This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773330

Deliverable report for

GAIN

Green Aquaculture Intensification in Europe

Grant Agreement Number 773330

Deliverable D2.6
Title: Eco-efficient disposal of aquaculture mortalities

Due date of deliverable: 30/04/2021
Actual submission date: 11/10/2021

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WP: 2

Dissemination Level:		
PU	**Public**	**Y**
PP	Restricted to other programme participants (including the Commission Services)	
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CO	Confidential, only for members of the consortium (including the Commission Services)	

Document log

Version	**Date**	**Comments**	**Author(s)**
Version 1	09/10/2020	Table of contents	Hallstein Baarset
Version 2	22/10/2020	First draft	Hallstein Baarset
Version 3	29/10/2020	Updated version	Hallstein Baarset
Version 4	19/04/2021	Updated version	Hallstein Baarset
Version 5	11/05/2021	Updated version	Hallstein Baarset, Christian Bruckner, Marta Castrica
Version 6	24/09/2021	Revision	Silvio Cristiano
Version 7	11.10/2021	Final version	Hallstein Baarset

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Recommended Citation

Baarset, H., Bruckner, C., Cristiano, S., Kreiss, K., Castrica, M. – Eco-efficient disposal of aquaculture mortalities. Deliverable 2.6. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 773330. 35 pp.

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GLOSSARY OF ACRONYMS

Acronym	Definition
AMB	Animal By-Product
DM	Dry Matter
DSG	Dried Spent Grain
EC	European Commission
GAIN	Green Aquaculture Intensification in Europe
GHP	Good Hygiene Practices
GMP	Good Manufacturing Practices
HSE	Health, Safety and Environment
IBC	Intermediate Bulk Container
LCA	Life Cycle Assessment
MFM	Mortalities Fish Meal
MV	Multivector
RAS	Recirculating Aquaculture System(s)
RH	Relative Humidity
SD	Standard Deviation
SHS	SuperHeated Steam
TRL	Technology Readiness Level

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Executive summary

Fish mortalities and discarded fish (in the following labelled as "mortalities"), being among main side streams from aquaculture operations, limit the eco-efficiency of current mortality disposal practices. Waister has developed an innovative method for the improvement of eco-efficiency in disposal of these side streams in terms of economic and environmental sustainability performances, as well as for the elimination of crucial hazards linked to their usual treatment.

The current predominant method consists in ensiling the mortalities and transporting them by tanker trucks and boats. The innovative method proposes to dry fish mortalities at the site of origin through an innovative method, thus allowing to eliminate the need of formic acid while still complying with EU and Norwegian regulations on mortalities disposal. The innovative method allows the dried product to be transported in bags or containers with ordinary trucks or boats.

The drying of mortalities at the site of origin also eliminates the need to transport substantial amounts of water, as the ensilage today typically contains 80-85 % water. Transport of the dried product in bags compared to tank transport cuts transport monetary costs with typically another 50 %. The environmental savings are presented in D4.4.

The development of the drying technology applicable to fish mortalities faced a number of challenges during the work in T2.1 'Valorisation of aquaculture side streams'. The technology was significantly improved through two major revisions of the machine, arriving at a Waister 15 Single-Loop machine as a well-functioning solution for mortalities drying.

For salmon and trout, with a high fat content, the work showed that some additive was necessary to ensure a stable drying process. The use of wood chips or dried spent grain as additives, in combination with the Single-Loop process, proved to be successful in the final innovative technology, performing with consistent stability over a 10-month period at Adriatic Farming in Croatia.

Microbiological safety was examined by analysing the dried mortalities product. No content of enterobacteriaceae and salmonella was found. There are currently no specific limits for amounts of sulphite-reducing bacteria in feedstuff. Measured values were generally considered "satisfactory" for feedstuff and certainly safe for storage and transport, as an alternative to ensilage.

Furthermore, a comparison with the sustainability of the current process for disposing mortalities, based on the application of LCA, shows that the innovation developed and tested in GAIN scores better in most impact categories, including the Global Warming Potential, and the terrestrial and marine eutrophication. The results are presented in detail in GAIN deliverable D4.4.

When entering the GAIN project, the technology was at TRL2 with a formulation of a technology concept assumed to be suitable for fish mortality drying. Throughout the GAIN project, the innovation passed to TRL7 with a system prototype being demonstrated in operational environment. Since December 2020 the first machine has been in daily operation at an aquaculture site. The solutions are now presented as a commercial product by Waister, meaning that the technology has entered TRL8 as a complete and qualified system. The economic sustainability and viability of this innovation is also corroborated by the results of the cost-benefit analysis, presented in detail in GAIN deliverable D6.9

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Table of Contents

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1. Introduction

GAIN is a collaborative project funded by the European Union (EU), designed to support the ecological intensification of aquaculture in the EU and the European Economic Area (EEA), with the dual objectives of increasing production and competitiveness of the industry, while ensuring sustainability and compliance with EU regulations on food safety and environment. GAIN aims to implement principles of circular economy in European aquaculture, by improving fish welfare and by reduction of environmental footprint, via the creation of co-products.

Particular focus is set on the increase of circularity in the aquaculture sector by the implementation of technologies to capture and valorise aquaculture side streams, specifically wastewater and mortalities (dead fish).

GAIN aims at identifying the most promising technologies and demonstrating their functionality and cost-effectiveness, thereby contributing to the scientific basis required to facilitate further development of relevant EU regulation. Various such technologies have been discussed earlier (Johansen et al., 2019), meeting the demands of different aquaculture facilities with respect to their typology, regional infrastructure and valorisation of end products.

In WP2, the enhancement of secondary outputs from aquaculture is investigated. T2.1 objective is to valorise aquaculture side streams. Among the major side streams investigated in T2.1 are mortalities and discarded fish, as they represent a significant amount of material with a potential of improved value in a circular economy perspective. This side stream consists of mortalities, i.e., fish that die from any reason during the production process, and discarded fish, i.e. fish that is deliberately removed from the production process. The main reasons for discarding fish are related to biological (low growth rate, physiological defects), capacity (limitations of space available in fish tanks and sea cages for the growing biomass) and market factors (i.e. overproduction compared to actual demand). D2.6 describes the outcome of valorisation of the side stream consisting of mortalities and discarded fish. As summarised in Baarset & Johansen (2019) and described in D2.5 (Bruckner et al., 2021), second to fish sludge, mortalities are the main side stream from the Norwegian aquaculture industry in terms of volume, cost of handling, HSE hazards and potential value. The major amount of mortalities is from sea-based production, while the landbased farms produce a smaller number. For landbased facilities the annual amount of deliberately discarded fish is almost equal to the amount of mortalities.

As happens in all farming activities, some individuals do not survive all the way from eggs to fully grown fish. The reuse of mortalities from aquaculture with minimal environmental footprint, maximum value and full compliance with EU regulations on food safety is important for achieving eco-intensification. In order to eco-intensify the aquaculture industry, mortalities and discarded fish should be reduced, by increasing fish welfare. On the other hand, losses are unavoidable and, therefore, their treatment is to be made more efficient and, if possible, circular processes for mortality reuse be implemented for the overall sustainability of the aquaculture sectors.

According to Barentswatch (2020), the mortality rate for salmon and rainbow trout in Norwegian sea-cages 2019 was 17 % in 2019, equalling 92.6 million fish. This represents an increase from 2018, when mortality rate was 16.4 %. On average, the typical loss in sea cages is 15-20 % of the number of individuals, which corresponds to 6–9 % of the biomass. This number includes escapes and is consistent with Bjørndal & Tusvik (2017). The annual amount of mortalities is currently increasing from year to year, as shown in **Figure 1**.

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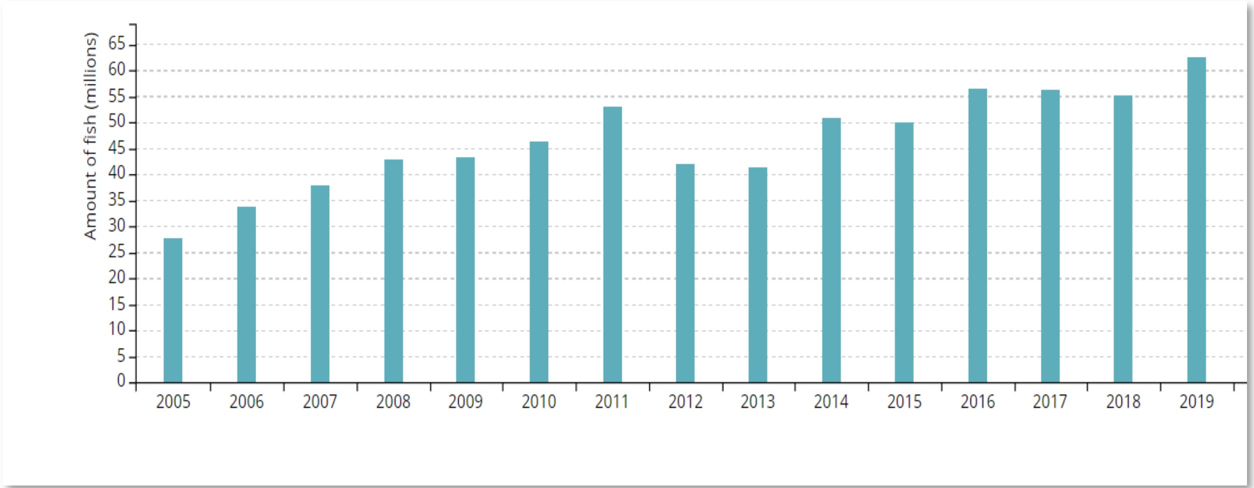

***Figure 1**: Development of mortality in Norwegian salmon and rainbow trout sea-cages (Barentswatch, 2020)*

In addition to the loss in sea cages presented in Fiskeridirektoratet (2020), the loss in landbased smolt production in Norway is shown in **Table 1**.

***Table 1**: Loss of juveniles in the production of Atlantic salmon, rainbow trout and trout by reasons. Numbers in 1000 individuals. (Fiskeridirektoratet, 2020). Updated 28.05.2020. Source: Norwegian Directorate of Fisheries.*

Year	2019				2018				2017			
County	Mortality	Fry destruction	Escapes	Others	Mortality	Fry destruction	Escapes	Others	Mortality	Fry destruction	Escapes	Others
Finnmark & Troms	6 561	7 077	179	602	8 507	5 069	-	2 282	7 337	5 194	-	58
Nordland	11 751	10 200	-	705	11 165	11 598	21	2 397	15 942	17 476	-	3 053
Trøndelag	8 883	7 604	-	760	10 396	9 164	-	1 012	11 294	9 206	-	770
Møre & Romsdal	11 219	4 840	-	80	5 445	5 327	-	203	10 094	7 957	-	1 565
Sogn & Fjordane	2 889	1 094	-	429	3 871	2 479	1	258	3 221	2 145	-	329
Hordaland	12 986	7 481	2	859	19 449	7 567	-	624	9 311	8 428	1	329
Rogaland	3 432	2 414	-	404	2 286	1 759	-	- 32	2 664	2 220	-	598
Other counties	464	2 086	-	- 15	283	692	-	-	155	305	-	28
Total	**58 185**	**42 796**	**181**	**3 824**	**61 402**	**43 655**	**22**	**6 744**	**60 018**	**52 931**	**1**	**6 730**

There is no available official data on the average size of smolt mortalities or smolts being discarded. To make a calculation of the biomass, we have used the average size of 13.5 g found at Helgeland Smolt, site Reppen where mortalities drying tests on salmon smolt were made (see **Section 4.1**, **Table 5**) as an assumption. **Table 2** shows the calculated biomass of Norwegian landbased aquaculture losses in the period of 2017-2019 based upon Fiskeridirektoratet (*ibid.*).

***Table 2**: Calculated biomass loss in Norwegian smolt production 2017-2019.*

Year	Mortalities and discarded fish (millions)	Calculated biomass loss (tons)
2017	112.9	1 524
2018	105.0	1 418
2019	101.0	1 364

A comprehensive report on legislation, regulation, and certification of aquaculture

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within the circular economy is given by Soula et al. (2019) in D3.1. The legal requirements for processing fish mortalities in Norway are found in national regulation FOR-2016-09-14-1064, which is based upon the corresponding EU regulations EC 1069/2009 and EC 142/2011. According to these regulations, mortalities must be sanitised at the location before transport. There are 6 standard methods where the product is processed in a combination of temperature, pressure, and residence time. In all these standard methods the particle size must also comply with a minimum measure stated in the requirements of each method.

EC Regulation 142/2011 concerning animal by-products not intended for human consumption is adapted by the Norwegian government (Lovdata, 2016). Mortalities and fish with signs of illness are classified as category 2 animal by-products (mortalities), while living fish with no signs of illness are classified as category 3 animal by-products (discarded fish). According to EC Regulation No 1774/2002, dried product from category 3 may be used in a multitude of applications including animal feed, while category 2 use is restricted to:

a. Bio-energy production
b. Technical applications
c. Fertiliser
d. Feed for fur animal breeding
e. Feed for zoo and circus animals
f. Other non-food producing animals

Aquaculture mortalities would in general be a category 2 product, limiting the application of the dried product according to the above list. Discarded fish and by-products from processing plants would be a category 3 product. In the definition of categories, it is not clear if intestine content can be included if the product is a category 3 product.

If the process in not compliant to any of the pre-approved methods mentioned above, the regulation introduces method 7, which is also labelled “alternative method” applicable for processing of category 3 material. Approval of this alternative method requires a verification by a research institution that the method produces a sanitised product. Verification relies upon compliance with requirements for three bacteria: *Clostridium*, *Salmonella* and *Enterobacteriaceae*. A national point of contact is appointed for approval of an alternative method in each EU country, as well as Norway. In Norway, the national point of contact is the Norwegian Food Safety Authority *Mattilsynet*[1].

[1] https://www.mattilsynet.no/

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2. Methodology

As described in D2.2 (Baarset & Johansen, 2019) the current practice of ensilage faces challenges regarding HSE hazards and operating costs. Initial work on innovative processes for mortality disposal in aquaculture (Baarset & Johansen, 2019) outlined the potential use of a drying process for stabilising mortalities. Indeed, existing drying technologies were experiencing quite hard challenges when trying to supply cost-efficient equipment for autonomous daily processing of wet mortalities. Therefore, a need was detected to verify a safe and cost-efficient drying technology that would require minimal operator attention, as well as transforming the mortalities – now seen no longer as a hazardous waste but rather as a raw material – into a microbiologically stable product reusable under the principles of the circular economy.

Based on the drying process with mechanical fluidisation of the raw material and superheated steam as drying media described in (Nygaard & Hostmark 2008), Waister improved this drying technology in a Waister generation 1 machine for the drying of mortalities.

The eco-intensification of fish mortalities requires a well-functioning drying technology, compliance with regulations and an assessment of the dried product, as shown in **Figure 2**. These areas constituted the work in D2.6.

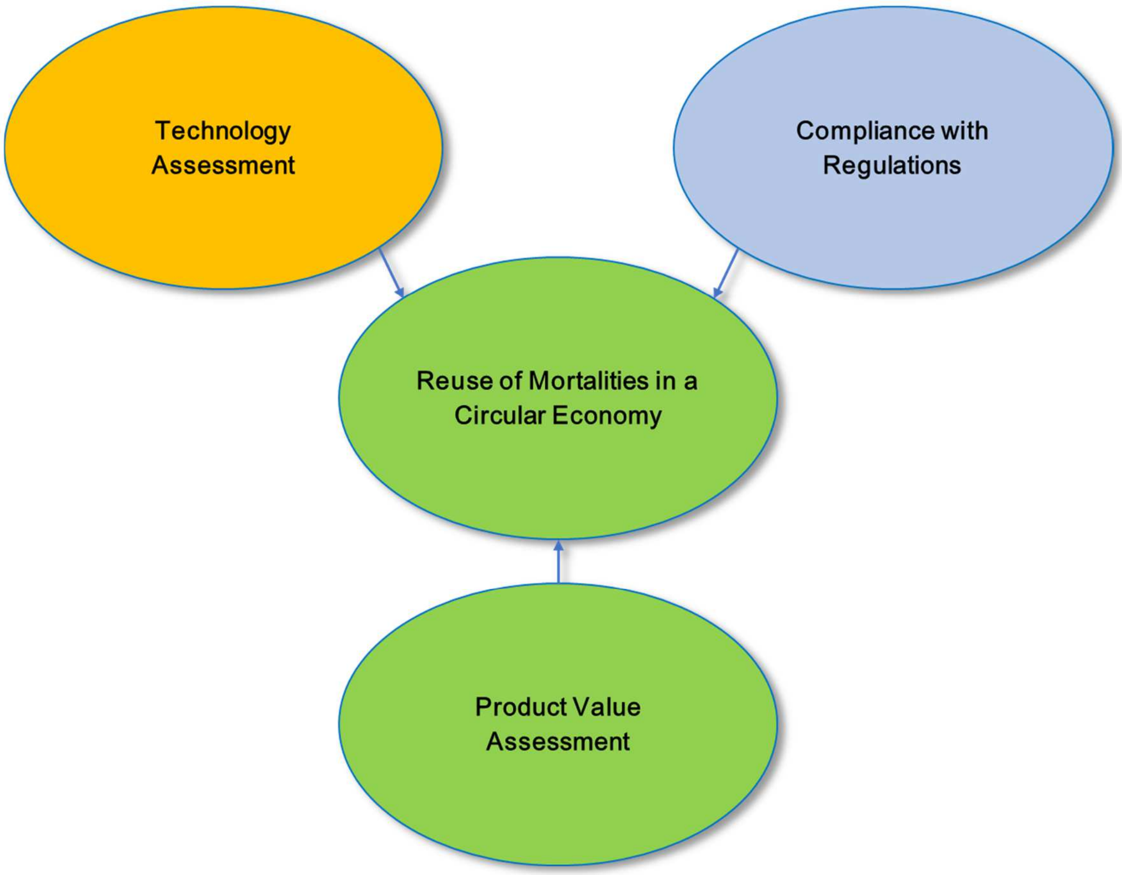

***Figure 2**: Framework for valorisation of aquaculture mortalities*

The work of Baarset & Johansen (2019) showed that there was a need of technology improvements of the Waister 15 generation 1 drying machine to become a well-functioning

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solution for mortalities. The work in D2.6 was targeted at solving the 7 main issues shown in **Table 3**.

***Table 3**: Listing of issues targeted main issues in development of a well-functioning drying machine for mortalities disposal.*

Issue	Description
1	Clogging of steam manifolds
2	Clogging of space between paddle and discharge conveyor
3	Instability in the drying process
4	Corrosion on fins in the condenser
5	Overfilling the drying chamber
6	Excess water entering the drying chamber
7	Collapsing fluidisation

Mortalities drying tests with the innovative technology was performed at Helgeland Smolt. They have two sites – Sundsfjord (**Figure 3**) and Reppen (**Figure 4**). The Sundsfjord site, located in Gildeskål municipality, is a combination of a flow-through and RAS hatchery for salmon smolt up to 250 g with a recently added post-smolt production for salmon up to 1000 kg. The Reppen site, located in Rødøy municipality, is a RAS hatchery in operation since 2017 for salmon smolt up to 250 g.

***Figure 3**: Helgeland Smolt, site Sundsfjord*

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***Figure 4**: Helgeland Smolt, site Reppen*

Design improvements were implemented in a final Waister 15 Single Loop dryer prototype which was installed at Helgeland Smolt, site Reppen for assessment, with the goal of a stable processing of actual mortalities from the hatchery. Each of the implemented improvements are presented in Section 3.

Initial work in M1-M18 (May 2018-October 2019) on drying of mortalities was made at Helgeland Smolt, site Sundsfjord in 2018 using a "MV BioWaste 20" machine. This machine was originally developed for drying mixed food waste. Based upon such experience with mixed food waste, it was assumed that the machine would work well on fish mortalities too. Waister developed an improved machine for drying food waste – "Waister 15 generation 1" – which was assumed to solve the instability issues experienced with the MV BioWaste 20 machine. Work was continued in M19-M23 (November 2019-March 2020) with the Waister 15 generation 1 at Helgeland Smolt, site Sundsfjord. However, it became evident that the drying process on mortalities still was not improved sufficiently. The remaining issues summarised in **Table 3** generated the development of a "Waister 15 Single Loop" at Helgeland Smolt, site Reppen being tested from M27-M28 (July 2020-August 2020).

Unfortunately, the implementation of the Waister 15 Single Loop was significantly delayed due to the complete closure of the Waister factory in Italy when the Covid-19 situation hit in M23-M26 (March 2020-June 2020). All subsequent activities were impacted by this delay.

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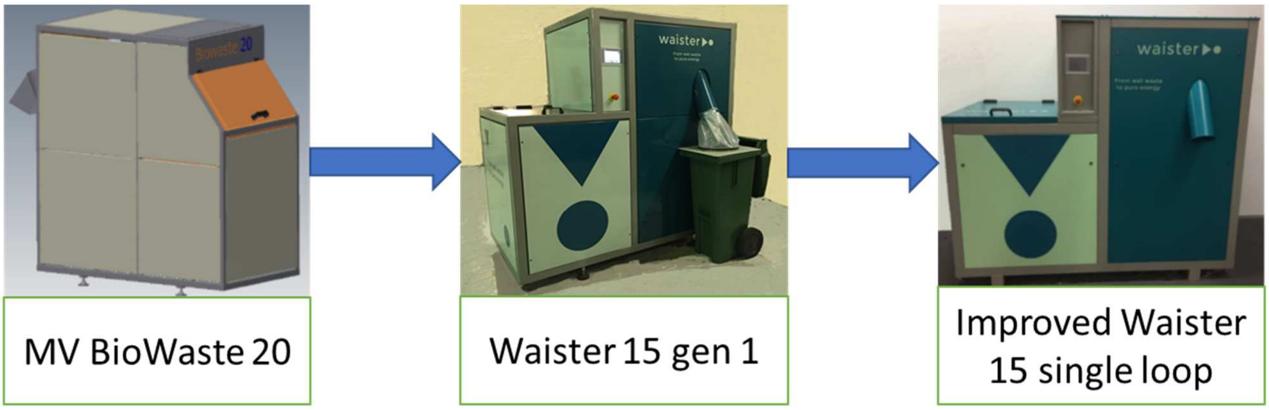

Figure 5*: Development stages in drying technology for mortalities towards the final Waister 15 Single-Loop machine*

An overview of legislation, regulations, and requirements for the application of aquaculture by-products in the circular economy is given in D3.1 by Soula et al. (2019). The assessment of the compliance with European regulation No 1774/2002 (EC, 2002) was investigated by analysing dried mortalities with a focus on three bacteria - *Clostridium perfringens*, *Salmonella* and *Enterobacteriaceae*. Results from the analysis are presented in Section 4.2.

An economic analysis of the operational costs of the innovative method for mortality disposal compared with current practices, together with an assessment of the dried product applications and values, were done for validation of the proposed innovative method for drying mortalities. A summary of the characteristics of the fish mortalities and discarded fish as input raw materials and the dried product as an output is presented in Section 4. The various end-of-life valorisation of the output dried product are presented in Section 5.

2.3 Sampling from drying tests on mortalities and discarded fish

During M27-M28 (July 2020-August 2020) a comprehensive testing and sample collection was made using the Waister 15 Single Loop machine at Helgeland Smolt (location Reppen). Waister operated the machine in this period to achieve maximum experience from the test operation. To observe the consequences of the improvements made on the Waister 15 Single Loop machine, it was opened occasionally to observe the fluidisation and to collect samples directly from the drying chamber.

A collection container was made from an IBC container for receiving the mortalities close to the Waister 15 Single Loop location. Mortalities were collected using a net, as shown in **Figure 6** to avoid receiving too much excess water with the mortalities.

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Figure 6*: Collection of mortalities from a container with a net*

The mortalities were carried in a bucket as shown in **Figure 7** and added to the inlet container of the Waister 15 as shown in **Figure 8**.

Figure 7*: Mortalities of salmon smolt prior to drying*

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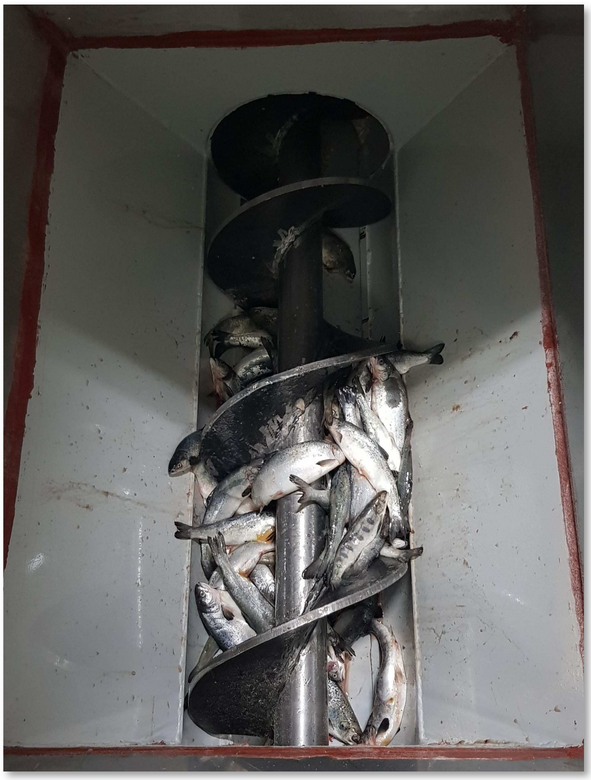

***Figure 8**: Mortalities in inlet buffer of Waister 15*

A variation of mortalities freshness and fish size was processed, e.g. fresh discarded fish from deliberate sorting, mortalities collected in each water tank within 24 hours, and dead fish several days old from fish tanks that were emptied for maintenance and which had already deteriorated.

All the above-mentioned raw material was processed in the Waister 15 Single Loop drying machine. Samples of dried product were collected from the drying chamber at different points in time. At time of sample collection, the DM of a representative sample was measured using a Mettler Toledo HE53 Moisture Analyzer (https://www.mt.com/gb/en/home/products/Laboratory_Weighing_Solutions/moisture-analyzer/entry_moistureanalyzer/HE53_230V.html#documents).

When mortalities were available in the inlet buffer, the Waister 15 machine was started. The machine immediately started fluidising the dried material residing inside the drying chamber, continuing to do so during the warm-up state. As soon as the temperature has reached a temperature setpoint, the Waister 15 goes into drying state. In this state it allows feeding wet material into the drying chamber, feeding small portions of the mortalities from the inlet buffer into the drying chamber. Each feeding sequence was of 6 s duration, then waiting for the relative humidity (RH) to start declining, before allowing the next feed. After a total feeding time was reached, the machines entered a finalisation drying state. The duration of this state was 12-30 minutes, with the RH gradually being reduced to approximately 10 %. No mortalities entered the drying chamber during the finalisation drying state. It is possible to ensure a minimum duration of the finalisation drying state by parameters settings.

The drying process was kept in automatic mode with the inlet drying media temperature set point of 125 °C. The relative humidity (RH) in the drying chamber was oscillating in the interval of 52-54 % during the drying process.

Dried product samples were packed in sterile bags using gloves to avoid

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contamination. Sample bags were sealed and marked with unique identification numbers. The level of DM was measured to ensure that samples were taken from a material with ≥90% DM. Samples were sent to a laboratory for analysis.

During the test operation period at Helgeland Smolt (location Reppen), samples were collected for analysis of bacterial levels and nutritional content of the resulting product.

In order to check that the drying method is compliant with the legislation, dried product samples from mortalities were analysed for the presence of:

- *Clostridium perfringens*
- *Salmonella*
- *Enterobacteriaceae*

Subsequent to the finalised test operation at Helgeland Smolt, a Waister 15 Single Loop machine was also installed at Adriatic Farming in Croatia for processing more fish mortalities. This location receives mortalities of trout smolt from a hatchery and trout up to 4.5 kg from sea cages.

As the use of wood chips as fluidising material limits the potential use of the dried product for certain applications, Waister installed a Waister 15 at Adriatic Farming in Croatia in M32 (December 2020) for the processing of trout mortalies. Spent grain from a local brewery in Croatia was also available for use as additive, replacing wood chips. This would allow for further analysis and assessment of optimal processing of a mix of spent grain and fish mortalities of different size, as well as collection of samples for analysis of a dried product containing a mix of mortalities and spent grain.

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3. Improvements of innovative technology for mortalities disposal

Each of the seven listed technical issues (see **Table 3**) experienced with the Waister 15 generation 1 dryer were analysed and relevant improvements were made in the design of the Waister 15 Single Loop machine to eliminate the experienced obstacles in the second reporting period (May 2019-October 2020). The different issues encountered were interconnected, as one issue influenced several of the others. Issues 1-4 were met by upgrading the design from Waister 15 generation 1 to the Waister 15 Single Loop.

The Waister 15 Single Loop machine was installed in Helgeland Smolt, site Reppen in M27 (July 2020), and operated for one month. Mortalities were manually fed into the inlet hopper of the Waister 15.

Waister used the experience from using Waister machines for drying of mixed food waste from hotel restaurants as a starting point for setting parameters for the drying process. From the assumption that mortalities have similarities with the mixed food waste in terms of dry matter and fat content, the Waister 15 Single Loop machine was set up. Then parameters were adjusted to improve the performance of the machine until it reached the capacity of 15 kg/h water removal.

3.1 Clogging of steam manifolds

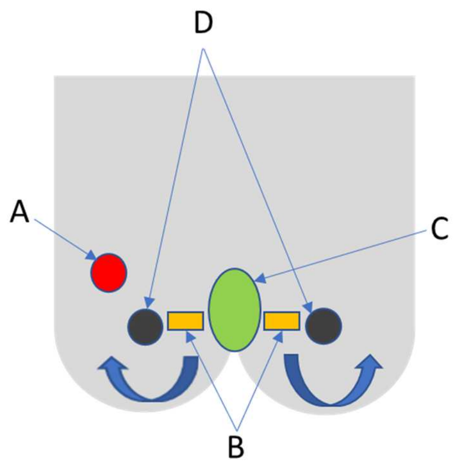

Figure 9*: Place of steam inlet manifold seen from inside the drying chamber (A = BioWaste 20, B = Waister 15 generation 1, C = Waister 15 single loop, D = rotor shafts)*

Figure 9 shows the evolution of the placement of the steam manifold for the superheated steam (SHS) to enter the drying chamber. In the BioWaste 20 machine, the steam manifold was placed to one side above the product bed inside the drying chamber with a slight slope. The single steam inlet manifold in the drying chamber was pointing to the top area of the fluidised material.

The steam inlet was split into two horizontal rectangular manifolds. This improved the mixing of steam with the material in the drying chamber, easing the fluidisation. The manifolds allowed the superheated steam to be inserted horizontally into the fluidised product. However, when agglomeration of mortalities occurred, the horizontal manifolds started to clog partially. As the amount of product inside the drying chamber increased, the manifolds clogged totally. Clogging of the inlet steam manifolds caused the superheated steam not to

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enter the drying chamber, and hence not to dry the material inside the drying chamber. To solve this issue, Waister developed a new manifold in the centre of the drying chamber of the Waister 15 single loop machine, arranging it steeply angled into the fluidised product. The flow of superheated steam is targeted to follow the bottom section of the drying chamber in the same direction as the paddles of the two rotors.

3.2 Clogging of space between paddle and discharge conveyor

Another issue experienced in the Waister 15 generation 1, when the product inside the drying chamber became agglomerated, was that the space between the discharge conveyor and the closest paddle was also clogged, forming a wall of material constricting the discharge of material. The result was a gradual accumulation of material inside the drying chamber. This eventually enforced the clogging of the inlet steam manifolds.

Adjustments of the design of the Waister 15 single loop machine included narrowing the distance between the discharge screw and the closest paddle to avoid a “dead zone” where accumulation of material may occur. The new design has proved to prevent clogging of the area between paddle and discharge conveyor. An additional improvement was to allow the discharge screw conveyor to do a short reverse feeding of the discharge conveyor. By running the discharge conveyor for a short time in reverse, any blocking material between paddle and discharge conveyor was pushed back and re-fluidised in the drying chamber.

3.3 Instability in the drying process

The instability of the drying process of the Waister 15 generation 1 machine was a result of the split of the saturated steam leaving the drying chamber into two separate streams – one leading the main steam to the condenser causing the moisture to leave the machine in form of a condensate, and the other leading a smaller stream to the heater for reheating to superheated steam.

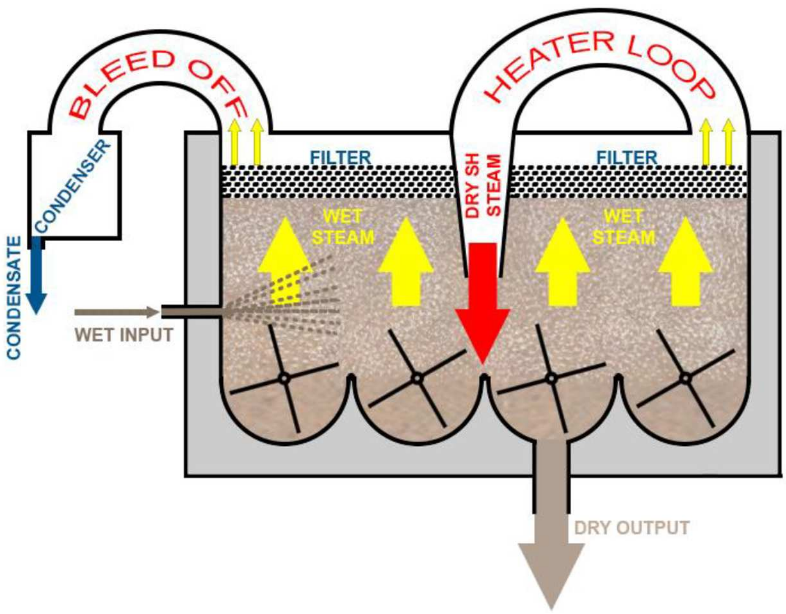

***Figure 10**: Waister original dryer design (MV BioWaste 20 and Waister 15 generation 1) with mechanical fluidisation and superheated steam. The flow of saturated steam leaving the drying chamber was split into two separate streams – one for reheating and one for condensing (bleed off).*

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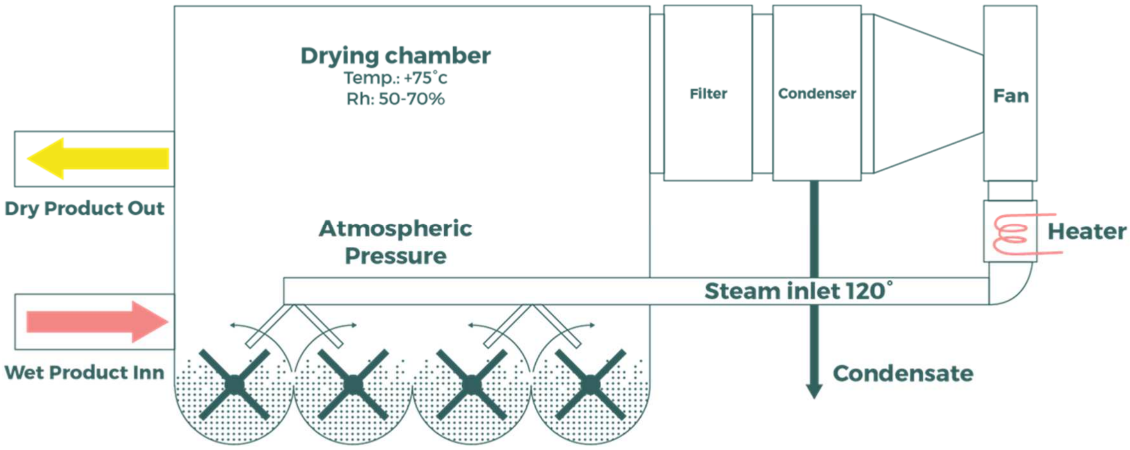

***Figure 11**: Waister 15 Single Loop drying with mechanical fluidisation and superheated steam. The flow of saturated steam leaving the drying chamber is forced to pass the condenser and heater in the single loop.*

Figure 10 shows the initial design with a split of the saturated steam leaving the drying chamber into two separate streams. In both the MV BioWaste 20 and Waister 15 generation 1 machine there was a separation of the saturated steam flow into two individual paths – the heater loop and the bleed off for condensation.

When encountering restrictions of the steam flow into the drying chamber, the steam will follow the path of less resistance. In the case of partial clogging of steam manifolds, this means less flow to the heater and more flow to the condenser. Since less steam flows through the drying chamber, less moisture from the product is collected. This in turn leads to less moisture in the saturated steam and less condensation. With increasing clogging of manifolds this eventually led to a complete stop of flow and no condensation.

The major improvement of the Waister 15 single loop machine was leading the complete steam flow through the condenser, keeping a partial moisture in the flow passing the heater becoming superheated steam. The design of the patented single loop concept used int the latest Waister 15 machine was developed in cooperation with professor Odilio Alves-Filho from the Norwegian University of Technology and Science (NTNU). A schematic description of the single loop drying process is shown in **Figure 11**.

Since the steam has one single path to follow, it is forced to enter the drying chamber. In combination with the improved design of the inlet steam manifold, this ensured that the manifold was always kept open. This improvement in the Waister 15 Single Loop design has proved to produce a more stable and predictable drying process on all products, and fish mortalities in particular.

3.4 Corrosion on fins in the condenser

An upgrade of material specifications on all surfaces in contact with the superheated steam as well as the saturated steam inside the Waister 15 eliminated the corrosion experienced in the two first machines.

In particular, the copper and aluminium fins inside the condenser were heavily corroded on the MV BioWaste 20 and Waister 15 generation 1 machines. The Waister 15 single loop machine fins are made of AISI 304 stainless steel. This has proved to be resistant to corrosion, maintaining the function of the condenser over time.

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3.5 Overfilling the drying chamber with wet material

Occasionally, the RH sensor measuring the saturated steam indicated low RH, even if the material in the drying chamber was quite humid. The superheated steam inlet into the machine was disrupting, resulting in heavily reduced amount of saturated steam leaving the drying chamber. This happened because the steam inlet into the drying chamber was partially or completely blocked by the wet product.

Since the saturated steam was unable to flow past the combined RH and temperature sensor, the readings from this sensor showed low RH, while the moisture in the drying chamber was high. On several occasions this resulted in feeding more wet product into the drying chamber with an already high amount of wet product. This resulted in a loss of fluidisation. During the work with the Waister 15 generation 1 machine, the drying process stopped even if there were remaining mortalities in the inlet container due to this issue.

As already explained in section 3.3, the Waister 15 Single Loop machine solved this issue, since it forces the steam to flow into the drying chamber, securing that the RH sensor always receives a flow of saturated steam. This secured that the RH sensor always gave reliable measures of the moisture level in the saturated stead leaving the drying chamber. The feed of new raw material into the drying chamber was now only allowed when levels of humidity were below the parameter set point and declining, with a resulting stable and satisfactory drying process.

3.6 Excess water entering the drying chamber

In other cases when the machine had stopped with the assumption of the inlet container being empty, when it actually contained substantial amounts of mortalities, we experienced to find the machine flooded with excess water the next day. This excess water is gradually released by the mortalities allowing it to leak through the grinder into the drying chamber.

Similarly, when adding larger quantities of mortalities into the inlet container, some excess water was released from the mortalities while waiting to be processed. This was experienced to make a leakage of excess water into the drying chamber creating a collapse of the fluidisation.

Waister assessed some solutions to separate mortalities from excess water, in search for an industrial solution with automatic receipt of mortalities from a transport system. While the tests were performed with manual feeding of the mortalities into the Waister 15 machine, an automated industrial solution would require a solution like the one shown in **Figure 12**, which shows a well-functioning device to separate fish mortalities from excess water used at Helgeland Smolt, site Reppen. This device can be placed where the mortalities arrive for separation before the mortalities enter the inlet buffer of the Waister 15 Single Loop.

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***Figure 12**: Device for separating fish mortalities from excess water*

3.7 Collapsing fluidisation

Using wood chips as start feed, the fluidisation of the material inside the drying chamber worked well in the beginning. As the ratio of mortalities to wood chips increased, the fluidisation eventually started to collapse. As an effect of this collapsing fluidisation, the material inside the drying chamber started to agglomerate. When the drying chamber filled up with an increasing amount of product, this led to clogging of steam inlet manifold as described above with the initial MV BioWaste 20 and Waister 15 generation 1 machines. Even with the Waister 15 single loop machine we experienced this agglomeration of product inside the drying chamber and collapsing fluidisation. The main reason for this phenomenon was the fat content in the product inside the drying chamber.

Fluidisation inside the drying chamber was deteriorating after a while. An optimal incorporation of wood chips in a ratio of 1 kg woodchips to 12.5 kg mortalities was experienced to solve this issue. At this ratio we experienced sustained fluidisation in the drying chamber. Other additives containing fibres to enrich fluidisation are assumed to yield the same effect.

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4. Analysis of mortalities and discarded fish

4.1 Characteristics of mortalities and discarded fish at Helgeland Smolt

The characteristics of the mortalities and discarded fish from Helgeland Smolt was analysed from detailed statistics of the daily amount and size of mortalities and discarded fish from a 401-day period (01.06.2019 – 06.07.2020) at Reppen site. The average and median daily amounts and the average size of the fish were estimated.

Further investigations were made on the events concerning the highest amounts of mortalities and discarded fish through interviews with staff on each of the event.

Measured data on the average size of juveniles lost at Helgeland Smolt were received in Excel format from their official reporting, containing number of fish, size of fish and total mass of mortalities and daily discarded fish. An overview of the distribution of total daily biomass of mortalities and discarded fish is shown in **Table 4**.

Table 4*: Number of days within each interval of mortalities and discarded fish biomass at Helgeland Smolt, site Reppen in the period 01.06.2019 – 06.07.2020.*

Biomass of mortalities and discarded fish	# days
< 5 kg	7
5-9.9 kg	85
10-19.9 kg	164
20-49.9 kg	80
50-99.9 kg	27
100-199 kg	15
200-499 kg	13
>500 kg	10
Total	**401**

Data from the analysed period shows that a total of 14.1 tons of mortalities and 21.3 tons of discarded fish were processed at Helgeland Smolt, site Reppen (**Table 5**). At two occasions, the site reported incidents causing mass mortality and deliberate destruction of biomass. These two occasions accounted for 14.7 tons, while 20.7 tons originated from the day-to-day operations. The availability of mortalities shows substantial variations from day to day. The median amount available was 15.3 kg/day, while the average amount was 51.7 kg.

Table 5*: Data for calculation of average weight of mortalities and discarded fish at Helgeland Smolt, Reppen site.*

	Biomass [kg]	# fish	Average weight [g]
Mortalities	14 142	1 002 149	14,1
Discarded fish	21 347	1 625 485	13,1
Total	35 489	2 627 634	13,5

These data show that, at the selected smolt farm, the average size of single mortalities was 14.1 g in a 401-day period (01.06.2019 – 06.07.2020), while single discarded fish was 13.1 g in average, giving an overall average size of 13.5 g per fish.

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Data from Helgeland Smolt (location Reppen) are shown in **Figure 11**, indicating that the variation in daily amount is substantial. While most days the mass is low, there are some shorter periods with high amounts of mortality biomass and discarded fish.

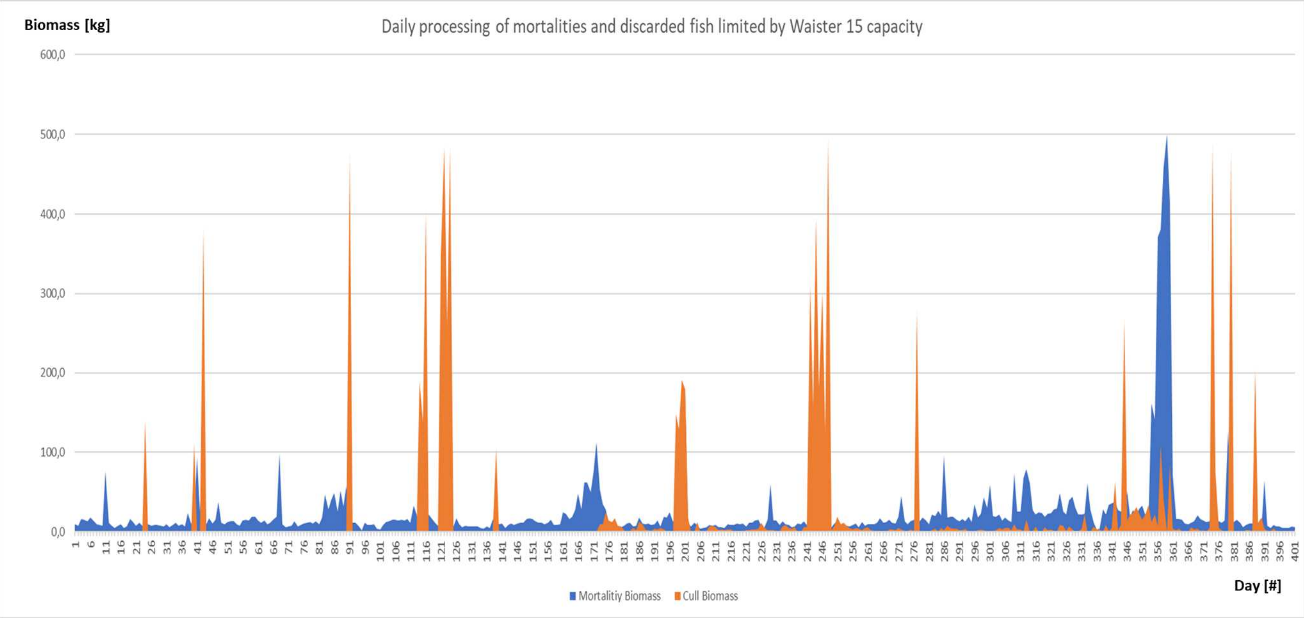

***Figure 13**: Daily amount of mortality biomass and discarded fish (cull biomass)*

Daily value shown in **Figure 13**. The Y-axis is cut at 500 kg for improved visualisation. In the 401-day period there were two separate incidents that produced above 1,000 kg of biomass in a timespan of two consecutive days. In both these cases an external service of ensiling of the biomass was applied.

The total amount of mortalities in the 401 days period was 35.4 tons, while the amount from special incidents was 14.7 tons.

4.2 Dried product characteristics

For starting up the dryer, we used wood chips as fluidising material. When feeding the Waister 15 repetitively with mortalities, the resulting ratio of mortalities *vs* wood chips was gradually increased. Initially, the drying process worked well. After some time, the fluidisation was deteriorating. The product was forming lumps, becoming increasingly sticky. Samples of product extracted from inside the drying chamber showed that the product was >98 % DM, meaning that the stickiness was caused by fat rather than moisture. Adding wood chips into the product proved to regain the fluidisation of the product. This proved that there is a need of a fluidising structure material when processing mortalities from salmon smolt, caused by the high fat content of the raw material. By testing different mixes of wood chips and mortalities, a 12.5:1 ratio was found to ensure stable processing.

From a total of about 500 kg of fish mortalities and 40 kg of wood chips was obtained about 210 kg of dry product. From the total, 30 different batches were randomly collected and placed in sterilized plastic bags. Each plastic bag contained about 300 g of dried product. All samples were sent to the laboratory of food inspection of the University of Milan and have been subjected to chemical (proximate) and microbiological analysis. The chemical composition of samples was analysed in duplicates according to the Association of Analytical Chemists methods (AOAC, https://www.aoac.org/), specifically were assessed the moisture

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(method 950.46), fat (method 960.30), ash content (method 923.03) and the nitrogen content carried out using Kjeldahl (1883) (method 992.15). Carbohydrate was determined by “difference” (FAO, 1998). Means and standard error (±) of means were calculated for two independent determinations of each proximate component except for total carbohydrate which was by “difference”.

Regarding the microbiological analysis, on each sample of Mortalities Fish Meal (MFM) were evaluated the *Enterobacteriaceae*, sulphite-reducing clostridia and *Salmonella* spp. To perform the analysis were aseptically collected 10 g, except for *Salmonella* spp. where the analytical unit was 25 g, from each sample and mixing it with 90 mL of sterile buffered peptone water (Oxoid, Basingstoke, UK). After homogenization in a Stomacher 400 (Stomacher 400 circulator; Seward Ltd., Norfolk, UK) for 60 s, decimal dilutions were prepared for the subsequent determinations. In particular, *Enterobacteriaceae* were enumerated using Violet Red Bile Glucose (VRBG) agar (Biolife, Milan, Italy) and were incubated at 37 °C for 24 h, while sulphite reducing bacteria were performed using Iron Sulphite agar (Biolife, Milan, Italy) and the plates were anaerobically incubated at 37 °C for 48 h. Presence of *Salmonella* spp. was detected according to the UNI EN ISO 6579-1:2017, the analysis procedure for *Salmonella* spp. is summarized in the **Table 6**.

Table 6*: Culture technique (media and agar), incubation conditions biochemical confirmation tests used for Salmonella spp. microbiological testing.*

Parameters	Culture Technique	Incubation		Culture media and agar	Biochemical confirmation test	Reference method
		Time (h)	Temp(°C)			
Salmonella spp. detection	Pre-enrichment	18	37	Buffered Peptone Water[1]	API 20 E NE[4]	ISO 6579:2017
	Selective Enrichment	24	41.5	Rappaport-Vassiliadis Broth[1]		
		24	37	Muller-Kauffmann-Tetrathionate-Novobiocin Broth [1]		
	Plate	24	37	Xylose Lysine Desoxycholate Agar [1]		
		24	37	Brilliant Green Agar[1]		

[1] Thermo Fisher, Waltham, USA.
[2] Bio-Rad, Marne la Coquette, France.
[3] Microgen Biproducts, Camberley, England.
[4] bioMérieux, Marcy l´Étoile, France.

The analyses were performed in duplicates and the results were expressed as Log colony forming unit (CFU)/g, except for *Salmonella* spp. where the results were expressed as presence/absence in 25 g of sample.

The results of proximate analyses are reported in **Table 7**.

Table 7*: Composition of mortalities fish meal (MFM) sample (n=15x2; mean ±*

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standard deviation).

	MFM	S.D.
Moisture (%)	**1.74**	**0.23**
Protein (%)	**30.31**	**0.29**
Lipids (%)	**31.24**	**0.34**
Total carbohydrates[1] (%)	**31.19**	**-**
Ash (%)	**5.52**	**0.10**

[1] Calculation by difference

Regarding microbiological results, all samples were negative for *Salmonella* spp. (absence in 25 g/sample) and *Enterobacteriaceae* (<1 Log CFU/g of sample), while for colonies of sulphite reducing bacteria all (*n*=14) were found negative, except for one sample (*n*=1) where the result was 1.47 Log CFU/g.

From a microbiological point of view, there are no real acceptance limits for specific microorganisms in feed raw materials. However, as regards feed materials composed of animal by-products (ABP), previously repealed by the EU Regulation No. 1069/2009 there was the EU Regulation No. 1774/2002 laying down health rules concerning ABP not intended for human consumption, which provided two microbiological limits which can be still kept as a reference. In particular, it defined the limit for *Salmonella* spp. absence in 25 g/sample and a count of *Enterobacteriaceae* less than 300 CFU/g sample (2.47 Log CFU/g sample). However, it should be noted that the feed raw materials for pet food production are first mixed, either manually or mechanically, to have balanced compositions, and in subsequent stages, the mixtures are processed using extruders.

The operation of this equipment is based on the ability to generate heat (from 120 to 178° C), using only a system of pressure (80-150 bar). The process takes no longer than 30 seconds, during which time a partially dehydrated finished product (moisture ranged from 5 to 12%) is obtained, with the desired shape and above all sterile.

In our study, the results showed a situation of total conformity from a microbiological point of view for the raw feed materials, specifically for MFM, also thanks to the heat treatment carried out which stabilizes the fish meal inside the drying chamber of the Waister 15 Single Loop dryer. However, it is of fundamental importance to monitor the transformation process and to implement Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) to obtain a feed raw material free of contamination or with limited contamination so that the whole feed chain is controlled. The limited presence of certain microorganisms such as *Enterobacteriaceae*, sulphite reducing bacteria, total viable count, *etc.,* in feed raw materials or finished products (pet food) can be attributed to poor hygienic conditions during production or subsequent contamination (processing, handling, packaging, and subsequent storage). This type of contamination is not a cause for concern from a health point of view, as these microorganisms are ubiquitous and incapable of causing disease in animals. In many cases, however, the presence of a high microbial load of these bacteria leads to odour changes caused by bacterial lipolytic and proteolytic action, which may result in a loss of palatability for the animal. For other pathogenic micro-organisms such as *Salmonella* spp., *Listeria monocytogenes*, *etc.,* especially in the finished product, they may be the cause of foodborne diseases in domestic animals which can also be a carrier for humans (Castrica et al., 2020), for these reasons the microbiological safety, in this case, of pet food is very important because it could be a potential source for several zoonotic pathogens and a public health concern (Weese

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et al., 2005; Nemser et al., 2014). Contamination and bacterial growth are much easier in raw and/or moist pet food; this consideration also applies to food for human consumption. Very often, dry pet food with a low water activity (aw: about 0.5, moisture of 10%) remains stable with inhibited bacterial growth until it is accidentally or voluntarily rehydrated (e.g., food rewetted accidentally during storage or by the pet owner to increase the food's softness or palatability or by the pet's drinking water or saliva).

The addition of water in contact with the rich substrate of pet food at room temperature can potentially create conditions favourable to bacterial growth. To avoid such contamination, especially during production, the European Pet Food Industry Federation (FEDIAF) and the American Feed Industry Association (AFIA) have issued good manufacturing guidelines to minimize microbiological risks in animal feed during manufacturing. It might be prudent to extend this approach to the postproduction phases.

A further fundamental aspect that must be considered is that when it comes to animal by-products, European legislation (EU Regulation No. 1069/2009) only allows the use of category 3 as feed material and excludes Animal By-Products (ABPs) of category 2. In particular, category 3 include:

- Carcasses or body parts passed fit for humans to eat, at a slaughterhouse
- Products or foods of animal origin originally meant for human consumption but withdrawn for commercial reasons, not because they are unfit to eat
- Domestic catering waste (with certain restrictions)
- Shells from shellfish with soft tissue
- Eggs, egg by-products, hatchery by-products, and eggshells
- Aquatic animals, aquatic and terrestrial invertebrates
- Hides and skins from slaughterhouses
- Animal hides, skins, hooves, feathers, wool, horns, and hair that had no signs of infectious disease at death.

Therefore, it is important to emphasize that in this specific case ungutted fish (with digestive tract content) cannot be used as feed material. However, the dried end product can be used in other contexts such as organic fertilisers/soil improvers/composting or anaerobic digestion, in any case, our preliminary study, aimed to verify the potential of the treatment to make our product microbiologically safe despite the presence of gut content.

However, for full applicability and compliance with current European legislation, only category 3 animal by-products and only edible matrices can be processed. Therefore, edible materials such as spent grains, and not wood chips, must be used as an initial starter and additive for the Waister 15 technology transformation process. The choice of the initial material must also take into consideration the chemical composition of the incoming wet product to avoid problems such as gelation or agglomeration that do not allow the finished product to dry completely.

Regarding the chemical analysis of MFM compared to a standard fish meal on the market (table 8), these showed lower protein percentages. The result could be associated with a dilution factor verified by the presence of wood chips. However, these results need further investigation.

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***Table 8**: Typical proximate compositions of some commercially available fishmeal used in the pet food industry (*B&F Italia SRL, 2021).

Feed Raw Material	Protein (%)	Lipids (%)	Ash (%)	Moisture (%)
Fish meal	60-64	8-14	22-18	3-6
Herring meal	68	12	12	6
Salmon meal	67	14	18	4
Tuna meal	62	11	22	6
Trout meal	62	12	20	6
Squid meal	75	6	10	9
Shrimp meal	50	11	22	11
Krill meal	53	15	12	8
Cod meal	62	12	18	6

In conclusion, the reuse of MFM in the animal feed industry is a sustainable option in line with the Goals of the 2030 Agenda and according to EU Regulation No. 999/2001 laying down rules for the prevention, control, and eradication of certain transmissible spongiform encephalopathies, it is possible to reuse fishmeal to feed different animal species as non-ruminants, fish, pets, and fur animals. Finally, thanks to their high protein value the fish meal has also high market value.

4.3 Mass and energy balance calculation

A mass balance calculation for processing of 1,000 kg of fresh fish mortalities with the Waister 15 single loop machine is shown in **Figure 14**. The input 1,000 kg of mortalities produces 278 kg of dried product at ≥90% DM and 722 kg of condensate. The additional input into the process is 614 kWh electric power as well as 10.8 m³ of cooling water. Recovery of heating energy as hot water at 50-60 °C can utilise a maximum of 368 kWh, reducing the net power consumption to 246 kWh.

In D4.3 an elaborated mass and energy balance for a typical salmon hatchery is presented.

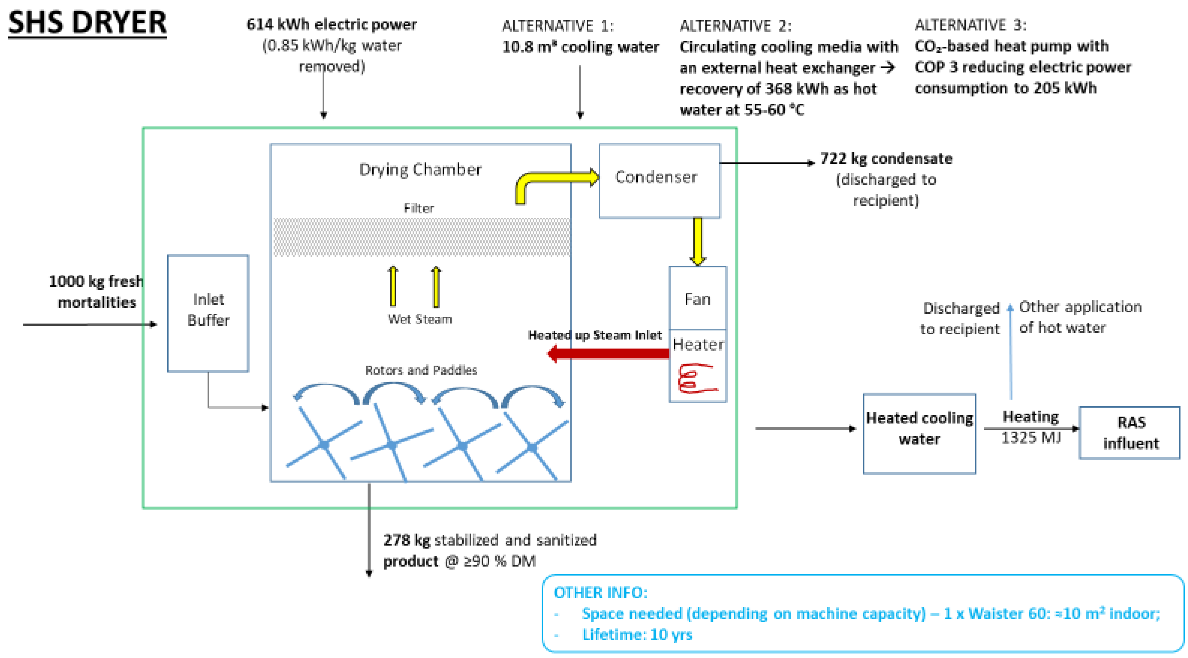

***Figure 14**: Mass and energy balance for processing of 1,000 kg of mortalities*

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5. Mortalities Valorisation

Validation of the dried product disposal was made for the most promising alternatives ranked from highest to lowest potential value:

1. **Feed for animals** (best value, requires high degree of safety assessment in use).

Table 9 below shows the animal species that can be fed with fishmeal according to the EU Regulation No. 999/2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

Obviously, as described above, only category 3 animal by-products can be used for feed purposes and without the presence of wood chips or any other non-food additives.

Table 9: Possible uses of fishmeal as feed.

	Ruminants	Non-Ruminants	Fish	Pets and fur animals	Reference
Fishmeal	Not allowed	Allowed	Allowed	Allowed	EU Reg. No. 999/2001

2. **Bio-fertiliser** (some potential value, requires safety in use).
3. **Bio-energy** product (low value, widely available application).
4. **Biogas substrate** (zero value, available application in some regions).

Use of the dried product as feed for animals, e.g. pet food, is only applicable, as already described, when raw material is category 3, while the other applications apply to both category 2 and category 3 materials.

As described in Section 3, a need was experienced for an additive product to obtain good fluidisation inside the drying chamber. Wood chips were used as an additive product, since it was the most easily available one at the Helgeland Smolt, Reppen site. Dried spent grain has later proved to work equally well as an additive material, as applied at Adriatic Farming in Croatia.

The dried product samples (**Figure 13**) therefore contained a mix of mortalities and wood chips. Wood chips as additive are only applicable if the dried product is used as a fertiliser, in bio-energy production or as biogas substrate. For use as feed for non-food producing animals, dried spent grain would be a preferred additive product.

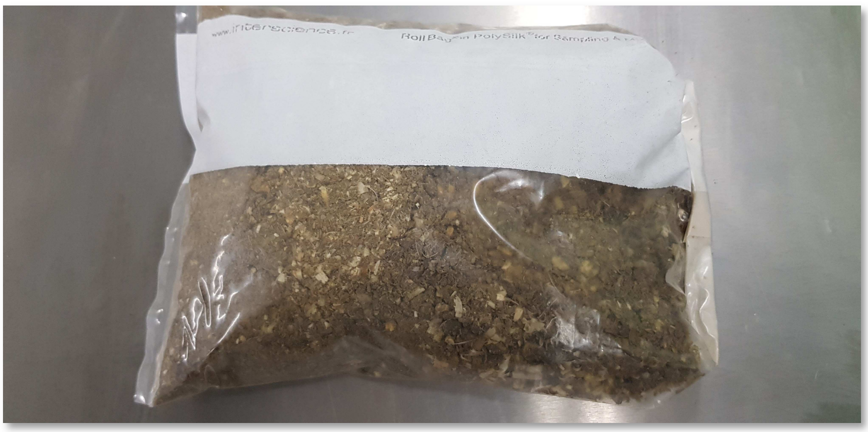

***Figure 15**: Product sample of dried mortalities*

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Dried mortalities and discarded fish will have the following market value if allowed for use as:

1. **Feed for animals: The economic value of fishmeal**

The value as a potential pet food ingredient based upon initial indications from Feed from Food s.r.l. (https://feedfromfood.com/) and the quotation provided by Borsa Merci di Bologna in Italy (Listino settimanale Borsa Merci Bologna, https://www.agerborsamerci.it/), states that the value is approximately € 1.60/kg for fish meal or fish pellets with a protein content between 64/66%, and about €1.90/kg for fish meal or fish pellets with a protein content between 70/72% when sold to feed industries that use fishmeal in the unaltered state or as an ingredient for inclusion in the formulation of croquettes or snacks. If proven as a safe product in compliance with government regulations, this will be the best application of dried mortalities and discarded fish. Dried spent grain as additive is applicable for use as pet food ingredient.

2. **Bio-fertiliser**

As fertiliser the substrate can have a limited value. However, there is a resistance to use mortalities in agriculture due to potential spread of disease to local recipients.

3. **Bio-energy product**

The value is approx. €40-45 per ton when delivered to cement factories replacing fossil fuel, based upon the energy content of the product and the price of equal amount of energy in terms of coal as fuel. However, cement factories typically require a minimum annual amount of 5000 tons to receive products at a regular basis (information given in meetings with Heidelberg Cement Group).

4. **Biogas substrate**

As biogas substrate the value of the product may be €0 as gate fee. There might be a willingness for biogas plants in Denmark to pay a minor amount for the dried product for use as biogas booster.

The environmental sustainability of such innovations was studied through a Life Cycle Assessments (LCA) of the innovative mortality disposal method compared to the current ensilage technology, as presented in D4.4 (Cristiano et al., 2021), publicly available. There, three scenarios were addressed:

- Scenario A: Ensilage
- Scenario B: Drying with Waister 15 using circulating water as cooling media
- Scenario C: Drying with Waister 15 using a closed loop of cooling media

Recovered heat from the drying process can be used for increasing temperature of inlet water to the fish farm, which would be relevant for fish farms with low temperature inlet water. Increased temperature on inlet water is favourable for the growth rate of the fish until the temperature reaches the optimal temperature for the fish species.

Further investigations were made on valorisation of the two applications most easily available – bio-energy product and biogas substrate, as well as the potentially most valuable valorisation path – animal feed ingredient for the pet food industry. Each of scenario B and C were analysed with the chosen end-of-life product valorisations, creating the following sub-scenarios:

- Sub-scenario 1: Animal feed ingredient for pet food
- Sub-scenario 2: Bio-energy at cement factory
- Sub-scenario 3: Biogas substrate

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Innovations in mortality treatment show good environmental performance for both scenario B and C for all three sub-scenarios, with scenario C1 – drying with Waister 15 using a closed loop of drying media and valorisation of the dried product for pet food – being the best performing one.

The cost-benefit analysis, based on the typical farm approach presented in GAIN deliverable D4.1, also indicates that the process for mortality disposal developed in GAIN is more sustainable than the ensilation from the economic point of view: the results are presented in detail in GAIN deliverable D6.9. Both deliverables are publicly available.

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6. Further Research

The outlined scenarios can be further developed to explore economic and environmental gains based on different transport distances from origin to disposal of the dried product, as a dried product allows a prolonged transport of the product achieving improved eco-efficiency in reuse of the product as a valuable new raw material, under the principles of the circular economy.

As future steps, further studies are needed to find the optimal food additive to add to the category 3 fish by-product to obtain a meal that can be reused in the animal feed sector. Heat treatment (treatment specification 125°C inlet drying media temperature, drying time between 12 min to 20 min) has already been shown to make the product microbiologically safe, but further studies testing different times and temperatures could further improve understand the possible limits and strengths of the heat treatment and the overall sanitizing process.

As already mentioned, dried spent grain (DSG) could be the food additive to be used during treatment. Moreover, DSG has good protein values and a dried product with DSG as additive could increase the protein levels of the finished product. This product will be a potential pet food ingredient, crucial for releasing the potential full value in the mortalities value chain. For these reason, further research for valorisation of the mixed product of mortalities and spent grain from the site of Adriatic Farming is ongoing.

Primarily, focus will be on application of dried mortalities with the highest value in accordance with current government regulations. Secondarily, potential applications that prerequisite changes in government regulations will be pointed out.

An approval of compliance with food safety needs to be made for an actual installation site. Waister will work closely with the aquaculture sites on collection and documentation of compliance.

Protein quality in dried fish meal is well preserved by using mechanical fluidisation and superheated steam according to Nygaard & Hostmark (2008). The protein quality of dried mortalities would be anticipated to be better than of the ensiled product. However, this was beyond the purposes of the present work and therefore could be further investigated.

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7. Conclusions

Mortalities are an inevitable side stream from all varieties of fish farming – sea cages, RAS and flow-through systems, as well as in transport vessels for live fish. As described in Baarset & Johansen (2019), mortalities and discarded fish are substantial side streams from the aquaculture industry in terms of both volumes, costs of operation, HSE hazards and potential value. Waister has investigated an innovative technology for mortality disposal which is more sustainable, cost-efficient, and less hazardous compared with the current one.

The eco-intensification of the aquaculture industry requires a cost-efficient and safe handling of mortalities and discarded fish in compliance with current regulations. The innovative technology for mortalities disposal based on using the Waister 15 Single-Loop machines for drying facilitates eco-intensification of the aquaculture industry.

Processing grinded mortalities with a 125 °C inlet superheated steam drying process and mechanical fluidisation minimum 12 minutes in a Waister 15 Single-Loop machine, produces a microbiologically stable product, enabling a safe storage, transport, and use. This innovative method eliminates the HSE hazards of ensilage processing.

Waister developed a well-functioning solution for mortalities drying, being a safe and cost-efficient alternative to the current method of ensilage. However, processing mortalities of salmon smolt and other species containing substantial amounts of fat, requires an additive for maintaining the mechanical fluidisation during the drying process. Product samples were analysed for microbial inactivation as well as nutrition value. The analysis results of microbial inactivation in the drying process indicates that it is a safe alternative to ensilage. No content of *Enterobacteriaceae* and *Salmonella* was found in the analysed samples. There are currently no specific limits for amounts of sulphite-reducing bacteria in feedstuff. The measured values are generally considered to be at "satisfactory" level for feedstuff.

Table 10 shows an overview of the challenges experienced with the initial prototypes for mortalities drying, which were solved in the final Waister 15 Single-Loop dryer.

Table 10*: Waister 15 Single-Loop solutions to experienced issues with the initial prototypes for mortalities drying.*

Issue	Solved by Single-Loop design	Solved by additive	Other solutions and comments
Clogging of the steam manifolds	X	X	
Clogging of space between paddle and discharge conveyor		X	
Instability in the drying process	X		
Corrosion of the fins in the condenser			Material quality upgrade
Overfilling the drying chamber with wet material	X	X	
Excess water entering the drying chamber			Manual filling; devise for separation of excess water from mortalities
Collapsing fluidisation		X	

The final prototype is operating with a Single-Loop flow of steam, securing a steady flow of superheated steam into the drying chamber as well a continuous flow of saturated steam from the drying chamber to the condenser. By introducing wood chips or dried spent

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grain as additive, fluidisation in the drying chamber was achieved, enabling the Waister 15 Single-Loop drying to process mortalities of salmon and trout with high fat level. The final Waister 15 Single-Loop machine has proved to work well on both small fish (salmon and trout smolt) as well as full-size fish from sea cages (trout).

The improvements which have been developed and tested in the work of T2.1 has given a robust design of the machine and its parameters. Waister has improved knowledge of the process of drying of mortalities, in particular of the necessity of an additive material to obtain proper fluidisation in the drying process.

The results show that the potential of the Waister innovative method of mortalities drying is promising in terms of cost-reduction compared to the current state-of-the-art method of ensilage, as well as a potential for increasing the value of mortalities as a dried product by compliance with government regulations on reuse of animal by-products and food safety**.**

The continued work in WP2 of the GAIN project will advise on the best solutions for eco-intensification of mortalities disposal in a circular economy perspective. The expected environmental benefits are assessed in T4.3 in the GAIN project.

The increased value of dried mortalities for use as feed ingredient requires the formal approval of the method as an alternative to ensilage in the current EU and Norwegian regulations.

The dried mortalities classified as a category 2 or category 3 product are applicable as a bio-energy products, creating heat energy from incineration or as biogas substrates in anaerobic digestive plants.

Mortality drying systems are applicable at all aquaculture locations where by-products are produced:

- Hatcheries (landbased fish farms)
- Feeding barges at sea cage sites
- Onboard work boats for aquaculture

In fish processing plants, the off-cuts and other side streams may also be processed using this novel drying technology. At such sites, it is easier to separate category 3 materials being transformed into animal feed ingredients for e.g. pet food or fish feed.

The TRL level on mortalities drying prior to GAIN project was TRL 6. The innovative solution with use of Waister 15 Single Loop for processing of mortalities is currently at TRL 8 with a complete and qualified system. The order for the first commercial installation is received and will be installed at Adriatic Farming in Croatia August/September 2021 as replacement for the current Waister machine used to verify stable operation with spent grain as additive.

Results show compliance of the viability for the innovative method of mortalities drying in terms of microbial inactivation.

Entering the GAIN project, the technology was at TRL 2 with a formulation of a technology concept assumed to be suitable for mortalities drying. The innovation passed to TRL 7 with a system prototype being demonstrated in operational environment. Since December 2020, the first machine has been in daily operation at an aquaculture site. The solutions are now presented as a commercial product by Waister, meaning that the technology has entered TRL 8 as a complete and qualified system.

The project has received funding from the European Union's Horizon 2020 Framework Research and Innovation Programme under GA n. 773330

References and useful links

Association of Official Analytical Chemists (AOAC, 2000). *Official Methods of Analysis*. 17th ed. Association of Official Analytical Chemistry Gaithersburg, Maryland, U.S.A.

Baarset, H., & Johansen, J. (2019) - Innovative processes for mortality disposal in aquaculture. Deliverable 2.2. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 773330. 14 pp.

Barentswatch (2020): Sustainability in Aquaculture (Nofima, SINTEF Ocean and BarentsWatch), What impact does Norwegian aquaculture have on the environment, the economy and society? https://www.barentswatch.no/en/ (accessed 07.10.2020)

Bjørndal T., & Tusvik A. 2017: Land based farming of salmon: economic analysis. NTNU Working paper series No. 1/21017. ISSN 2464-3025. https://www.ntnu.no/documents/1265701259/1281473463/WPS+1+2017.pdf/6ee4cd65-e3b0-44a6-aa42-d017cb42d020

Bruckner, C.G., Johansen, J., Ferreira Novio, M., Svenningson, L., Baarset, H., Conceição, L. & Soula, M. (2021): Eco-efficient solutions for reusing aquaculture side stream. Deliverable 2.5. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 77330. 18 pp.

B&F Italia SRL (04.08.2021), Raw Materials For Animal Feed. https://www.bfitalia.net/public/content/Catalogo%20Prodotti%20PETFOOD_1.pdf.

Castrica, M., Menchetti, L., Panseri, S., Cami, M., & Balzaretti, C. M. (2021). When Pet Snacks Look Like Children's Toys! The Potential Role of Pet Snacks in Transmission of Bacterial Zoonotic Pathogens in the Household. *Foodborne Pathogens and Disease*, *18*(1), 56-62.

Cristiano, S., Newton, R., Baarset, H., Regueiro, L., Bruckner, C., Svenningsson, L., Royer, E., & Pastres, R. (2021). Report on the application of LCA. Deliverable 4.4. GAIN – Green Aquaculture Intensification in Europe. EU Horizon 2020 project grant no. 773330. 93 pp.

EC – European Commission (2002). Regulation No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. *Official Journal L 273, 10/10/2002 P. 0001 – 0095.* https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002R1774:EN:HTML.

FAO – United Nations' Food and Agriculture Organisation (1998). Carbohydrates in human nutrition, Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutritional Papers No. 66. Rome.

Fiskeridirektoratet (2020): Settefiskproduksjon av laks, regnbueørret og ørret /

The project has received funding from the European Union's Horizon 2020 Framework Research and Innovation Programme under GA n. 773330

Statistics of loss in landbased Norwegian aquaculture, May 2020 updates. https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Laks-regnbueoerret-og-oerret/Settefiskproduksjon

Kjeldahl, J. (1883) "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances), Zeitschrift für analytische Chemie, 22 (1) : 366-383.

Lovdata (2016), Forskrift om animalske biprodukter som ikke er beregnet på konsum (animaliebiproduktforskriften). https://lovdata.no/dokument/SF/forskrift/2016-09-14-1064

Møretrø T., Hjeltnes B., Lunestad B. T., & Nesse L. L. (2010): Assessment of the Fish Silage Processing Method (FSPM) for treatment of category 2 and 3 material of fish origin. https://vkm.no/download/18.2994e95b15cc545071682a93/1500465602428/360b21640f.pdf

Nemser, S.M., Doran, T., Grabenstein, M., McConnell, T., McGrath, T., Pamboukian, R., et al. (2014). Investigation of Listeria, Salmonella, and toxigenic Escherichia coli in various pet foods. *Foodborne Pathog Dis*, 11:706–709.

Nygaard, H. & Hostmark, O. (2008) Microbial Inactivation during Superheated Steam Drying of Fish Meal, *Drying Technology*, 26:2, 222-230, DOI: 10.1080/07373930701831648.

Soula, M., Newton, R., Ruiz, C., Regueiro, L., Mendez, D., Ferreira, M., Johansen, J., & Little, D. (2019). Report on legislation, regulation, and certification of aquaculture within the circular economy. Deliverable 3.1. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 773330. 73 pp.

Weese J S, Rousseau J, & Arroyo L. (2005) Bacteriological evaluation of commercial canine and feline raw diets. *Can Vet J*,46: 513–516.

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List of tables

List of figures

The project has received funding from the European Union's Horizon 2020 Framework Research and Innovation Programme under GA n. 773330