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Report

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Optimization of Dover sole reproduction

Final report for task 1.2 Upscaling of sole fingerling production: Production of Dover sole eggs

Design and development of commercial scale farming technologies for sole

SOLEMATES
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1. Introduction

In aquaculture unpredictable and variable reproductive performance is an important limiting factor for the successful mass production of juveniles (Izquierdo, Fernandez Palacios et al. 2001). Therefore a reliable supply of sufficient numbers of good quality eggs and larvae is essential for the establishment of commercial scale production of Dover sole. Dover sole are reported to spawn readily and naturally in captivity and the buoyant, fertilized eggs are collected easily from the spawning ponds (Baynes, Howell et al. 1993). Fertilization rates are however rather variable (Howell 1997). To sustain a large scale commercial industry the reproductive performance of Dover sole in captivity is insufficient and improvement is clearly needed.

In general, maturation of fish is influenced by a multiplicity of factors and environment plays a very important role (Bromage, Porter et al. 2001). This means that broodstock management practices potentially greatly affect successful reproduction of sole in captivity. The first step towards a more reliable production of fertilized eggs is therefore the establishment of best practices for broodstock management. The procedure for Dover sole reproduction is relatively simple and consequently most published accounts of sole spawning in captivity are descriptive rather than analytical (Imsland et al., 2004).

This study aims at increasing our understanding of the effects of different aspects of broodstock management on the production of fertilized eggs. These aspects include delayed and postponed spawning by photoperiod and temperature manipulation, broodstock nutrition, bottom substrate, F1 males and tank size.

Spawning out side the natural spawning season enables the continuous supply of juveniles for stocking of ongrowing facilities and displacement of spawning will therefore be an important element in broodstock management. The spawning season of Dover sole has been successfully displaced by several months by manipulation of photoperiod and temperature but in advanced broodstock the amount of eggs and the viability of the eggs was observed to be adversely affected (Imsland, Foss et al. 2004).

Production of Dover sole juveniles in captivity is still dependent on collection of wild breeders. This dependency on natural stocks hampers the set up of selective breeding programs in order to improve production performance. So far all attempts to involve the first generation off spring of wild breeders (F1) in juvenile production have failed.

The first objective of this study was to make recommendations for Dover sole broodstock management that improve and secure the production of fertilized eggs.

Bottom substrates are hardly used in broodstock tanks for Dover sole. Most likely this is the result of practical disadvantages such as poor self cleaning capacity and spreading of substrate through culture systems. However, burying in bottom substrate is an important aspect of the natural behaviour of Dover sole. It is not unlikely that offering sole breeders a bottom substrate improves reproductive performances as captive conditions then correspond more to natural conditions.

Improvement of broodstock nutrition and feeding has been shown to greatly improve egg and sperm quality in aquaculture. Lipid and fatty acid composition and vitamin E and C content of broodstock diets have been identified as major dietary factors that determine successful reproduction (Izquierdo, Fernandez Palacios et al. 2001). Our broodstock were fed three kinds of fresh, natural feeds every week: fresh mussel meat (*Mytilus edulis*), ragworm (*Nereis virens*, farmed) and lugworm (*Arenicola marina*). It is however unknown whether this diet covers all nutritional requirements. Therefore a treatment was installed in this study in which breeders were additionally fed with an enriched feed to test the effect of supplying a surplus of essential nutrients associated with fecundity, egg development on egg production and fertilization rate.

It is unknown whether the size of currently used broodstock tanks limits Dover sole in displaying their spawning behaviour and thereby limits its reproductive performance. Therefore a large tank was installed as a treatment in this study.

The second objective of this task was the production of eggs for project partners to be used to produce the juveniles required for experiments within this project.

2. Materials and Methods

2.1 Experimental period

The study started in November 2002. The actual experimental period started on the first of January 2003. This day is referred to as Day 1. The experimental period lasted for 161 days. On this day the last batch of eggs was spawned.

2.2 Treatments in broodstock management

At RIVO a broodstock of approximately 250 animals with a total biomass of 79.8kg was used to install the different treatments of broodstock management. About 10% of this broodstock was collected at sea in 1997/1998; the rest was collected at sea during the summer of 2002. These animals were PIT-tagged, sexed and weighed and randomly distributed over 7 broodstock tanks. Generally a sex ratio of 1:1 and a density of 3 ind/m^2 was established. In total seven different treatments were implemented to test effects of broodstock management on egg production and fertilization rate.

2.3 Advanced spawning (tank 1)

In this study photoperiodic controlled spawning tanks (tank 1 and 2) were first of all installed to lengthen the juvenile production period. In addition, these tanks provide the opportunity to study the effects of out of season spawning on reproductive performance.
Spawning of the fish in tank 1 was advanced by two months by compression of the annual cycle of day-length and water temperature. The temperature was increased at the end of January from 7°C to 10°C, the day-length was increased weekly and increased from 8 hours to 16 hours of light by which spawning was advanced 2 months.

2.4 Postponed spawning (tank 2)

Spawning of the fish in tank 2 was postponed by two months by lengthening the annual cycle of day-length and water temperature. Initially the day-length was kept constant at 8 hours of light until the end of March. The temperature was kept at approximately 10°C and decreased to approximately 8°C at the beginning of March.

2.5 Gonad sampling (tank 3)

In this tank a double density was stocked to have fish available for destructive sampling for gonad tissues. Samples were taken on day 80 and 163 of the experimental period. Seven males and seven females were sampled at each occasion. The results of the histological studies of gonad tissue are reported in a separate report. The reproductive performance of this group is reported in this report.

2.6 F1 males (tank 4)

A group of breeders was comprised of F1 males and wild females to test the reproductive performance of farmed male Dover sole. Gonad tissue and blood samples were taken form the F1 males to investigated the reproductive performance of these fish.
The results of the histological studies of gonad tissue are reported in a separate report. The reproductive performance of this group is reported in this report.

2.7 Sand substrate (tank 5)

In this tank the bottom was covered with a 10cm layer of sand to test the effect of a bottom substrate on reproductive performance.

2.8 Enriched diet (tank 6)

Once a week lugworms were enriched with DHA Selco. In order to facilitate injection of Selco, it was diluted with water in a 3:1 ratio. Each worm was injected with 0.6 ml Selco-water suspension

2.9 Large tank (tank 7)

To investigate the potential positive effects of a large broodstock tank on Dover sole reproduction in captivity, a large broodstock tank was installed. This tank was circular and had a bottom surface area of 16m^2.

2.10 Location of the tanks

Tank 1 and 2 were located at RIVO. The tanks contained full strength seawater and were operated in a large and low loaded recirculation system with a total volume of 50 to 100m^3, depending on the amount of seawater in stock. Water was treated in gravel beds. Photoperiod was controlled by covering the tanks with black plastic and installing timer controlled electric light. The water temperature was controlled by installing cooling units.

Tanks 3, 4, 5 and 6 were located at the pilot system in IJmuiden. In these tanks borehole water (25 ppt) was used, which was for 60%/day renewed. As the system was located in a green house there was a natural photoperiod. In general, this system followed the outside air-temperatures. In January 2003 two cooling machines were added to the system for better temperature control. The system showed a diurnal variation in water temperature of 1°C maximum. The temperature was recorded automatically every 10 minutes with a logging system.

Tank 7 was located at Zeeland Vis and was flown through with surface water from the Oosterschelde estuary and had a natural annual light and water temperature cycle.

Table 1 provides an overview of the treatments.

Table 1: Overview of broodstock management treatments

Tank	Size (m^2)	Stock	Treatment
1	7	11M/10F	2 months advanced
2	7	11M/10F	2 months postponed
3	10	26M/33F	Sampling of gonad tissues
4	10	15 Farmed M/15F	Effect farmed males
5	10	14M/16F	Sand substrate
6	10	14M/14F	Enriched feed
7	16	28M/26F	Effect tank size

2.11 Feeding

The fish were fed three different diets. Each diet was fed once a week, resulting in three feeding days per week. The feeding level was 1% BW on a wet weight basis per feeding. The different diets were: fresh mussel meat (*Mytilus edulis*), ragworm (*Nereis virens*, farmed) and lugworm (*Arenicola marina*). The fish in tank 6 were fed the same with the exception that the lugworms were injected with DHA Selco as described above.

2.12 Egg collection

In all tanks eggs were collected from the outflowing water outside the tank in a collector equipped with a fine mesh net. The system in the greenhouse (tanks 3 to 6) initially yielded poor quality eggs, which was believed to be due to the long residence time of the eggs in the pipes leading to the egg collectors. The system was therefore adapted and equipped with an airlift. This resulted in improved egg quality from these tanks.

2.13 Determination of fertilization rate

Each batch of eggs was weighed and a sub-sample of the floating eggs of approximately 100 eggs was taken to determine fertilization rate. Fertilization rate was determined by counting the number of fertilized eggs and the total number of eggs in the sample using a light microscope. In some samples fertilization rate was easily determined as the multi-cellular stage was easily observed. In other samples, direct determination of fertilization proved difficult. Those samples were incubated individually in a Petri-dish at room temperature (16°C on average). Fertilisation rate was determined after one day of incubation when embryos could be easily detected.

2.14 Determination of the time of spawning

In order to get an idea about the timing of the spawning and the number of different spawns within one batch of collected eggs, an apparatus was developed that could sample spawned eggs at discrete time intervals continuously during the night. This 'spawning timer' was made from a belt-feeding machine which slowly pushed a cassette with small boxes with a mesh bottom through a flow of water. Approximately 10% of the tank overflow was pumped over the spawning timer. The apparatus was started every day at 17:00 and stopped at 8:00-9:00 the next morning. The content of each container was weighed and recorded. Photos of the spawning timer are shown in Appendix I.

2.15 Video of spawning

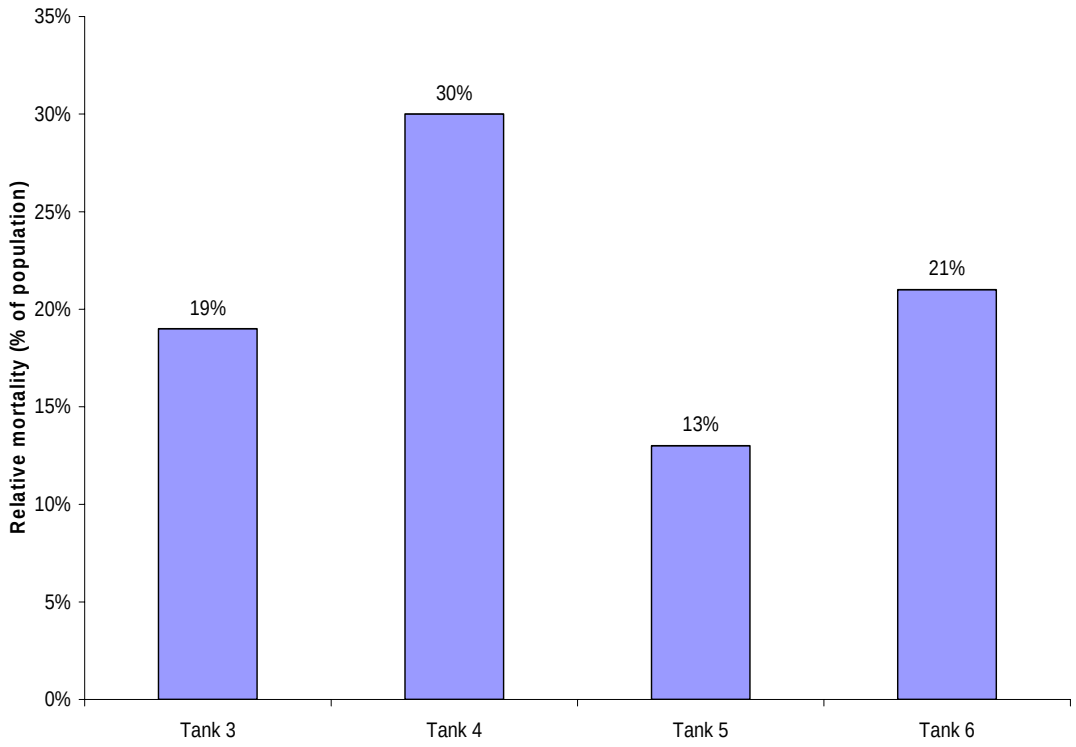
Visual observation of spawning behaviour could give important clues with regard to reasons for low fertilisation. On 25 February 2003 an underwater-camera was installed in the tank 1. The camera was connected to a monitor and a recorder and installed halfway the water column giving a view of app. 50% of the tank volume. In order to make recordings in the dark period, 3 blue lights were installed over the water surface producing roughly 20 lux at the water surface. A number of recordings was made before dark with normal light (100 lux at surface) in the period of app. 17.00 to 20.00 hour and subsequently for 3 hours with the blue light on. The recordings in the 'dark' with the blue light were stopped after a day for fear of disturbing the spawning behaviour as it could not be excluded that the fish couldn't see the blue light.
On 6 March 2003 the video observations were abandoned all together because during these observations production of fertilised eggs had stopped completely.

3. Results

3.1 Mortalities

During the period of November 2002 until March 2003 two mortalities were registered in the total broodstock of 189 fish (excluding ZLV). However, at the end of March a heavy mortality was observed in tanks 7 to 8. Over a period of a few days 17 fish died of a total stock of 132 animals in these tanks. Especially ripe females seemed to be affected and died (14 females against 3 males; average GSI females 10.6%). Fish were examined by a pathologist and seemed to suffer from an infection of the gut. The fish were successfully treated with a bath treatment of Flumequine at a concentration of 17 mg/l during 24 hours.
The mortality in the tanks 3 to 6 is presented in Figure 1.
Almost total mortality was observed in tank 7 at Zeeland Vis at the beginning of March. These fish were kept on flow-through with surface water from Oosterschelde. This water had cooled down to 2°C at this time of the year which is lethal for Dover sole. Dover sole have been reported to be sensitive to cold winters and can show significant mortality in the North Sea during severe winters. As a result all fish in tank 7 were lost and no results were obtained from this treatment.

Figure 1. Mortality in Tanks 3 to 6 during the experimental period



3.2 Egg production and fertilization rate

The eggs collected daily from each broodstock tank throughout the experimental period are presented in Figures A till E in Appendix II. Per day the amount of fertilized and unfertilized eggs are presented.
Table 2 and 3 provide overviews of the results of the different treatments in this study. Table 2 presents spawing characteristics in terms of time, temperature and day-length at the onset and end of spawning for all broodstock tanks. Table 3 presents the reproductive performance of all broodstock tanks in this study. More detailed results are presented below for each treatment.

Table 2: Spawning characteristics

Tank	Treatment	Start of spawning			End of spawning		
		Day	T (°C)	Day length (hrs)	Day	T (°C)	Day length (hrs)
1	Advanced.	36	9.6	14.25	134	11.1	16.5
2	Postponed	61	10.0	8	161	11.6	13
3	Gonad sampling	110	8.8	14.25	158	15.0	16.75
4	F1 males	110	8.8	14.25	156	15.0	16.75
5	Sand	107	8.8	14.25	160	15.0	16.75
6	Enriched feed	109	8.8	14.25	156	15.0	16.75

Table 3: Overview reproductive performance

Tank	Treatment	N batches	N batches fertilized	% batches fertilized	Total N of eggs	Overall fertilization rate (%)[1]	Highest fertilization rate (%)[2]	Average fertilization rate of fertilized batches (%)[3]	Fecundity (g eggs/kg female)
1	Advanced.	82	28	34.1	3.422.967	8.7	67	18	735
2	Postponed	57	10	17.5	2.123.450	5.2	31	13	474
3	Gonad sampling	30	14	46.7	1.177.050	11.9	90	23	285
4	F1 males	32	0	0	1.033.620	0.0	0	0	448
5	Sand	40	27	67.5	1.725.360	18.8	72	26	440
6	Enriched feed	22	7	31.8	690.550	7.3	57	26	365
TOTAL		263	90	34.2	10.172.997	9.1		17.7	478

[1] Total number of fertilized eggs collected as a percentage of the total number of eggs collected during the entire spawning period.
[2] Highest fertilization rate obtained in a single batch
[3] Total number of fertilized eggs collected as a percentage of the total number of eggs in all collected batches that contained fertilized eggs during the entire spawning period.

Advanced spawning, tank 1

The day-length and water temperature for treatment 1 are presented in Figure 2. From Figure 2 it is clear that the onset of spawning in this treatment coincides with the rise in water temperature from 7°C at day 23 to 9.6°C at day 36.

Postponed spawning, tank 2

The day-length and water temperature for tank 2 are presented in Figure 3. In tank 2 some small batches of eggs were collected at day 61 to 67 after a temperature rise from 9.6°C at day 36 to 10.0 °C at day 61. At the onset of spawning at day 61 the water temperature was immediately reduced and reached 8.2°C at day 72. This successfully altered spawning for some time but could not prevent the restart of spawning two weeks later at day 86 at 8.2°C. Another direct drop in temperature to 7.8°C reached at day 88 altered spawning again. At day 107 at a water temperature of 8.3°C spawning of large batches started. Spawning of good quality batches of eggs started at day 128.

Gonad sampling, tank 3

The day-length and water temperature for tank 3 are presented in Figure 4. Spawning started at day 110 after a rise in temperature from 8.8°C at day 101, peaking at 13.5°C at day 108 and dropping to 8.8°C at day 110.

F1 males, tank 4

The day-length and water temperature for tank 4 are presented in Figure 4. Spawning started at day 110 after a rise in temperature from 8.8°C at day 101, peaking at 13.5°C at day 108 and dropping to 8.8°C at day 110.

Sand substrate, tank 5

The day-length and water temperature for tank 5 are presented in Figure 4. Spawning started at day 107 after a rise in temperature from 8.8°C at day 101, peaking at 13.5°C at day 108 and dropping to 8.8°C at day 110.

Enriched feed, tank 6

The day-length and water temperature for tank 6 are presented in Figure 4. Spawning started at day 109 after a rise in temperature from 8.8°C at day 101, peaking at 13.5°C at day 108 and dropping to 8.8°C at day 110.

Figure 2 Day-length and water temperature in tank 1, advanced spawning during the experimental period.

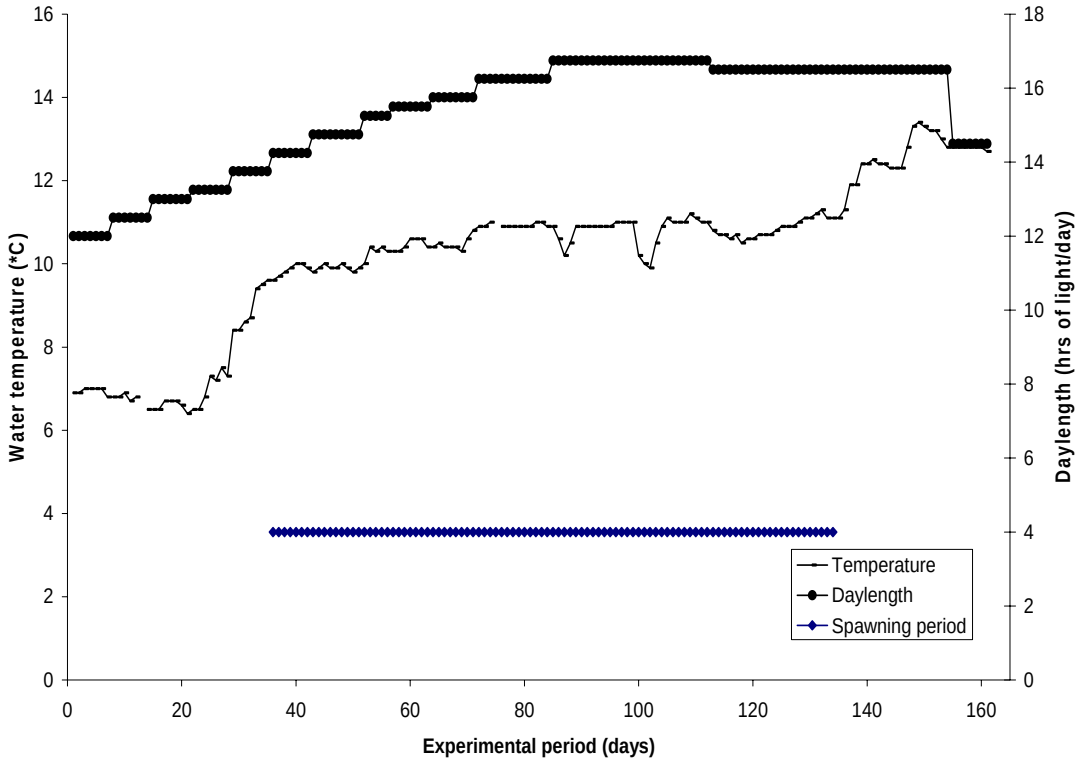


Figure 3 Day-length and water temperature in tank 2, postponed spawning during the experimental period.

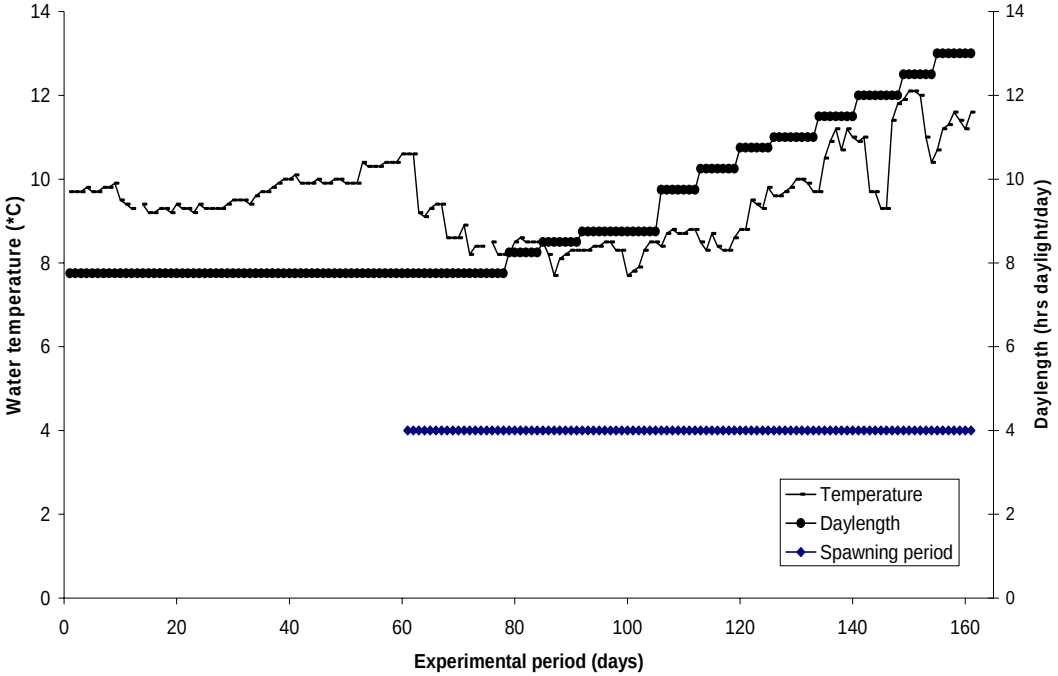
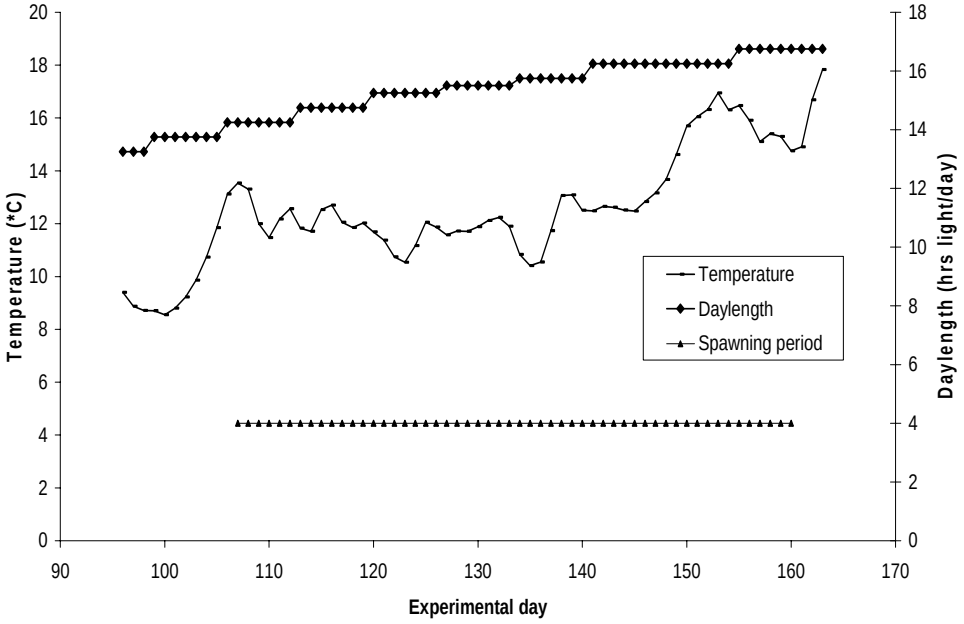


Figure 4 Day-length and water temperature in tank 3, 4, 5 and 6 during the experimental period.



Overall results

In Figure 5 the relative number of batches containing fertilized eggs is presented for the different treatments. In Figure 6 the overall fertilization rate of all batches collected in each broodstock tank is presented for the different treatments. In Figure 7 the number of fertilized and not fertilized eggs per kg of female body weight during the whole spawning season is presented.

Figure 5 Relative number of batches containing fertilized eggs for each of the broodstock tanks

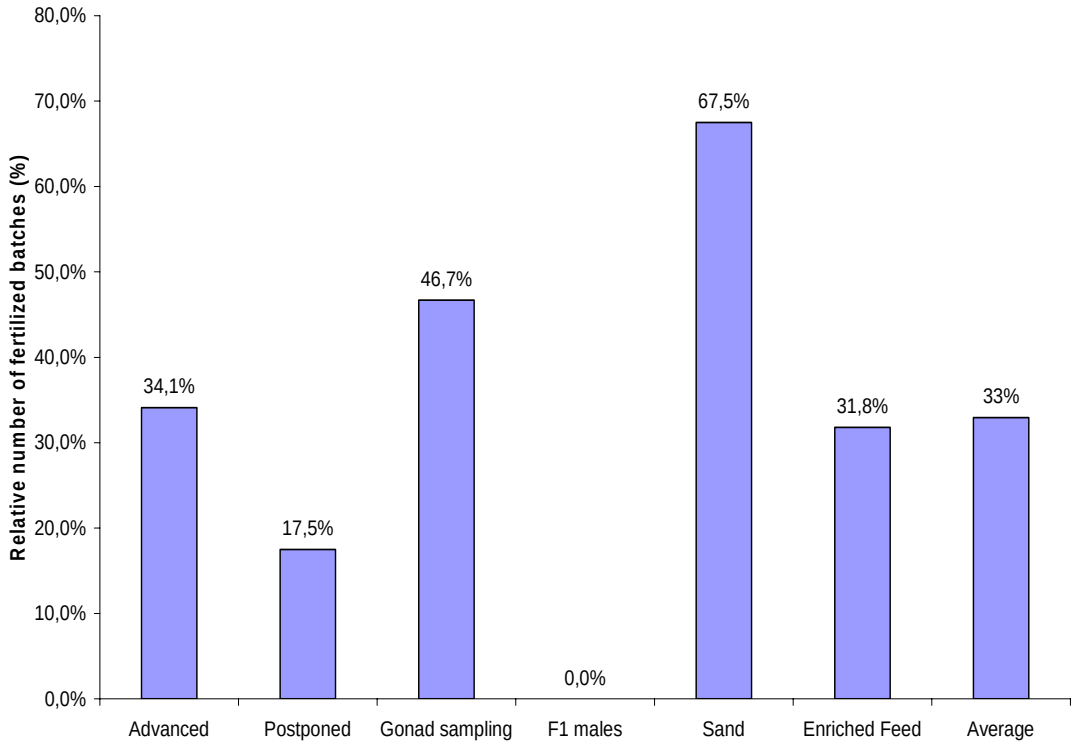


Figure 6 Overall fertilization rate of all batches collected for each broodstock tank.

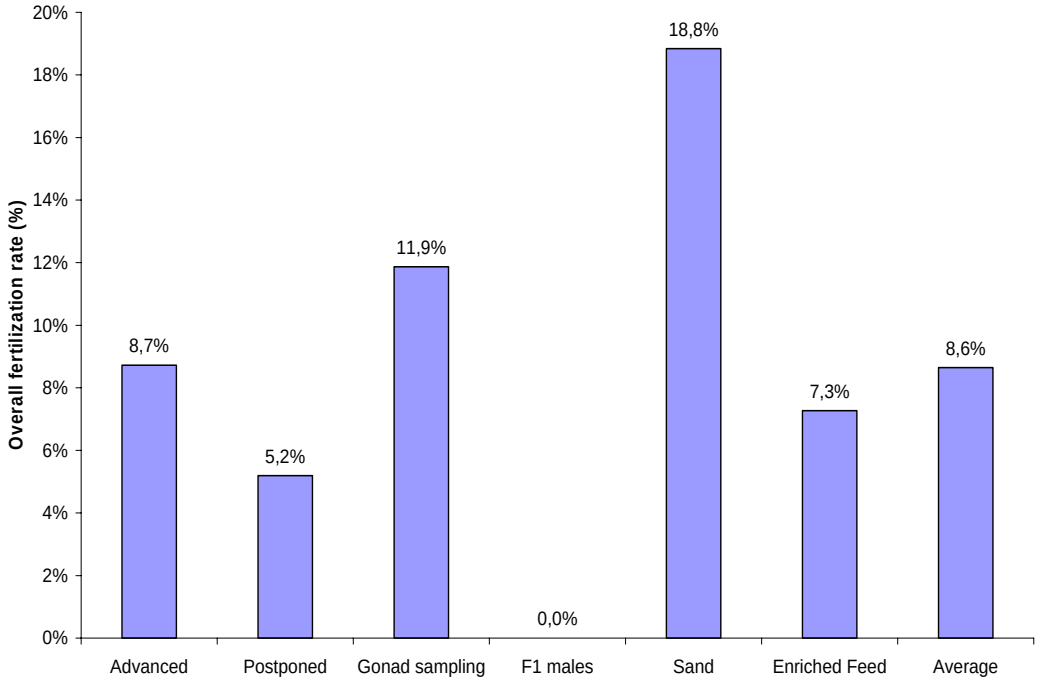
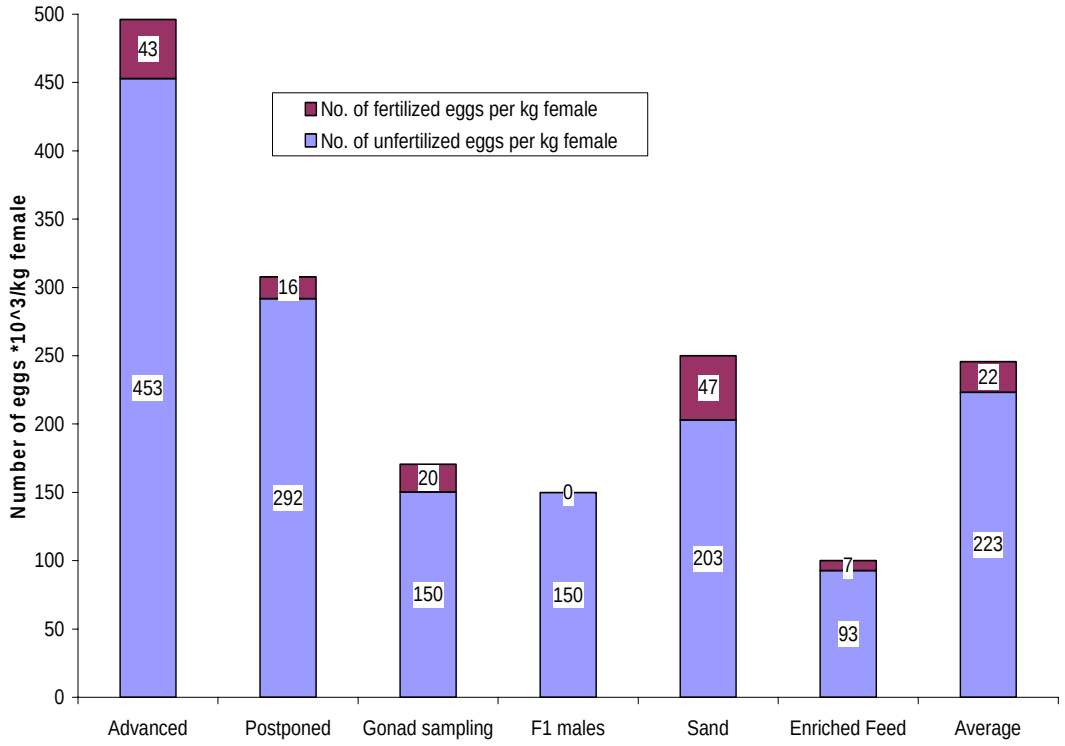


Figure 7 Relative number of fertilized and not fertilized eggs collected during the whole spawning season for each broodstock tank



3.3 Determination of the time of spawning

The time of spawning was measured on 17 occasions, all in tank 1. The Figures 8a, b and c present examples of the results. The amounts of eggs collected are plotted to the time intervals in which they were collected. This provides insight in the timing of spawning. In 4 out of the 17 samplings a single peak could be detected. On all other occasions there is more than one peak which indicates multiple spawning.
The grey area indicates the dark period. In Figure 9 presents pooled data of the 17 individual samplings. From this figure it can be derived that 70% % of the batches are produced after dark and mostly between 22.00 and 02.00 hour. However, it is clear that spawning does not exclusively take place during the dark hours. It is found that there is already considerable spawning activity late afternoon and during the early evening.

Figure 8 a, b and c. Examples of results obtained with the spawning timer.

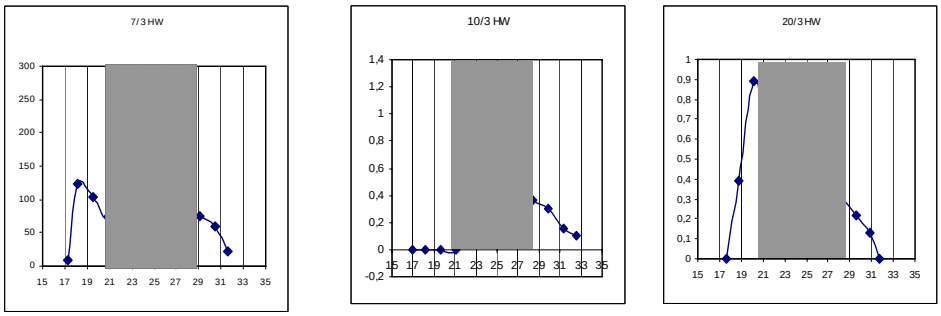
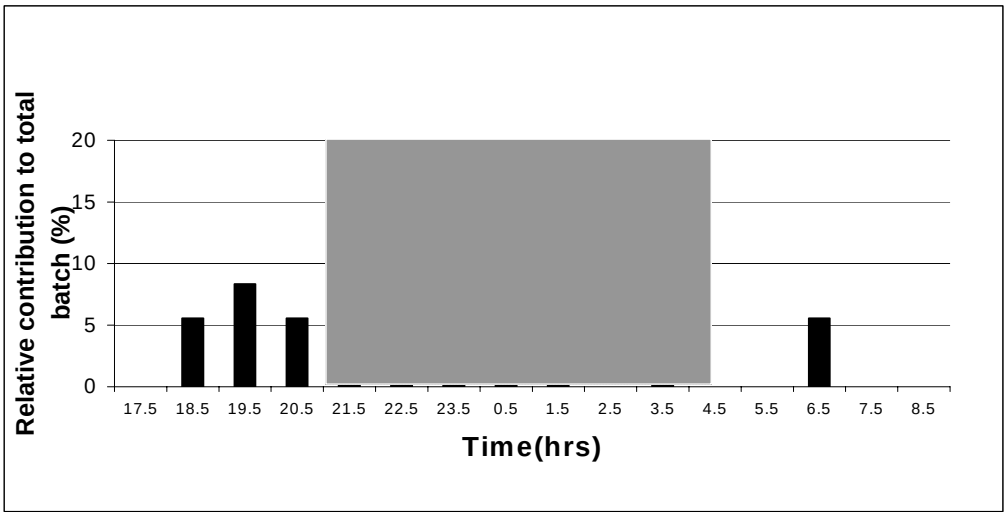


Figure 9 Pooled data of the 17 individual samplings



3.4 Video recording of spawning behavior

The recordings showed continuous activity of male fish 'checking' the females. A few occasions were observed in which a male fish chased a female from the bottom and swam synchronous under the female to the surface. In those cases egg expulsion was never observed. In two cases expulsion of eggs by a female lying on the bottom was observed without any interference from a male fish. This release of eggs results in the production of unfertilized batches of egg or, in case of multiple spawning, in low fertilization rates. The reasons for this release of eggs without male interference and subsequent fertilization remain obscure.
On one occasion a visual observation was made (not on video) of spawning fish during daylight (1800 h). In this case a male and a female were hanging vertically under the water surface while the female was shedding eggs. Expulsions of sperm could not be observed in this case. The next morning fertilised eggs were detected in the collector.
In order to get observations during peak-spawning, an infra-red camera should probably be used.

3.5 Production of eggs for the project partners

Shipment of whole batches of eggs probably would have led to deterioration of the water due to the presence of dead eggs and ultimately poor quality larvae upon arrival. Therefore batches of eggs selected for shipment were incubated until hatch to separate fertilized eggs. Separation of floating eggs from not floating eggs proved to be an unsuccessful method for this purpose. During the spawning season, it was found that there's no clear relationship between the floating and fertilization rate. There were many batches of eggs with high floating rate in full strength seawater but with low or even zero fertilization rate. There were some occasions when most of the sinking eggs proved to be fertilized and the corresponding batches of larvae hatched and proved to be healthy and active. Therefore sinking eggs were only discharged from the incubators in the afternoon of second day after the start of hatching.
A total of 262.205 larvae were sent to project partners in Greece, Portugal and Wales. Table 4 provides an overview of the batches of larvae send. Prior to larger batched, small test batches were sent in most cases to check whether the larvae arrived in good order.

Table 4: Overview of batches of larvae sent to project partners

Batch	No of larvae	Date	Country
HW21/3	8820	26-3-03	Portugal
HW2/4	8686	7-4-03	Greece
HW3/4	2125	7-4-03	Greece
HW16/4	21561	22-4-03	Greece
HW17/4	13685	22-4-03	Greece
HW18/4	5751	24-4-03	Wales
HW 20/4	5000	24-4-03	Wales
S9 24/4	17000	28-4-03	Wales
HW8/5	2233	13-5-03	Portugal
HO8/5	14268	13-5-03	Portugal
HO12/5	17902	19-5-03	Greece
HO15/5	22050	21-5-03	Belgium
S9 25/5	15912	27-5-03	Wales
S7 26/5	47330	27-5-03	Wales
S9 26/5	19882	27-5-03	Wales
S7 30/5	40000	3-6-03	Wales
total	262.205		

4. Discussion

4.1 Mortality

From Figure 1 it is clear that the lowest mortality occurred in Tank 5 with the sand substrate. This suggests that sand might have a beneficial effect on survival. Unfortunately the effect could not be confirmed statistically due to lack of replications of treatments. Remarkably, a similar effect of sand on mortality was found in the sand trial within Task 3c Ongrowing in circular tanks of this project.

4.2 Egg production and fertilization rate

Advanced spawning, tank 1
In this treatment the rise in temperature triggered the onset of spawning. This is consistent with previous experience gained at RIVO and Baynes et al. (1993) who reported that a rise in temperature is an important environmental trigger for spawning.
Under natural North sea conditions Dover sole spawn in early spring; March to April (Fonds 1975). This means that in this trial spawning was effectively advanced by 1 to 1.5 months.

Postponed spawning, tank 2
In this treatment spawning was not effectively postponed. This is probably due to the relatively high winter temperature of 9.6°C. In previous experiments at RIVO it was found that spawning can be effectively postponed by extending the winter period at water temperatures of approximately 8°C. Spawning is then induced by raising the temperature to 10-11°C. Postponing spawning in this treatment was also attempted by keeping the fish at a short day-length of 8 hours. Clearly this short day-length did not prevent the onset of spawning in this tank. In this study temperature was found to be a stronger environmental trigger for spawning than day-length. This is in contrast with Bromage et al. (2001) who reported that in flatfish it is probably the seasonally changing pattern of day-length that initiates spawning. Displacement of the natural spawning period of Dover sole by temperature manipulation alone has been reported by Lenzi and Salvatori (1989)(Imsland, Foss et al. 2004)

Gonad sampling, tank 3
An effect of double stocking on fertilization rate cannot be derived from this trial as a total of 14 fish was removed on day 80 of the experimental period, making the density similar to all other treatments. Significant numbers of fertilized eggs were only produced after day 146 of the experimental period. This means that this treatment yielding the second best relative number of fertilized batches and overall fertilization rate, cannot be attributed to a larger number of male fish present in the tank. It is however remarkable that removing and sedating all fish during the spawning period did not seem to have a negative impact on fertilization rate and egg production.

F1 males, tank 4
The poor fertilization rate in this treatment is probably due to some kind of incapability of the F1 males. Blood samples and testis tissue samples where taken from these fish to investigate their reproductive functionality. The results will be reported separately. Active sperm was observed by light microscopy in semen obtained form testes dissected from F1 males in this treatment.

Sand substrate, tank 5
The overall fertilization rate obtained in this tank is the highest of all broodstock tanks in this study.

Enriched feed, tank 6

From the egg production and fertilization rate obtained in this tank it is clear that enrichment of the feed with DHA Selco did not result in improved reproductive performance. Reduced fecundity as a result of poor broodstock nutrition has been reported for several marine fish species (Izquierdo et al. 2001). It is therefore quite remarkable that in this study the fecundity found for the enriched feed treatment was the second lowest among all other treatments.

The lack of improvement of reproductive performance of the broodstock fed with an enriched feed could be due to the high variation in good quality diets fed to the fish in all tanks including three types of live or fresh feeds as described above. As a result the nutritional value of the total diet was already more than adequate and supply of additional DHA Selco did not provide any added value to the diet.

Reproductive performance

From all these results it is clear that Tank 5 with the sand bottom substrate is the best performing broodstock tank. Tank 5 yields the highest relative number of batches containing fertilized eggs, the highest overall fertilization rate, the highest total number of fertilized eggs and the highest number of fertilized eggs per kg female body weight. Apparently providing Dover sole a more natural environment such as a sand substrate on the tank bottom is beneficial for reproductive performance.

Tank 1 yields the highest number of batches and number of eggs produced of all treatments. In total number of fertilized eggs and highest number of fertilized eggs per kg female body weight Tank 1 is the second best performing tank after Tank 5. This however is due to the high egg production in Tank 1, the relative number of fertilized eggs is below average. Tank 1 is characterized by the lowest winter temperature of all broodstock tanks. In Tank 1 the water temperature reached 6.5°C whereas Tank 2 and the Tanks 3 to 6 reached minimal temperatures of 7.7°C and 8.5°C respectively.

Although nothing definitive can be concluded from the current results, it seems worthwhile to investigate the effect of minimal winter temperature on total egg production. Certainly winter temperatures below 7°C are recommended for Dover sole broodstock.

It is quite remarkable that the egg production in the four tanks (3, 4, 5 and 6) which were subject to the same photoperiod and temperature regime, takes places in a very similar period.

The effect of salinity was not systematically studied in this trial. However, different salinities existed between treatments. In tank 1 and 2 full strength seawater was used (33ppt) and the salinity was thereby the same as under natural conditions. In the tanks 3, 4, 5 and 6 the salinity was 25ppt and thus rather low compared to natural conditions for Dover sole (33ppt). Although the current results do not provide conclusive evidence for an effect of salinity on reproductive performance, it seems that a low salinity (25 ppt) does not have a negative effect on reproductive performance of captive Dover sole compared to conditions with natural salinity (33ppt).

5. Conclusions and recommendations

As no replications for the treatments were present, results cannot be tested for significant differences. However, the current results are valuable and strong recommendations for broodstock management of Dover sole that improve reproduction can be made

The following is concluded:

- Spawning of Dover sole can be successfully advanced by temperature and photoperiod manipulation
- A sand substrate in broodstock tanks for Dover sole leads to lower mortality among breeders, results in the highest relative number of batches containing fertilized eggs, the highest overall fertilization rate, the highest total number of fertilized eggs and the highest number of fertilized eggs per kg female body weight.
- Under the current feeding regime, feeding Dover sole breeders with DHA selco enriched feed did not result in improved reproduction.
- Short daylength (8 hours) alone is insufficient for postponing of spawning in Dover sole.

Based on the results of the current trials the following recommendations can be made with respect to broodstock management for Dover sole

- Given the high output in terms of number of eggs and batches of tank 1 and the fact that Tank 1 was the only tank with a winter temperature below 7.7°C, it is recommended that winter temperature for Dover sole broodstock reaches at least 7°C.
- Based on the current results it is strongly recommended to apply a sand substrate on the bottom of broodstock tanks for Dover sole.

6. Literature

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Appendix / The spawning timer

Appendix II Figures A - E

Figure A Daily egg production during the experimental period in tank 1, advanced spawning.

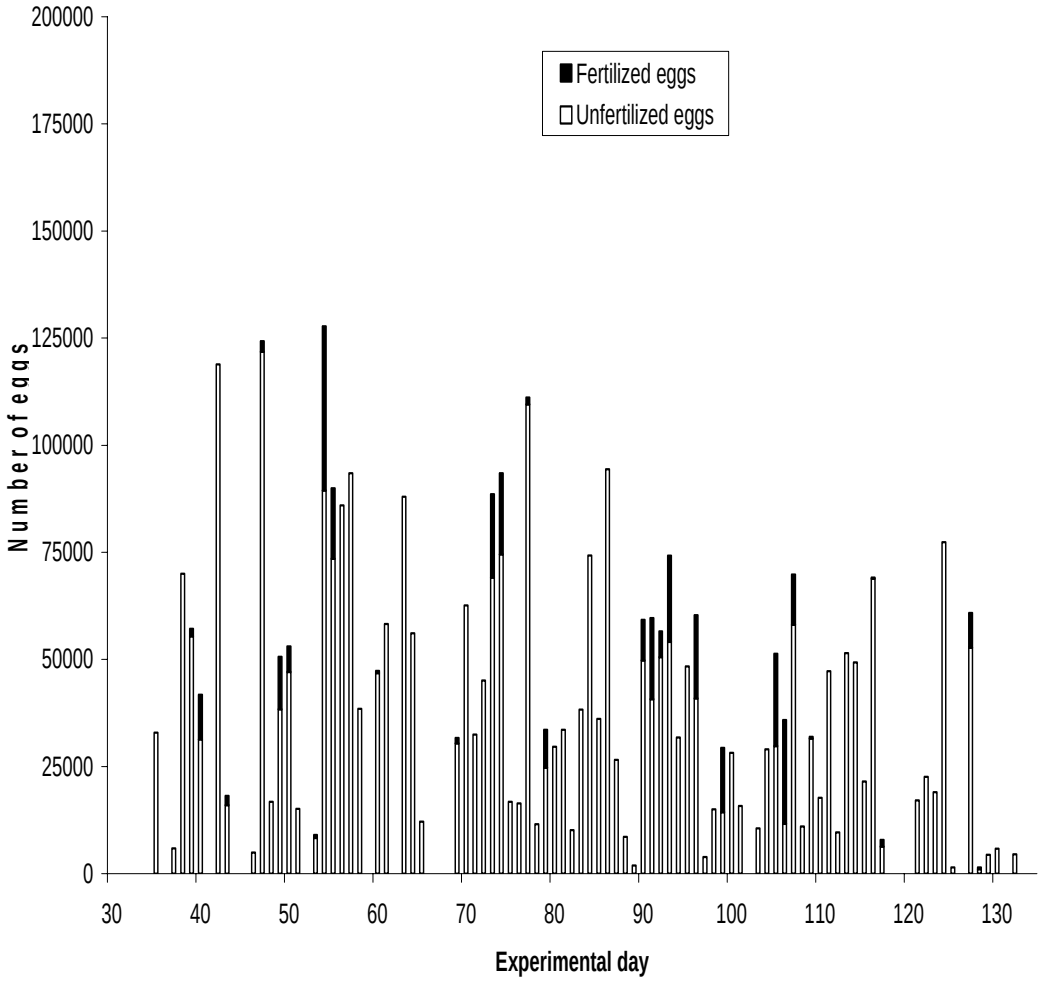

Figure B Daily egg production during the experimental period in tank 2, postponed spawning

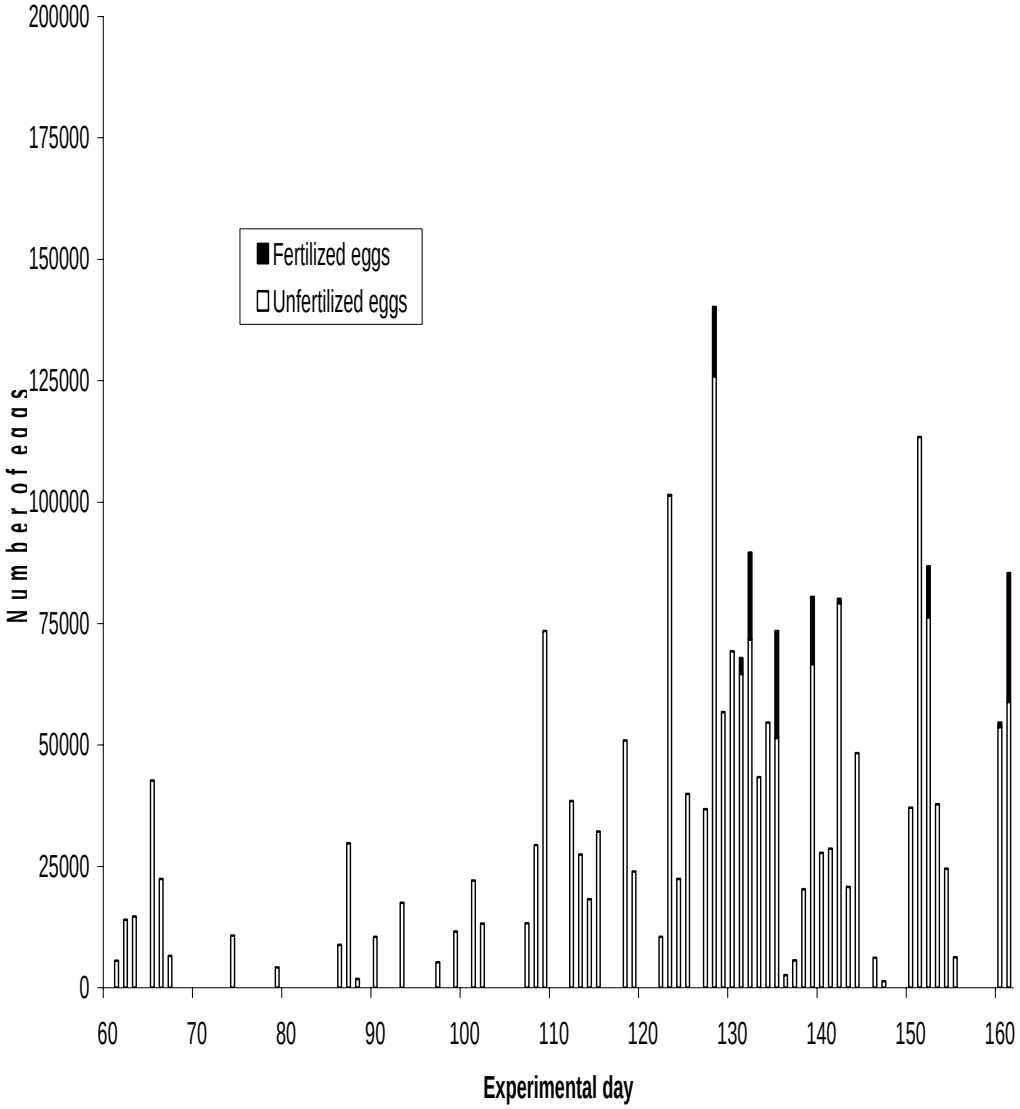

Figure C Daily egg production during the experimental period in tank 3, Gonad sampling.

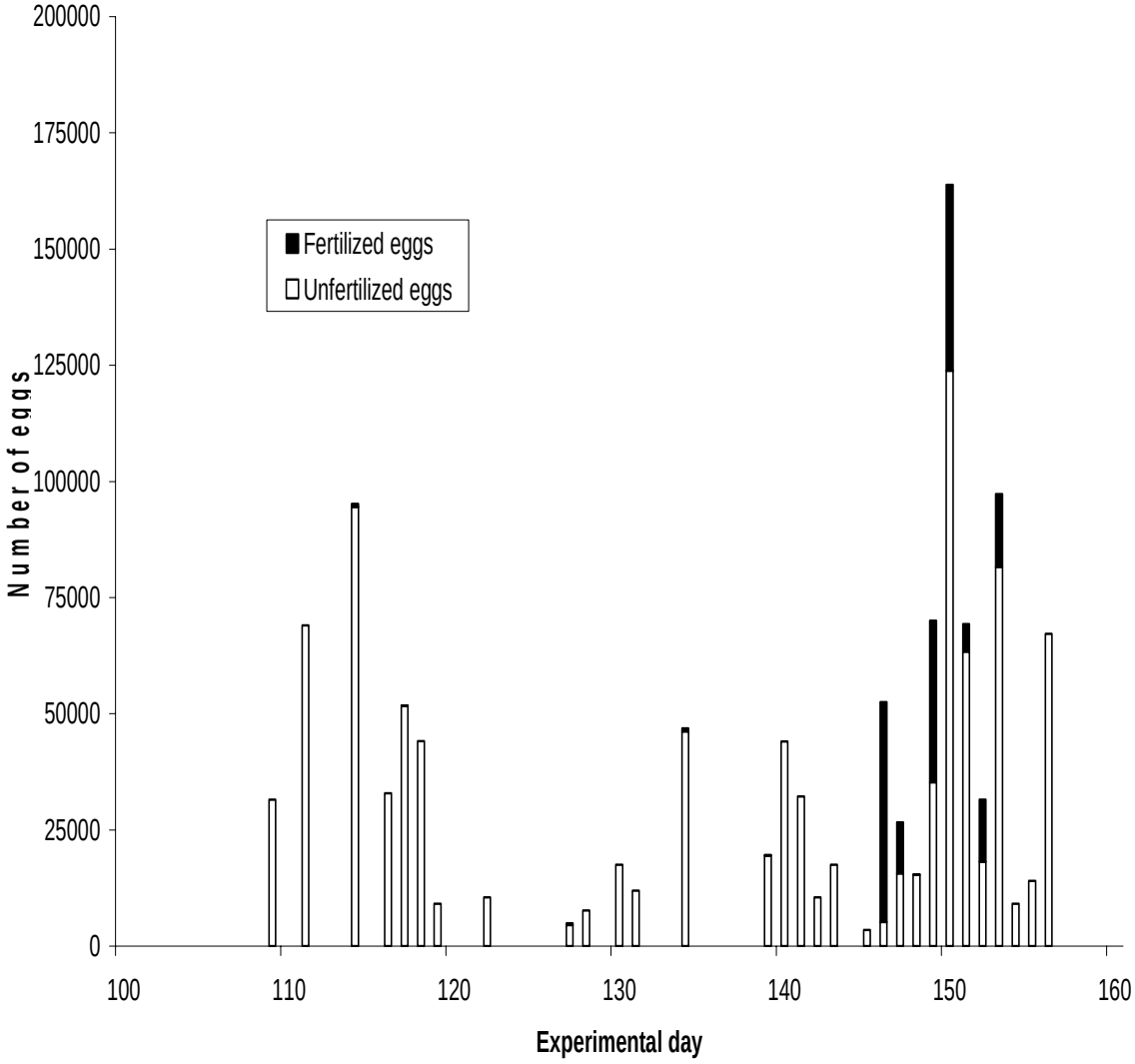

Figure D Daily egg production during the experimental period in tank 5, Sand substrate.

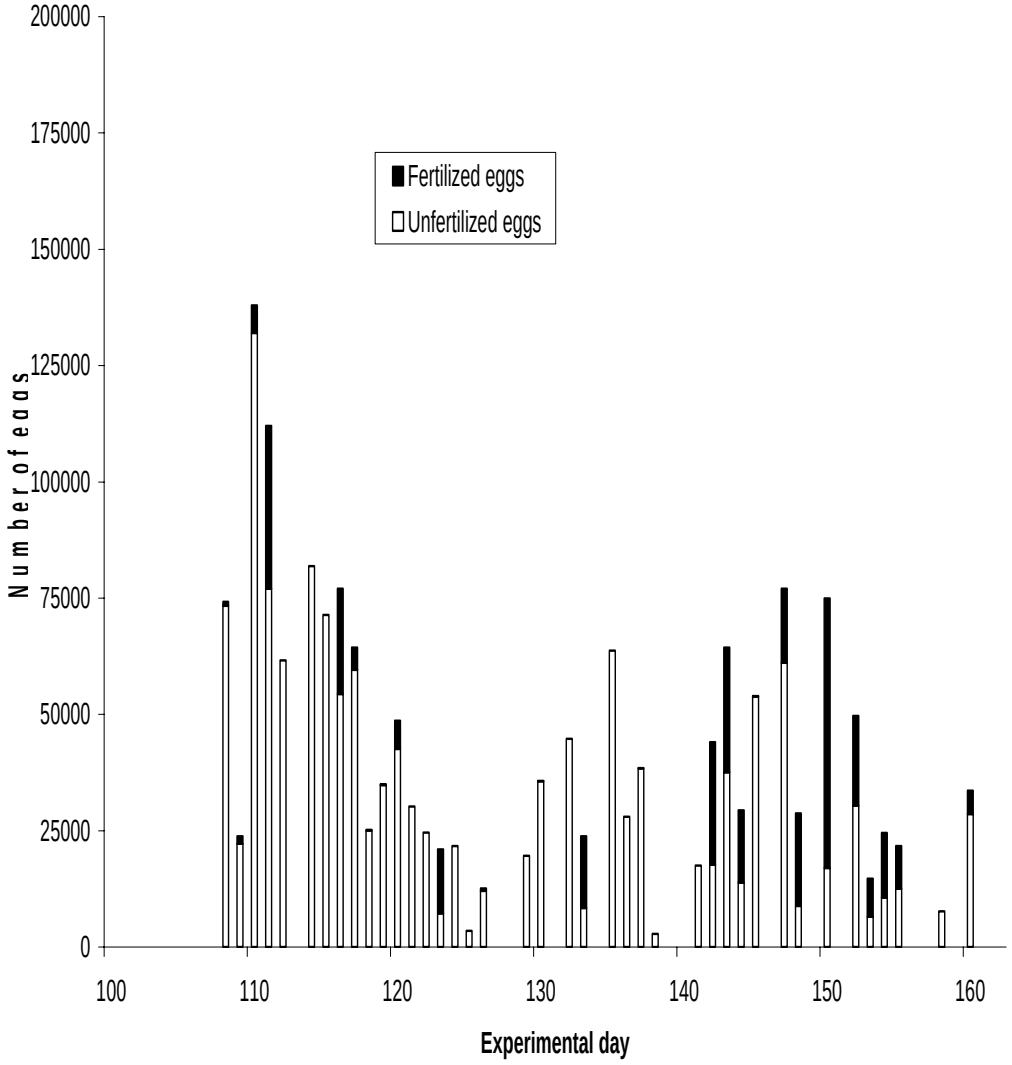

Figure E Daily egg production during the experimental period in tank 6, Enriched feed.