2	29.7 ± 0.0	37.1 ± 1.9	35.7 ± 0.1

Samples were obtained from Machrihanish in 2009. Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different ($P < 0.05$). [1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.4 %; [2], Predominantly n-9 isomer; [3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.5 %; [5], Totals include 20:3n-3 present in some samples at up to 0.2 %; [6], Includes C16 PUFA; PUFA, polyunsaturated fatty acids.

Table 10. Lipid class composition (percentage of total lipid) of *Lepeophtheirus spp*. from Atlantic salmon, sea trout and halibut

Lipid class	Atlantic salmon	sea trout	halibut
PC	13.9 ± 1.5 [b]	14.2 ± 1.7 [b]	18.6 ± 0.7 [a]
PE	14.1 ± 2.6 [b]	13.7 ± 1.8 [b]	20.0 ± 1.4 [a]
PS/PI/PA/CL	5.2 ± 3.0	7.6 ± 3.7	12.4 ± 5.1
Sphingomyelin	3.7 ± 0.8 [b]	5.3 ± 1.6 [ab]	8.1 ± 1.3 [a]
LPC	0.3 ± 0.2 [b]	1.3 ± 0.2 [a]	0.7 ± 0.2 [b]
Total polar	37.2 ± 5.3 [b]	42.0 ± 7.4 [b]	59.7 ± 5.7 [a]
Total neutral	62.8 ± 5.3 [a]	58.0 ± 7.4 [a]	40.3 ± 5.7 [b]
Cholesterol	10.5 ± 0.7	12.1 ± 3.1	11.6 ± 1.4
Triacylglycerol	42.2 ± 7.5 [a]	39.9 ± 12.1 [a]	18.2 ± 3.1 [b]
Free fatty acid	10.1 ± 1.5	6.1 ± 1.6	10.5 ± 1.4
Steryl/wax ester	Trace	Trace	Trace

Results are means ± SD (n = 3). Values within a row with different superscript letter are significantly different ($P < 0.05$). CL, cardiolipin; LPC, lyso-PC; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

Table 11. Fatty acid composition (percentage total fatty acids) of *Lepeophtheirus spp*. from Atlantic salmon, sea trout and halibut.

Fatty acid	Atlantic salmon	sea trout	halibut
14:0	2.0 ± 0.1 [a]	1.0 ± 0.3 [b]	0.5 ± 0.2 [b]
16:0	18.7 ± 0.7 [a]	17.4 ± 1.0 [ab]	15.0 ± 2.0 [b]
18:0	3.8 ± 0.1 [b]	4.9 ± 0.7 [ab]	5.1 ± 0.4 [a]
Total saturated[1]	25.1 ± 1.1	24.9 ± 1.0	21.6 ± 3.2
16:1n-9	1.7 ± 0.3	1.6 ± 0.1	1.4 ± 0.1
16:1n-7	2.7 ± 0.1 [a]	2.4 ± 0.2 [a]	0.9 ± 0.2 [b]
18:1n-9	25.0 ± 1.2 [a]	18.8 ± 4.3 [b]	8.8 ± 0.4 [c]
18:1n-7	1.5 ± 0.1 [b]	1.8 ± 0.1 [a]	0.8 ± 0.1 [c]
20:1[2]	2.2 ± 0.2 [a]	1.9 ± 0.1 [a]	1.4 ± 0.2 [b]
22:1[3]	1.0 ± 0.0	0.8 ± 0.3	0.5 ± 0.2
24:1n-9	1.1 ± 0.1	2.3 ± 1.1	3.6 ± 1.7
Total monoenes	35.3 ± 1.2 [a]	29.7 ± 2.8 [b]	17.6 ± 2.1 [c]
18:2n-6	0.4 ± 0.1	0.8 ± 0.4	0.9 ± 0.2
20:4n-6	1.2 ± 0.3	1.1 ± 0.1	0.9 ± 0.1
Total n-6PUFA[4]	2.3 ± 0.1 [b]	3.3 ± 0.4 [a]	3.8 ± 0.4 [a]
18:3n-3	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
20:4n-3	0.4 ± 0.1 [a]	0.5 ± 0.0 [a]	0.3 ± 0.0 [b]
20:5n-3	5.1 ± 0.2 [a]	5.7 ± 0.1 [a]	4.2 ± 0.4 [b]
22:5n-3	4.3 ± 0.3 [a]	2.5 ± 0.2 [b]	1.4 ± 0.4 [c]
22:6n-3	22.1 ± 1.2 [b]	22.2 ± 2.1 [b]	35.8 ± 4.9 [a]
Total n-3PUFA[5]	32.2 ± 1.6 [b]	31.5 ± 2.1 [b]	42.1 ± 5.6 [a]
Total PUFA	34.5 ± 1.6 [b]	34.8 ± 2.4 [b]	45.9 ± 5.5 [a]

Results are means ± SD (n = 3). Values within a row with a different superscript letter are significantly different ($P < 0.05$). [1], Totals include 15:0, 20:0 and 22:0 present in some samples at up to 0.7 %; [2], Predominantly n-9 isomer; [3], Predominantly n-11 isomer; [4], Totals include 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.3 %; [5], Totals include 18:3n-3 and 20:3n-3 present in some samples at up to 0.2 %; PUFA, polyunsaturated fatty acids.