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Knowledge Gateway Scheme

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Bord Iascaigh Mhara Service Project

To Secure a Sufficiently Large Irish *C.gigas* Tetraploid Broodstock Pool for Selective Breeding Programmes for Irish Oyster Hatcheries

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To Secure a Sufficiently Large Irish *C.gigas* Tetraploid Broodstock Pool for Selective Breeding Programmes for Irish Oyster Hatcheries Cartron Point Shellfish Ltd

Final Report

BIM put the proposed 2016 BIM tetraploid programme to tender in late 2015 and Cartron Point Shellfish Ltd was awarded the contract to carry out a programme of work to build on securing a sufficiently large tetraploid broodstock pool for selective breeding programmes for Irish oyster hatcheries. The work was carried out by Iarfhlaith Connellan at Cartron Point Shellfish, New Quay, Co. Galway.

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

Overview of Project

Since 2011 the State, through funding from BIM and the Marine Institute, has supported a programme to develop founding populations of tetraploid oysters, produced from certified disease free stocks in a hatchery situated in a disease free area. These first generations of tetraploids were successfully crossed with Irish diploid *C.gigas* broodstock at three different hatcheries in September 2015 to produce the first Irish natural triploid spat. In order to build on this programme for the benefit of the whole Irish *C.gigas* sector it is necessary to increase the number of tetraploid stock available to the Irish hatcheries to many thousands. This will facilitate the development of broodstock selection programmes in Irish hatcheries based on growth performance of their own triploid progeny.

The work of the programme has concentrated on 4 objectives that had been previously identified as:

- Provision of tetraploid gametes to Irish hatcheries.
- Increasing the stock of putative tetraploid oysters.
- Maximizing the genetic input of RB1 triploid stock to the pool of tetraploid oysters.
- Provision of second generation tetraploids

Provision of tetraploid gametes to Irish hatcheries

The provision of sufficient tetraploid gametes to enable the natural production of triploid oysters in each Irish hatchery has been the most pressing requirement of the programme. The method employed consisted of providing each hatchery with 4 to 5 ripe male tetraploid oysters with sufficient gametes to fertilize 400 – 700 million diploid eggs for each production run. This primary aim has been achieved in both Spring and Autumn spawning seasons of 2016.

Spring Spawning

Stocks introduced in February were stripped in March and provided sufficient gametes to stock three Irish hatcheries with an initial batch of tetraploid oysters. Lissadell Hatchery received the tetraploid gametes on the 17th of March and Tralee Bay Hatchery was supplied from a second batch of tetraploids stripped on the 29th of March. Both of these hatcheries successfully produced batches of triploid oysters. These oysters were reared in both upwelling and downwelling nursery holding systems and have been put to sea inter-tidally in bags on trestles or in nursery trays.

Stocks of triploid *Crassostrea gigas* spawned and fertilized using tetraploid males at Cartron Point Shellfish Hatchery were contemporaneously reared in an early upwelling nursery system and subsequently placed in an on-site FLUPSY. Triploid oysters from this group, now growing in Galway Bay have exhibited growth rates far exceeding that of similar sized diploid oyster seed deployed during the same period i.e. June to September. Triploid oysters grew from a mean weight of 0.015g to a mean of 12g (Figure 1). Further growth trials in Aughinish Bay of triploid oysters from this and subsequent production runs are at present being undertaken.

Autumn Spawning

At the beginning of September 2016 tetraploid oysters were again sampled from both the site at Eannish in Aughinish Bay and from those retained tetraploids from earlier conditioning experiments and those conditioned in the FLUPSY system.

The oysters conditioning naturally in oyster bags on trestles at Eannish exhibited the highest gamete fecundity as well as presenting the most advanced gonadal development stages. The gametes harvested from these oysters were used to produce batches of triploid oysters by crossing with RB1 diploids[1]. These oysters, fertilized in 3 Irish hatcheries and from which commercial batches of triploid oysters were produced have gone to sea as spat in Sligo, Galway, Clare and Kerry.

[1] RB1 refers to a closed population of oyster broodstock derived from importation of *C. gigas* from British Columbia in the 1970s by MAAF in Conway. As such, this brood stock is distinctly of different genetic origin than most other *C. gigas* used on commercial farms in Ireland.

Figure 1. Triploid oyster seed reared in a swinging oyster bag on a site in Inner Galway Bay

Increasing the stock of putative tetraploids

Rationale

To ensure an adequate supply of tetraploid oysters for future commercial production runs and to maintain as broad a genetic spectrum as possible from the founder population of oysters (RB1), it is necessary to create a new batch of tetraploid oysters by the suppression of the extrusion of the 1st polar body on a continuing basis.

Method

On 1st September 2016 batches of triploid oyster growing in Aughinish Bay were brought into the hatchery where they were opened and graded by gender using an optical microscope.

Only the female oysters were retained and from these a further selection after microscopic examination was undertaken to establish the fecundity level and to eliminate all oysters showing presence of both sets of gametes. All selected oysters were then subjected to a flow cytometry ploidy assessment.

Strip spawning of the most fecund was then undertaken and the gametes collected in individual polyethylene containers.

Male diploid gametes from 5 oysters were used for individual successive fertilizations of the triploid eggs.

Results

Table 1 below outlines the number of triploid eggs per batch and the treatment applied during fertilization.

Table 1. Summary of Tetraploid induction of 25 million triploid eggs

Batch No.	# Triploid eggs	1st PB (minutes)	Cessation of CB treatment (minutes)
1	870,000	15	15
2	200,000	20	23
3	3,800,000	18	32
4	2,000,000	16	28
5	11,000,000	21	26
6	1,500,000	19	34
7	4,000,000	20	38
8	2,000,000	16	35

Larval Rearing

As was expected there was a very high attrition rate throughout larval development (see Table 2 below).

Table 2. Summary of development of 25 million induced tetraploid larvae

Day	Treatment	Counts
Day 1	gentle aeration	Trochophore larvae
Day 2	water change	D larvae Feeding : T.isochrysis
Day 3	water change	2 x 10^6 Veliger larvae 90µm
Day 5	water change	1.8 x 10^6 Veliger larvae
Day 11	water change	1.0 x 10^6 Veliger larvae 6% at 280 µm
Day 16	water change	0.6 x 10^6 Veliger larvae range 90--280 µm
Day 21	water change	0.3 x 10^6 Veliger with 20% pediveligers larvae
Day 21	water change	0.2 x 10^6 pediveligers into settlement trays
Day 35	water change	0.0025 x 10^6 spat at 800 µm

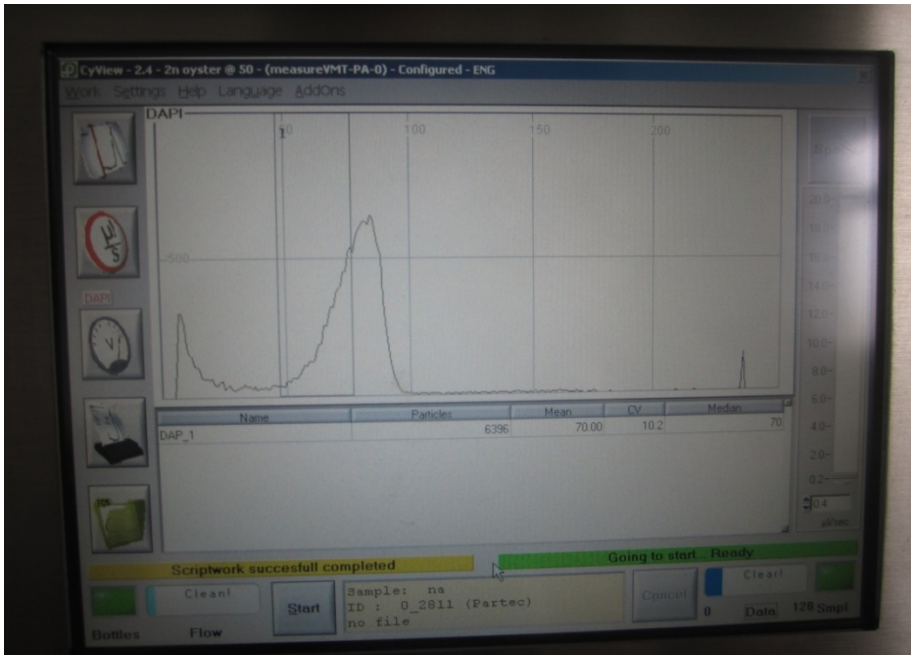

Figure 2: Flow cytometry graph of 24 hour putative tetraploid larvae (trochophores)

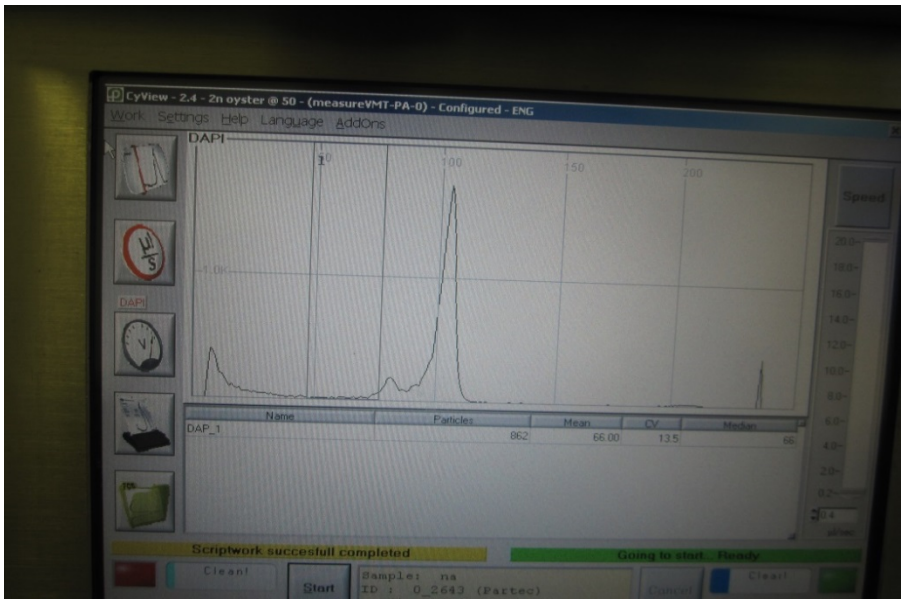

Figure 3: Flow cytometer graph of putative Tetraploid larvae of *Crassostrea gigas* day20

The provision of second generation tetraploids

Methods

Tetraploid oysters were collected from Aughinish Bay (See below). From this cohort the motile gametes of suitable male oysters were used to create commercial batches of triploid oysters. The oysters without gametes and those containing both sets of gametes were discarded as well as any oyster regardless of developmental stage of gonads exhibiting polyploidy on examination with a flow cytometer.

Table 3. Tetraploid oysters collected from Aughinish

Tetraploid Batch	Number	Date
4N (Eannish M)	32	31/8/16

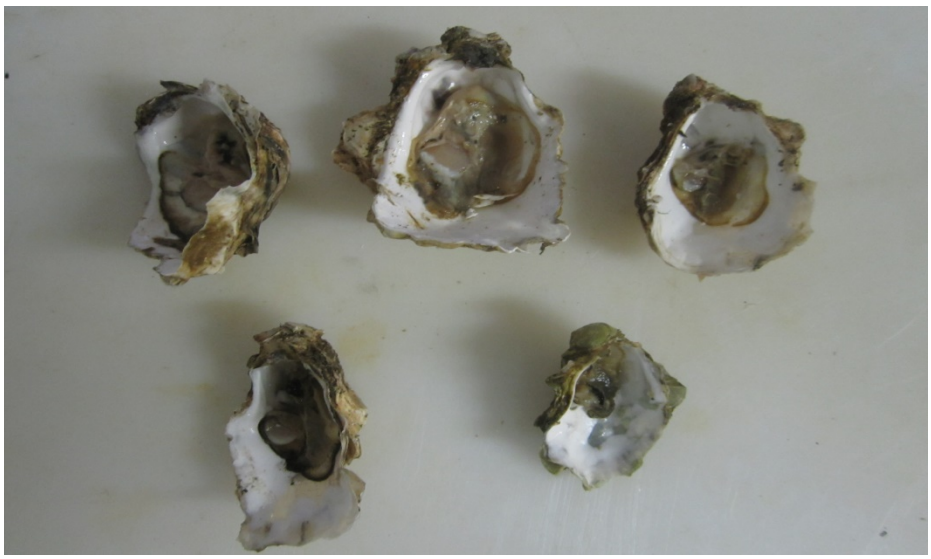

Figure 4. Tetraploid male *Crassostrea gigas* (tetraploid tetraploid crosses 2016)

Four male and four female suitable tetraploid oysters were isolated and strip spawned into individual polyethylene 2 litre containers.

The eggs were mixed and divided into 4 aliquots. Fertilization of each egg aliquot was achieved using sperm from one individual male tetraploid oyster. This method ensures that a maximum number of individual oysters contribute to the gene pool and obviates as far as possible the dangers of inbreeding. After fertilization had been ascertained the developing embryos were pooled and placed in larval rearing bins.

A total of 24 million fertilized eggs were placed in larval bins on day one. On day 7 the remaining veligers were reared in a single larval bin and the total number of survivors had reduced to approximately 0.8million.

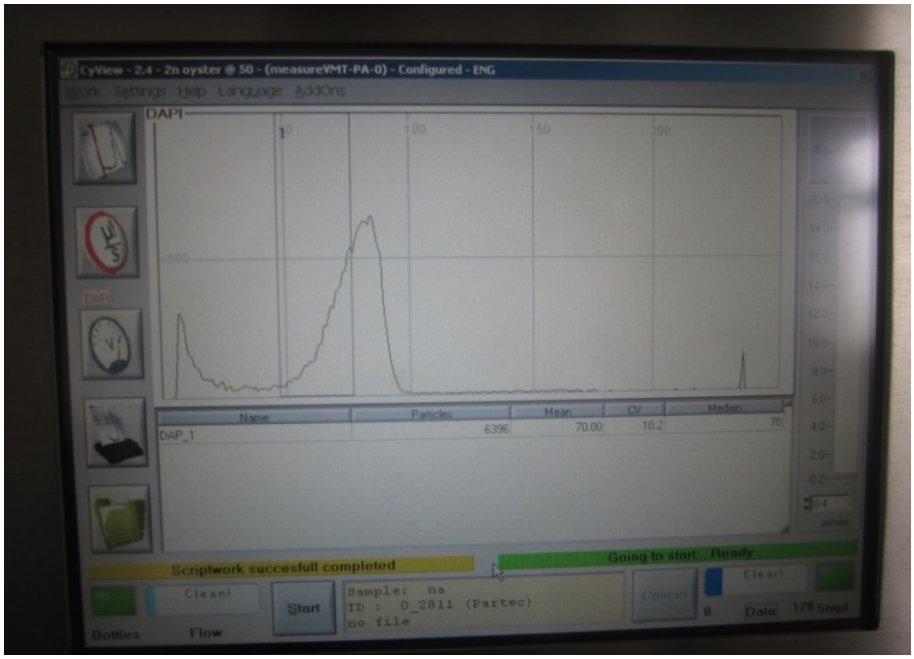

Figure 5. Flow cytometer graph of tretraploid larvae from tetraploid tetraploid cross 2016

Larval rearing

The tetraploid – tetraploid crosses were reared initially in a 5,000l larval bin and water changes were performed at intervals of 24 hours initially and subsequently at intervals of 48 hours. Throughout the larval rearing period microalgae *T.isochrysis* and *Chaetoceros muelleri* were fed from a mixture of continuous and batch cultured microalgae. Algal density was maintained at approx. 50 cells per ml in the culture bins and feeding was effected 2/3 times per day.

Table3. Summary of development of larvae from tetraploid tetraploid cross

Day	Treatment	Count
Day 4	Water change	10 x 10^6 D larvae 40 µm
Day 11	Water change	1.2 x 10^6 Veliger larvae (18% 250 µm)
Day 14	Water change	0.8 x 10^6 Veliger larvae
Day 15	Water change	0.8 x 10^6 Veliger larvae (300 µm see Fig 6)
Day 18	Water change	0.7 x 10^6 Veliger larvae
Day 21	Water change	0.6 x 10^6 Veliger larvae (90% settled spat)
Day 30	Water change	0.004 x 10^6 settled spat of putative tetraploids)

From day 17 additional microalgae (*Tetraselmis suecica*) was added to the larvae in culture as the veligers approached metamorphosis

Figure 6. Day 15 Pediveliger of tetraploid C.gigas (300µm)

Settlement

Pediveliger activity was observed on day 18 of the larval rearing period and settlement trays of 170µm sieve size with ground and graded sterilized oyster shell (350µm sieve size) was presented to pediveliger larvae. Metamorphosis and attachment was observed from day 20 onwards.

Figure 7. Settlement trays (120µm mesh) with ground oyster shell cultch and pediveliger

Attachment to shell particle was observed in 68 % of early metamorphosed larvae however smaller larvae were seen to go through metamorphosis without attachment to shell

particles. Inevitably some larvae attached to the tray meshes as well as the PVC side walls. These settlers were removed on a daily basis using a razor blade or by brushing while immersed in seawater.

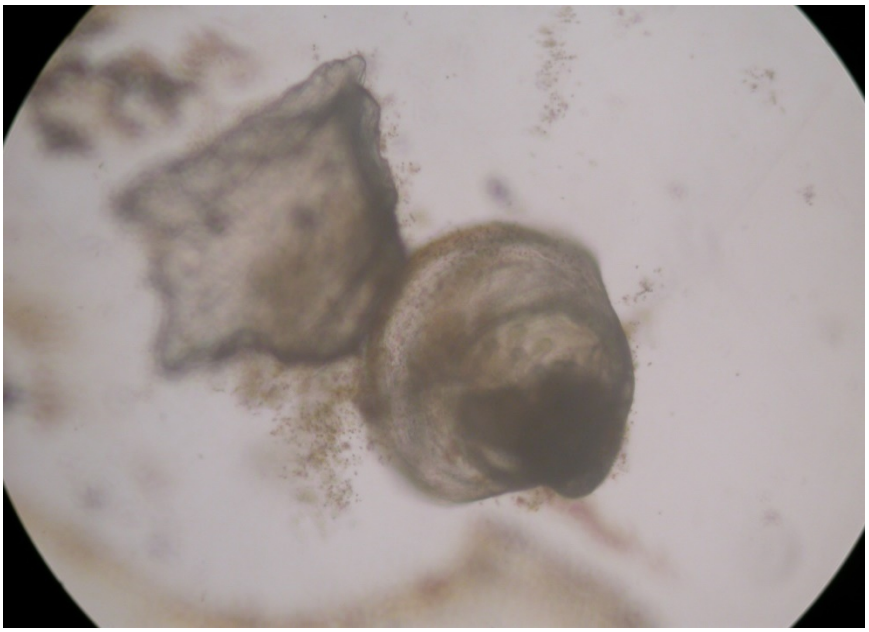

Figure 8. Metamorphosed tetraploid spat of C.gigas(350µm) attached to shell particle

Figure 9. Settlement bin showing airlift pump and microalgal mixed ration

Spat were reared on a mixed diet of *T.isochrysis, Skeletonema costatum and Chaetoceros muelleri.* Initially, microalgae cultivated in batch culture cylinders was used. Subsequent feeding of spat in excess of 1mm shell length was accomplished using microalgae from a continuous culture polyethelene bag chemostat system. Additional *Skeletonema* from outdoor mass cultures was used in feeding when spat had reached approx. 2mm shell length.

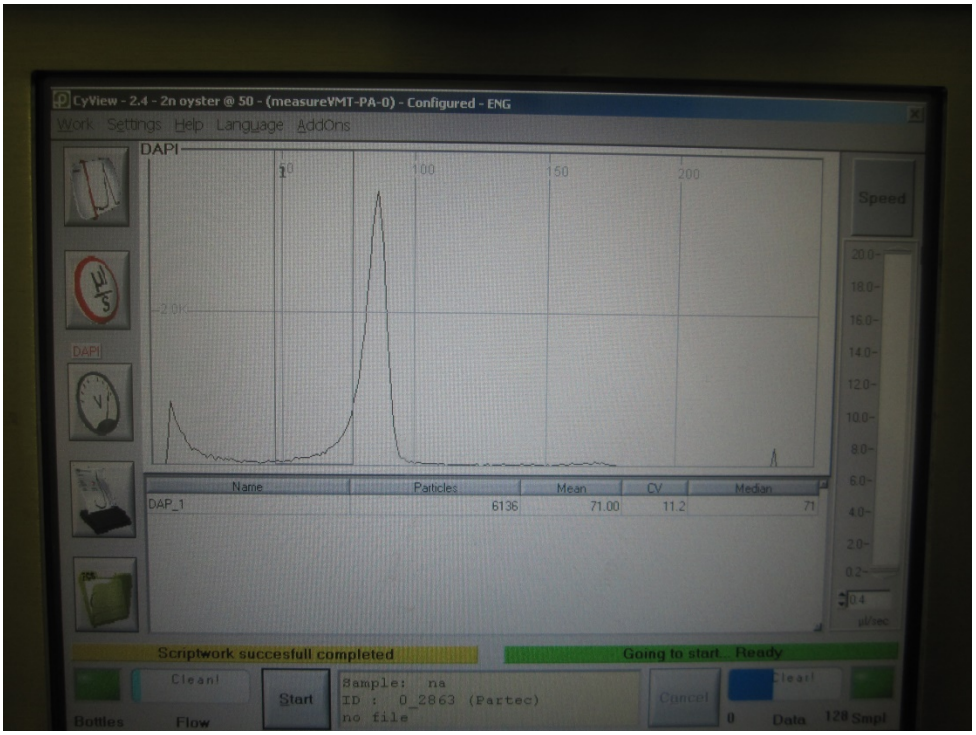

Figure 10: Flow cytometer graph of Tetraploid larvae day 70 (tetra x tetra crosses)

Conclusions

The aims of the programme have all been addressed and despite continuing difficulties in the development of a successful out of season conditioning regime for adult tetraploid *Crassostrea gigas* it has been possible to supply all Irish Hatcheries with tetraploid gametes both in Spring and in Autumn. The successful induction of a new stock of tetraploid oysters now brings the stock of putative tetraploids to several hundred. The primary aim of the project and one that remains an objective for the future is the building up of a diverse viable reservoir of tetraploid *Crassostrea gigas.* A secondary aim of the project, to incorporate a greater genetic input of the RB1 stock into the tetraploid gametes available for distribution has begun with the present year class of induced tetraploids. The cross breeding of tetraploid oysters begun in 2016 will further enhance this aim.