VIE ET MILIEU - LIFE AND ENVIRONMENT, 2019, 69 (1): 47-52

POTENTIAL IMPACT OF AQUACULTURE EFFLUENTS IN LOCH CRERAN, SCOTLAND

*L. NIPPARD, C. CIOCAN**

School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, BN2 4GJ, East Sussex, UK
** Corresponding author: C.Ciocan@brighton.ac.uk*

MARINE POLLUTION
MYTILUS EDULIS
AQUACULTURE
BIOINDICATOR
HISTOLOGY

ABSTRACT. – The aquaculture industry is developing at a fast pace, growing from 1 million tonnes a year harvested in the 1950s, to more than 75 million tonnes nowadays. However, there are rising concerns over the environment impacts. The aim of this study is to investigate the impacts of salmon aquaculture in Loch Creran, Scotland, using mussels *Mytilus edulis* (Linnaeus, 1758) as a bioindicator. Water samples and mussel tissues were collected from two different sites along the loch shore and were analysed for contaminant bioaccumulation and biological effects, using gas chromatography and histology techniques. The results showed high levels of heavy metals and organic compounds (n-hexadecanoic acid, 1-Heptatriacontanol, Isophorone, Cholestan-3-ol and oleic acid) in mussel tissues close to the salmon farms. The histology results indicate that the mussels from the aquaculture site are in poor health condition, with 90 % of tissue samples exhibiting one or more signs of pollutant induced stress, such as haemocyte infiltrations, lipofuscin accumulation, parasites and possible neoplasms.

INTRODUCTION

Over the last 60 years, production, investment and employment in aquaculture industry has grown significantly, with approximately 75 million tonnes of aquaculture product harvested in 2014 (FAO report 2016). Salmon is farmed in over 24 countries (Burridge *et al*. 2010) and only recently Scotland became the third largest producer globally, shipping salmon to over 50 countries. As a result of the development of aquaculture, rural and coastal employment opportunities have increased considerably (Peel & Lloyd 2008). Aquaculture in Scotland contributes to approximately 8000 jobs; 35 % related directly to production, 60 % in the supply chain and 5 % from other associated employment (Marine Scotland, 2015).

The western Scottish islands and coastline are the preferred location for fish and shellfish farms due to the area providing adequate shelter, deep enough water, and ease of access to facilities (Peel & Lloyd 2008). However, salmon farming in Scotland also has a negative impact on the environment. For 18-24 months salmon are reared in seawater, in large floating net pens, which will allow for all aquaculture waste to be released directly into the environment (Cao *et al*. 2007). In areas of shallow water and weak currents, an accumulation of waste from aquaculture can occur on the seabed close to the discharge point, potentially impacting benthic macro-fauna (SEPA, 2017).

The Scottish Environmental Protection Agency (SEPA) regulates the chemicals (including medicines) used in aquaculture, by setting site specific limits (SEPA, 2017). Any new aquaculture operation requiring licencing, SEPA is able to assess the risk posed to the environment by such activities, through seabed surveys conducted every two years.

Nevertheless, the aquaculture effluents are often released onto the natural environment. They consist of pesticides, fish feed waste, faecal matter, and chemical residue, therefore rich in nutrients such as nitrogen and phosphorus, which can cause eutrophication, anoxia and increased turbidity (Read & Fernandes 2003, Russel *et al*. 2011).

There is much consideration for the health of the farmed species and Scotland's farmed salmon is recognised internationally as having high health standards (Marine Scotland, 2015). However, the environmental impacts of aquaculture are mostly related to the persistence of the contaminants in the environment and their long term effects on local aquatic communities, especially the vulnerable populations of shellfish (Marine Scotland, 2009, 2015).

Mussels have been frequently used as bioindicators for environmental pollution as they are sessile, filter-feeding species, able to accumulate a large array of contaminants from the water column (Ciocan *et al*. 2012), with significant consequences on their physiology and survival (Viarengo *et al*. 1990, Aarab *et al*. 2011).

The aim of this study is to investigate the impact of salmon aquaculture in Loch Creran, Scotland using *Mytilus edulis* as a bioindicator. The levels of contaminants in water and animal tissue will be assessed and the pollutant induced effects on mussel histology will be examined and discussed.

MATERIALS AND METHODS

Sample site characterization: Loch Creran is located on the west coast of Scotland in the Argyll and Bute region. The Loch

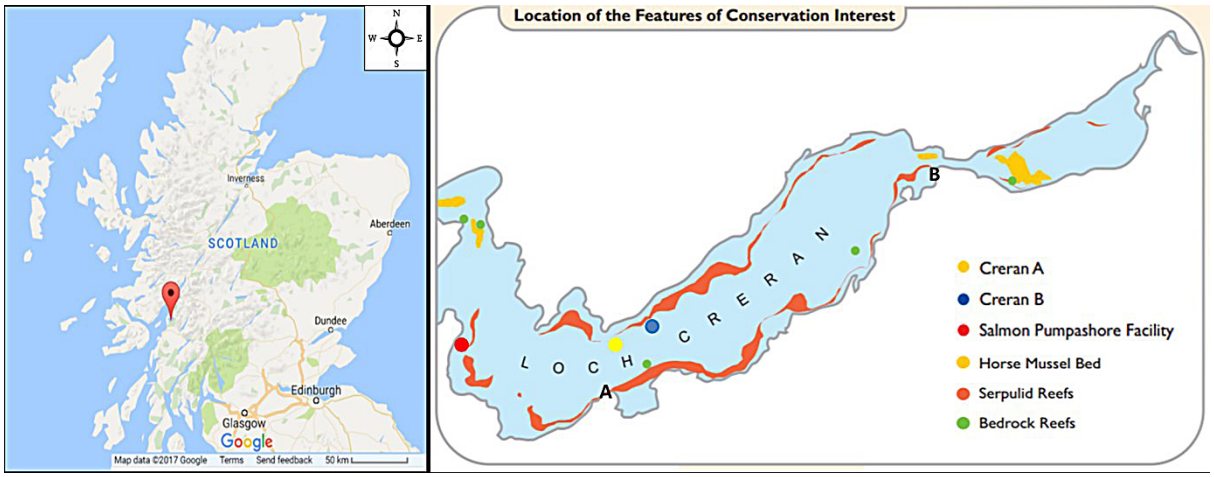

Fig. 1. – Loch Creran located on the west coast of Scotland. Orientated NE-SW with the Scottish Sea Farms Ltd processing plant labelled in Red and the two sea cages in yellow (currently active) and blue. **A**: Sample site and **B**: Control site (Google Maps, 2017) and (Argyll Marine Special Areas of Conservation, 2005).

is approximately six miles long, orientated NE-SW and is typical of a fjordic sea loch (Fig. 1). Due to its unique bedrock and slow growing biogenic reefs (*Serpula vermicularis* and *Modiolus modiolus)*, Loch Creran has been designated a marine Special Area of Conservation (mSAC) under the EU Habitats Directive (92/43/EEC). The slow growing reefs are extremely sensitive to physical damage and provide habitat for a wide range of marine species, especially sessile invertebrates. Therefore, salmon cages are placed in deeper water away from the biogenic reefs, preventing physical damage to the reefs, thus mitigating the effects of the aquaculture structure.

Sample collection: Water samples and mussels were collected from area A, within 600 m from the salmon sea cages and approx. 2 km from the Scottish Sea Farms Ltd salmon processing plant (Fig. 1). A potentially control sample area (B) was identified approximately 5 km SW from site A, at the eastern end of the loch. In June 2016, 500 ml water samples were collected from each site, in triplicates, whilst 20 mussels (45 ± 4 mm length) were sampled at a spring low tide. The mussels were kept in containers filled with seawater and surrounded with ice and transported to the laboratory facilities for analysis.

ICP Analysis: Flesh from 10 mussels were pooled and analyzed for their elemental content alongside water samples. An inductively coupled plasma optical emissions spectrometer (ICP-OES Perkin Elmer Inc. Optima 2100 DV) was used, and the method was adapted from Giltrap *et al*. (2016) and Amachree *et al*. (2013). *Mytilus edulis* samples from each site were placed in oven at 85° until a constant weight. The dried tissues were homogenised and ground into a fine powder using a pestle and mortar, which were washed between samples to avoid cross contamination.

An aliquot of 0.1 g of powder from each site was weighed out and digested by 3 ml of nitric acid (HNO_3), in triplicates. After 3 hours of acid digestion, 7 ml of analytical grade water was added to bring the solution up to the 10 ml necessary for ICP-OES analysis. The 3 × 10 ml of water samples from both sites were centrifuged and analyzed using the ICP-OES, which was calibrated to measure for Cu, As, Cd, Pb, Cr, Fe, Mn, Zn, and P.

Gas chromatography analysis: An aliquot of 5 g of the dried tissue samples for each site was placed into a 20 ml extraction vial containing 20 ml of 1:1 hexane/acetone mix. The extraction was carried out using a CEM Mars 6 microwave digester Agilent 7820A GC with 5977B MS. Over a 15 minute period the samples were heated to 115° C and maintained at this temperature for 20 minutes. After the samples had cooled the supernatant was removed and bottled for analysis.

Targeted analysis was carried out for traces of organic contaminants: n-hexadecanoic acid, 1-heptatriacontanol, oleic acid, eicosapentaenoic acid, cholestan 3, ethyl iso allocholate, isophorone, diethyl phthalate.

The equipment, Agilent 7820A gas chromatograph with 5977B mass spectrometer, employed a DB-5MS 30 m × 250 µm × 0.25 µm column, filled with 1 ml/min of He. The oven was heated to 50° C and increased at a rate of 50° C/min to 320° C and held for 10 minutes. The mass spectrometer full scan was set at 50-600 m/z.

Histology analysis: Fragments of 0.5 cm^2 gonad, gill and hepatopancreas were sampled from each mussel (10 mussels per site) and placed into 1 ml of formaldehyde solution 4 %. The tissue sections were processed using Leica TP 1050 Tissue processor and 7 microns slices have been cut using a rotary microtome (Leica). The samples were then stained using haematoxylin and eosin, and examined using a Nikon Eclipse E200. Images were captured using a GXCam Hichrome Lite camera via GX capture software.

Statistical analysis: Data has been processed using Minitab 17. To test the differences between samples, normality tests have been applied followed by nonparametric Mann Whitney test. Confidence limit has been set at 95 %.

RESULTS

Metal contaminants in mussel tissue and seawater

The level of metal contaminants in mussel flesh collected from the aquaculture site A showed significantly higher concentrations of Cd (0.018 mg/L), Pb (0.121 mg/L) and Zn (8.636 mg/L), compared to the control site B (0.011 mg/L for Cd, 0.103 mg/L for Pb and 4.301 mg/L for Zn). The elemental concentrations in the mussel tissues were noticeably higher than the concentrations in the water, probably due to the bioaccumulation ability of *M. edulis*. Only Zn showed a significantly higher level in area A (0.172 mg/L) compared to B (0.132 mg/L).

Gas chromatography – Analysis mussel tissue

The targeted analysis for n-Hexadecanoic acid, 1-Heptatriacontanol, Oleic acid and Isophorone showed that the bioaccumulation of those respective compounds in mussel flesh was significantly higher in site A (closer to aquaculture site). In water samples, only 1-Heptatriacontanol and n-Hexadecanoic acid were detected at very low concentrations, in the area A (Fig. 2).

Histopathology of mussel tissues

Most of the gill tissue samples from mussels in area A showed lipofuscin accumulation, haemocyte aggregations or both. No such histopathologies have been recorded in the control site B (Fig. 3).

Nine of the ten hepatopancreas samples from the area A showed histological damage. Lipofuscin droplets have been observed in 40 % of the samples, whilst more than half of mussels exhibited sclerotic cells within the digestive tubule walls. Other pathologies included haemocyte infiltration in the digestive tract, parasite infestation and atrophy of the digestive tubule walls (Fig. 4).

40 % of the mussels in area A showed histopathologies of the gonad: haemocyte infiltration, atretic oocytes and even two potential gonad neoplasms (Fig. 5). All ten mussels from control area B showed healthy gonad tissues.

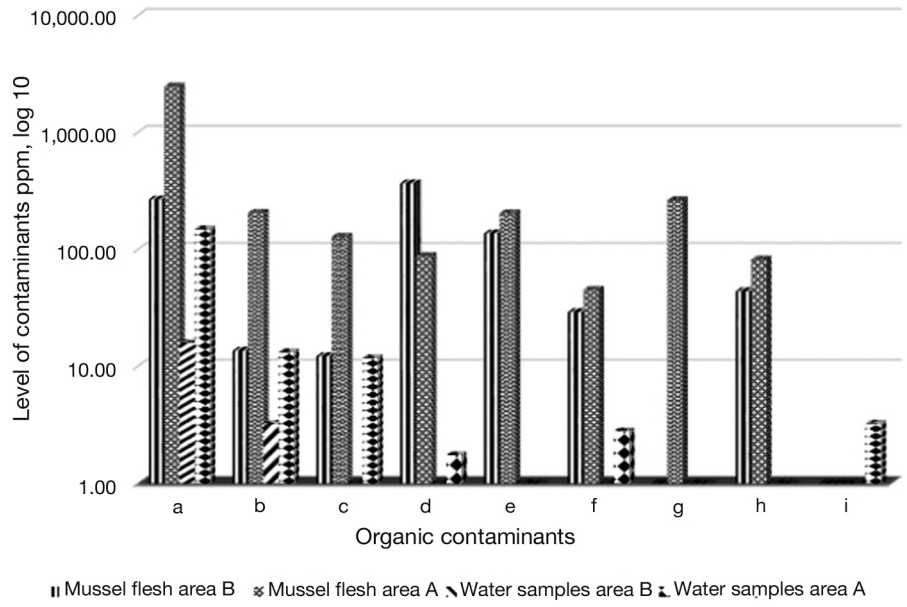

Fig. 2. – Levels of organic compounds (log scale) detected in mussel flesh and water samples, from two locations (A and B) in Loch Creran. The targeted analysis was carried out using an Agilent 7820A GC-MS. a: n-Hexadecanoic acid; b: 1-Heptatriacontanol; c: Oleic acid; d: Eicosapentaenoic acid; e: Cholestan-3-ol; f: Ethyl iso-allocholate; g: Isophorone; h: Cyclohexyl(methyl)silane; i: Diethyl Phthalate.

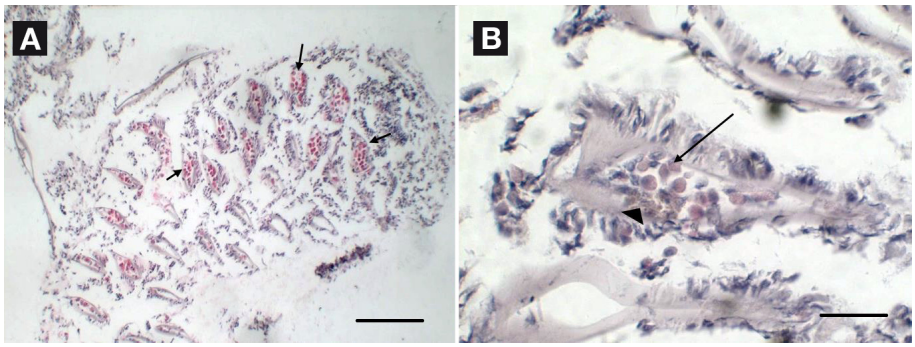

Fig. 3. – Gill tissue sampled from the aquaculture site A. **A**: Multiple gill filaments infiltrated by haemocytes (arrows). **B**: Gill filament containing haemocytes (arrow) and lipofuscin accumulation (arrow head). Scale bars = 200 µm (A) and 10 µm (B).

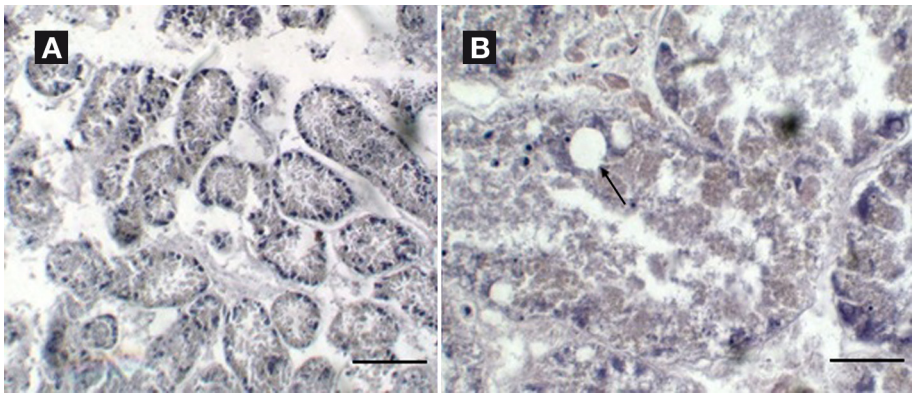

Fig. 4. – Histopathology of digestive gland in mussels collected from area A. **A**: Sclerotic cells and atrophy in the digestive tubules; **B**: Vacuolisation in the hepatopancreas. Scale bars = 200 µm (A, B).

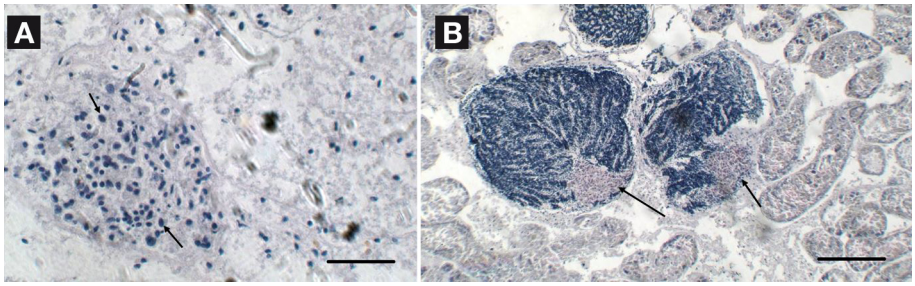

Fig. 5. – Gonad histopathology. **A**: Possible neoplasm in a mussel from site A, gonad in resting stage. Abnormally large nuclei (arrow); **B**: Haemocytic infiltration in a male follicle, gonad from a mussel in site A. Scale bars = 200 µm (A, B).

DISCUSSION

The level of contaminants in mussel tissues and water samples have been compared with background assessment criteria (BAC) developed by OSPAR (2010). The levels of Cu, Pb, and Zn exceeded the BAC in both areas. According to Sunila, 1988, prolonged exposure to Cu results in haemocyte infiltration and lipofuscin accumulation, especially in gills and digestive gland. Our results showed that 9 out of the 10 mussel samples from the aquaculture site A exhibited those pathologies, compared to zero in the control area B. Antifouling paints used in open water fish farms are the potential source of Cu pollution. However, Loch Creran has the second greatest mixing depth when ranked against other sea lochs, which results in a 60 % water exchange occurring every 3 days (Wilson *et al*. 2006). The water mixing can therefore explain the high levels of Cu recorded at both sites.

The levels of Cd and Pb in the mussel tissue samples were significantly higher in area A compared to control area B. Previous studies into the biological adverse impact of Cd on *M. edulis* have reported inflammations leading to haemocyte infiltration in the digestive tissue (Amachree *et al*. 2013, Sunila 1988). Interestingly, Giltrap *et al*. (2016) reported Cd contamination in the Irish Sea, not related to aquaculture impact, with mussels exhibiting similar pathologies to those observed in our study.

Our results showed increased levels of Zn in both mussel tissues and water samples from area A. Whilst antifouling paints can be the source of heavy metal leakage into the Loch, research into the effects of elevated levels of Zn on *M. edulis* appears to be limited. A study by Nadella *et al*. (2013) suggested that Zn has no significant effects on mussel embryos.

n-Hexadecanoic acid is a naturally occurring fatty acid in animals that is produced during lipogenesis. Fatty acids are known for their antibacterial and antifungal properties and further research by Aparna *et al*. (2012) found that the fatty acid n-hexadecanoic acid also has anti-inflammatory agents. In our study, the concentration of n-hexadecanoic acid was almost 10 times higher at the aquaculture site A in the mussel tissues and seawater compared to the control. It is suspected that fish feed waste could contribute to the high levels of n-hexadecanoic acid present in area A. Similarly, the concentration of 1-heptatriacontanol is 15 times higher in the mussel tissue and 4 times higher in seawater at the aquaculture site A. The compound can be used for treating dermatological disorders, as well as fungal infections and parasite infestations (Gohar 2001).

New studies have suggested that fishmeal has increasingly become enriched with vegetable and animal derived fatty acids, such as oleic acid (White *et al*. 2017); therefore the fatty acid composition of modern salmon fishmeal differs considerably from the natural food sources. Moreover, Redmond *et al*. (2010) showed that terrestrial plant and animal derived fatty acids from aquaculture feed are assimilated into the body tissue of mussels, *M. edulis,* from natural populations. Our results are in accordance with Redmond *et al*. (2010), with the concentration of oleic acid in the mussel tissue from area A ten times higher compared to control. Oleic acid was also detected in seawater samples from area A, but absent from control

area B. These findings suggest that the salmon farm in Loch Creran is using terrestrial plant or animal derived fatty acids in the fish feed and some compounds are bioaccumulated in mussels.

Cholestan-3-ol also known as Coprostanol is a bioactive lipid compound, present in fish feed, known for its chemotherapeutic, antibiotic, antiparasitic, and antiseptic properties (Luo *et al*. 2015). In a study by Frena *et al*. (2016), Coprostanol was used as an indicator of anthropogenic pollution, including aquaculture, in an estuarine system in northeast Brazil. Elevated concentrations of Coprostanol in aquatic environment were associated with severe signs of stress in mussels (Sherwin *et al*. 1993). Our results showed that Coprostanol concentrations were 1.5 times higher in the mussel tissues at the aquaculture site A, in contrast to the control samples.

Ethyl iso-allocholate is a steroid derivative compound found in rice and has antimicrobial properties (Kullappan *et al*. 2016). In our study, this compound was found in higher concentrations in mussel tissues from area A, compared to control.

Cyclohexylmethylsilane and isophorone are mainly used in coating surfaces (metal or plastic) and the commercial compounds also contain herbicide and pesticide agents. Isophorone was not detected at the control site and was present at 263.94 ppm at the aquaculture site A, implying that this compound is potentially used in coating products for the sea cages in Loch Creran. The levels of Cyclohexylmethylsilane are twice as higher at the aquaculture site compared to the control but are in relatively low concentrations compared to isophorone.

Histopathology

Increased lipofuscin accumulation and haemocyte aggregations in the gills filaments, especially in mussels from area A, demonstrate that the natural population of *M. edulis* are affected by the pollutants present in the water. Aarab *et al*. (2011) and Svärdh & Johannesson (2002), showed that PAHs and metal pollution can induce such histopathological damage in mussel gills. Moreover, high levels of haemocytes invading the digestive tract have been observed on mussels from area A, as well as large number of vacuoles in the hepatopancreas in the same samples. The presence of sclerotic cells in the digestive tubules in mussels from area A are considered a sign of a chronic long term stress, similar to the results obtained by Sunila (1988). Interestingly, there appeared to be a skewed sex ratio at the polluted site with a large proportion of males (4:1 males to females), which could be an indicator of environmental stress. According to Giltrap *et al*. (2016), over exposure to chemicals in pesticides can cause feminization in male mussels. Also, male mussels are likely to be more susceptible to neoplasms due to the males undergoing multiple divisions during gametogenesis (Maloy 2001). Mussels in our study exhibited possible gonadal neoplasms in two samples from area A.

CONCLUSION

The observed histopathology in the mussel samples from the aquaculture site are an indicator that the mussels in this area are under environmental stress. Many individuals exhibited signs of stress induced by metal pollution. The overall heavy metal levels were above BAC in the tissues and EU standards in the water at both sites, suggesting that the aquaculture activities are impacting on the health of the entire loch.

The chemicals routinely monitored by SEPA were not found in the water or mussel tissue samples in either area. However, most of the organic compounds identified by GC analysis were found in the mussel tissue and seawater samples in area A, close to aquaculture site. Our results indicate that the typical organic pollution encountered on the developed coastlines is exacerbated by aquaculture activities. Further research is needed to establish the impact of these compounds on the health status of natural populations of *M. edulis*.

REFERENCES

Aarab N, Godal BF, Bechmann RK 2011. Seasonal variation of histopathological and histochemical markers of PAH exposure in blue mussel (*Mytilus edulis* L.). *Mar Environ Res* 71(3): 213-217.

Amachree D, Moody AJ, Handy RD 2013. Comparison of intermittent and continuous exposures to cadmium in the blue mussel, *Mytilus edulis*: accumulation and sub-lethal physiological effects. *Ecotoxicol Environ Safety*: 19-26.

Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chem Biol Drug Design* 80(3): 434-439.

Argyll Marine Special Areas of Conservation 2005. Loch Creran Marine SAC Management Plan.

Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture* 306(1-4): 7-23.

Cao L, Wang W, Yang Y, Yang C, Yuan Z, Xiong S, Diana J 2007. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environ Sci Poll Res Int* 14(7): 452-462.

Ciocan C, Cubero-Leon E, Peck MR, Langston WJ, Pope N, Minier C, Rotchell JM 2012. Intersex in *Scrobicularia plana*: transcriptomic analysis reveals novel genes involved in endocrine disruption. *Environ Sci Technol* 46(23): 12936-12942.

FAO 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome: 200 p.

Frena M, Souza MRR, Damasceno FC, Madureira LAS, Alexandre MR 2016. Evaluation of anthropogenic contamination using sterol markers in a tropical estuarine system of northeast Brazil. *Mar Poll Bull* 109(1): 619-623.

Giltrap M, Ronan J, Tanner C, O'Beirn FX, Lyons BP, Mag Aoidh R, Rochford H, McHugh B, McGovern E, Wilson J 2016. Application of a weight of evidence approach utilising biological effects, histopathology and contaminant levels to assess the health and pollution status of Irish blue mussels (*Mytilus edulis*). *Mar Environ Res* 122: 33-45.

Gohar AA 2001. Heptatriacontanol and phenolic compounds from *Halochris hispida*. *Nat Product Sci* 7(3): 68-71.

Luo X, Su P, Zhang W 2015. Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Mar Drugs* 13(7): 4231-4254.

Kullappan M, Anbarasu A, Ramaiah S 2016. Ethyl iso-allocholate from a medicinal rice karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: a molecular docking and dynamics study. *Indian J Pharm Sci* 78(6): 780-788.

Maloy A 2001. Gametogenic Cycles of Marine Mussels, *Mytilus edulis* and *Mytilus trossulus* in Cobscook Bay, Maine. Univ Maine: 42.

Marine Scotland 2009. A Fresh Start. The renewed Strategic Framework for Scottish Aquaculture. *The Scottish Government*.

Marine Scotland 2015. Scotland's National Marine Plan: A Single Framework for Managing Our Seas. [online] http://www.gov.scot/Publications/2015/03/6517/0

Nadella SR, Tellis M, Diamond R, Smith S, Bianchini A, Wood CM 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: critical tissue residues and effects of dissolved organic matter and salinity. *Comp Biochem Physiol C Toxicol Pharmacol* 158(2): 72-83.

OSPAR Commission 2010. Agreement on CEMP Assessment Criteria for the QSR 2010.

Peel D, Lloyd MG 2008. Governance and planning policy in the marine environment: regulating aquaculture in Scotland. *Geo J* 174(4): 361-373.

Read P, Fernandes T 2003. Management of environmental impacts of marine aquaculture in Europe. *Aquaculture* 226(1-4):139-163.

Redmond KJ, Magnesen T, Hansen PK, Strand Ø, Meier S 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). *Aquaculture* 298(3-4): 202-210.

Russell M, Robinson CD, Walsham P, Webster L, Moffat CF 2011. Persistent organic pollutants and trace metals in sediments close to Scottish marine fish farms. *Aquaculture* 319(1-2): 262-271.

SEPA (2017). Aquaculture. https://www.sepa.org.uk/regulations/water/aquaculture

Sherwin MR, Van Vleet ES, Fossato VU, Dolci F 1993. Coprostanol (5-cholestan-3-ol) in lagoonal sediments and mussels of Venice, Italy. *Mar Poll Bull* 26(9): 501-507.

Sunila I 1988. Acute histological responses of the gill of the mussel, *Mytilus edulis*, to exposure by environmental pollutants. *J Invert Pathol* 52(1): 137-141.

Svärdh L, Johannesson K 2002. Incidence of hemocytes and parasites in coastal populations of blue mussels (*Mytilus edulis*) – Testing correlations with area, season, and distance to industrial plants. *J Invert Pathol* 80(1): 22-28.

Viarengo A, Canesi L, Pertica M, Poli G., Moore MN, Orunesu 1990. Heavy metal effects on lipid peroxidation in the tissues of *Mytilus galloprovincialis* lam. *Comp Biochem Physiol C: Comp Pharmacol* 97(1): 37-42.

White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Kutti T, Dempster T 2017. Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. *Sci Total Environ* 586: 1170-1181.

Wilson A, Nickell T, Cromey C, Black K 2006. ECASA Study Site Report; Loch Creran, Scotland. Internal Document.

Received on January 17, 2018
Accepted on March 18, 2019
Associate editor: G Tita