An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Food Institutional Research Measure

Final Report

Marine sourced peptides for glycaemic management. MaraPep

DAFM Project Reference No: 13F467

Start date: 01/01/2014

End Date: 31/05/2018

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Collaborating Research Institutions and Researchers:
Ulster University: Prof. Finbarr O'Harte, Dr. Emeir McSorley & Dr. Philip Allsopp
Kerry Foods Ltd.
BioMarine Ingredients Ireland Ltd.

Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental → Applied → Pre Commercial

1	2	3 x	4	5	6	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	'***Food and Health***' and '***Sustainable Food Production and Processing***'.

Key words: *(max 4) anti-diabetic, marine proteins, protein hydrolysate, Type 2 diabetes mellitus*

1. Rationale for Undertaking the Research

The global demand for high-quality protein is rapidly increasing. This is driven primarily by a growing world population, which is expected to reach 9.8 billion by 2050 and 11.2 billion by 2100. This demand is further underpinned by emerging economies in developing countries, increased urbanisation, the recognition of the role of proteins in a healthy diet and an increased need for high quality protein intake by the elderly.

Type 2 diabetes mellitus (T2DM), the most predominant form of the diabetes, is a complex chronic metabolic condition characterised by insulin resistance and insufficient pancreatic insulin production, resulting in high blood glucose levels (hyperglycaemia). Progression of the disease can lead to numerous long-term micro- and macro-vascular complications which can increase the risk of premature death (Fowler, 2011). The most recent data indicates that approximately 415 million people are currently living with T2DM globally and predictions indicate that if immediate interventions are not taken that this could rise to 642 million by 2040. Therefore, the successful prevention and management of T2DM is a major public health priority globally.

Marine sources such as macroalgae (*Palmaria palmata*), along with low value underutilized fish species (e.g., blue whiting and boarfish which are landed due to recent changes in EU Common Fisheries Policy) and fish processing by-products (e.g., salmon trimmings and skin) contain significant quantities of high quality protein. These sources therefore represent good candidate raw materials for sustainable protein supply and for the mining of bioactive peptides with applications as biofunctional ingredients.

The beneficial effects of food-derived proteins/peptides, in health promotion and disease prevention, including T2DM, are being increasingly recognized. Outcomes from the previously funded NutraMara project identified that specific *Palmaria palmata* and salmon muscle protein and salmon skin gelatin hydrolysates showed promising anti-diabetic activity, i.e., dipeptidyl peptidase (DPP)-IV inhibitory activity *in vitro*. The proposed research aimed to build on findings from this project through assessment of the potential of marine-derived peptides as sources of anti-diabetic functional ingredients using a combination of *in vitro* cell-based, small animal and human studies.

2. Research Approach

The specific objectives of this research were to (1) extract proteins from *Palmaria palmata,* boarfish, blue whiting, and gelatin from salmon muscle and fish skin, (2) generate marine derived protein hydrolysates at laboratory scale, (3) investigate the *in vitro* anti-diabetic properties of hydrolysates using enzyme inhibition and cell-based assays, (4) identify protein hydrolysates displaying stability to simulated gastrointestinal digestion (SGID), (5) investigate the effects of acute and chronic administration of protein hydrolysates on energy metabolism and antidiabetic potential in normal and obese diabetic *ob/ob* mice, respectively, (6) produce the lead protein hydrolysate at semi-pilot scale, (7) assess the effect of administration of the lead hydrolysate on biomarkers of glycaemic function and satiety in acute and chronic studies with normal healthy and overweight/ obese adults, respectively, (8) characterise the physicochemical properties of the hydrolysates/fractions and the technofunctional properties of the lead protein hydrolysate, and finally (9) fractionate, identify and characterize the peptides potentially responsible for the observed activity in the lead protein hydrolysate.

A number of protocols for the extraction of highly pure protein isolates from various marine sources were developed/adapted during the course of the project. These include development of a method for

extraction of an essentially carbohydrate-free protein isolate from the macroalga *Palmaria palmata*, a more industrially relevant protocol for extraction of highly pure gelatin from salmon skin and a protocol for the extraction of protein from boarfish and blue whiting. Protein hydrolysates were generated from extracted protein isolates using specific food-grade proteolytic enzyme preparations.
All hydrolysates generated were assessed for *in vitro* DPP-IV inhibitory, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP). Furthermore, the anti-diabetic activity of hydrolysates were assessed in numerous cell models these include insulin secretory activity from cultured pancreatic BRIN-BD11 cells, glucagon-like peptide-1 (GLP-1) secretory activity from enteroendocrine GLUTag cells, GLP-1 and PYY secretory activity from enteroendocrine GLUTag cells STC-1 cells, and basal and insulin-stimulated glucose uptake in 3T3-L1 adipocytes. Furthermore, mechanistic studies (membrane depolarisation, intracellular calcium and intracellular cAMP assays) were performed with specific hydrolysates to determine the potential pathways by which the hydrolysate mediates insulin secretion from BRIN BD11 cells.

Selected hydrolysates showing positive activity in *in vitro* assays were then assessed for acute glucose-lowering and insulin releasing properties *in vivo* using 10-12 week old male NIH Swiss mice. Delayed persistent glucose lowering/insulin secretory effects (half-life), where hydrolysates were administered 4, 8 or 12 h prior to the glucose challenge, was also assessed in these mice.
The chronic effect of 2 protein hydrolysates which showed highest anti-diabetic activity in acute animal studies, on biomarkers of glycaemic control and energy metabolism were assessed in ob/ob mice. In addition, the *Palmaria palmata* protein hydrolysate was assessed for *in vivo* antidiabetic activity in streptozotocin (STZ) induced diabetic mice.

The lead candidate hydrolysate (boarfish protein hydrolysate) as selected based on outcomes from the chronic *in vivo* small animal studies and sustainable supply considerations for the future was generated at semi-pilot scale in conjunction with BioMarine Ingredients Ireland Ltd. (BII). Following sensory studies the hydrolysate was incorporated into an appropriate delivery vehicle and the acute effects of the hydrolysate (3.5g) on biomarkers of glycemic control, metabolic syndrome and appetite were assessed in a placebo controlled crossover study in normal healthy individuals. The effect of the hydrolysate (3.5g) on biomarkers of glycemic control, metabolic syndrome and appetite were subsequently assessed in a randomised parallel placebo controlled 12 week dietary intervention study in overweight and obese individuals. The hydrolysate (3.5g) was administered in capsule format.

The boarfish protein hydrolysate generated at semi-pilot scale was subjected to SGID and fractionated using semi-preparative reverse phase-high performance liquid chromatography (RP-HPLC). Potent fractions identified by bioassay-guided fractionation (DPP-IV inhibition and insulin secretory activity from BRIN BD11 cells) were analysed by mass spectrometry and numerous peptide sequences were identified. Seventy one peptides were selected and confirmatory studies were performed with synthetic peptides.

The technofunctional properties (solubility, clarity and heat stability) of the boarfish protein hydrolysate generated at semi-pilot scale was also assessed. Furthermore, the effect of conditions, which mimic those commonly used in industry, and *in vitro* simulated digestion on *in vitro* anti-diabetic activity and peptide profile of tomato based products fortified with the boarfish protein hydrolysate generated at semi-pilot scale was performed. This study was not part of the original approved grant application,

however, we believe that such a study was required if the lead candidate hydrolysate is to be used as a functional food ingredient. Ultimately, the outcomes from the study provide industry with evidence that the lead anti-diabetic boarfish protein hydrolysate, generated at semi-pilot scale, can withstand commonly used food processing conditions and has potential applications as a functional food ingredient.

3. Research Achievements/Results

Task 1: Extraction of proteins and protein fractions from marine sources

- A number of food friendly industrially relevant protocols for the extraction of highly pure protein isolates from various marine sources were developed/adapted. These include development of a method for extraction of an essentially carbohydrate-free protein isolate from the macroalga *Palmaria palmata*, a more industrially relevant protocol for extraction of highly pure gelatin from salmon skins and a protocol for extraction of protein from boarfish and blue whiting.
- Outcomes from this task have identified that the underutilised low value fish species, boarfish and blue whiting, for which Ireland has significant quotas, are a rich source of high quality protein.

Task 2: Optimisation of enzymatic hydrolysis strategies for the release of antidiabetic peptides

- A set of hydrolysis conditions and specific proteolytic enzyme preparation combinations for the generation of marine derived protein hydrolysates with good anti-diabetic activity were identified
- An industrially relevant integrated protocol for the generation and separation of highly pure marine protein hydrolysates from salmon, boarfish and blue whiting muscle was developed.
- The protocol for generation of the lead protein hydrolysates (boarfish protein hydrolysate) was successfully transferred from laboratory (0.5 L) to semi-pilot scale (1,200 L).

Task 3: *In vitro* biological activity assessment of marine protein hydrolysates

- A set of hydrolysates, peptide fractions and synthetic peptides with potent *in-vitro* anti-diabetic (DPP-IV inhibition, and insulin, GLP-1, and PYY secretory activity from various cells types) have been identified.
- Marine derived protein hydrolysates (*Palmaria palmata*, salmon skin gelatin and salmon, boarfish and blue whiting muscle) were shown to have the most promising anti-diabetic activity *in vitro.*
- The DPP-IV inhibitory activity of the lead marine protein hydrolysate incorporated into selected food model systems was shown to be retained following treatment with a number of commonly used food processing treatments and simulated gastrointestinal digestion.
- The potential mechanism(s) by which marine protein hydrolysates mediate insulin secretory activity from BRIN-BD11 cells has been identified using *in vitro* studies.

Task 4: Physicochemical, technofunctional, food formulation and sensory characterisation of hydrolysates

- All hydrolysates and fractions were fully characterized in terms of their physicochemical properties (% degree/extent of hydrolysis, molecular mass distribution and hydrophobicity profiling)
- The lead protein hydrolysate is a good source of all essential and non-essential amino acids.
- The lead protein hydrolysate was shown to be highly soluble at higher pH values (pH 7-10) and had lower solubility at pH values between 2 and 4.

- Standard industrial treatments had no effect on the DPP-IV inhibitory activity or peptide profile of the lead protein hydrolysate incorporated into the product
- The DPP-IV inhibitory actvity and peptide profile of the lead protein hydrolysate incorporated into a tomato based food product was stable.
- The DPP-IV inhibitory actvity and peptide profile of the lead protein hydrolysate incorporated into a tomato based food product was similiar prior to and after simulated gastrointestinal digestion.

Task 5: *In vivo* testing of hydrolysate activities in mouse studies

- Marine protein hydrolysates (Salmon muscle and skin gelatin, boarfish and *Palmaria palmata*) showed improved glucose lowering effects compared to saline control in Normal NIH Swiss mice. A blue whiting protein hydrolysate showed improved glucose lowering effects in normal NIH Swiss mice.
- Salmon skin gelatine and boarfish protein hydrolysates were shown to have an anti-diabetic effect in obese diabetic *ob/ob* mice.

Task 6: An investigation of the effect of the protein hydrolysate with greatest activity on short and long-term markers of glycaemic control, metabolic syndrome and appetite in humans.

- The results of the acute and chronic human studies where 3.5g of the boarfish protein hydrolysate generated at semi-pilot scale, showed no significant difference in the levels of the glycaemic (glucose, insulin, glucagon-like peptide 1 (GLP-1) and satiety (ghrelin, leptin) biomarkers compared to controls. Although an equivalent dose of boarfish protein hydrolysate demonstrated anti-diabetic properties in murine models, the same effects were not observed in humans.

Task 7: Development of a strategy for enrichment and identification of bioactive peptides

- Specific protocols for the separation of boarfish protein-derived peptides using semi-preparative RP-HPLC were developed and along with bioassay driven fractionation was used to identify peptide fractions with potent *in vitro* anti-diabetic activity.
- Numerous peptide sequences were identified by mass spectrometry.
- Information on the primary sequence of boarfish peptides with promising *in vitro* anti-diabetic activity was obtained.
- A bank of boarfish-derived peptides with promising *in vitro* anti-diabetic activity was generated.
- Further information on the structural features associated with DPP-IV inhibitory activity was identified.

4. Impact of the Research

Outcomes provide industry with

- Food-friendly industrially relevant protein extraction and peptide generation protocols for high quality protein/gelatin extraction and peptide generation from various marine sources.
- Highly pure marine-derived protein/gelatin isolates and peptides which are both halal and kosher compliant.
- Substrates for the generation of protein-derived health enhancing ingredients
- A set of hydrolysis conditions and specific proteolytic enzyme preparation combinations for the generation of marine-derived protein hydrolysates with high anti-diabetic activity
- A protocol for the generation of marine protein hydrolysates at semi-pilot scale
- Increased commercial value of marine resources, in particular boarfish and blue whiting

- Scientific evidence that marine protein hydrolysates can beneficially modulate biomarkers of type 2 diabetes *in vitro and in vivo*
- Evidence that marine protein hydrolysates when incorporated into a food system are stable during certain food processing conditions.

Outcomes provide society with
- A range of marine-derived health-enhancing functional ingredients for the prevention and/or control of certain metabolic diseases.
- A alternative source of high quality protein which is both halal and kosher compliant

Outcomes provide the scientific community with:
- Enhanced scientific knowledge in the area of marine protein content and composition
- Identification of critical parameters which affect protein extraction yields.
- Increased scientific knowledge in the area of marine protein hydrolysate generation.
- Determination of hydrolysis conditions and specific proteolytic enzyme preparation combinations for the generation of marine derived protein hydrolysates with promising anti-diabetic activity
- Identification of critical parameters for the transfer of a protocol for generation of a marine protein hydrolysate from laboratory to semi-pilot scale
- Significant enhancement of scientific knowledge in the health enhancing effects of marine protein hydrolysates/peptides *in vitro* and *in vivo.*
- Potential mechanism by which marine derived proteins/peptides mediate anti-diabetic *activity in vitro*
- Identification of peptide sequences potentially responsible for the observed biological activity *in vitro*
- Identification of potential food matrixes for hydrolysate delivery
- Increased knowledge and identification of conditions under which the hydrolysate is stable.
- Training of postgraduate and undergraduate students and postdoctoral researchers with advanced technical skills and scientific knowledge
- Leveraging of further funding to advance scientific knowledge and technical skills

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

Ongoing collaboration with Prof. Finbarr O'Harte, Dr. Emeir McSorley and Dr. Philip Allsopp at UU and with R&D personnel at Kerry have been augmented by this research.
New linkages have been developed with personnel from BioMarine Ingredients Ireland.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

A number of food friendly industrially relevant protocols for the extraction of highly pure protein isolates from various marine sources were developed/adapted. These include development of a method for extraction of an essentially carbohydrate-free protein isolate from the macroalga *Palmaria palmata,* and a more industrially relevant protocol for extraction of highly pure gelatin from salmon skins and a protocol for extraction of protein from boarfish and blue whiting.

Furthermore, new approaches have been developed and successfully transferred to semi-pilot scale for the generation of marine-derived functional food ingredients.

(iii) Outcomes with economic potential

The exploitation of marine raw materials as sources of high quality proteins and health enhancing ingredients can add value to these low-value underutilised fish species and co-products of the marine processing industry.
Reduces the cost of disposal of such by-products.

(iv) Outcomes with national/ policy/social/environmental potential

This project has advanced our knowledge and innovation capacity in (a) the development of multifunctional health enhancing ingredients and (b) constitutes an environmentally sustainable benefit by dealing with discard that now has to be landed due to changes in the Common Fisheries Policy and converting the major by-product obtained from the marine processing industry into health enhancing food ingredients.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

- Harnedy, P.A. & FitzGerald, R. J. (2015) Extraction and enrichment of protein from red and green macroalgae. In Stengel, D.B. and Connan, S (Eds): Natural Products from Marine Algae, Methods in Molecular Biology, Springer. 1308, 103-108.
- Neves, A.C., Harnedy, P.A. & FitzGerald, R. J. (2016) Marine processing proteinaceous by-products: A source of biofunctional food ingredients. In: Dhillon, G.S. (Ed): Protein Byproducts: Transformation from environmental burden into value-added products. Academic Press, 63-68.
- Neves, A.C., Harnedy, P.A., O'Keeffe, M.B. & FitzGerald, R.J. (2017) Bioactive peptides from Atlantic salmon (*Salmo salar*) with angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory, and antioxidant activities. Food Chemistry. 218:396-405.
- Neves, A. C., Harnedy, P. A., O'Keeffe, M. B., Alashi, M. A., Aluko, R. E., & FitzGerald, R. J. (2017). Peptide identification in a salmon gelatin hydrolysate with antihypertensive, dipeptidyl peptidase IV inhibitory and antioxidant activities. Food Research International. 100 (1), 112-120.
- Harnedy, P.A., O'Keeffe, M.B. & FitzGerald, R.J. (2017) Fractionation and identification of antioxidant peptides from an enzymatically hydrolysed *Palmaria palmata* protein isolate. Food Research International. 100 (1), 416-422.
- Harnedy, P. A., Parthsarathy, V., McLaughlin, C. M., O'Keeffe, M. B., Allsopp, P. J., McSorley, E. M., O'Harte, F. P. M., & FitzGerald, R. J. (2018). Atlantic salmon (*Salmo salar*) co-product-derived protein hydrolysates: A source of antidiabetic peptides. *Food Research International* 106, 598–606.
- Harnedy, P. A., Parthsarathy, V., McLaughlin, C. M., O'Keeffe, M. B., Allsopp, P. J., McSorley, E. M., O'Harte, F. P. M., & FitzGerald, R. J. (2018). Blue whiting (*Micromesistius poutassou*) muscle protein hydrolysate with *in vitro* and *in vivo* antidiabetic properties. *Journal of Functional Foods*. 40,137-145.

- Le Gouic, A.V., Harnedy, P.A. & FitzGerald, R. J. (2018) Bioactive peptides derived from fish protein by-products. In Mérillon, J.M. & Ramawat, K.G. (Eds): Reference Series in Phytochemistry. Bioactive Molecules in Food. Springer. 1-35.
- Parthsarathy, V., McLaughlin, C.M, Harnedy, P.A., Allsopp, P.J, Crowe, W., McSorley, E., et al. (accepted). Boarfish (*Capros aper*) protein hydrolysate has potent insulinotropic and GLP-1 secretory activity *in vitro* and acute glucose lowering effects in mice. *International Journal of Food Science and Technology*.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

- Harnedy, P.A. & FitzGerald, R.J. Biologically active peptides from marine protein sources. NutraMara Conference and Expo, Harnessing Marine Bioresources for Innovations in the Food Industry, 29-30th June 2015, Dublin, Ireland.
- Parthsarathy, V., McLaughlin, C.M., Mullan, C., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte, F.P.M. Boarfish protein hydrolysates stimulate both insulin and GLP-1 secretion from cultured cells in vitro, NutraMara Conference and Expo, Harnessing Marine Bioresources for Innovations in the Food Industry, 29-30th June 2015, Dublin, Ireland.
- McLaughlin, C.M., Parthsarathy, V., Mullan, C., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., FitzGerald R.J. & O'Harte, F.P.M. Palmaria palmata protein hydrolysates have potent in vitro effects upon insulin and glucagon like peptide-1 secretion from cultured cells, NutraMara Conference and Expo, Harnessing Marine Bioresources for Innovations in the Food Industry, 29-30th June 2015, Dublin, Ireland.
- Mullan, C., Harnedy, P.A., Mc Laughlin, C.M., Parthsarathy, V., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte, F.P.M. Synthetic peptides isolated from the macroalga Palmaria palmata demonstrate dipeptidy-peptidase-4 inhibitory actions on GLP-1 degradation using a HPLC assay system, NutraMara Conference and Expo, Harnessing Marine Bioresources for Innovations in the Food Industry, 29-30th June 2015, Dublin, Ireland.
- McLaughlin, C.M., Parthsarathy, V., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., Fitzgerald, R.J. and O'Harte, F.P.M. Blue whiting protein hydrolysates display potent in vitro secretory effects upon insulin and glucagon-like peptide-1 release and acute glucose lowering effects in mice. Irish Endocrine Society 40th Annual Meeting, October 2016, Belfast, Northern Ireland. (in Irish Journal of Medical Science, 2016 Vol. 185, pp. 405-405).
- O'Harte, F.P.M., Mullan, C., Harnedy, P.A., McLaughlin, C., Parthsarathy, V., Allsopp, P.J., McSorley, E.M. & FitzGerald, R.J. Synthetic peptides from marine origin enhance both incretin hormone stability and insulin secretion *in vitro*. Diabetes UK Professional Conference, March 2016, Glasgow, UK. (In Diabetic Medicine, 33: 59).
- McLaughlin, C., Parthsarathy V, Allsopp P, McSorley E, FitzGerald R.J., Harnedy P.A., O'Harte FPM Protein hdrolysates from the red seaweed *Palmaria palmata* enhanced both insulin and GLP-1 secretion in cultured cells and improved acute glucose tolerance in mice. Diabetes UK Professional Conference, March 2016, Glasgow, UK. (In Diabetic Medicine, 33: 82).
- Parthsarathy, V., McLaughlin, C., Mullan, C., Harnedy, P.A., Allsopp, P., McSorley, E., FitzGerald, R.J. and O'Harte, F.P.M. Boarfish (C*apros aper*) protein hydrolysates show potent insulinotropic and GLP-1 secretory activity *in vitro* and acute glucose lowering effects *in vivo*. Diabetes UK Professional Conference, March 2016, Glasgow, UK. (In Diabetic Medicine, 33: 60).
- Laughlin, C.M., Parthsarathy, V., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J., Harnedy, P.A. & O'Harte, F.P.M. Twice daily oral administration of boarfish (*Capros aper*) protein hydrolysate improves lipid parameters and glycaemic control in obese diabetic ob/ob mice Diabetes UK Professional Conference. 8-10th March 2017, Manchester, UK.

- O'Harte, F.P.M., Mullan, C., Harnedy, P.A., McLaughlin, C.M., Parthsarathy, V., Allsopp, P.J., McSorley, E.M. & FitzGerald, R.J. Synthetic peptides from marine origin enhance both incretin hormone stability and insulin secretion *in vitro*. Diabetes UK Professional Conference. 8-10th March 2017, Manchester, UK.
- Mc Laughlin, C.M., Parthsarathy, V., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J., Harnedy P.A. & O'Harte F.P.M. Twice daily oral administration of boarfish (*Capros aper*) protein hydrolysate improves lipid parameters and glycaemic control in obese diabetic *ob/ob* mice. Irish Endocrine Society 41st Annual Meeting, 13-14th October 2017, Dublin, Ireland.
- Harnedy, P. A., Parthsarathy, V., McLaughlin, C. M., Allsopp, P. J., McSorley, E. M., O'Harte, F. P. M. & & FitzGerald, R. J. Evaluation of the *in vitro* and *in vivo* anti-diabetic potential of a salmon trimmings protein hydrolysate. 47th Conference of the Western European Fish Technologists' Association (WEFTA), 9-12th October 2017, Dublin, Ireland.
- Crowe, W., McLaughlin, C.M., Allsopp, P.J., Slevin, M.M., Harnedy, P.A., Cassidy, Y., Baird, J., Devaney, M., Fitzgerald, R.J., O'Harte, F.P.M. & McSorley, E.M. The effect of boarfish protein hydrolysate on postprandial glycaemic response and satiety in healthy adults. Nutrition society, Irish section annual meeting, 20-22nd June 2018, Coleraine, Northern Ireland.
- Crowe, W., Baird, J., Allsopp, P.J., McLaughlin, C.M., Harnedy, P.A., Fitzgerald, R.J., O'Harte, F.P.M. & McSorley, E.M., The effect of consuming boarfish protein hydrolysate on metabolic health in overweight adults. Nutrition society, Irish section annual meeting, 20-22nd June 2018, Coleraine, Northern Ireland.
- McLaughlin, C.M., Parthsarathy, V., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte F.P.M. Synthetic peptides from marine origin enhance both incretin hormone. Nutrition society, Irish section annual meeting, 20-22nd June 2018, Coleraine, Northern Ireland.
- McLaughlin, C.M., Harnedy, Parthsarathy, V., P.A., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte F.P.M. Administration of protein hydrolysates from *Palmaria palmata* reduced food intake, improved glycaemic parameters and lipid profile in streptozotocin diabetes induced mice. Nutrition society, Irish section annual meeting, 20-22nd June 2018, Coleraine, Northern Ireland.
- Harnedy, P.A., McLaughlin, C.M., Parthsarathy, V., O'Keeffe, M.B., Allsopp, P.J., McSorley, E.M., O'Harte, F.P.M. and FitzGerald, R.J. *Palmaria palmata*: a potential source of bioactive peptides for management of hyperglycaemia. Seaweed 4 Health Conference, 24-27th June 2018, GMIT, Galway, Ireland.
- McLaughlin, C.M., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte F.P.M. Administration of protein hydrolysates from *Palmaria palmata* reduced food intake, and improved glycaemic parameters in streptozotocin induced diabetic mice. Seaweed 4 Health Conference, 24-27th June 2018, Galway, Ireland.
- McLaughlin, C.M., Parthsarathy, V., Harnedy, P.A., Allsopp, P.J., McSorley, E.M., FitzGerald, R.J. & O'Harte F.P.M. Synthetic peptides derived from *Palmaria palmata* show promising antidiabetic actions in cultured cells, as well as in acute in vivo studies in mice. 35th European peptide synposium, 26-31st August 2018, Dublin, Ireland.
- Harnedy, P.A., Parthsarathy, V., Mclaughlin, C.M., O'Keeffe, M.B., Allsopp, P.J., McSorley, E.M., O'Harte, F.P.M. & FitzGerald, R.J. Evaluation of the *in vitro* and *in vivo* antidiabetic potential of a blue whiting (*Micromesistius poutassou*) protein hydrolysate. 2nd Food Bioactive and Health Conference, 26-28th September 2018, Lisbon, Portugal.

(iii) National Report

n/a

(iv) Workshops/seminars at which results were presented

See conference details above

(v) Intellectual Property applications/licences/patents

n/a

(vi) Other

n/a

5. Scientists trained by Project

Total Number of PhD theses: 1

McLaughlin C.M., Ulster University, Coleraine, Exploring the antidiabetic potential of protein hydrolysates derived from underutilized marine sources. July 2018. (DEL NI funded)

Total Number of Masters theses: 0

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
University of Limerick	1	0.2
Ulster University	3	0.6
Total	**4**	**0.8**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	4	6.66
PhD students	1 DEL NI Funded	3.00
Masters students		
Temporary researchers	5	1.18
Other		
Total	**10**	**10.84**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
0	

9. Project Expenditure

Total expenditure of the project: € 561,669.26

Total Award by DAFM: € 581,117.07

Other sources of funding including benefit in kind and/or cash contribution(specify):

Benefit in kind: The Good Fish Company:–Salmon trimmings and salmon skin: €2,500
Kerry Ingredients: Mineral analysis.
BioMarine Ingredients Ireland Ltd.: Semi-pilot scale trial: hiring of external GMP facilities, supply of raw material and processing aids.

Breakdown of Total Expenditure

Category	University of Limerick Institution 1	Ulster University Institution 2	Total
Contract staff	0.00	0.000	0.00
Temporary staff	0.00	18,182	18,182
Post doctorates	159,767.94	139,539	299,306.94
Post graduates	0.00	0.00	0.00
Consumables	44,028.54	62,589.38	106,617.92
Travel and subsistence	4,969.06	5,054.46	10,023.52
Sub total	208,765.54	225,364.66	434,130.20
Durable equipment	912.66	0.00	912.66
Other	0.00	0.00	0.00
Overheads	59,017	67,609.40	126,626.40
Total	**268,695.20**	**292,974.06**	**561,669.26**

10. Leveraging

Additional funding was awarded by DAFM, MI and EI:

DAFM Project Reference No: 14/F/873- 'Mining marine materials for novel functional ingredients that modulate immune response for benefit in inflammation and allergy.' (MarineMod)

DAFM Project Reference No: 17/F/260 - 'Extraction and exploitation of bioactive fish components for health enhancement.' (MaraBioactive)

MI Project Reference No: PBA/MB/16/01- National Marine Biodiscovery Laboratory of Ireland (NMBLI)

MI Project Reference No: IND/18/20 - Development of marine functional food to support muscle health and healthy aging in older adults

EI Project Reference No: IP/2015/0400/E - Assessment of the potential of boarfish and blue whiting skin and bone co-products as a source of gelatin/protein

11. Future Strategies

To investigate the development of reproducible and scalable protocols for the generation of prototype blue whiting protein-derived ingredients with demonstrated sensory (taste and appearance) acceptability along with appetite modulating/satiety (initially in small animals) activity. (DAFM Project Reference No: 17/F/260 - 'Extraction and exploitation of bioactive fish components for health enhancement.' (MaraBioactive))

Incorporation of fish protein hydrolysates into a market-ready prototype and performance of a human intervention study to determine the role of ingestion of the hydrolysate on biomarkers of metabolic syndrome in overweight and obese subjects. (Funding application recently accepted).

To develop a marine functional food containing a blue whiting protein hydrolysate to support healthy aging with a focus on skeletal muscle health through a human intervention study with a medically-stable older adult (>65 y) population and an *ex vivo-in vitro* cell culture platform with serum conditioned by *in vivo* feeding of the hydrolysate. (MI Project Reference No: IND/18/20 - Development of marine functional food to support muscle health and healthy aging in older adults)

Selective large-scale cultivation for the sustainable production of protein-rich macrolagal species for exploitation as a source of high quality protein and health enhancing bioactive peptides (functional food ingredients). (Disruptive Technology Innovation Fund, approved for funding Dec 10th 2018).