# Commercial-scale evaluation of advanced microbiological control techniques for marine finfish larval rearing in the UK

**OSR 06 10**

## TECHNICAL REPORT

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# TECHNICAL REPORT

## Executive Summary

The background to the project relates to the ongoing difficulty in finding a consistently successful larval rearing protocol for the two main species being cultivated in the UK: Atlantic halibut and Atlantic Cod.

There are several types of stress in marine finfish larval rearing, including water chemistry, microbiology, nutrition and physical handling. A newly available product, Protex®, based upon a natural extract from the prickly pear, has been found to be effective in accelerating the production of Heat Shock Protein (HSPs) in a wide range of animals, including fish. This effect has been demonstrated to be beneficial to the animals in resisting stress.

A pilot scale study of the use of the product at Otter Ferry Seafish (OFS) in 2006 had demonstrated some potential for beneficial use of the product in halibut larval rearing. The present project was designed to test this on a large commercial scale, using replicated experiments with two treatment concentrations and controls. The phase of halibut larval rearing targeted for an experimental approach is 'first feeding' – the stage at which larvae start to feed exogenously on live planktonic organisms cultured in the hatchery.

The experimental protocol was straightforward, and the project was able to generate sufficient material, initially in the form of stripped and fertilised halibut eggs, to fulfil the original proposed project specifications. Achievement of experimental objectives within the project was demonstrably high.

The results of the project suggested that there was no overwhelming evidence to suggest that Protex® can assist with first feeding success with halibut larval rearing, under the conditions applied to this project's experimental protocol.

It is nevertheless concluded that further work with Protex® might be justified in the broader context of marine larval rearing, and that accelerated healing, observed in the broodstock, should be investigated further.

## 1. Overall Objectives of the Project

### *1.1 Background*

The ‘marine species’ sector of UK and Scottish aquaculture continues to offer excellent market-led opportunities for further development, especially with the two species of fish best suited for cultivation in our cooler waters: Atlantic cod and Atlantic halibut. Both species have now been tested in the market place, and have been well received in their respective niches. These niches are well-researched, and there is little doubt that 30,000 tonnes of cod and around 5,000 tonnes of halibut per year, from farming, would be readily absorbed by the UK market alone – a £60 million per annum sector.

Unfortunately development of full-scale cultivation of both species has not been as fast as expected – nor as fast as the market would wish. Many technical challenges in the hatchery and ongrowing of both species have been overcome, but the reliable large-scale production of juvenile fish from hatcheries remains a problematic area for the industry, and has been the ‘pinch point’ for the sector.

The 2006 season demonstrated this difficulty very clearly. Otter Ferry’s halibut production was less than anticipated, but so too was the cod production from Machrihanish and the Isle of Man. Cod production at Ardtoe and in Shetland stayed on track, but the problems experienced at the other hatcheries were entirely unexpected, and could occur again elsewhere. More consistency within this sub sector is urgently required.

Juvenile fish can be sourced from hatcheries overseas, but this places the UK industry at a strategic disadvantage in terms of ‘whole production chain’ control. It also raises some issues about long term fish health integrity for UK Ltd. Supplies from overseas hatcheries can also be erratic, and this has implications for any full-scale production programme.

The project was designed to investigate one of the very few practical opportunities currently available which might address this persistent bottleneck in our hatchery production. Although it was centred on the halibut hatchery unit at Otter Ferry, its core work plan was equally relevant to the cod hatcheries at Machrihanish, Ardtoe, Shetland and Isle of Man.

### *1.2 Vulnerabilities in Marine Larval Rearing*

Marine finfish larval rearing is inherently more complex than the rearing of salmonids, due to the requirement to provide live first-feeding prey organisms to the very small larvae, and the difficulties in mimicking the environmental conditions in which these delicate creatures undertake their first feeding period. Salmonids are able to be first-fed directly on artificially formulated inert feeds.

The use of cultured rotifers (*Brachionus plicatilis*) and hatched brine shrimps (*Artemia salina*) in marine finfish larval rearing is well established globally, and

for some species of fish, such as seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), their use has become relatively consistent and successful. However, these are living organisms that must be introduced into the larval rearing environment at a time when the marine larvae themselves are very small and vulnerable. The prey organisms, and also the cultured phytoplankton that is also often added into the system, bring many microbial contaminants into the larval rearing tank. These tend to find the environment conducive to further growth, and the larval rearing tank thus becomes a stressful complex 'ecology' of phytoplankton, zooplankton, fish larvae – and a wide range of micro-organisms.

Various techniques have been developed to try to control this complex ecology within larval rearing tanks, but experience over more than three decades shows that the techniques are often only applicable to the fish species for which they have been developed.

There is little doubt that widespread use of antibiotics has been practised within the industry in the past, but this is not acceptable in terms of modern production systems, modern regulations, and modern consumer preferences. Furthermore, as a solution it has often proved to be lacking – micro-organisms can quickly develop resistance to antibiotics.

This project approached the problem from a different direction in order to ensure that the larval fishes have the best capacity to resist the stresses of environmental and microbial inadequacies of the hatchery facilities. Larval fish have very little capacity for inflammatory or immune or hormonal resistance to stress, but like all cellular animals they have the capacity to produce inducible chaperones (heat shock proteins) (HSPs) given enough time. These are the basic protective molecules of life, but fish take many hours to produce them naturally.

### *1.3 The Test Product - Protex®*

Protex® contains the chaperone stimulating factor which switches on the production of HSPs, before the fish is under any stress. With small aquarium fish and sea bass it has been shown to reduce mortalities by up to 70% in a wide variety of stressor situations ranging from transportation without adequate oxygen, high ammonia concentrations, and *Vibrio* bacterial infections. When used every three days in the water it maintains HSPs at a much higher tissue concentration than in the natural situation. This has parallels with the way a vaccine results in increased antibodies, but is totally non-specific in the case of HSPs.

A recent pilot study at Otter Ferry Seafish produced results suggesting 3 to 4 fold improvements in halibut larval survival to metamorphosis, when Protex® is available in the water. If this improvement could be replicated on a commercial scale it would provide the much needed consistency in production that will ensure the viability of marine fish hatcheries in the UK.

Protex® contains as its active principle TEX-OE which is a highly concentrated extract of the skin of a cactus fruit which is registered as a foodstuff in the EU and as an organic product. In another form it is widely used in the USA, France, and Russia as a human stress-reducing nutriceutical. There is therefore no problem with its use in the food chain and no therapeutic claims are made by the manufacturer, requiring veterinary registration.

### *1.4 Specific Project Objectives*

The objectives of the project were to:

1. Undertake replicated and controlled full-scale larval rearing trials using constant dosing with Protex®, at two different dose rates recommended by the UK distributor of the product.
2. Develop protocols from this data to allow it to be used in standard methodology in halibut culture and in cod culture

## 2. Description of methodology employed

### *2.1 Administration and Dose Rates*

Protex® is delivered as a water-soluble compound within absolute alcohol.

The product supplier recommended that the two experimental treatment dose rates should be:

- 1 ml Pro-Tex per 250L system volume (standard) - P1
- 2 ml Pro-Tex per 250L system volume (high) - P2

Fish should be exposed to the product at the specified dose for a minimum of 2 hours and treated every third day throughout the first feeding period.

During the first feeding stage, the larvae are held in 12,000L tanks (3.9m dia. X 1m deep). Fresh seawater is introduced tangentially at the bottom of the tank and exits via 2 banjo filters on the surface of the tank at the side. Flow rates range from 4L/min – 20L/min. Before the addition of the product, to ensure the 2 hour minimum exposure time is met, the tank level was first lowered (by siphon) by 30cm. This ensured, that even at maximum flow rate, dilution below the treatment level would not take place during the first 2 hours following treatment.
Where treatment took place in the 2m weaning tanks, following transfer from first feeding but before 650ºD, the water supply was turned off for 2 hours and oxygen levels maintained with a central aeration stone.

The quantity of Protex® to be used in the treatment (up to 100ml) was measured using a disinfected 100ml measuring cylinder. In order to aide the distribution round the tank, the Protex® was first mixed into 10L of fresh seawater in a disinfected plastic watering can. A 40cm length of 10mm I.D. silicon tubing was attached to the spout of the watering can. This was used to dispense the dose under the surface, round the circumference of the tank up

to 1m in from the edge. Mixing was achieved by the action of a single aeration stone at the centre of the tank. Using a suspension of kaolin as a visible substitute for the Protex®, this was shown to provide full mixing throughout the tank within 15 minutes. Any unforeseen failure of mixing or dilution would be covered by the double dose of the second experimental treatment. Treatments were started at 170ºD post hatch.

### *2.2 Pre-Experimental Phase – Egg Incubation and Yolk Sac*

Halibut larval rearing has 4 distinct phases:

- Egg incubation (after stripping and fertilisation), which takes place in 450 L conical vessels and which lasts for 10 days at a constant water temperature of 6 ºC. Eggs are collected and moved from incubators to yolk sac silos before hatching
- Yolk sac rearing, which takes place in 1700 L deep vessels, at a constant temperature of 6 ºC. Total darkness is maintained until the end of this phase, which normally lasts 28 days. At the end of this phase, larvae are attracted to the surface of the vessel using light, and gently collected and transferred to first feeding tanks
- First feeding, takes place in 12,000L tanks (3.9m dia. X 1m), at a temperature of 9 ºC - 12 ºC and with low levels of surface illumination (100 - 1200 lux). This is the phase where the first exogenous food is supplied to the larvae, in the form of live planktonic *Artemia salina*. There are a variety of water treatments and feed enrichment protocols that are essential during the first feeding phase, but these were identical for all the treatments within the project. The first feeding phase normally lasts 60 days before weaning commences. Larvae are transferred into the first feeding tanks from the yolksac system at 150ºD. They are kept in complete darkness under a black insulated cover. The water temperature is 6ºC at a flow rate of 4L/min. Between 210ºD - 250ºD increasing amounts of ambient temperature water replace the chilled supply, gradually raising the temperature over a 3 – 4 day period. At 150ºD - 160ºD the cover is removed, aeration started and artemia and kaolin added. Protex® treatments begin at this point.
- Weaning is the start of the process of offering inert dry pelleted diets to the larvae, and slowly withdrawing the daily additions of live *Artemia*. Weaning is considered to be complete when all the surviving larvae are exclusively eating dry diets, at which point they have also undergone the typical flatfish larval metamorphosis process.

The experimental phase of this project is completely focused on the first feeding stage, since this was identified (from the pilot project and from experience with other fish species) as the stage where Protex® would be most likely to be beneficial.

Nevertheless, Braden Ltd suggested that Protex® might also have a beneficial effect on the broodstock and subsequent egg quality. Consequently, Protex® was used non-experimentally to treat the broodstock throughout the

stripping season. Treatment was at the P1 level (1ml per 250L) in an enclosed oxygenated area within the tank, for a minimum of 1.5 hours every 3 days.

### *2.3 First Feeding Experimental Phase*

The experimental phase commenced with the first stocked first feeding tank on 02.04.07. Product dosing was based on either control (none), standard (P1) or high (P2) treatments once every three days.

With regular broodstock egg strippings throughout a season, batches of larvae do not all become ready for stocking into first feeding tanks at the same time. In order to minimise any temporal effect on the experiment, tanks were stocked and treated sequentially, i.e. P1; Control; P2; P1; Control; P2; etc. Twelve tanks in total were stocked according to the experimental protocol, giving 4 replicates each of P1, P2 and Controls.

At the end of the larval stocking period, there was sufficient material to stock a $13^{th}$ tank. It was decided to treat this single tank using a new product containing 10 times the concentration of Protex®. Treatment was at the equivalent of the P2 level (2ml/2500L).

Husbandry related procedures were the same for all tanks. Flow rate was increased from 4L/min – 20L/min over the first feeding period. Enriched artemia was fed to appetite 3 times per day. A kaolin suspension was drip fed from a 10L bucket to maintain clouded water conditions, essential for normal feeding behaviour. The flow of air to the single, central aeration stone was progressively increased in line with the increasing size and strength of the larvae. Tank bases were siphoned daily and mortalities recorded.

### *2.4 Assessing Results*

Eggs transferred to the yolksac silos were measured volumetrically and egg numbers then calculated on the basis of 40,000 eggs per litre.
After 28 days, the number of larvae in a yolksac silo was estimated by eye and the larvae from up to 4 silos were transferred into 1 first feeding tank. This was taken as the initial number. This judgement is based on over 10 years' experience, including years in which all the larvae were counted. Counting was stopped across the industry as it was considered to be damaging to the larvae. From time to time individual silos are counted as a check on the estimation.

The final number was the number of larvae remaining at 650°D post hatch (≅ 80 days). Although not weaned, these larvae are considered to be through first feeding with daily mortalities low (generally <10/day) and recordable. The number was taken as the number of larvae counted out of the first feeding tanks into the weaning tanks at 450Ɗ - 550 Ɗ, less the mortalities from that point up until 650°D. This was confirmed by an accurate count when the tank was first graded (800°D - 1000°D) with mortalities added back to 650°D. This is the figure used in the results.

% survival is the difference between the initial number and the final number, expressed as a percentage of the initial number.

## 3. Detailed description of scientific / technical achievements

### *3.1 Results*

Overall production through the initial key stages of larval rearing is shown in Table 1, and compared with results from 2006. Higher survival through egg incubation and first feeding has been the main feature of 2007.

Table 1. Numbers produced and percentage survival at each larval stage for the 2006 and 2007 seasons.

| LARVAL | TOTAL NUMBERS | | % SURVIVAL | |
|---|---|---|---|---|
| STAGE | 2007 | 2006 | 2007 | 2006 |
| Eggs Incubated | 5.3M | 6.9M | | |
| Larvae Hatched | 1.8M | 1.9M | 34.1 | 26.7 |
| Nos to First Feeding | 456,000 | 507,000 | 25.3 | 27.4 |
| Nos To Weaning | 47,628 | 25,000 | 10.4 | 4.9 |

The survival of individual experimental treatments of larvae during 2007 is shown in Table 2. The batches are listed in tank-number order, which reflects chronological order of stocking during the season.

Table 2. Results from the experimental first feeding period in chronological order.

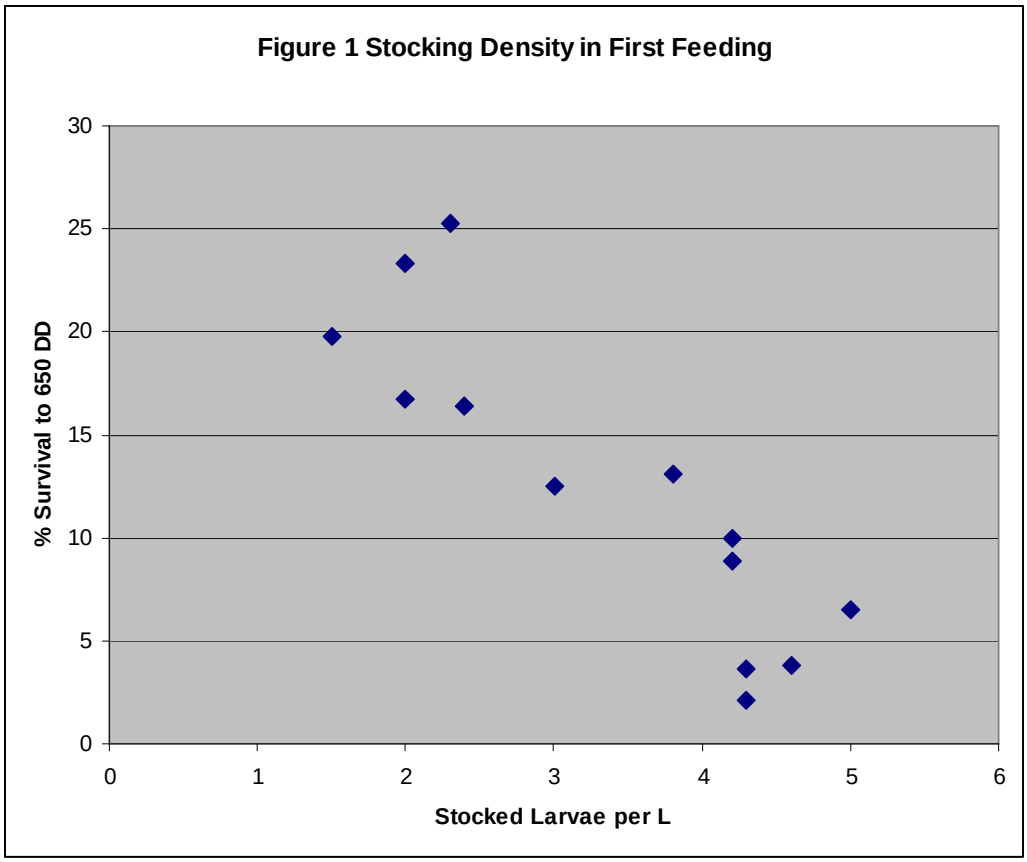
| Tank No | Treatment | Start No | No 650ºD | % Survival |
|---|---|---|---|---|
| 1 | P1 | 42,000 | 3753 | 8.9 |
| 2 | Control | 20,000 | 4654 | 23.3 |
| 3 | P2 | 43,000 | 920 | 2.1 |
| 4 | P1 | 43,000 | 1560 | 3.6 |
| 5 | control | 46,000 | 1729 | 3.8 |
| 6 | P2 | 50,000 | 3267 | 6.5 |
| 7 | P1 | 23,000 | 5811 | 25.3 |
| 8 | control | 38,000 | 4984 | 13.1 |
| 9 | P2 | 42,000 | 4200 | 10 |
| 10 | P1 | 30,000 | 3738 | 12.5 |
| 11 | control | 15,000 | 2964 | 19.8 |
| 12 | P2 | 24,000 | 3938 | 16.4 |
| 13 | P2(x10) | 20,000 | 3334 | 16.7 |

Table 3 presents the experimental results grouped according to treatment regimes. No clear advantage from using Protex® is evident, at either concentration.

Table 3. Results from the experimental first feeding period by treatment.

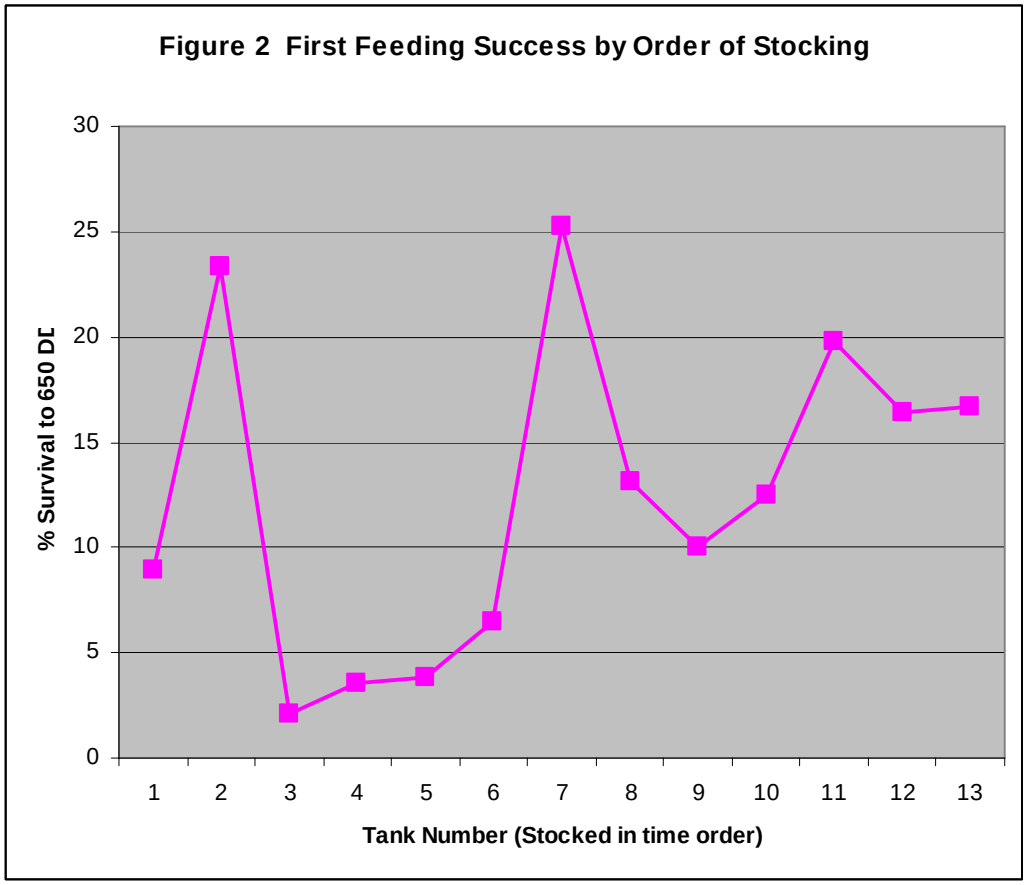
| Tank No | Treatment | Start No | No 650ºD | % survival | Wtd. Average |
|---|---|---|---|---|---|
| 1 | P1 | 42,000 | 3753 | 8.9 | |
| 4 | P1 | 43,000 | 1560 | 3.6 | |
| 7 | P1 | 23,000 | 5811 | 25.3 | |
| 10 | P1 | 30,000 | 3738 | 12.5 | |
| | Total: | 138,000 | 14862 | | 10.8 |
| 3 | P2 | 43,000 | 920 | 2.1 | |
| 6 | P2 | 50,000 | 3267 | 6.5 | |
| 9 | P2 | 42,000 | 4200 | 10 | |
| 12 | P2 | 24,000 | 3938 | 16.4 | |
| | Total: | 159,000 | 12325 | | 7.8 |
| 2 | control | 20,000 | 4654 | 23.3 | |
| 5 | control | 46,000 | 1729 | 3.8 | |
| 8 | control | 38,000 | 4984 | 13.1 | |
| 11 | control | 15,000 | 2964 | 19.8 | |
| | Total: | 119,000 | 14331 | | 12 |

Larval experimental tanks were stocked as material became available on a batch basis during the season. Numbers coming through batches are not commonly identical, and it was impossible to stock each experimental tank with exactly the same number of larvae. Consequently, the initial stocking densities of experimental and control tanks were quite different. Figure 1 shows the relationship between survival to weaning and initial stocking density. It does suggest a relationship between stocking density and survival.



Performance of halibut larvae through all stages of production, including first feeding, is extremely variable and apparently subject to a wide range of influences. The exact time of year or month when larvae are passing through critical phases might be important, since coastal water quality parameters might be changing constantly. Figure 2 shows larval survival compared with tank number, i.e. with date of stocking (Tank 1 being the first one of the season).

Figure 2 does not appear to demonstrate any clear trend in seasonality.



Whilst not strictly part of the experimental programme, the broodstock fish were also dosed with Protex® during the 2007 stripping season. Regular hand stripping of large halibut is a stressful operation, and mortalities as well as serious physical damage leading to poor condition and resultant poorer breeding performance, occur each season. Table 4 shows the mortalities for 2006 and 2007.

Table 4. Broodstock mortality for the 2006 and 2007 stripping seasons.

| Year | Feb | Mar | Apr | May | Jun | Total |
|---|---|---|---|---|---|---|
| 2006 | 2 | 1 | 1 | 1 | 2 | 7 |
| 2007 | 0 | 1 | 1 | 0 | 0 | 2 |

### *3.2 Discussion*

It is clear from the results shown in Table 3, that there was no positive effect of the Protex® on halibut larval survival during the first feeding stage with the current dose rates and method of delivery. Average percentage survival in the control group was 12%, against 10.8% and 7.8% in the P1 and P2 groups respectively.

From Table 1 it can be seen that quantities and survival rates at the different stages were similar to last year and that sufficient material was produced to successfully complete the trial. Originally, only 3 tanks per treatment were planned, but this was increased to 4, due to the availability of larvae at the start of first feeding.

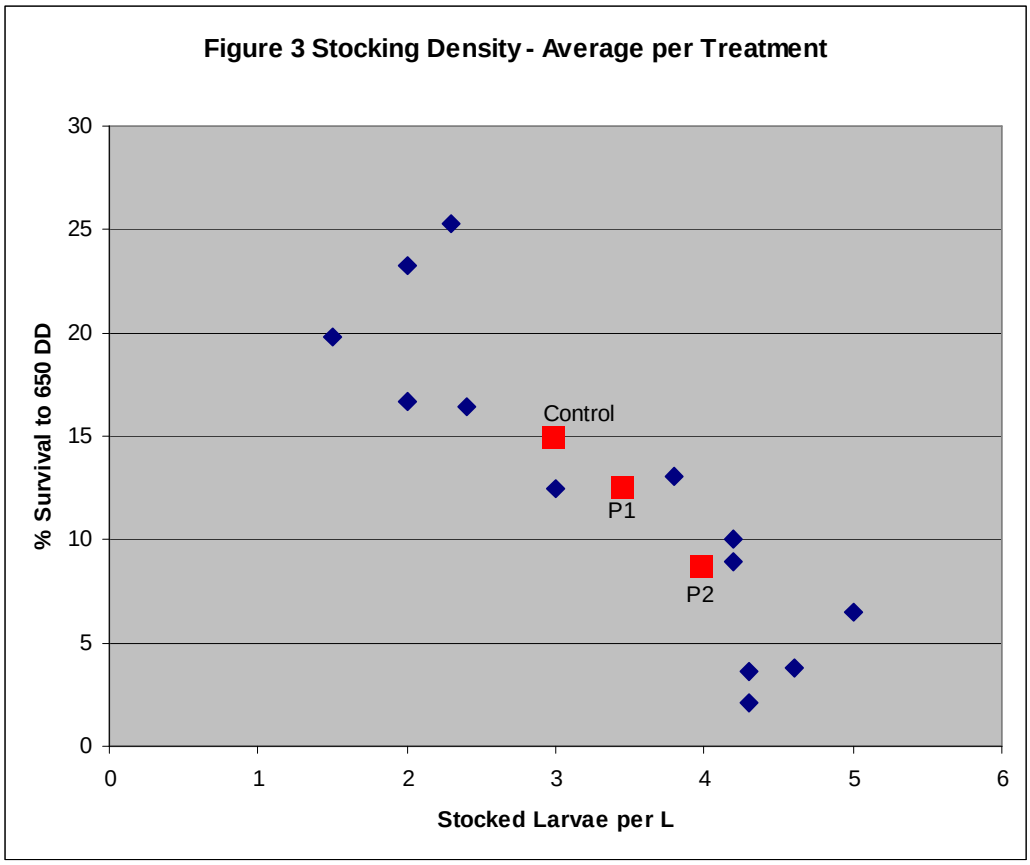
Table 2 shows the treatments in chronological order. There was normally 5 days between the start of each tank.

A significant drop in percentage survival can be seen in Tanks 3, 4, 5 and possibility 6. This was because most of the larvae in these tanks failed to start feeding. Normally 50% - 70% of larvae start to feed within 5 days of live food being introduced, as occurred in all other tanks in this study. In tanks 3, 4, and 5 only between 10 – 20% of the larvae commenced feeding. This is generally thought to be due to poor larval quality but could also be due to pathogens or water quality problems and is not fully understood. The larvae in these tanks appeared to be healthy and behaved normally when the cover was removed. But when the light above tank was switched on the behaviour appeared to be erratic and unfocussed and after 2 hours mortalities began to appear on the surface.
Turning on the light soon after removing the cover (or transferring from yolksac in Norway) is a standard Norwegian protocol when start feeding with

*Artemia* and is thought to stimulate feeding behaviour. After the third tank in a row had exhibited what appeared to be an adverse reaction to the light, the standard protocol was slightly altered. Following a procedure used 5 years previously when feeding copepods, the cover was removed 3 days before the light was turned on to allow the larvae to acclimatise to low levels of reflected light. This appeared to return start feeding percentages to normal and was adopted for the rest of the tanks in the study.
Fortunately, one tank from each treatment was affected to a similar extent by the poor start feed, thus not significantly affecting the balance of the trial. However, by the time the decision to change the protocol was made, Tank 6 (P2 treatment) only had 18 hours with the cover off before the light was switched on as opposed to 72 hours for the rest of the treatments. Although the start fed was significantly better than in Tanks 3, 4 and 5, it may not have obtained the full benefit of the light acclimatizing period and this may be reflected in the still relatively poor results from this tank.

As stated in section 2.4, the final number of larvae remaining at 650ºD was physically counted whereas the initial number, at the start of first feeding, was estimated by eye. What effect errors in this estimation could have on the results is now discussed.
A 20% error on an averagely stocked tank of 35,000 larvae would make a difference of 7000 larvae above or below the estimated figure. This would cause a 25% difference in the final percentage survival around the average of 12%. This would appear to be significant. However, this is the difference between a final survival figure of 12% and 15% and is well within the variation seen within each experimental group. Based on past experience, a minimum improvement of 50% over and above the 12% survival seen in the controls (i.e. 18%) would have been required to demonstrate an improvement attributable to the Protex® treatment. With successful industry survivals now 30% - 40% this was still a relatively modest target.
It is very unlikely that the error in estimation would be greater than 20%. Errors would also tend to be balanced across the treatments.

As stated earlier, no positive effect on percentage survival can be attributed to the Protex® treatments. In fact, the average survival from the P2 treatment is 35% lower than the control group indicating the possibility of a negative effect. This, however, is almost certainly due to differences in initial stocking densities across the treatments. When all the batches, irrespective of Protex® treatment are considered in relation to initial larval stocking number (and therefore density), there is an apparent trend towards improved survival related to lower initial stocking density. The data is shown in a graph in Figure 1. (Tanks 3, 4 and 5 are excluded as unrepresentative due to the start feeding problems discussed earlier.)
Applying the initial stocking densities of the P2 treatment (average 3.9/litre) and the controls (average 2.4/litre) to the graph fully explains the difference in final percentage survival between the 2 groups. The P1 group was also affected by higher densities but to a lesser extent. Figure 3 illustrates this apparent density effect on experimental results.




There has always been a ceiling to the stocking densities in halibut first feeding above which percentage survivals were drastically reduced. A pattern of falling survivals as the densities approach that ceiling has also been observed in previous years. However, that relationship is not hard and fast and tanks with “higher” densities can have percentage survivals as high as tanks with lower densities, producing large numbers of juveniles. The position of the “ceiling” is also not fixed, and over the years, as the process has become better understood, it has gradually moved up. Successful hatcheries across the industry are currently using initial stocking densities of 12 – 15 larvae/litre, over twice the highest density used in this study. In a given first feeding facility, with a fixed number of tanks, there is little to be lost by using “higher” densities in terms of the absolute numbers of juveniles being produced and everything to gain if the system is working well. It was hoped that the Protex® treatment would help to achieve better survivals at all of the densities used in the trial, none of which were high compared with current industry norms.
With hindsight it may have been better to balance the stocking densities over the treatments rather than using a fixed chronological pattern. However, the variances caused by stocking density differences can be accounted for and do not affect the overall validity of the results.

Following each Protex® treatment, a film was observed to form on the tank surface. This could persist for up to 24 hours at the early larval stages when

water flows, and therefore exchange rates, were low. It is assumed that this was caused by the alcohol carrier and its effect on surface tension. This was not observed to have a negative effect on the larvae. However, following discussions with Bradan limited, a small quantity of Protex® was supplied at 10 times the normal concentration and therefore containing 10 times less alcohol for any given dose. This was trialled at the P2 level on a final batch, outside the experimental program (see tank 13, Table 2). The surface effect was greatly reduced and it is recommended that this be used for all future treatments of larvae during the early stages.

Protex® was also used non experimentally to stimulate HSP production in the broodstock throughout the stripping season. Treatment was at the P1 level (1ml/100L) for a minimum of 1.5 hours, every 3 days. A comparison of the egg volume and fertilisation rate of each female for the 2006 and 2007 stripping seasons is shown in Table 5. Although fertilisation rate and fecundity were similar to that in the 2006 season, it was observed that the overall condition of the fish was better than in previous years.
Normally, because of the constant handling, lack of feeding and low water temperature (6℃), the condition of the broodstock gradually deteriorates as the season progresses. In particular, minor damage can develop on fin and tail edges, which does not heal until water temperatures are raised and feeding recommenced at the end of stripping. This year, overall, there was noticeably less damage, and when specific damage was observed, significant healing was seen to take place within 6 – 9 days. This has never been seen before during the stripping season. Furthermore, if secondary infection of such damage takes place fish almost invariably succumb. In 2007, only 2 fish were lost, possibly because the lesions healed up so promptly.

Table 5. Comparison of egg volume and fertillisation rate in 2006 and 2007

| Fish I.D. | Vol 2006 | Vol 2007 | Fert % 06 | Fert % 07 | Fert Eggs 06 | Fert Eggs 07 |
|---|---|---|---|---|---|---|
| OO73 | 14650 | 12750 | 43 | 69 | 6300 | 8798 |
| 19EF | 5500 | 2000 | 33 | 57 | 1815 | 1140 |
| 1E3D | 8500 | 8100 | 69 | 28 | 5865 | 2268 |
| 22C4 | 4900 | 4500 | 33 | 15 | 1617 | 675 |
| 2742 | 8000 | 6100 | 26 | 55 | 2080 | 3355 |
| 280F | 7850 | 4500 | 40 | 28 | 3140 | 1260 |
| 2C6F | 4300 | 4100 | 12 | 7 | 516 | 287 |
| 333B | 2100 | 3500 | 12 | 48 | 252 | 1680 |
| 335C | 2800 | 5900 | 61 | 37 | 1708 | 2183 |
| 3E2F | 8650 | 8200 | 50 | 64 | 4325 | 5248 |
| 517F | 3400 | 5450 | 55 | 61 | 1870 | 3325 |
| 5828 | 7000 | 2900 | 20 | 12 | 1400 | 348 |
| 5C8E | 8300 | 9600 | 32 | 44 | 2656 | 4224 |
| 698C | 20100 | 25600 | 20 | 20 | 4020 | 5120 |
| 6CCA | 11300 | 13600 | 35 | 23 | 3955 | 3128 |
| 7369 | 7300 | 7200 | 28 | 24 | 2044 | 1728 |
| 74C2 | 5650 | 6950 | 24 | 20 | 1356 | 1390 |
| 7710 | 7500 | 10100 | 31 | 47 | 2325 | 4747 |
| 7BFA | 5800 | 13450 | 75 | 58 | 4350 | 7801 |
| 80A4 | 6000 | 9150 | 13 | 41 | 780 | 3752 |
| 8347 | 3450 | 3100 | 15 | 4 | 518 | 124 |
| 8D04 | 6000 | 3100 | 50 | 16 | 3000 | 496 |
| 8EB0 | 12400 | 4100 | 46 | 22 | 5704 | 902 |
| 9177 | 2500 | 1900 | 18 | 18 | 450 | 342 |
| 91F4 | 12850 | 6650 | 41 | 12 | 5269 | 798 |
| 9F12 | 2650 | 3600 | 41 | 86 | 1087 | 3096 |
| 9E2D | 3000 | 6800 | 19 | 41 | 570 | 2788 |
| A56A | 5250 | 11500 | 13 | 24 | 683 | 2760 |
| A0E3 | 5600 | 8400 | 46 | 79 | 2576 | 6636 |
| B209 | 5850 | 3600 | 28 | 41 | 1638 | 1476 |
| B75A | 19800 | 9950 | 25 | 28 | 4950 | 2786 |
| C226 | 16300 | 10600 | 36 | 36 | 5868 | 3816 |
| C60A | 5000 | 5200 | 24 | 23 | 1200 | 1196 |
| CBE8 | 7000 | 11200 | 29 | 27 | 2030 | 3024 |
| D3A4 | 5950 | 5500 | 38 | 29 | 2261 | 1595 |
| E1A3 | 5200 | 4150 | 58 | 21 | 3016 | 872 |
| E53D | 7500 | 9800 | 35 | 46 | 2625 | 4508 |
| EA07 | 9600 | 10050 | 45 | 39 | 4320 | 3920 |
| Total: | 285500 | 282850 | | | 100139 | 103592 |
| Overall Fertillisation Rate: | | | 35.1 | 36.6 | | |

This increased robustness of the broodstock is reflected in the comparison of the mortality figures for the 2006 and 2007 stripping seasons, shown in Table 4. A total of 7 fish died between February and July in 2006 compared with 2 fish in the same period in 2007.
Earlier this year, ongrowing salmon on a sea farm in Shetland, showing severe chronic coldwater ulcer disease skin lesions, were treated with Protex®. Significant and rapid healing of the ulcers was observed. A second stock of fish on another farm with the same symptoms was treated 2 weeks later with the same results.
The initial hypothesis on why the Protex® treatment should have this effect is that it somehow facilitates the amoeboid flow of epidermal cells over an open wound, normally inhibited by cooler temperatures and the presence of bacteria. (Prof. R.J. Roberts. pers.comm.)

## 4. Conclusions

The Protex® treatments used in 2007 had no positive effect on halibut larval survival during the first feeding stage, as had been observed in the pilot trial in 2006.
Given the growing amount of evidence from other studies that Protex® stimulates protection from stress at the cellular level, it could be concluded that the relatively poor percentage survivals seen in first feeding at Otter Ferry are not then primarily stress related. The negative relationship seen between survival and increasing stocking density could instead point to bacteria or water quality problems as the more likely reason for the poor results.

A 10 times concentrated Protex® solution should be used when treating larvae at the early stages to limit the effect of the alcohol carrier on the tank surface.

Protex® treatments appeared to accelerate healing in damaged broodstock. This could be of great benefit across the Aquaculture Industry and should be investigated further.

## 5. References

Formal scientific references are not included, since this experimental programme was based upon Otter Ferry's own expertise. Personal communication was maintained with other halibut hatchery operators during the season, as well as with the UK supplier of Protex® and a halibut grower in Shetland.

## 6. Acknowledgements

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