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Thematic Priority

D6. Report on relative performance of triploid and diploid *Mytilus edulis* and *Mytilus galloprovincialis* larvae and spat

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Draft **1.**

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Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Report on relative performance of triploid and diploid *Mytilus edulis* and *Mytilus galloprovincialis* larvae and spat.

Summary

- All four R&D institutes conducted trials to produce triploid mussels and compare their larval performance with diploid controls (*Mytilus edulis*: UWB, IFREMER, IMARES and *Mytilus galloprovincialis*: CIMA).
 - Out of eleven trials, seven concluded that triploid mussel larvae grew significantly faster than diploids although most of these trials were compromised by either lack of replication or uncontrolled density.
 - In no trials did diploid larval growth performance exceed that of triploids.
 - It is safe to conclude that triploid mussel larval growth performance is similar to or better than that of diploid larvae during culture.
 - Often the survival of triploid larvae during culture was significantly lower than survival of diploid controls, but this was not always the case. Good trials demonstrate that this need not be a barrier to production of ready-to-settle triploid larvae
- Four comparative trials of diploid and triploid spat growth were conducted, each using a different on-growing system.
 - In one trial, diploid spat appeared to grow faster than triploid spat in a down-welling system although this could not be demonstrated statistically.
 - In one trial, one particular cohort of triploids grew significantly faster than all other spat but other triploid cohorts in the same trial were not significantly different from diploid controls.
 - It is safe to conclude that growth performance of triploid spat/seed is probably similar to that of diploid spat/seed.
- Consumer testing of cooked triploid and diploid mussels suggests that colour and texture may differ slightly between the two, but that odour and taste are similar.
 - It is safe to conclude that triploid mussels would not have a market disadvantage due to consumer acceptability.

Part I Larvae

Larval rearing trials were carried out on *M. edulis* at UWB, IFREMER, IMARES and on *M. galloprovincialis* at CIMA.

UWB

Trial UWB-1

Triploid *M. edulis* larvae were produced using the chemical 6-DMAP at two different concentrations (300 μ M and 600 μ M) to block second polar body formation during meiosis II (details in WP3 report). Triploid (300 μ M and 600 μ M) and diploid (control) veliger larvae were initially held for 3 days in 8-litre vessels at a concentration of approximately 45 larvae ml⁻¹.

The comparative growth trial was begun at days 6 post-fertilisation and larvae were reared in triplicated 2-litre plastic jars of filtered seawater at 10 larvae ml⁻¹. All larvae were fed a diet of *Pavlova lutheri* and *Rhinomonas reticulata* at a 4:1 ratio equal to 25,000 cell ml⁻¹ day⁻¹. Three times a week the larvae were sieved, inspected, measured, the containers cleaned and the seawater replaced. The survival of larvae was estimated weekly from counts of a fixed volume. Digital image sampling of the larvae was conducted using a Nikon Coolpix 4500 camera mounted on a compound microscope. From the images the shell lengths (µm) of larvae were measured with the aid of the computer based software ImageJ.

In this trial larval densities were adjusted after 13 days to 5 larvae ml⁻¹ in each jar because of significant larval mortalities.

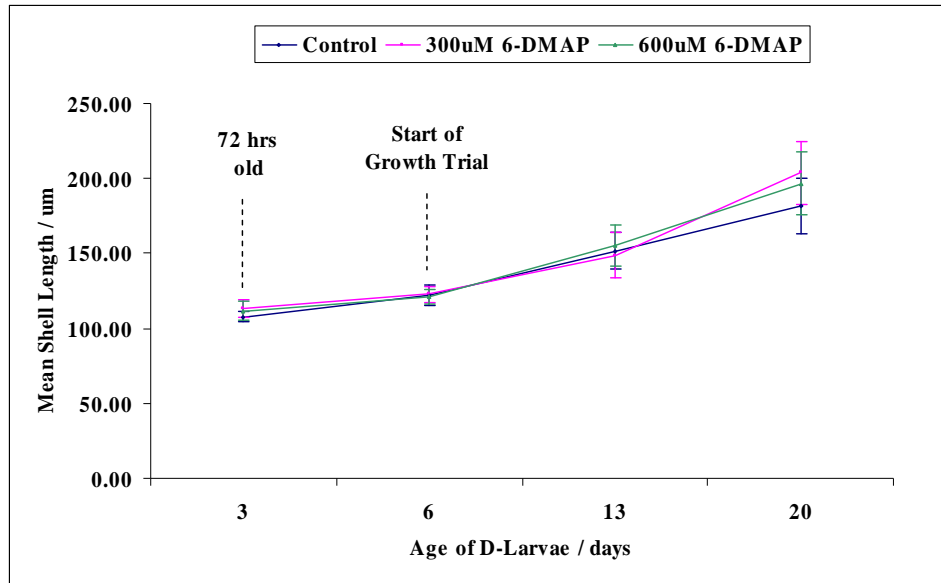


Figure 1. Mean shell length (µm) of putative *Mytilus edulis* triploid and control diploid veliger larvae reared for up to 20 days at 15°C. Error bars indicate ± standard deviation

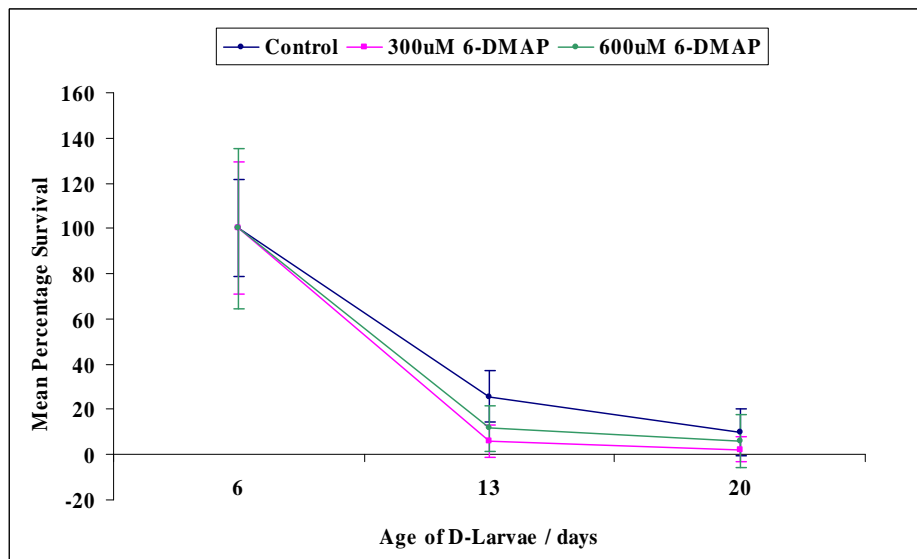


Figure 2. Mean percentage survival of putative *Mytilus edulis* triploid and control diploid veliger larvae reared for up to 20 days at 15°C. Error bars indicate ± standard deviation.

After 20 days, triploid larvae were significantly larger than diploid larvae (Figure 1, 2N, mean shell length 182 µm: 3N [300µM], 204 µm and 3N [600µM], 197 µm). However,

larval mortalities were very high in all treatments with only 10% survival of diploids and between 2% and 5 % survival of triploids (Figure 2). Therefore this trial cannot be regarded as safe evidence of better growth performance of triploid larvae.

Conclusion:

- **Triploid larvae grow significantly faster than diploid larvae**
- **Treat conclusion with caution due to very low survival in both 2N and 3N cultures**

Trial UWB-2

Triploid *M. edulis* larvae were produced using 6-DMAP at concentrations of 300 μ M and 600 μ M litre⁻¹ to block second polar body at meiosis II (details in WP3 report).

Two groups of eggs (group 1 from 2 females = Fg and group 2 from one female = F1) were fertilised with a pool of spermatozoa from three males. There were slight differences in triploid induction treatments between the two groups (details in WP3 report) and three groups were finally reared: F1 family (2N control), Fg family (2N control) and Fg family 300 (3N treatment).

Veliger larvae from these three groups were divided into three pseudoreplicates each and reared at 10 larvae ml⁻¹ in 5-litre plastic containers and fed on a diet of *P. lutheri* and *R. reticulata* at a 4:1 ratio equal to 25,000 cell ml⁻¹ day⁻¹. Three times a week the larvae were sieved, inspected, measured, the containers cleaned and the seawater replaced. The survival of larvae was estimated weekly from counts of a fixed volume. When inspection revealed that approximately 50% of the larvae were 'eyed', in any one of the cultures, the trial was concluded.

Figure 3 shows the mean increase in shell length (μ m) of the three groups of *M. edulis* larvae grown for 36 days.

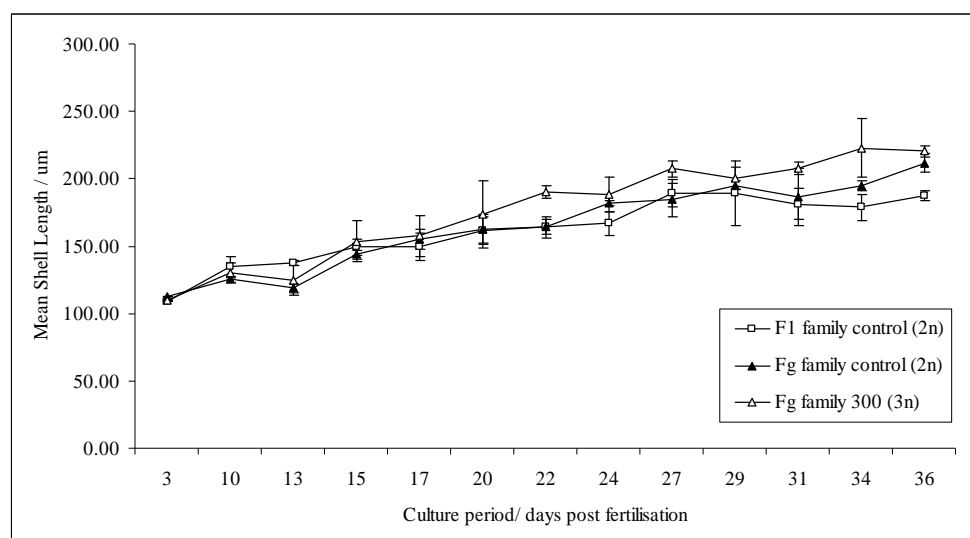


Figure 3. Mean shell length (μ m) of putative *Mytilus edulis* triploid (Fg family 300) and control diploid veliger larvae (Fg family control and F1 family control) reared for up to 36 days at 15°C. Error bars indicate \pm standard deviation.

The average growth rate for the three treatments was 3.09 μ m day⁻¹ and factorial regression analysis showed a significant difference between the growth rates of at least two groups. The F1 control treatment showed a significantly lower than average growth rate ($t=-3.56$, $p=0.001$), treatment Fg control was not significantly different from the average growth rate ($t=0.24$, $p=0.83$), and the Fg 300 treatment show a significantly higher growth rate from

the average ($t=3.32$, $p=0.002$). This indicates that triploid larvae grew significantly faster than diploid larvae.

There was generally good survival of larvae during the trial (Figure 4: almost 100% in F1 2N, but between 40% and 50% in the other groups) and some larvae in all three groups were reared to metamorphosis. The difference in survival between the two diploid control groups (F1 2N and Fg 2N) is probably a reflection of the different parentage of these two groups.

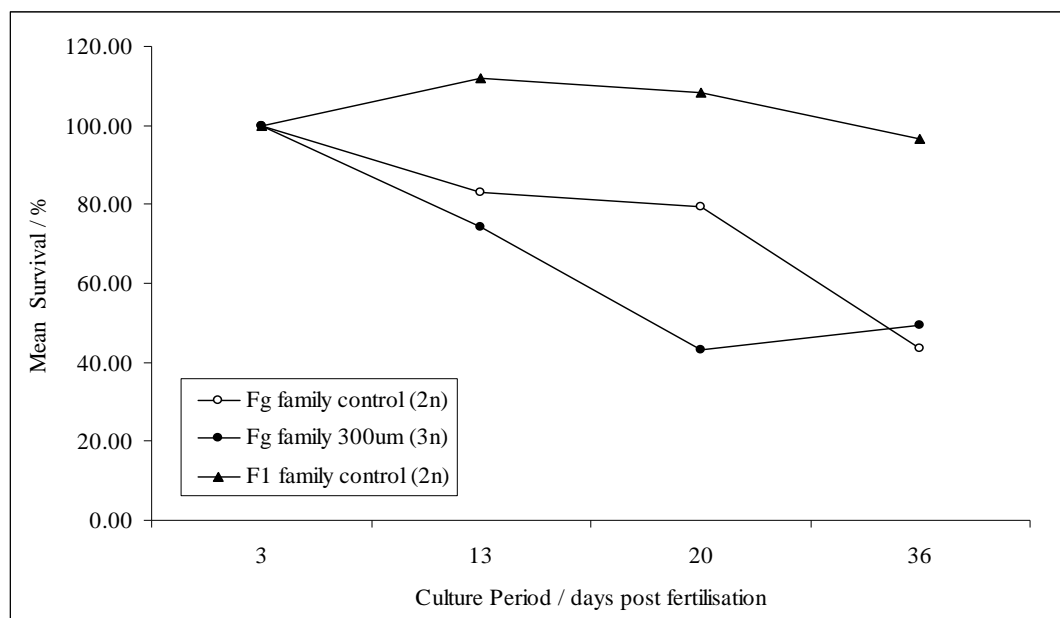


Figure 4. Mean percentage survival of putative *Mytilus edulis* triploid and control diploid veliger larvae reared for up to 36 days at 15°C. Error bars indicate \pm standard deviation.

Conclusion:

- **Triploid larvae grew significantly faster than diploid larvae**
- **There is a significant effect of genetic parentage on performance of diploid larvae.**

Trial UWB-3

Triploids *M. edulis* larvae were produced using 6-DMAP at concentrations of 300 μ M and 600 μ M to block second polar body at meiosis II (details in WP3 report). Eggs were from 2 females, fertilised by spermatozoa from 3 males.

The veliger larvae from the treated and untreated groups were divided into three pseudo-replicates each and reared at 10 larvae ml^{-1} in 5-litre plastic containers. All rearing was undertaken as described for Trial UWB 2. The volume of cultures was modified weekly to maintain rearing densities of 10 larvae ml^{-1} . The trial was concluded once 50% of the larvae were 'eyed', in any one of the cultures.

Table 1 shows the mean shell length (μ m) of treated (94% triploid, as determined by flow cytometry) and untreated diploid *M. edulis* larvae grown up to 36 days after fertilisation. There was no significant difference between mean shell length of larvae within pseudo-replicates (Kruskal-Wallis tests: Treated: $H= 4.78$, $P=0.092$, $DF = 2$; Untreated $H= 1.62$, $P=0.446$, $DF = 2$) so data were pooled. Analysis of the pooled mean shell length (μ m) using a student t-test after 34 days, and again at 36 days, identified significant differences in growth

between diploid and triploid larvae (34 days: t-value=4.63, P=0.044, DF=2, 36 days (t-value=4.30, p = 0.013, DF=4). The results indicate that triploid larvae grow faster than diploid larvae.

Table 1. Mean shell length (μm) and standard deviation (μm) of *Mytilus edulis* larvae reared for 34 and 36 days post-fertilisation. Pooled data from 3 pseudo-replicate cultures in each treatment

Treatment	Days after fertilisation (standard deviation)	
	34	36
Untreated	192.0 (3.9)	202.5 (2.9)
Treated	222.3 (10.6)	224.0 (8.2)

Larval survival is shown in Figure 5 and indicates similar mean survival (around 50%) but large variances around the means in both treatments.

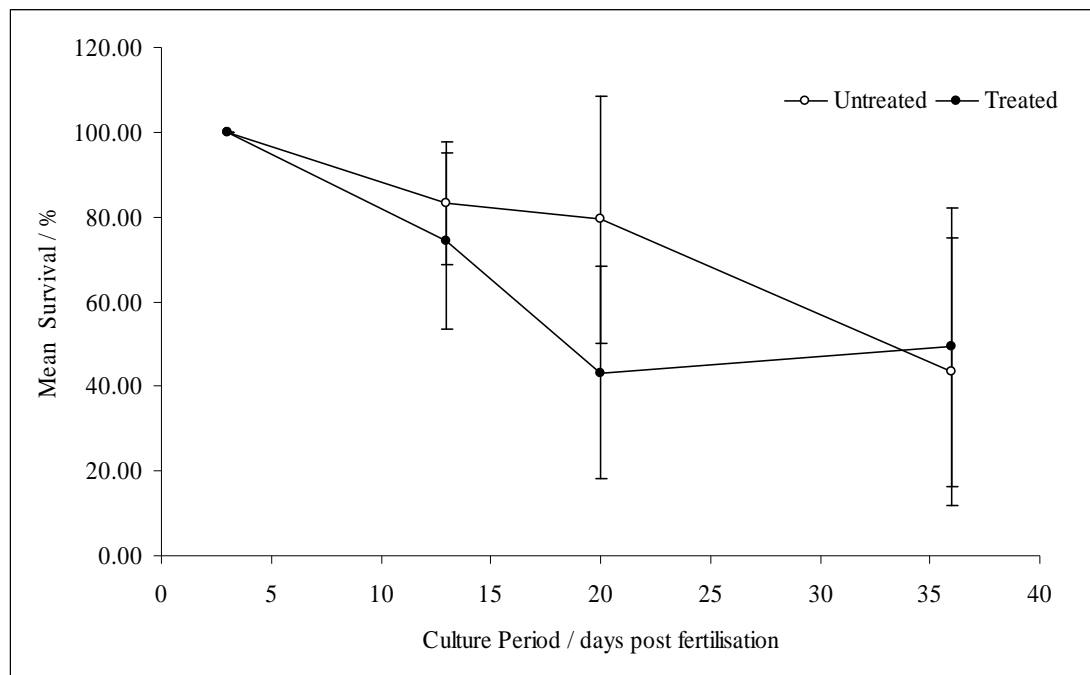


Figure 5. Mean survival of *M. edulis* triploid and diploid control veliger larvae in cultures reared for 36 days

Conclusion:

- **triploid larvae grew significantly faster than diploid larvae**
- **survival of triploid and diploid larvae was similar**

Trial UWB-4

Eggs from a single female were fertilised by spermatozoa from three different male mussels to give three groups (Families 1, 2 and 3). Batches of the fertilised eggs from each family were treated with 6-DMAP at 300 μM for 20 minutes at 16-17 $^{\circ}\text{C}$ to induce triploidy and a batch from each family remained untreated as diploid controls (details in WP3 report).

A single diploid culture (control) and single (>96% 3N) triploid culture of D-larvae was reared for each family at a density of 10 larvae.ml⁻¹ in 5-litre plastic vessels. All rearing was undertaken as described for Trial UWB 2. When inspection revealed that approximately 50% of the larvae were ‘eyed’, in any one of the cultures, the trial was concluded.

After 31 days growth the triploid larvae in all 3 families had a higher mean shell length than their diploid counterparts although these differences are probably not significant because their standard deviations overlap (Figure 6). Because replication of cultures within families was not undertaken (due to insufficient numbers of larvae) it is not possible to test for the significance of these differences in mean shell length between 3N and 2N larvae within families.

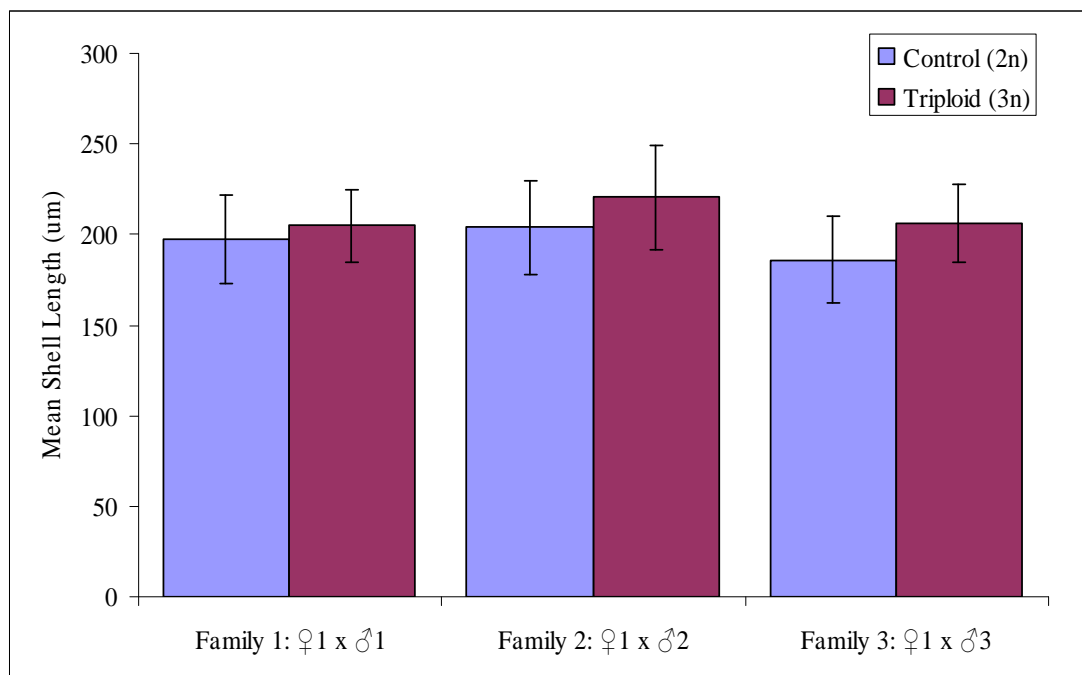


Figure 6. Mean shell length (µm) of diploid and triploid *Mytilus edulis* veliger larvae after 31 days. Error bars indicate ± standard deviation.

Conclusion:

- **There is no significant difference in mean shell length between diploid and triploid larval cultures**

IFREMER

TRIAL IFREMER-1

Mussels (*M. edulis*) were spawned in January 2007 and around 2 million eggs were treated with 300 µm 6-DMAP for 20 min starting 15 min after fertilisation to induce triploidy. A diploid control of untreated eggs was also fertilised. The putative triploid cohort was later estimated (by flow cytometry of ground samples of 100 larvae) to consist of 73% triploids. Larvae were reared using the standard conditions at the IFREMER hatchery at La Tremblade (details in WP2 and WP3 reports) and good settlement was obtained. Triploid and diploid spat were on-grown at Grainocean and La Tremblade although comparative data on spat performance are not available.

Mean shell length of 20 day old triploid and diploid larval cohorts differed (Figure 7) and it can be seen that the 95 % C.I. around the means of the two cohorts do not overlap. A t-test showed a significant difference between 2n and 3n.

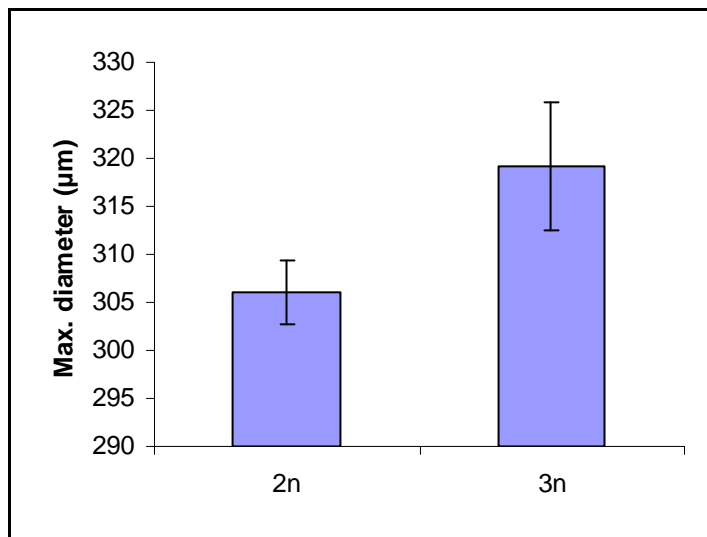


Figure 7. A comparison of *M. edulis* larval size between diploid (2n) and triploid (3n) batches at 20 days of age, based on Samba technologies image analysis measurement of 50 larvae from each ploidy class.

Conclusion:

- **Triploid *M. edulis* larvae grow significantly faster than diploid larvae**
- **The conclusion must be treated with caution because there was no replication in the trial**

IMARES

TRIAL IMARES 1

Wadden Sea *M. edulis* were induced to spawn on 16 April 2007 [R0704] Two batches of fertilised eggs were divided into two one with half subjected to treatment with 300 µM 6-DMAP to induce triploidy and the other half acting as a control. A further untreated control batch of eggs was fertilised.

Treated (putative triploid) and untreated (diploid) embryos were placed at 80-100 eggs ml⁻¹ in 50 litre containers with 0.2 µm filtered and UV treated seawater and gentle aeration for 48 hours and veliger larvae were collected. One triploid and two diploid batches produced a suitable number of veliger larvae for a growth trial.

Veliger larvae were reared at 17 °C in 0.2 µm filtered seawater with gentle aeration in 50 litre tanks at a density of 10 larvae ml⁻¹. Larvae were fed 12.500 cells ml⁻¹ of *P. lutheri* and 12.500 cells ml⁻¹ of *Chaetoceros gracilis* or *Chaetoceros calcitrans* day⁻¹ and cleaned two times a week.

Larvae showed poor growth and survival in all treatments (Figures 8 and 9).

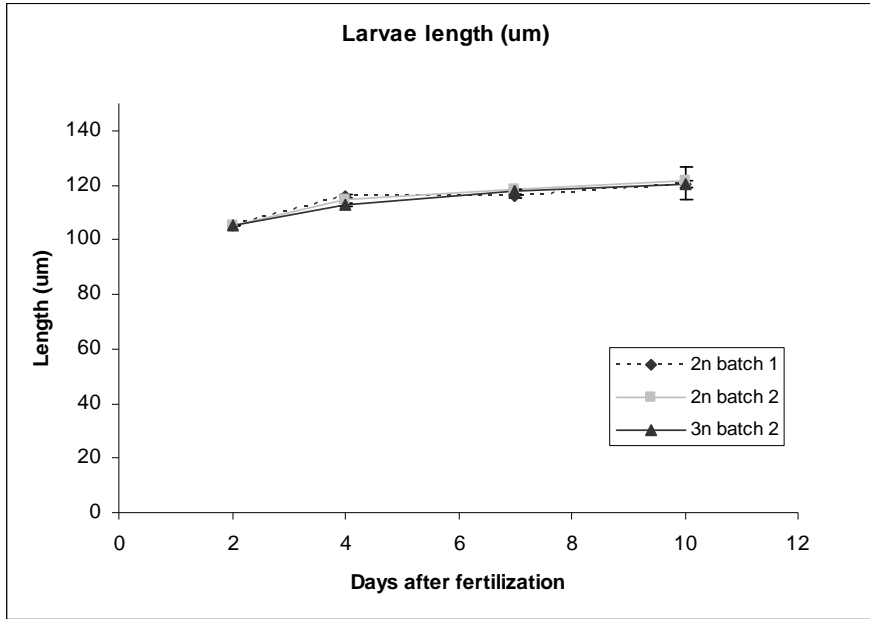


Figure 8. Growth of diploid and putative triploid veliger larvae of *M. edulis* larvae over 12 days. The average with standard deviation is presented, n=2 for 2n batch 1, n=4 for 2n batch 2 and n=1 for 3n batch 2 .

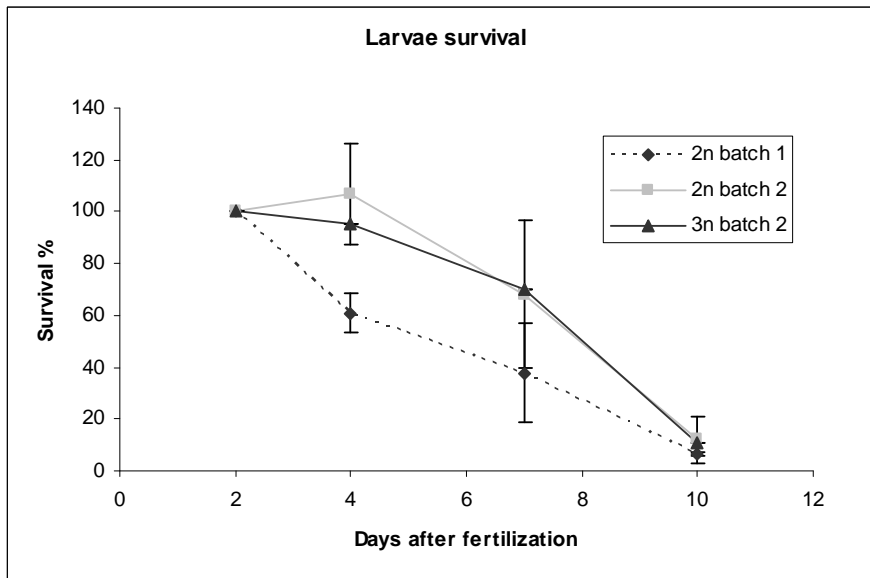


Figure 9. Survival of diploid and putative triploid veliger larvae of *M. edulis* larvae over 12 days. The average with standard deviation is presented, n=2 for 2n batch 1, n=4 for 2n batch 2 and n=1 for 3n batch 2.

Conclusion:

- **Poor growth and high mortalities mean that no conclusions should be drawn from this trial**

TRIAL IMARES 2

Mussels (*M. edulis*) from the Oosterschelde estuary were induced to spawn on the 9th and the 16th of July 2007 (Batches R0707 and R0708) and eggs were treated with 6-DMAP to induce triploids (details in WP3 report).

Treated (putative triploid) and untreated (diploid) embryos were placed at 80-100 eggs ml⁻¹ in 50 litre containers with 0.2 µm filtered and UV treated seawater and gentle aeration for 48 hours and veliger larvae were collected. Batch R0707 produced approximately 70% triploids while only 1-4% of batch R0708 were triploids. Although the diploid and “triploid” cultures from batch R0708 were reared through the larval stage separately, we do not compare growth rates between them here because of the very low percentage of triploids. Details are given in the WP3 report.

Veliger larvae from batch R0707 were reared at 17 °C in 0.2 µm filtered seawater with gentle aeration in 50 litre tanks at a density of 10 larvae ml⁻¹. Larvae were fed 12.500 cells ml⁻¹ of *P. lutheri* and 12.500 cells ml⁻¹ of *Chaetoceros gracilis* or *Chaetoceros calcitrans* day⁻¹ and cleaned two times a week.

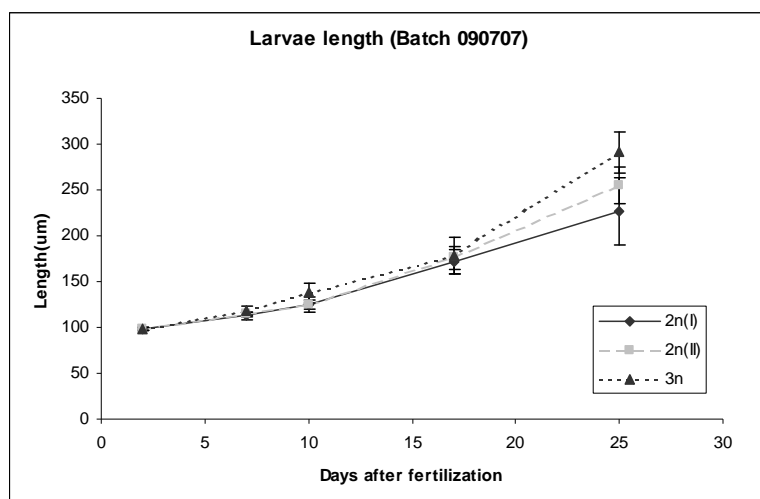


Figure 10. Growth of triploid and diploid cohorts of *M. edulis* veliger larvae from batch R0707, IMARES Trial 1.

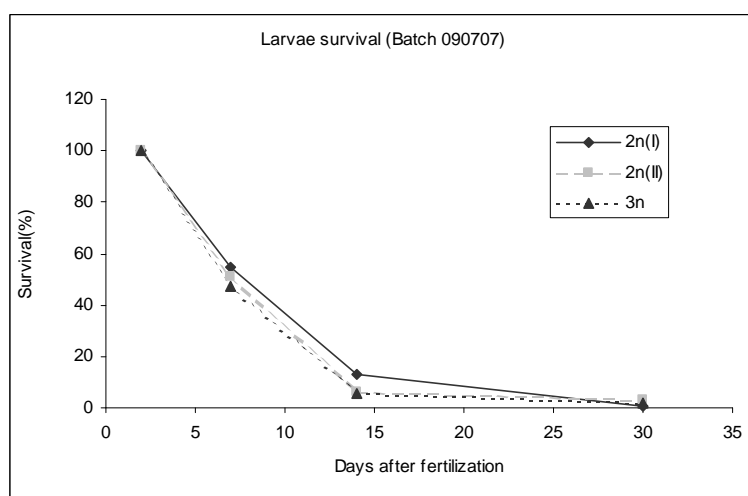


Figure 11. Survival of triploid and diploid cohorts of *M. edulis* veliger larvae from batch R0707, IMARES Trial 1.

Larvae showed growth in all treatments (Figure 10) with a significantly higher mean shell length observed in the triploid cohort treatment compared with at least one of the diploid cohorts ($p < 0.001$). However, survival of larvae was low in all three cohorts and most larvae were dead by day 30 (Figure 11).

Conclusion:

- **Triploid larvae grew significantly larger than diploid larvae over 30 days.**
- **Heavy mortalities during larval culture mean that conclusions drawn from this trial should be treated with caution**

TRIAL IMARES-3 (Roem van Yerseke Technology Transfer Workshop)

A large scale triploid induction trial was carried out on the premises of Roem-van-Yerseke by a team of staff drawn from all partner R&D institutions. Details of methodologies are given in the 18 month activity Report (WP3 and Annex 1) and it was estimated that 32.5 million triploid veligers with normal morphology were produced. Because larval culture took place in a commercial background any rigorous scientific analysis of comparative growth data between triploid and diploid larvae in this trial is severely compromised by two key features. Firstly, the smallest larvae were discarded at every water change. Secondly, batches of triploid larvae were separated during the sieving process into cohorts of different size ranges. Nevertheless, there are data about the percentage of triploid larvae within each of the various size-separated cohorts (Figure 12).

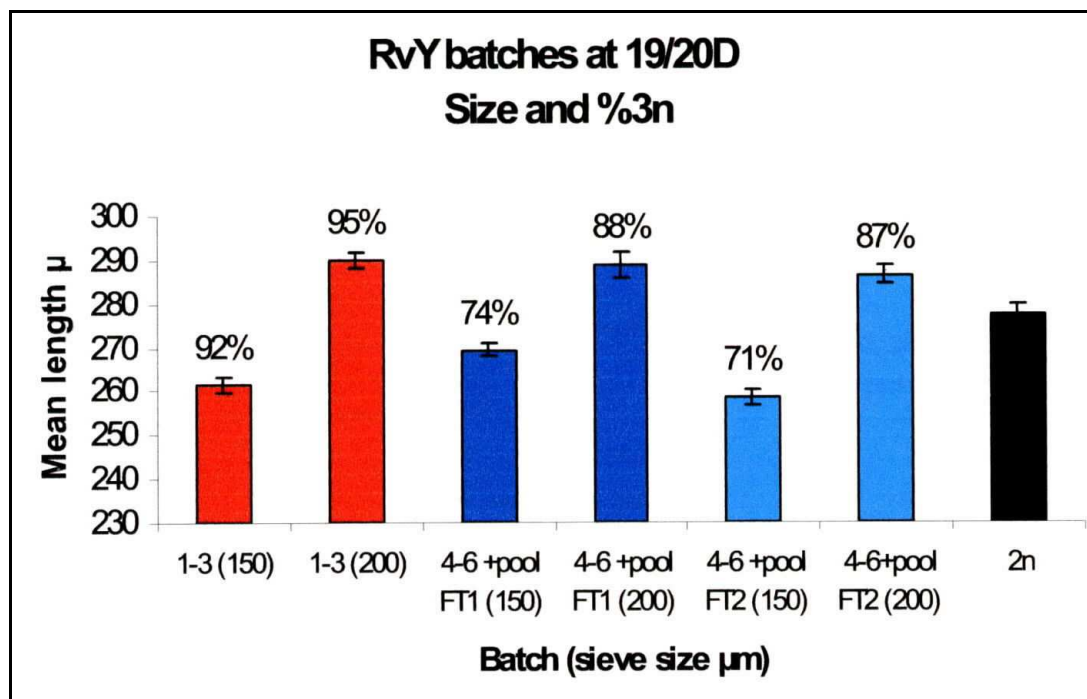


Figure 12. Mean shell lengths of size-sorted cohorts of *M. edulis* triploid larvae reared in an experimental commercial-scale hatchery. Comparative diploid larvae are shown at the right (2n). Each colour represents a pooled batch of triploid larvae that has been size-sorted into a small group and a large group at an early stage of larval culture. Numbers at the head of the columns indicate the percentage triploidy of the group.

When the mean shell length of all groups of larvae containing triploids (3 batches, each with a small and large group) is compared with the mean shell length of the diploid group there is no significant difference between them ($p>0.05$). In spite of the compromised nature of the size data in this trial, this simple analysis of all triploids vs all diploids supports the conclusion that there is no significant difference between the growth rates of triploid and diploid *M. edulis* larvae.

Conclusion:

- **Triploid *M. edulis* larvae can be grown successfully at a semi-commercial scale**
- **There is no significant difference in larval growth rates between triploid and diploid veliger larvae.**

CIMA

Trial CIMA-1.

Mussels (*M. galloprovincialis*) from Galicia were spawned and triploid induction using 300 μ M 6DMAP was carried out (for details see WP3 report). Veliger larvae were reared in pseudo-replicated containers (3 cultures of the triploid cohort [mean 76%] and 3 cultures of untreated diploids) under standard CIMA conditions (details in WP2 report) and fed *P. lutherii* and *I. galbana*.

Comparative larval sizes were measured at 2, 7 and 14 days after fertilisation. There was no significant difference in size between treatments nor between replicates within treatment after 2 days ($p>0.05$, Figure 13), but by 14 days there were significant differences between treatment and between replicates within treatments ($p<0.05$, Figure 14). The diploid larvae were larger than the triploids.

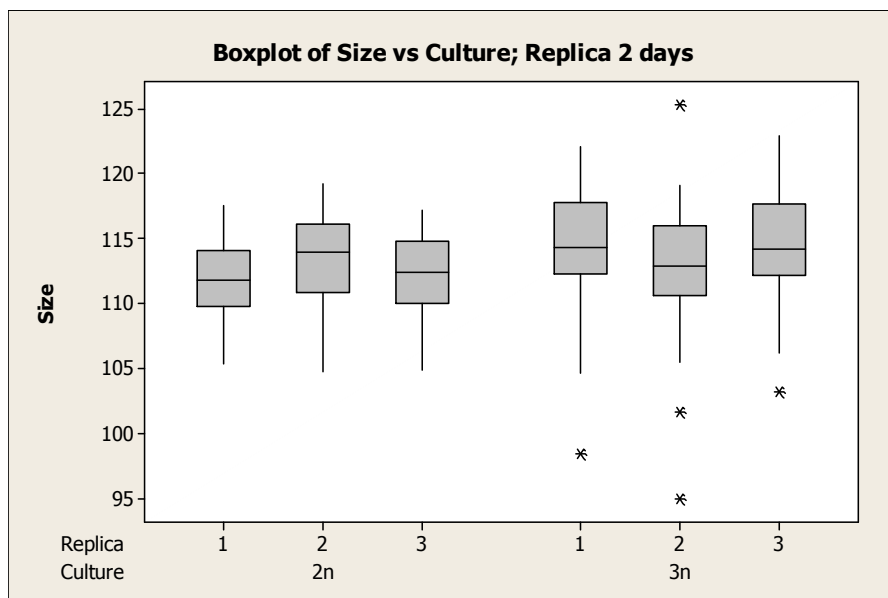


Figure 13. Sizes of comparative cultures of triploid and diploid *M. galloprovincialis* veliger larvae at 2 days post-fertilisation.

After 14 days there was no significant difference between the size of triploid and diploid larvae ($p=0.144$), but significant differences in size between replicates within treatments were evident ($p<0.001$, Figure 14).

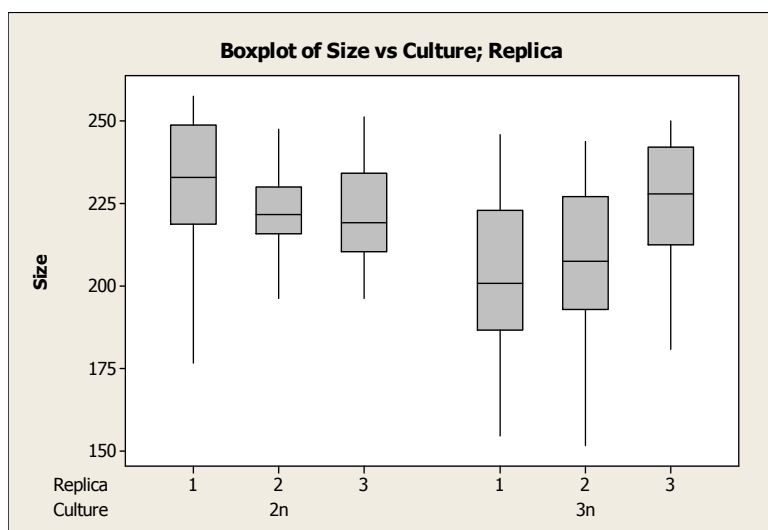


Figure 14. Sizes of comparative cultures of triploid (3n) and diploid (2n) *M. galloprovincialis* veliger larvae at 14 days post-fertilisation.

Mortalities during the larval phase were significantly higher in the triploid cultures, almost double that in the diploid cultures ($p=0.004$). This will have affected the availability of food to the larvae (number of cells of phytoplankton larva⁻¹) and would give an advantage to the triploid larvae in a food-limited situation.

Conclusion:

- **Significant differences in mean shell length between replicate cultures of *M. galloprovincialis* veligers generally outweigh any significant differences between diploid and triploid larvae**
- **Caution must be used in drawing conclusions from the trial because it was not possible to control larval densities.**

Trial CIMA-2.

Mussels (*M. galloprovincialis*) from Galicia were spawned and triploid induction using 300 μ M 6DMAP was carried out (for details see WP3 report). Veliger larvae were reared in pseudo-replicated containers (2 cultures of the triploid cohort [mean 79% triploid] and 2 cultures of untreated diploids) under standard CIMA conditions (details in WP2 report) and fed *P. luteii* and *I. galbana*.

Table 2. Values of p for significance of difference (ANOVA) between the mean sizes of triploid and diploid veliger larvae of *M. galloprovincialis* during 20 days of growth.

Day of sampling	Replicates within treatments	Between treatments	Sense of significant difference
3	0.443	0.026	Triploids larger
8	<0.001	0.030 (only the best replicates analysed)	Triploids larger
15	Not tested	0.003 (only the best replicates analysed)	Triploids larger
20	Not tested	<0.001	Triploids larger

Larvae were sampled at 3, 8, 15 and 20 days after fertilisation. At every sampling time the triploid larvae were generally larger than the diploids but there were also significant differences between replicate cultures within treatments from day 8 onwards. Also there were heavy mortalities from this time and subsequent statistical analysis was based on comparisons between the cultures with the best survival (Table 2). By day 20 the mean sizes of larvae in the triploid and diploid cohorts were very different: the approximate mean shell lengths of diploids was 260µm, and for triploids was 280µm.

Mortalities during the larval phase were significantly higher in the triploid cultures compared with the diploid cultures ($p=0.003$). This will have affected the availability of food to the larvae (number of cells of phytoplankton larva⁻¹) and would give an advantage to the triploid larvae in a food-limited situation.

Conclusion:

- **Triploid *M. galloprovincialis* larvae grow significant faster than diploid larvae**
- **Caution must be expressed in drawing conclusions from the trial because there were heavy mortalities and it was not possible to control larval densities.**

Trial CIMA-3.

Mussels (*M. galloprovincialis*) from Galicia were spawned and triploid induction using 300 µM 6DMP was carried out (for details see WP3 report). Veliger larvae were reared in pseudo-replicated containers (2 cultures of the triploid cohort [52% and 70% triploid] and 2 cultures of untreated diploids) under standard CIMA conditions (details in WP2 report) and fed *P. lutherii* and *I. galbana*.

Larvae were sampled at 3, 7 and 14 days after fertilisation. After this time heavy mortalities occurred in all cultures and no settlement was achieved. Replicate cultures within treatments were pooled for all analyses.

At day 3 and 7 the triploid larvae were significantly larger than the diploids but this difference was not evident at day 14 (Table 3). In this trial, in contrast to trials CIMA 1 and CIMA 2, there was no significant difference in survival between the larvae in the triploid cohorts compared with the diploid cohorts ($p=0.355$). However mortalities in the two replicate triploid cultures were very different and this compromises the test of significance between treatments.

Table 3. Values of p for significance of difference (ANOVA) between the mean sizes of triploid and diploid veliger larvae of *M. galloprovincialis* during 14 days of growth.

Day of sampling	Between treatments	Sense of significant difference
3	<0.001	Triploids larger
7	<0.001	Triploids larger
14	0.067	

Conclusion:

- **In this trial triploid *M. galloprovincialis* larvae grew significant faster than diploid larvae at an early stage, but by day 14 there was no significant difference between them.**
- **Caution must be expressed in drawing conclusions from the trial because there were heavy mortalities and it was not possible to control larval densities.**

Part II Spat

Comparative performance of triploid and diploid mussel spat

UWB

Trial UWB-2

Testing of a laboratory scale down-welling system

Initially groups of ready to settle diploid and triploid mussel larvae were settled as spat on 85 μ m nylon mesh sieves connected to a down-welling system for 51 days. The settled spat consisted of two diploid groups (D1 and D2) and two corresponding triploid groups (T1 and T2). Triploids were produced by exposing developing embryos to 300 μ M 6-DMAP litre⁻¹ for 20 minutes once 50% of eggs had expelled 1° polar bodies. All groups share the same female parent although the male parent differs. Groups D1 and T1 shared the same male parent and D2 and T2 share the same male parent.

After the initial settlement phase the triploid and diploid mussel spat were cultured in nylon mesh (120 μ m) sieves at a density of 100 spat per sieve, equivalent to 1.2 spat cm⁻², each reared in their own sieves, with four sieves per group. The sieves were suspended in temperature controlled holding tanks at 18°C, from which a submersible pump continuously circulated 10 μ m filtered seawater through each sieve at a rate of approximately 0.25 litres min⁻¹. Four sieves were held per holding tank, with one sieve being from each spat group. Every two days the seawater in the holding tanks was changed prior to the addition of a mixed live algae diet of *P. lutheri* and *R. reticulata* given *ab libitum* to the holding tanks. Furthermore, once a week the rearing sieves were rinsed with filtered seawater and the holding tanks cleaned to remove any waste and settled material. The mean initial shell length (μ m) of mussel spat was estimated at the start of the experiment for each group by measuring a random sample of 100 individuals. After 62 days, the number of surviving spat in each sieve was determined as well as the mean shell length which was calculated from a sample of 20 spat per sieve.

Table 4 shows the mean values of the shell length, at the beginning and at the end (62 days) of the experiment, the overall increase in shell length and the survival of the four groups. Although starting from a smaller mean size at settlement, the diploid spat reached a similar size to the triploid spat after 62 days.

However, observed differences in survival between groups with the same level of ploidy (D1 vs. D2 and T1 vs. T2) were approximately three times higher than differences between groups with different level of ploidy (D1 vs. T1 and D2 vs. T2). This means that differences in survival were due more to the male used to produce the half-sib families than to the ploidy of the mussels.

Table 4. Mean shell lengths (μ m) and survival of *Mytilus edulis* spat reared in a down-welling system

Group	Shell length, μ m		Shell length increase, μ m	Survival % (s.d.)
	Initial size	Size after 62 days (s.d.)		
D1	506	1376 (39.98)	870	56.25 (8.0)
D2	660	1603 (194.02)	943	71.75 (8.0)
T1	694	1332 (163.09)	638	51.75 (3.0)
T2	716	1473 (243.16)	757	67.00 (12.0)

Conclusion:

- Diploid spat appeared to grow faster than triploid spat in a laboratory-scale down-welling system, but statistical proof was not possible
- Survival of spat was more dependent on the genetic background of the spat than on their ploidy

IMARES

Trials IMARES-3 and IMARES-2 (IMARES, Roem van Yerseke and Neeltje Jans)

Testing of an indoor micro-nursery (IMARES), an outdoor land-based nursery (Roem van Yerseke) and a rope culture (Neeltje Jans).

IMARES-3

A large scale triploid induction trial was carried out in March 2007 on the premises of Roem-van-Yerseke by a team of staff drawn from all partner R&D institutions. Details of methodologies are given in the 18 month activity Report (WP3 and Annex 1) and it was estimated that 32.5 million triploid veligers with normal morphology were produced. Details of performance of triploid and diploid larvae are given earlier in this report.

On May 16th 2007, the 2n and 3n settled spat were divided into 3 size classes: small, medium and large. Half of the 2n and 3n batches of the medium size were transferred to the rope culture of Neeltje Jans, while the other half remained in the land-based nursery at Roem van Yerseke. The complete 2n and 3n set of the small size class stayed at Roem van Yerseke and the complete 2n and 3n set of the large size class went to Neeltje Jans. Shell length and survival were monitored in all these batches.

Following transfer from Roem van Yerseke to Neeltje Jans, the medium size spat increased in size to approximately 7 mm and the large size spat to around 15 mm by the beginning of November (Figure 15). The largest measured individual was 22 mm at an age of 200 days after fertilisation. Sizes of spat did not differ significantly between treatments (ANOVA, $P > 0.05$), except for the medium sized 3n spat of batch RvY040506 (ANOVA, $P < 0.01$).

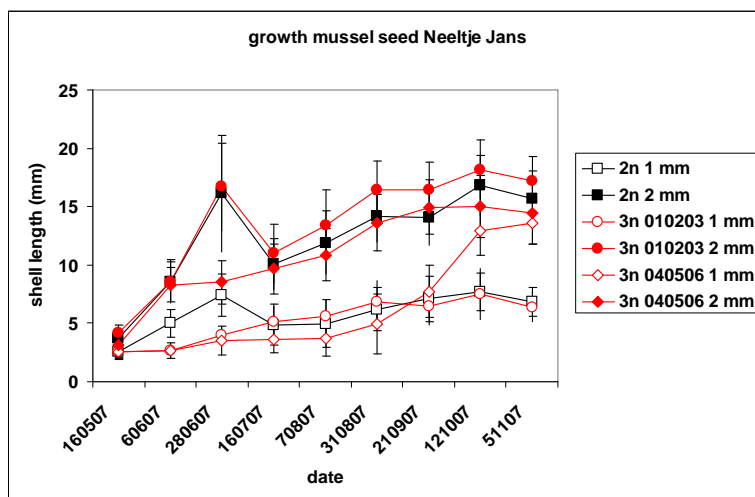


Figure 15. Shell lengths of diploid and triploid spat (mean \pm s.d.) of *M. edulis* from the RvY010203 and RvY040506 batches transferred to rope culture at Neeltje Jans.

IMARES-2

In a further trial at IMARES (R0707 and R0708 – see **Trial IMARES-2** in larval section of this report), in August 2007, ready-to-settle 2n and 3n larvae were introduced into cylinders and cultured in a down-welling system at 17°C. All diploids were pooled in one cylinder and all triploids in another. It should be noted that the percentage of triploids was estimated to be extremely low in R0708 – less than 5% - so pooling these with the 70% triploids from R0707 experiment will have reduced the pooled percentage of triploids down to around 35-40%.

Spat were fed a mixture of *Phaeodactylum tricoratum*, cultured in outdoor basins, and *P. lutheri* cultured indoors in 25 l plastic bags. Algal mixture was pumped by a peristaltic pump from an algae holding container to the down-welling system. Filtered seawater was introduced into the down-welling system to keep the concentration of the algal cells below the pseudo-faeces threshold. The mussels were fed 0.4 g dry weight of algae for each g of wet weight of spat per week.

Although in all sampling events from August to November 2007 the 3n individuals were larger than the 2n (Figure 16), these differences were not significant (ANOVA, $P > 0.05$). The fact that at the beginning of November, the total biomass of the 2n spat in the cylinder (48 g) was two times higher than that of the 3n spat (22 g), could suggest that spat growth is density (biomass)-dependent and not, in this instance, ploidy-dependent. In addition, the percentage of triploid spat in the “triploid” batch was probably less than 50% so any potential growth advantage of triploids would have been more difficult to detect. The results of this trial are therefore entirely unreliable as a comparative measure of triploid and diploid spat performance.

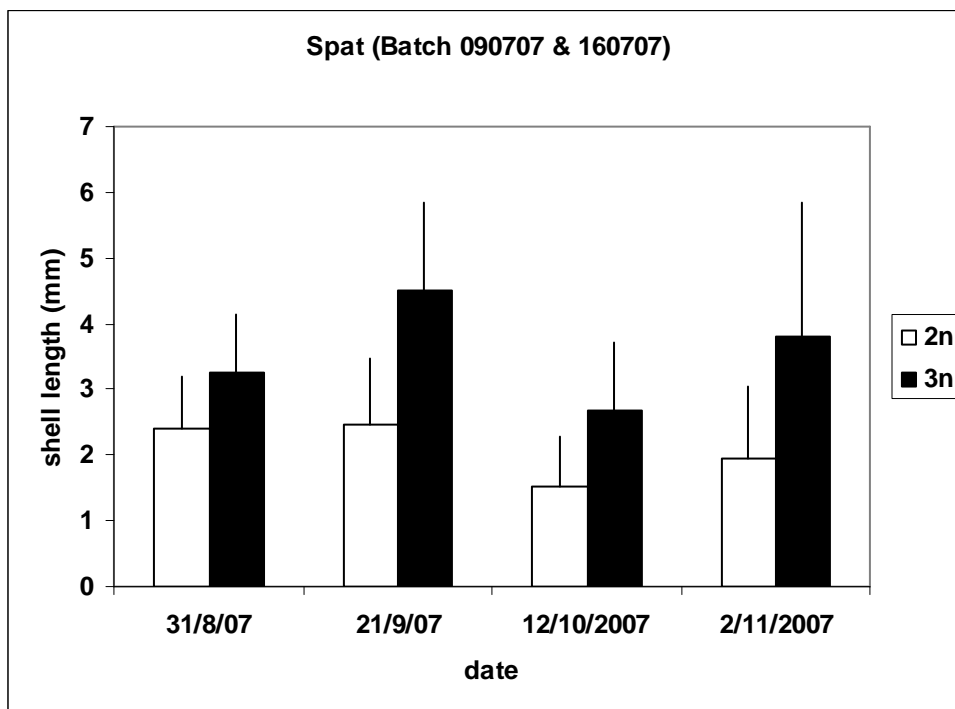


Figure 16. Shell lengths of diploid (2n) and triploid (3n) spat (mean \pm s.d.) of trials R0707 and R0708 kept at the indoor nursery of IMARES.

Conclusion:

- **One size-selected batch of triploid spat grew significantly faster than all others in rope culture at Neelte Jans**

- Growth of other triploid and diploid batches of spat were not significantly different from one another
- Any differences in growth between batches of “triploid” and diploid spat grown in this down-welling system trial are entirely compromised by the low percentage of triploids and density effects.

CIMA

Testing of an indoor micro-nursery at CIMA.

Trial CIMA-1.

Mussels (*M. galloprovincialis*) from Galicia were spawned and triploid induction using 300 μ M 6DMAP was carried out (for details see WP3 report). Veliger larvae were reared in pseudo-replicated containers (3 cultures of the triploid cohort [mean 76%] and 3 cultures of untreated diploids) under standard CIMA conditions (details in WP2 report) and fed *P. lutherii* and *I. galbana*. For details of comparative triploid and diploid veliger larval performance see the larvae section

Settlement was obtained by moving ready to settle 2n and 3n pediveliger larvae to separate down-welling units consisting of PVC cylinders provided with nylon mesh screens of 150 μ m. Settled diploid and triploid spat were transferred into two new down-welling units which were introduced into a large flow-through rectangular tank type raceway. Spat were fed a mixture of the microalgae *Skeletonema costatum* and *Tetraselmis suecica* which was continuously supplied by a pump to the incoming seawater. Four months after fertilization, one sample of 30 individuals was taken from each down-welling unit (two samples in total: 2n and 3n) and the spat shell length (maximum anterior-posterior size) measured (Figure 17).

Mean value of the 3n spat (7.6 mm) was not significantly different from that of the 2n spat (7.0 mm). However, the shell length distribution of 3n spat was considerably wider (c.v. = 39.8) than that of 2n spat (c.v. = 20.25)

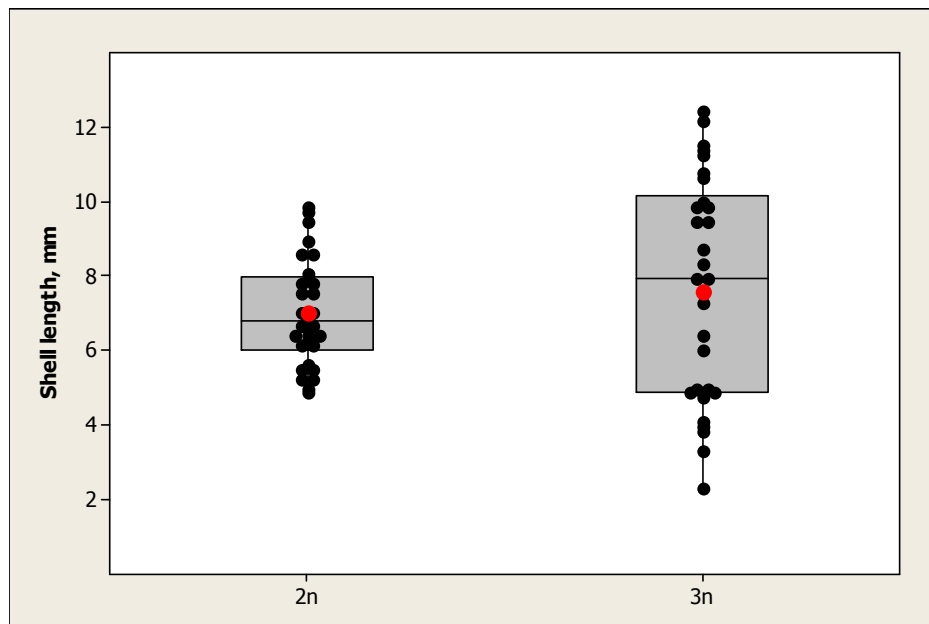


Figure 17. Box-plot diagram of the shell length of 2n and 3n spat, four months after fertilization. Red dots: mean values. Black dots: individual values

Conclusion

- **There was no significant difference between the growth of triploid and diploid spat in down-welling culture IFREMER/Grainocean**

Subsequent testing of an indoor micro-nursery, an outdoor land-based nursery, and a offshore long-line system at Grainocean.

In January 2007, 2n and 3n mussel larvae were produced in the IFREMER hatchery at La Tremblade (batch BS0704). Half a million of ready-to-settle pediveliger larvae of each ploidy type were sent to the Grainocean farm facilities in 180 µm sieves where they were settled and grown on mesh screens in downwelling units in a micro-nursery. After this, mussels were pre-ongrown up to seed size on square net frames in an outdoor land-based nursery and then moved to a seashore environment where they were ongrown into lantern nets in a long-line system for three months. A part of these 2n and 3n mussels were harvested on November 2007 and their relative performance compared.

The results of this experiment are summarised in Table 5. First at all, we have to highlight that all the putative triploid mussels tested were really triploid mussels (100% of triploids in the Triploid group) and no contamination between triploid and diploid individuals was detected (0 % of triploids in the Diploid group). Secondly, size of 2n and 3n mussels was very similar and not significant differences in length, width, thickness and shell weight were detected between the two types of mussels. Small but significant differences were detected in the total weight and the fresh flesh weight of mussels, being the 2n individuals heavier than their 3n counterparts. Finally and very important, no mature individuals were detected within the triploid group.

Table 5 Variables measured in 2n and 3n mussels. Significant differences are indicated in blue colour.

Variable	Diploid	Triploid
% of triploids	0	100
Average length (mm)	57.07	55.53
Average width (mm)	26.9	27.07
Average thickness (mm)	21.12	20.74
Average shell weight (g)	3.49	3.28
Average fresh flesh weight (g)	6.37	5.38
Average weight (g)	15	13
% of mature individuals	100	0

Conclusion

- **There was no significant difference length, width, thickness and shell weight of triploid and diploid spat**
- **2n individuals were significantly heavier than their 3n counterparts**
- **no mature individuals were detected within the triploid group**

DISCUSSION

A summary of the variables measured, systems used and main results of the three lab-scale experiments conducted by UBW, IMARES and CIMA and the two large-scale trials carried out by IMARES/Neeltje Jans and IFREMER/Grainocean is shown in the Table 6. The three lab-scale experiments were carried out under similar cultivation systems: laboratory-scale or micro-nursery down-welling systems. However, the results were inconsistent between experiments. In two cases, IMARES and CIMA, 3n spat was bigger than 2n, but the differences were not statistically significant. In the case of UBW the result was the opposite: 2n spat grew faster than 3n.

When the spat produced by IMARES in the Roem van Yerseke hatchery was moved, within net cages, to a long-lines system of Neeltje Jans, no significant differences in size were detected between 2n and 3n seed after five and half months of ongrowing in the field. Results obtained in the other large-scale experiment, conducted by Grainocean, confirm this conclusion since no important differences between the two types of mussels were detected after several months of ongrowing within lantern nets in a long-lines system. A relevant result of this last experiment was to observe that while all sampled diploid mussel were mature (presence of gametes) their triploid counterparts were not.

Therefore, from the four experiments carried out in this project a relevant (with commercial impact) growth advantage of triploid mussels with respect to their diploid counterparts can not be deduced.

Table 6 Summary of the growth and survival experiments of 2n and 3n spat carried out by UBW, IMARES/Neeltje Jans, CIMA and IFREMER/Grainocean

Partner	Activity	System	Main results
UBW	Comparison of growth and survival of 2n and 3n spat	Laboratory scale down-welling system. 120 µm nylon mesh cylindrical downwellers suspended within rectangular tanks	After 62 days, 2n spat grew faster than 3n spat. No differences in survival were detected
IMARES Neeltje Jans	Comparison of growth of 2n and 3n spat and seed	Down-welling system in a indoor micro-nursery (IMARES) Nylon mesh cages in long-lines systems (NJ)	After several weeks, 3n spat was bigger than 2n, but differences were not significant After 5½ months (May to November) not significant difference in size between 2n and 3n mussels was detected
CIMA	Comparison of growth of 2n and 3n spat	Cylindrical downwellers in a raceway tank in an indoor micro-nursery	After 4 months, 3n spat was bigger than 2n spat, but differences were not significant
IFREMER Grainocean	Comparison of growth of 2n and 3n seed up to commercial size	Downwellers in an indoor micro-nursery Square net frames in a outdoor land-base nursery Lantern nets in long-lines system	Size of 2n and 3n mussels was very similar. No significant differences were detected for length, width, thickness and shell weight Small but significant differences were detected for fresh flesh weight and total weight No mature 3n individuals were detected

Part III Market size mussels

Comparative performance of triploid and diploid mussels in consumer sensory tests

An evaluation panel was conducted to compare the sensory properties (colour/appearance, odour, taste/flavour and texture) of diploid and triploid *M. edulis* mussels produced during the Blue Seed project.

Mussels (*M. edulis*) were spawned in January 2007 at IFREMER (See details in Larvae section under **TRIAL IFREMER-1**) and around 2 million eggs were treated with 300 μm 6-DMAP for 20 min starting 15 min after fertilisation to induce triploidy. A diploid control of untreated eggs was also fertilised. The putative triploid cohort was later estimated (by flow cytometry of ground samples of 100 larvae) to consist of 73% triploids. Larvae were reared using the standard conditions at the IFREMER hatchery at La Tremblade (details in WP2 and WP3 reports) and good settlement was obtained.

Diploid and triploid ready-to-settle larvae (500,000 of each type) were transferred on 180 μm sieves to the nursery facilities of the SME Grainocean (France) for settlement and spat culture. Settlement was carried out by spreading pediveliger larvae on mesh screens in circular downwellers. After detaching the spat from the mesh screens, they were held first in an indoor micro-nursery and then in an outdoor land-based nursery. Final on-growing of the 2n and 3n seed was carried out in lantern nets in a long-line system situated in a seashore environment. On November 9th 2007 2n and putative 3n mussels were transported on ice from Grainocean to Yerseke.

Table 7. Description and scale of the four sensory categories tested in the panel

SENSORY CATEGORY	DESCRIPTION	SCALE
Colour and appearance	Natural colour variation: off-white to light orange. Fresh, shiny and contrasting appearance	9
	As 9, still shiny.	8
	Less shiny, a bit pale	7
	No shine, pale and a bluish film.	6
	Colours are fading, slightly milky colour, dull and a bluish film.	5
	Faded colours, slightly grey (dead) colour, pale and green film.	4
	Faded colours, green, dull.	3
	Green colour, dead grey.	2
Odour	Distinct mussel odour, fresh, marine, boiled egg, creamy and butter.	9
	Typical mussel, fresh, boiled egg, neutral, sweet.	8
	Still some mussel odour, slightly boiled egg, slightly boiled potato, neutral.	7
	Slightly mussel odour	6
	Boiled potato, a bit musty, slightly earthy, slightly sour, just a little mussel odour.	5
	Boiled potato, musty, sour, earthy.	4
	Sour, slightly ammonia, musty, earthy/muddy	3
	Very sour, ammonia, bitter, Sharp odour of almonds, spoilage.	2
	Spoilage, rotten	1
Taste/flavour	Typical mussel, marine, fresh, sweet	9
	Boiled egg, liver, mussel	8
	Watery (neutral) Young cheese, sweet, creamy	7
	Boiled potato, metallic, plain.	6
	Boiled potato, slightly fishy, rubbery, slightly bitter.	5
	Tasteless, slightly fishy, green beans, no mussel taste.	4
	Watery, sour, astringent, bitter aftertaste, muddy.	3
	Sour, astringent, bitter, rancid.	2
Texture	Tender, smooth, juicy.	9
	Soft, juicy, elastic.	8
	Slightly dry or tough, easy to chew.	7
	Slightly dry or tough, less easy to chew.	6
	Tough, soft elastic.	5
	Slightly mealy, soft,	4
	Mealy, very soft,	3

The next day, during lunch time of the second day of the Final Meeting of the Blue Seed project, the sensory evaluation panel was conducted. Diploid and triploid mussels were steamed separately, but following exactly the same procedure, in the restaurant of a Social Centre in Yerseke near to IMARES. Mussels were delivery to each panellist in two separate plastic pans (Figure 18) labelled as A and B. Panellists (n = 14) were all of them members of the Blue Seed project (Figure 19), 8 men and 6 women, from four different countries (Wales, UK; France, The Netherlands and Galicia, Spain), 9 from RTDs (UBW, CIMA, IMARES and IFREMER), 4 from SMEs (Grainocean, OPMEGA and Deepdock) and 1 from AquaTT. Tasters rated the A and B mussels for colour/appearance (scale from 2 to 9), odour (scale from 1 to 9), taste/flavour (scale from 2 to 9) and texture (scale from 3 to 9) (Table 6). Data were analysed by a Friedman's test, which is a non-parametric test for randomized block experiments and is a generalization of the paired sign test. In this experiment each panellist was considered as a block (14 in total) and the type of mussel the treatment with two levels (2n and 3n).



Figure 18. 2n and 3n mussels in plastic pans



Figure 19. Tasting of mussels by panellists

RESULTS AND DISCUSSION

The mean ratings of the diploid and triploid mussels for the four sensory categories are shown in Table 8. Diploid mussels scored higher than triploid mussels for colour/appearance, taste/flavour and texture. For colour/appearance and texture, the differences between both types of mussels was clearly significant ($S = 6.40$; $P = 0.011$ and $S = 7.00$; $P = 0.008$). For taste/flavour the difference between 2n and 3n mussels was in the limit of significance ($S = 3.60$; $P = 0.058$). For odour, scores of diploid and triploid mussels were very similar (7.8 and 7.9, respectively) and not significantly different ($S = 1.00$; $P = 0.317$).

Table 8. Mean ratings (standard deviation) for the four sensory categories and the total score in diploid and triploid mussels

Sensory category	Mean rating (s.d.)		Friedman test
	Diploid mussels	Triploid mussels	
Colour/Appearance	8.4 (0.76)	7.6 (0.84)	$S = 6.40$; $P = 0.011$
Odour	7.8 (1.12)	7.9 (0.73)	$S = 1.00$; $P = 0.317$
Taste/Flavour	8.4 (1.08)	7.6 (1.78)	$S = 3.60$; $P = 0.058$
Texture	8.4 (0.51)	7.7 (1.06)	$S = 7.00$; $P = 0.008$
Total score	33.0 (2.45)	30.9 (3.60)	$S = 4.57$; $P = 0.033$

On the whole, both types of mussels got a good acceptance by the panellists. Diploid and triploid mussels obtained very high total scores: 33.0 and 30.9, respectively, of a maximum of 36. It was agreed by most of the panellist that diploid mussels show a slightly more strong marine flavour than triploid mussels. However, some of the panellist considered that the less intense and more neutral flavour of the triploid mussel could favour their acceptance by the consumers not accustomed to eating mussels or other marine products.

Conclusion:

- Triploid mussels appear to have a different colour and texture to diploid mussels
- No significant differences were detected in odour or taste of triploid and diploid mussels