



EMFF OP 2014-2020

Knowledge Gateway Scheme

2016

Bord Iascaigh Mhara Service Project

Report on BIM Cleaner Fish Programme 2016



2016 CLEANERFISH PROGRAMME NUIG CARNA RESEARCH STATION

FINAL REPORT

BIM put the proposed 2016 BIM cleaner fish programme to tender in late 2015 and NUIG Carna Research Station was awarded the contract to carry out a programme of work to develop techniques to produce a minimum of 200,000 juvenile lumpfish >8g (a size suitable for sea deployment). This work was carried out by a team headed up by Dr Majbritt Bolton-Warberg at the Carna Research Station facilities in Carna, Co. Galway.

This work is funded by the European Maritime and Fisheries Fund 2014-2020 (EMFF).

Overview of Project

The overall goal of the Cleaner Fish Programme 2016 was to develop techniques to produce 200,000 lumpfish (*Cyclopterus lumpus*) larvae from eggs using available best practice methods for delivery to sea for use in an integrated management plan to control lice. The Cleaner Fish Programme 2016 had a start date of April 1st 2016 and all work was undertaken at Carna Research Station (CRS), the National 2of Ireland Galway's base for marine research. Once juvenile lumpfish reach a suitable size (10-15 g+) they are deployed to sea for use as cleaner fish. This report provides an overview of all the work undertaken in 2016 under this project.

Summary of Previous Work

January to April 2016

2015 Cohort grow-out in 2016

In 2015, three distinct groups of lumpsucker were incubated as eggs and reared on site in Carna for the 2015 Cleanerfish Programme, two from Norway (juveniles imported late January 2015 and eggs imported in May 2015) and one from the UK. The Norwegian juveniles and eggs were imported from Flekkefjord, southern Norway. More than 100,000 lumpsucker were reared at CRS in 2015, with approximately 50,000 remaining on site in January 2016. These fish were not of sufficient size to be transferred from the hatchery facility to sea prior to Christmas transfer deadlines. At this time they were 6 months post

hatch, with the approximate time from hatch to sea transfer in lump sucker varying from 5 to 8 months. This highlights the variable growth in lump sucker and the further need to optimise rearing strategies as well as any other husbandry methods that would facilitate faster growth while maintaining high survival rates. From January – April 2016, the remaining juveniles from the 2015 cohort were transferred to sea (see Table 1).

Table 1. Details of 2015 cohort hatchery reared juvenile lump sucker transfers (date, destination, #'s, mean weight) to sea in 2016.

DATE	#	Mean Wt (g)	DATE	#	Mean Wt (g)
14/1/16	2,212	12	29/3/16	2,072	31
	2,730	19		1,285	43
	2,061	22		1,462	41
	2,448	23		1,144	59
	1,776	12		2,270	29
	2,991	13		1,554	44
	1,401	20		1,623	42
	1,806	22	19/4/16	1,333	37
	2,155	21		1,671	39
	1,601	11		2,068	36
18/3/16	1,200	56		2,275	37
	1,023	39		1,092	40
	1,186	39		1,309	36
				1,334	36
				1,177	34
				3,030	17
TOTAL	24,590			26,699	

Broodstock for 2016

In July and August 2014, juvenile lump sucker were captured on seaweed longlines in Ventry Bay, South West Ireland (ranging from 0.09 g – 15 g). These fish were on-reared at Carna Research Station and were maintained as potential native Irish broodstock (see Figure 1 for growth profile of these fish, error bars illustrates variation). The genetic makeup of these fish is unknown, although fin clips have been taken to be included as part of a study on population genetics of lump sucker in the Atlantic Ocean. Some of these fish (2014 cohort, N ~17) became mature during the 2016 spawning season and produced viable eggs. A small volume of eggs were fertilised by mature 2014 cohort males but very few males (~2) became mature during the season, necessitating the use of wild caught mature males (see below for information on spawning).

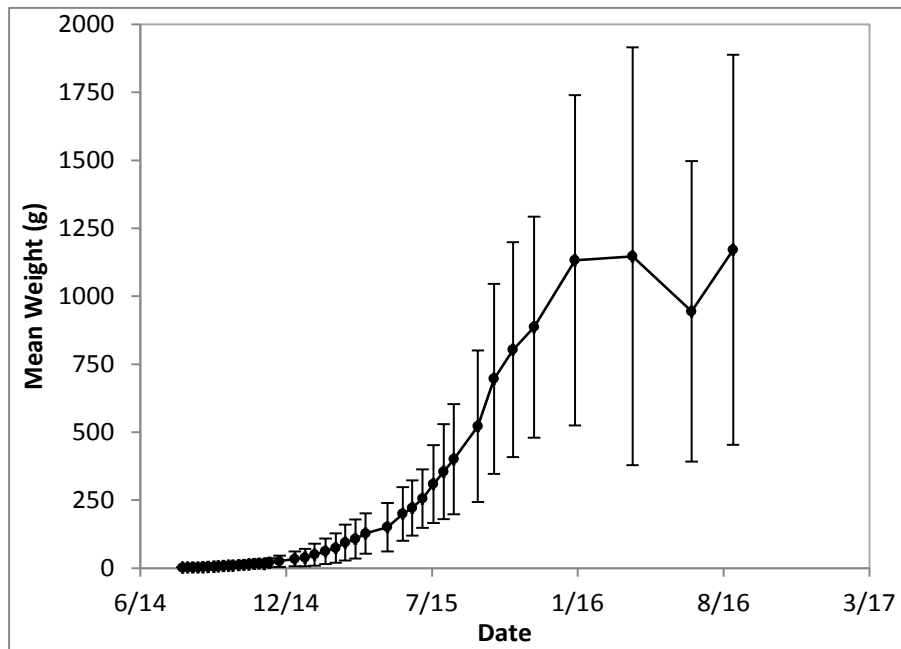


Figure 1. Growth profile (mean weight \pm SD) of 2014 wild collected lump sucker on-reared at Carna Research Station. The dip in growth observed in August 2016 was due to the death of several large fish. The remaining broodstock became infected with a fungus and were culled in October 2016, with the largest fish recorded as 3.2kg.

In December 2015, lump sucker broodstock (N = 53) were imported from the UK. These fish had undergone the mandatory health checks and all paperwork was in order prior to transport. Initially fish grew well and acclimated to their holding tanks; however, by the end of February 2016 it became clear that they had a fungal infection (different to the one isolated from 2014 broodstock which occurred later in the year). All fish were inspected by the designated veterinarian and in consultation it was deemed necessary to cull all remaining fish. A thorough processing of the fish was carried out on March 1st 2016 with samples taken by the Marine Institute for further analysis. Table 2 outlines some of the information taken from these fish during the processing. Fish remains were disposed of using a registered haulier, as is standard practice as per CRS’s Fish Health Management Plan.

Table 2. Information obtained from Swansea lump sucker Broodstock including length, weight, height, organ weights, sex and colour.

#	Length (cm)	Height (cm)	Weight (g)	Total gut (g)	Sex	Liver (g)	Gonad (g)
1	33.3	16.2	1701	251.38	F	64.04	66.19
2	36.6	18.0	2214	392.18	F	105.95	142.22
3	32.0	14.0	1336	239.63	M	50.54	109.94
4	38.5	19.1	2620	315.79	F	83.60	130.23
5	36.7	16.0	1775	604.95	F	130.32	334.43
6	35.2	16.9	1751	324.29	F	88.48	92.80

7	33.5	15.2	1674	335.46	F	88.30	132.98
8	35.5	18.0	2307	504.37	F	91.12	299.72
9	35.9	16.1	1885	338.91	F	101.54	120.37
10	25.9	10.6	715	120.28	M	16.15	62.30
11	36.6	16.0	1763	270.64	F	57.41	86.74
12	34.3	15.2	1576	259.11	F	68.08	91.42
13	35.6	16.9	1866	365.58	F	79.08	175.46
14	35.3	15.8	1769	380.01	F	96.68	179.95
15	30.0	14.2	1081	167.55	M	24.35	73.08
16	30.6	13.0	1004	157.68	M	23.41	76.76
17	30.0	14.0	1136	124.78	F	16.24	30.18
18	34.8	16.8	2048	434.81	F	85.43	243.67
19	35.3	17.2	2102	456.95	F	113.42	207.91
20	27.4	11.1	830	137.44	M	31.83	48.83
21	28.4	13.1	1035	107.30	F	26.86	12.52
22	37.6	18.1	2323	168.93	F	39.57	63.27
23	30.9	14.0	1343	411.05	F	108.12	150.53
24	35.3	15.4	1675	288.66	F	72.96	110.79
25	34.9	17.0	2095	419.77	F	109.61	196.57
26	36.0	18.3	2380	460.08	F	129.72	161.56
27	33.3	14.3	1283	231.83	F	57.11	80.61
28	31.0	12.0	944	144.00	F	24.00	35.00
29	39.0	18.0	2118	400.00	F	103.00	156.00
30	28.0	11.0	652	91.00	M	18.00	34.00
31	29.0	14.5	1077	155.00	M	15.00	65.00
32	30.0	14.0	1275	200.00	F	42.00	48.00
33	29.5	14.0	1093	139.20	F	24.85	49.20
34	35.0	15.5	1639	290.15	F	68.71	128.13
35	31.2	15.5	1476	221.89	M	33.53	78.88
36	37.3	18.5	2210	333.99	F	88.11	125.01
37	32.0	15.3	1443	238.52	F	57.25	68.74
38	36.8	16.8	2067	437.52	F	100.37	238.65
39	32.5	14.0	1277	202.57	M	36.05	93.43
40	28.0	13.0	918	164.43	M	35.78	74.17
41	35.5	18.0	2023	336.53	F	95.76	110.65
42	33.6	14.8	1523	336.08	F	85.02	169.07
43	36.0	16.0	1854	377.08	F	87.42	182.28
44	30.3	12.5	1038	170.91	M	32.78	85.35
MEAN	33.3	15.3	1589	284.28		66.09	118.70
SD	3.3	2.1	501	123.39		34.26	71.17
CV	9.95	13.93	31.53	43.41		51.84	59.96

In addition, two batches of Irish eggs were incubated, on-reared and a selection (N ~100) maintained as a broodstock source from the 2015 cohort (Figure 2). These fish will start to mature in the 2017 spawning season and were an average of 5-600 g at the end of September. The number of these fish has been reduced due to an outbreak of amoebic gill disease (AGD) but mortalities are currently negligible and treatment (freshwater baths) was undertaken to reduce the problem. If AGD had progressed too far, fish did not tolerate the freshwater baths; however, on the whole, the treatments worked well. They did require several treatments over the summer months to keep AGD at bay.

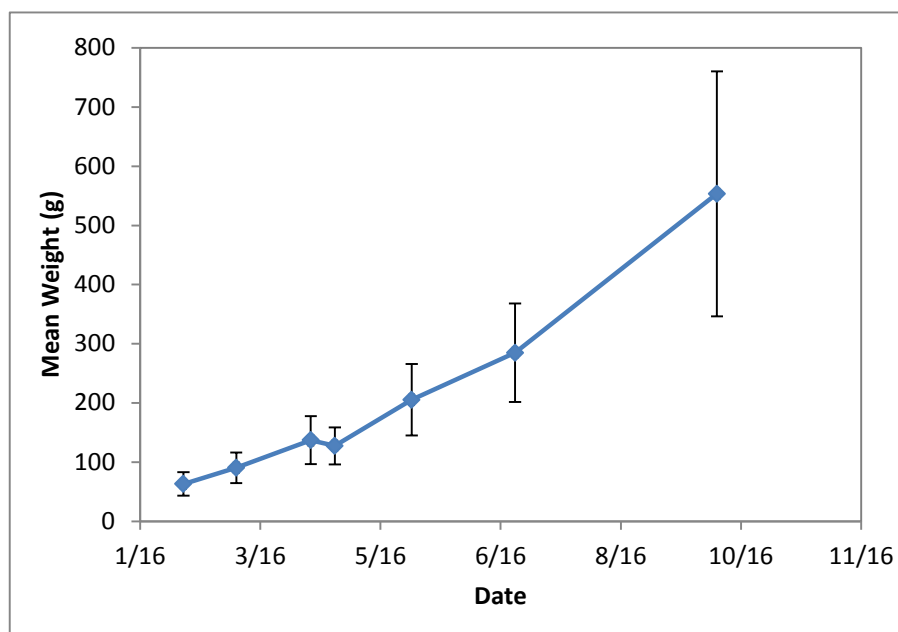


Figure 2. Growth profile (mean weight \pm SD) of 2015 Irish lump sucker broodstock, hatched in Carna Research Station. A proportion of this population will spawn for the first time in 2017.

Systems

All tanks and systems (egg cones, recirculation units, larval/juvenile tanks and on-rearing tanks) were prepared for the upcoming larval run during the first 3 months of 2016. This included thorough cleaning and disinfection of tanks, ensuring oxygen diffusers were operating sufficiently, cleaning and disinfection of standpipes, additional standpipes produced if required etc. A new incubation system (Figure 3) was set up and tested with cones purchased by a salmon producer (12 L Sterner Family Hatcher units). These were set up on a small recirculation system which included a sump (490L capacity) containing actively aerated biomedica, two holding tanks (150 L capacity each), a heater/chiller unit (to maintain

a constant temperature throughout the incubation), a small UV filter and a sock filter (50 µm). This system was topped up with incoming ambient seawater at <10% per day to compensate for evaporative loss of water from the system.



Figure 3. Lump sucker egg incubation system at Carna Research Station with holding tanks (150 L capacity) and sump (490 L capacity) filled with biomedica. System fitted to heater/chiller unit to control temperature (not pictured).

2016 EGG SOURCES

A number of lump sucker egg batches were incubated at Carna Research Station between February and May 2016. These came from a variety of sources (see Table 2 for more detail):

- Wild females crossed with wild males (caught Donegal and Carna)
 - 4 fertilised egg batches (4 different crosses)
 - 1.9 kg total biomass of eggs
 - 20-90% hatch rate (53% average)
- Own broodstock (females and males)
 - 9 fertilised egg batches (unknown number of crosses)
 - 2.2 kg total biomass of eggs
 - 6 batches unfertilised due to immature males (biomass not included here)
 - 24-44% hatch rate (32% average)
- Own females crossed with wild males (Donegal and Carna)

- 2 fertilised egg batches (2 different crosses)
- 0.5 kg total biomass of eggs
- 20-29% hatch rate (25% average)
- Fertilised ova imported from Norway May 12th 2016
- 5 egg masses
- <2-67% hatch rate

Table 2. Overview of lumpsucker egg batches incubated at Carna Research Station in 2016, including spawning date, weight, female and male parental origin, % hatch and comments. Each batch was placed in its own cone. Not all batches were incubated at the same time. Ventry fish were labs own broodstock caught in 2014 and held in Carna. Norwegian eggs were wild caught stock, though eggs were imported to Ireland under licence.

Batch	Spawning date	Weight (kg)	Female Parent	Male Parent	% hatch	Comments
1	23/02/2016	0.499	Donegal	Donegal	19.48	
2	03/03/2016	0.411	Donegal	Carna (1)	30.17	
3	07/03/2016	0.329	Ventry	Ventry	0.00	Unfertilised
4	08/03/2016	0.413	Ventry	Ventry	0.00	Unfertilised
5	16/03/2016	0.369	Ventry	Ventry	28.95	
6	21/03/2016	0.311	Ventry	Donegal	20.57	
7	31/03/2016	0.239	Ventry	Ventry	44.25	
8	05/04/2016	0.185	Ventry	Carna (1)	28.60	
9	15/04/2016	0.128	Ventry	Ventry	0.00	Unfertilised
10	20/04/2016	0.143	Ventry	Ventry	0.00	Unfertilised
11	22/04/2016	0.241	Ventry	Ventry	0.00	Unfertilised
12	24/04/2016	0.221	Ventry	Ventry	0.00	Unfertilised
13	08/05/2016	0.000	Ventry	Donegal	0.00	Unfertilised

14	23/04/2016	0.546	Donegal	Donegal	70.72	
15	25/04/2016	0.497	Donegal	Donegal	92.40	
16*	22/04/2016	1.185	Norway	Norway	48.38**	Very poor batch
17*	22/04/2016	1.130	Norway	Norway		Very poor batch
18	22/04/2016	0.970	Norway	Norway		
19	22/04/2016	0.990	Norway	Norway		
20	22/04/2016	1.195	Norway	Norway	67.94	
21	26/04/2016	0.160	Ventry	Ventry	24.08	Final egg mass

*very poor hatch rate (<2%) in batches 16 and 17 from Norway (see report below)

**this value is for batches 16-19 that hatched at the same time into the same holding tank, therefore not possible to distinguish hatch rate in each batch

All the eggs were disinfected prior to transfer to egg incubation systems whether being imported or moved from spawning tank to egg system. Pyceze is used as per manufacturer's instructions on each batch for 30 minutes. Egg counts and microscopy are also undertaken as standard practise to check the quality of eggs. Due to the inherent sticky nature of lump sucker eggs it is not possible to remove dead or unfertilised eggs from the batch for the duration of incubation. Microscopic checks are carried out to determine stage of development and to corroborate visual inspection on arrival. Dead and unfertilised eggs were not counted. It is also difficult to determine what was never fertilised compared to an egg that was fertilised and subsequently died. It was deemed inappropriate to tear apart the egg mass to look further inside the mass or to take further samples as this would damage eggs.

LUMPSUCKER EGG IMPORT FROM NORWAY MAY 2016

Fertilised lump sucker eggs (5L total) were transported from Norway on May 12th 2016. Two boxes of chilled eggs were collected and transported to Carna Research Station.

Upon arrival, the temperature in both boxes was measured (7.8°C). All batches of eggs were weighed and disinfected prior to stocking into the incubation system. A total of 5 batches were received (Batches 16 -20, 5.4 kg total). Notes were made on the condition of each batch and photographs are included for ease of reference. Batches 16 & 17 came in one box, while Batches C – E came in the second box.

Batch 16 (Figures 4 a-c)

- Large amount of mucous when handle
- Many dead eggs (confirmed via microscopy)

- When disinfected, a large amount of oil appeared on surface of water (from yolk sacs) and water became cloudy
- Eggs were 'soft' and broke apart easily when handled
- Some eyed ova, which appeared normal



Figures 4 a - c. Lumpsucker egg batch 16 showing many discoloured dead eggs (a), microscopic view of undeveloped eggs (b) and normally developing eyed ova (c).

Batch 17 (Figures 5 a & b)

- No mucous during handling
- Many dead eggs but less than Batch A, more eyed ova evident
- When disinfected a large amount of oil appeared on surface of water (from yolk sacs) and water became cloudy
- Eggs 'soft' and broke apart easily
- Noticed more 'debris' on eggs compared to other batches

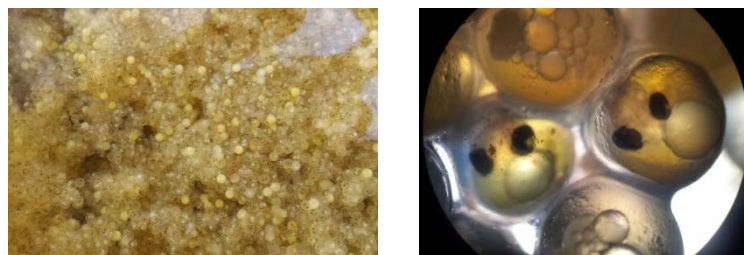


Figure 5 a and b. Lumpsucker egg batch 17 showing discoloured dead eggs (a) and microscopic view of both undeveloped and normally developing eyed ova (b).

Batches 18 – 20 (Figures 6 a - c)

- All batches of better quality than A & B with more eyed ova and less dead eggs
- Better 'feel' to the eggs, less mucous
- Water did not turn cloudy during disinfection and no oil droplets on surface
- Easy to see larval blood pumping and movement

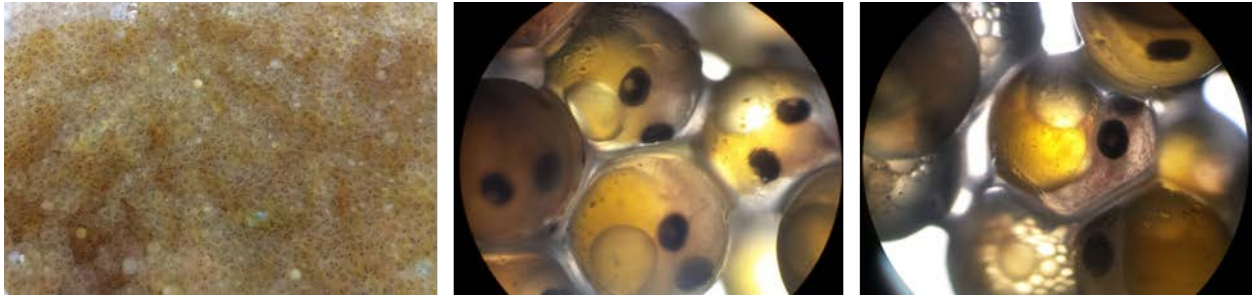


Figure 6 a - c. General photos for lumpsucker egg batches 18, 19 and 20 showing normally developed eggs with few dead discoloured ova (a) and microscopic views of both undeveloped and normally developing eyed ova (b & c).

Table 3 provides summary data on all Norwegian lumpsucker egg batches at Carna Research Station in May 2016. The two poor egg batches had the lowest hatch rate.

Table 3. Summary information on all lumpsucker egg batches from Norway, including total weight and hatch rate.

Batch	16	17	18	19	20
Total Weight (g)	1185	1130	970	990	1195
% Hatch	<2	<2	49	48	67

Production, Survival and Growth

Growth and survival are being monitored regularly to ensure fish are in good condition and feed utilisation is maximised. More than 250,000 juveniles were produced from the different sources outlined above. The majority of fish came from the Norwegian imported eggs (~180 - 200,000). From hatch to present there is >90% survival in juveniles. There were minor problems with tail nipping but this was overcome very early on through increased hand feeding, changes in photoperiod and ultimately the installation of belt feeders on each tank.

Growth for the older batches was in line with that seen in 2015 but the Norwegian fish have been slower growing (more uniform in size) due to increased stocking densities (see Figure 7 below). However, in 2015, it was noted that >50% of the Norwegian population was still <8 g (3-5 g) at ~7 months post hatch, while 5% of the UK population was <8 g (6 - 8 g) at ~8 months. This too reflects differences in stocking density and also rearing temperature

between these two stocks in 2015 (different hatch dates). The smallest fish (Norwegian stock) are currently at 6 months old, having hatched at the end of May.

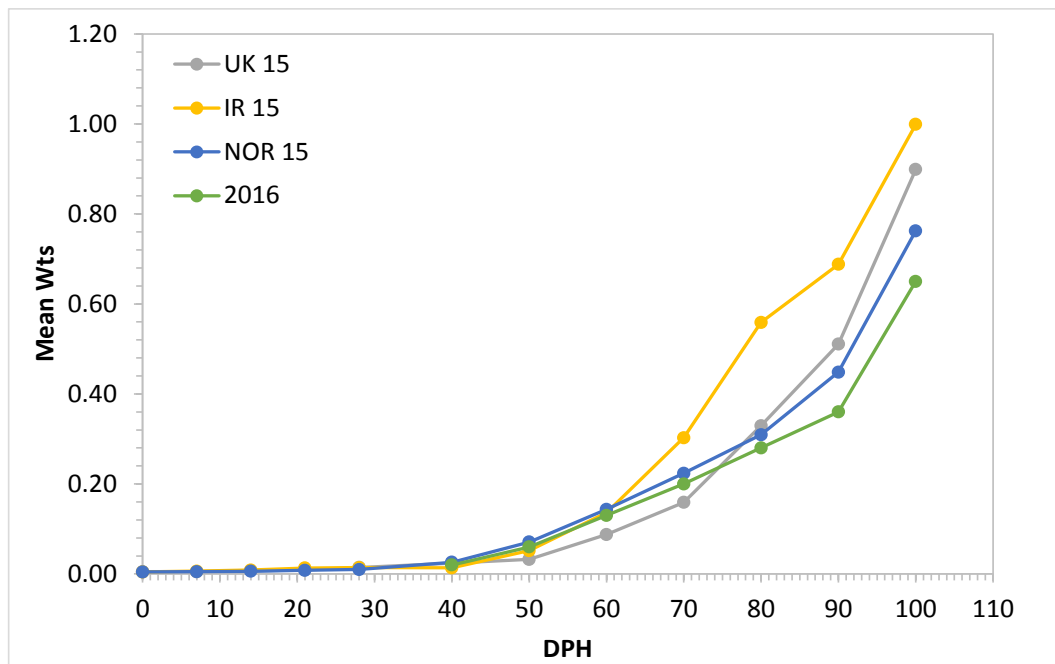


Figure 7. Growth profiles for 4 different lump sucker populations from hatch to 100 days post hatch (DPH). The green line denoted 2016 refers to the Norwegian stock. These fish were more highly stocked than all other cohorts graphed, particularly compared to the yellow line (Irish 2015 cohort) which had relatively few fish per tank. (Norwegian 2015: 20 fish per L, Irish 2015: 9 fish per L, UK 2015: 9 fish per L, Norwegian 2016: 37 fish per L)

A temperature controlled recirculation system (see above) was used during egg incubation and thus the temperatures experienced by the various egg batches were set during this period and were maintained at approximately 9°C. However, it is clear in Figure 8 that batches 16-20 experienced lower incubation temperatures than all of the other batches. This is due to the fact that these eggs (Norwegian in origin) were incubated for approximately 3 weeks at 7°C in Norway prior to transfer to Carna. This could have contributed to their reduced growth rate along with high stocking densities. The higher stocking densities (see Table 4) are the biggest contributors to the reduced growth in 2016.

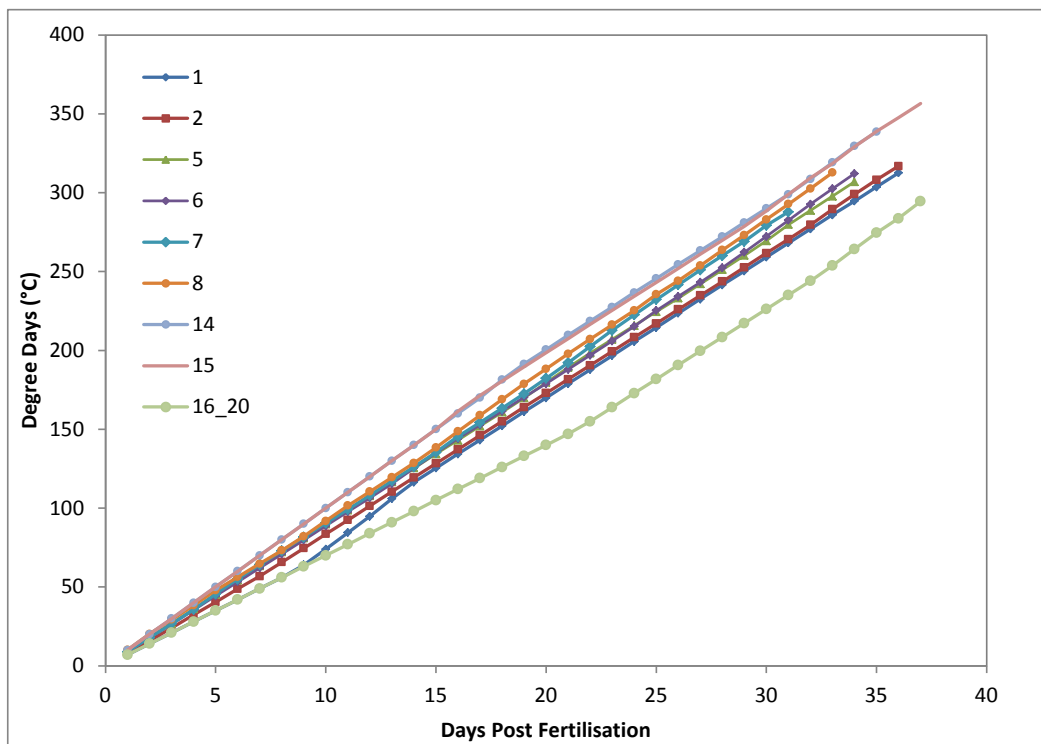


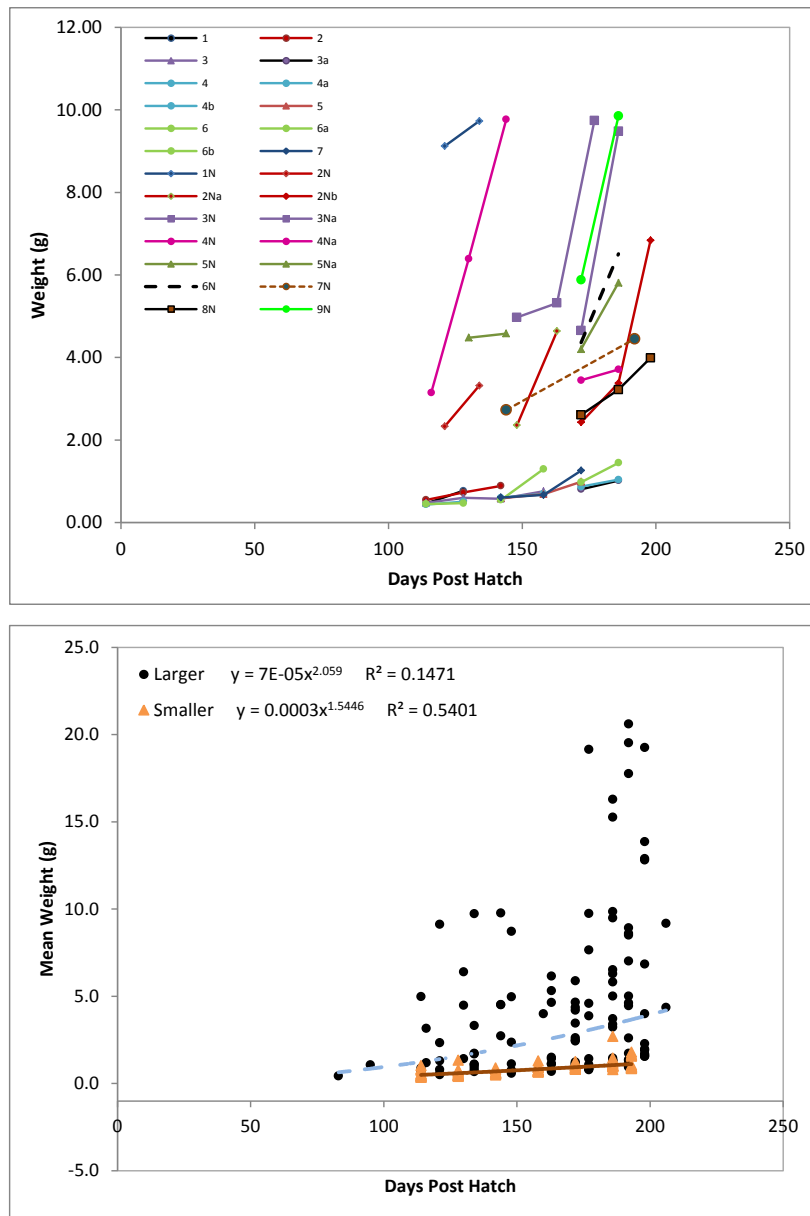
Figure 8. Degree days from spawning until Day 0 (hatch) for all lump sucker egg batches incubated at Carna Research Station in 2016

Table 4. Stocking densities, origin and hatch date of newly hatched lumpstickers in first rearing tanks at Carna Research Station in 2016. First rearing tanks are 440 L capacity.

Hatch Date	Origin	Stocking Density (fish/L)
29/3	Irish	22
7/4	Irish	28
18/4	Irish	24
24/4	Irish	15
30/4	Irish	24
7/5	Irish	12
27-28/5	Irish and Norwegian	15-53
1/6	Irish	9

Due to their fast growth rates and the rapid development of size hierarchies within a population, lumpstickers must be graded relatively regularly (e.g. every 2 - 3 weeks depending on size). This helps to reduce aggressive encounters and serves to split biomasses across tanks.

Due to the frequency of grading it is difficult to track a particular group of fish through from hatch to sea transfer. Figures 10 a and b illustrate the variability in size at age for a population of lumpstickers. It is clear from both graphs that there is a smaller grade that persists and these fish will take significantly longer than their siblings to reach transfer size.



Figures 10 a and b. Mean weight versus days post hatch for all tanks of lump sucker reared in 2016. Top graph shows information by tank, where possible to track a tank over several sampling periods (ie prior to grading). The bottom graph shows the mean weights for individual tanks (small grades indicated by orange triangles).

Finally, if we take the mean weights for tanks that are deemed as 'large' versus 'small' grades, we see the growth curves observed in Figure 11. The variation in sizes is evident from the error bars associated with the 'large' grade of fish from approximately 120 days post hatch. The error associated with the smaller grade is much reduced because these fish are constantly being graded out of the tanks and thus the numbers in these tanks are steadily being reduced. Lumpsucker grow relatively steadily and uniformly (given adequate feed and space and an optimum temperature) until they reach approximately 1 - 3 g, at which point, growth rate increases and we start to see larger and larger variations in sizes (hence the frequent grading required).

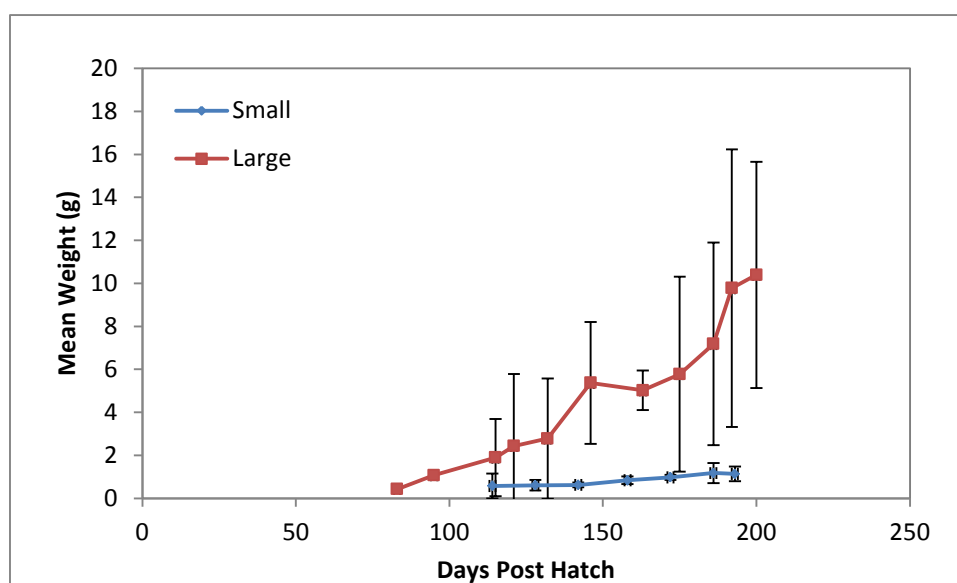


Figure 11. Growth (mean weight \pm SD) profiles of all tanks combined (with tanks designated as either 'large' (= faster growing fish) or 'small' (= slower growing fish)) for lumpsucker reared at Carna Research Station in 2016

Vaccination and Sea Transfer

Prior to sea transfer, fish are dip vaccinated twice at CRS and for their first dip fish must be a minimum of 1 g. In addition, there should be 300° days (time dependent on temperature: 30 days @10° C) between the second dip and sea transfer. The vaccine is used as follows: 1 L vaccine plus 9 L sea water to make a 10 L bath. This will vaccinate 10,000 fish @1 g or 50 kg of bigger fish. Thus, there must be a minimum of 10,000 1 g fish to ensure that a bath of

at least 10 L is used, anything less is too little water and will cause undue stress. The bath must be kept constantly oxygenated.

To date >38,000 fish have been transferred to sea (Table 4), with a further ~12,000 to be transferred on 20th December. As of December 2016 there were approximately 200,000 fish on site, equivalent to 376 kg of biomass. The following table (Table 5 – all fish accounted for including those transferred or awaiting transfer) outlines the current numbers, sizes and vaccination status of the stock being reared in Carna. Times for vaccination and sea transfer are approximate. As fish are moved to sea and stocks graded and redistributed, growth will improve (depending on temperature). Sea transfer may be to ongrowing cages (>5 g average), smolt nets (>10 g average) or broodstock cages (>20 - 25 g average).

Table 4. Details of fish transfer in November and December, including date, numbers and mean weight.

Date	#	Wt.(g)
2/11/16	240	7
9/11/16	1209	19
	2578	21
	1344	25
	3867	9
	4845	6
	3849	9
	3918	10
9/12/16	2550	18
14/12/16	13994	7
TOTAL	38394	

Table 5. Overview of current lump sucker stocks being held at Carna Research Station

# fish	Average Size	1 st dip	2 nd dip (+ 3 weeks)	Sea Transfer
5,131	>20g	Yes	Yes	9 th Nov
16,479	>5g	Yes	Yes	9 th Nov
2,550	>15g	Yes	Yes	9 th Dec
13,994	>5g	Yes	Yes	14 th Dec
11,865	10g	Yes	Yes	20 th Dec
50,019				

35,000	2g+	Yes	Jan
35,511	1g+	Yes	Jan-Feb
131,000	1g		Feb-Apr
201,511			

Lessons Learned in 2016

Growth was inhibited in the youngest fish due to the high stocking densities, despite improved feeding strategies. Future strategies will focus on the use of additional surface area and 'skimming' larger fish out of tanks at smaller sizes to reduce density dependent effects on growth. This will also facilitate vaccination but this requires a minimum of 10,000 fish to be carried out effectively (see above). Ideally, regular 'skimming' of tanks every few weeks should provide a more consistent and staggered production (potentially smaller numbers in each batch), rather than having larger numbers of fish ready but with longer periods of time between each batch. It was not possible to alleviate the stocking densities by simply splitting fish into additional tanks as no additional tanks were available. A study looking at the effects of stocking density on growth over a 6 week period revealed little difference in growth over a 10 fold difference in stocking density. These data are still being analysed and thus are not presented here. This highlights that for short term periods, high stocking density is tolerated but clearly (based on our production tanks), prolonged periods at high densities does effect growth.

Improvements over 2015

A greater hatch rate was achieved in 2016 (ranging from 20-92%, based on 100,000 eggs per kg) compared to 2015 (~20% estimated but no counts done). This was highly dependent on egg source with eggs from the 2014 Carna broodstock females having the lowest hatch rate (20-44%). These fish were not on a conditioning diet or photoperiod and were first-time spawners. The highest hatch rate was observed in two batches from Donegal wild broodstock with 70 and 92% hatch rate estimated. The good batches received from Norway (18 -20) had hatch rates of 48 – 68% and this is thought to have been affected by the delay in transport. Clearly, native, wild broodstock yielded the best quality and survival of eggs in 2016. Survival of juveniles was also greater in 2016 and tail nipping, which was a major problem in 2015, was almost non-existent. The installation of belt feeders (Figure 12) improved rearing and enabled greater use of time for optimising other husbandry procedures (e.g. grading, cleaning). A more rigid feeding schedule was employed in 2016, with strict weaning periods and well established times for moving onto larger feed. Recently, a new feed size/type has been released by Otohime, called S1. This diet is 1 mm average and readily replaces the C1 diet (840 – 1400 µm). Switching from C1 to S1 has

made a significant difference to the cleanliness of the tanks, with C1 (more like a crumb than a pellet) decomposing more rapidly (either as waste faeces or excess feed) than S1.



Figure 12. Belt feeders installed over larval/juvenile lump sucker tanks in Lab 2 at Carna Research Station.

Another improvement is the ability to take out fish >5 g (but <10 g) for on-growing. This is invaluable as it creates space in the hatchery and nursery areas and facilitates grading and splitting of tanks to reduce biomass, which will increase growth.