Science Series
Technical Report no.132

Research on finfish cultivation

A synopsis of research funded by the Department for Environment, Food and Rural Affairs (Defra) between 1990 and 2004

S.M. Baynes, D. Verner-Jeffreys and
B.R. Howell

Cefas

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Preface

This review provides a synopsis of the results of research on finfish cultivation funded by the Department for Environment, Food and Rural Affairs (Defra) since 1990.

Government has actively promoted research into aspects of finfish cultivation since the 1960s, recently through Defra and formerly through the Ministry of Agriculture, Fisheries and Food (MAFF). Commercial success of the UK trout and salmon farming industries over the last two decades has meant that there has been less need to develop these sectors through investigations funded exclusively from the public purse. Instead government research funds for this area of work have been, in part, made available through schemes set up to support collaborative investigations in which the industrial partners have also contributed resources. Wholly government funded research related to salmonid farming has been mainly directed towards developing various means of ensuring protection of fish welfare, natural stocks and the environment.

Government sponsored research in marine finfish cultivation in the 1970s led to advances that underpinned the development of the industry. Defra has continued to provide support to tackle aspects that have held back the development of the marine finfish farming industry in the UK. Priority has been given to investigations of the potential of new species and the constraints that affect the industry; these are the projects that make up most of this review.

The review begins with an Introduction that provides an overview of the development of marine finfish cultivation in the UK and a brief summary of Defra policy for support of R&D. Two sections follow that present the main findings of individual projects. The achievements have been drawn together to provide integrated accounts of the work. Part 1 deals with the technical constraints affecting marine fish cultivation through practical aspects of the rearing cycle and influences affecting the quality of the fish produced. Part 2 addresses issues of sustainability: management of water use, diet formulation, fish welfare and the quality of the product. Information on uptake of the research and full reference to published results are also provided.

Introduction

Overview of marine finfish cultivation in the UK

It is now over 100 years since the first marine hatcheries were built. In 1893 one was set up at Dunbar, on the east coast of Scotland, and this was followed by another at Piel, near Barrow in Lancashire in 1897 and one at Port Erin on the Isle of Man in 1902. All aimed to bring about a recovery of depleted wild stocks by the release of eggs and larvae into the sea, the main target species being plaice and flounder.

This was the start of marine finfish cultivation research in the UK. Although these hatcheries failed to realise their main aspiration and most had closed by the 1920s, they did generate a wealth of knowledge about the early life stages of these marine fish species. This provided a sound basis for the revival of interest in marine finfish cultivation that came in the 1960s and was stimulated by experimental work in support of ecological programmes on fish larvae at the MAFF Fisheries (now Cefas) Laboratory at Lowestoft, in Suffolk. The research demonstrated that plaice larvae could be successfully reared through all the larval stages in aquaria. This led to the construction of a pilot-scale hatchery alongside Liverpool University's Marine Biological Station at Port Erin, where artefacts from the first marine fish hatchery movement still remained. The attraction of Port Erin was access to a plentiful supply of clean seawater and captive stocks of mature plaice as a source of fertilized eggs.

This development programme was led by a MAFF scientist, but funded and staffed through the White Fish Authority (WFA, later to become the Seafish Industry Authority or SFIA). The work demonstrated that juvenile plaice could be mass-produced in marine hatcheries; the question then arose as to what use could be made of this new-found ability. The idea of enhancing depleted wild stocks was resurrected, but it was found that few reared fish survived after liberation in the sea. Release into an enclosed bay at Ardtoe on the west coast of Scotland met with a similar result; the fish were lost through either poor environmental conditions or predation by crabs.

By the 1970s detailed evaluations of technical and economic issues led to a shift of focus towards the development of intensive fish farming with an agreed division of responsibilities between the main agencies involved. The White Fish Authority were to concentrate on developing on-growing methodology at their site adjacent to the nuclear power station at Hunterston, Ayrshire (utilising a warmed water effluent) with hatchery systems at the Ardtoe site. MAFF took over the Port Erin facility with the brief of refining hatchery techniques and extending the methodology to other species such as Dover sole and turbot. Economic considerations clearly indicated that market prices needed to be high to recover the costs of farming and so the effort quickly focussed on these high value species.

Rearing turbot larvae proved extremely challenging, but once the bottleneck of juvenile supply was alleviated the high growth rates, which young turbot achieved on fish-based diets, encouraged commercial interests to quickly become involved. In contrast, sole could readily be reared through their larval stages, but on-growing proved problematic because the available fish-based diets were unsuitable; consequently there were no successful commercial developments with sole at that time.

Both species require temperatures higher than those commonly found around the British coast to achieve their maximum growth rates. The availability of waste warm-water effluent from power stations had been seen as a means of providing near-optimal conditions throughout the year, but for various reasons, this did not prove to be the most appealing option to the commercial companies that had become involved. Their preference was to locate their activities in southern Europe where water of the desired temperature could be obtained directly from the sea.

Commercial production of marine finfish really began during the early 1980s and by the end of the century annual production in Europe exceeded 100,000 tonnes. Sea bass and sea bream made up more than 90% of this, but production of turbot, mainly in Galicia, Spain, had reached a level approximately equal to the supply from wild fisheries.

In the UK, the main focus had switched to cage farming of salmon in the sheltered waters of the west coast of Scotland, as well as to trout in the fresh waters of England, Wales and Scotland. By the year 2003 production of farmed salmon had risen to more than 160,000 tonnes a year, appreciably increasing the availability of this quality fish at an affordable price while bringing important socio-economic benefits to the remote areas of Scotland.

Encouraged by the success of marine finfish farming elsewhere in Europe, aspirations to develop a marine fish farming industry in the UK have persisted and the search for a species suited to our environment has continued. Halibut, a high value cold-water species, was identified as a promising candidate though it was recognised that, because of its somewhat unusual life-history, rearing techniques established for other species would require considerable adaptation. In the first instance, the large size of brood fish and their requirement for low temperatures meant that specialist facilities were needed to house them

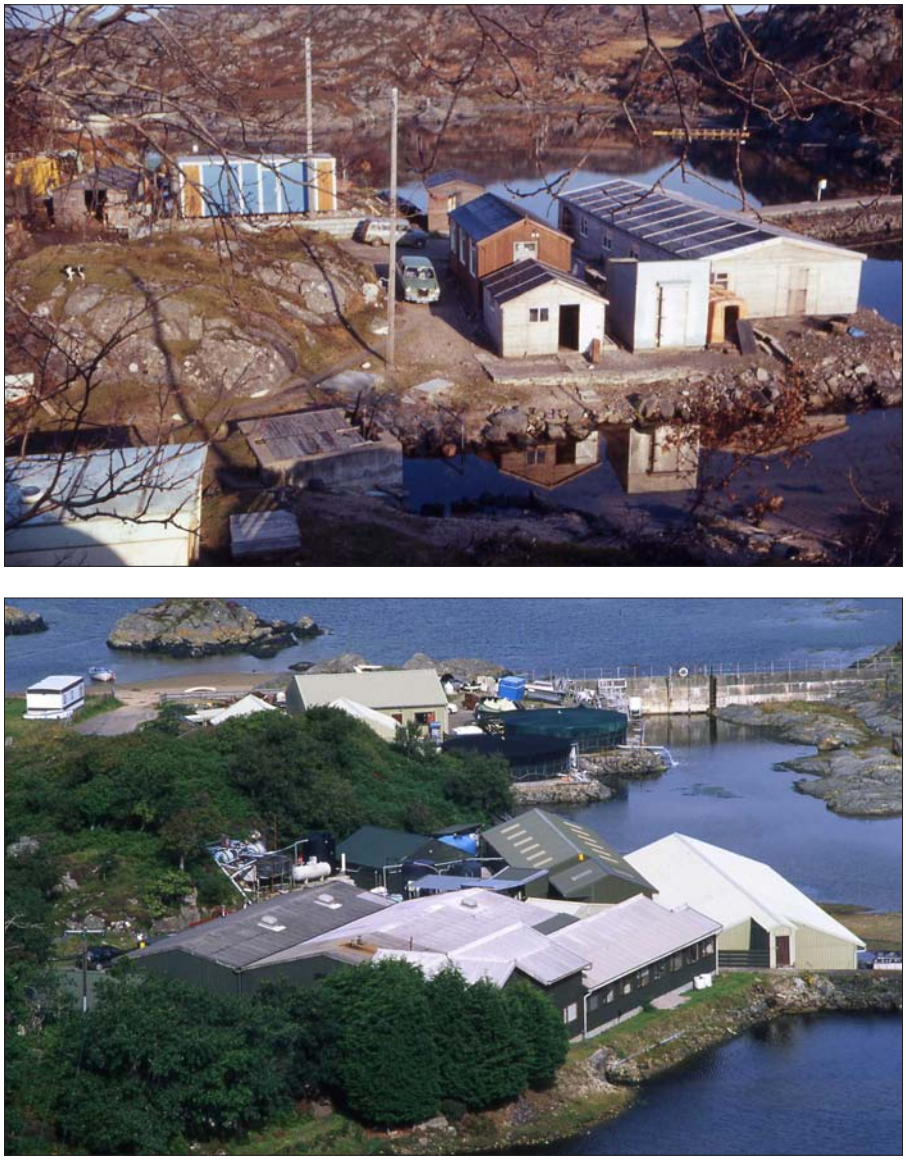

Figure 1. The SFIA Marine Farming Unit site at Ardtoe: from relatively small beginnings (the top photograph was taken in 1972) the Unit expanded to provide extensive and important facilities for halibut and cod cultivation work by the time of the bottom photograph in 1999.

in order to secure a reliable source of eggs. The SFIA Fish Cultivation Unit at Ardtoe was able to provide these facilities and later, as the Seafish Marine Farming Unit, became the focal point of the UK's effort with this species, the research programme complementing work already underway in Norway.

Meanwhile, global interest in assessing whether the availability of reared fish could have a role in rehabilitating declining stocks had been renewed by the developments in hatchery technology and it was decided to evaluate such potential using the Dover sole. There were valuable fisheries for this species around the UK coast that were coming under considerable pressure and though prospects for intensive farming were hampered by feeding problems, the species was one of the easiest to rear through its larval stages in large numbers. The work on Dover sole was largely undertaken at the MAFF/Cefas Laboratory at Conwy in North Wales.

The very positive commercial developments that have taken place over the last twenty years or so have not been without their difficulties. The success of the salmon industry in particular has raised important questions of sustainability and potential impact of intensive aquaculture systems on the environment. Throughout this period government has addressed public concerns provoked by the developing industry and has provided support for technical studies. Defra has been the principal sponsoring department with clear policy and scientific objectives that are outlined below. The finfish cultivation research that has been funded has largely been associated with addressing constraints to the development of a marine farming industry in the UK. In that respect new species and new techniques have been considered priorities. Only limited work has addressed further development of the established trout industry in the last decade, except where there has been active involvement of industrial partners or a perceived need to protect the environment or welfare of the animals.

More of the marine finfish cultivation research and development is now passing to the hands of the established industry. Seafish supported direct research into aquaculture at Ardtoe for over 30 years, the work focussing on technology transfer that provided a bridge between

Figure 2. The MAFF (subsequently Cefas) Laboratory at Conwy, North Wales in 1990. The buildings and tanks, adjacent to the River Conwy, were first established in the early 1900s for shellfish research and gradually extended during subsequent decades until closure in December 1999. From 1986 work on coastal ecology and finfish stock-enhancement studies with Dover sole were included in the Laboratory's research programmes.

academic research and industry application. Their work on halibut and cod in recent years made a major contribution to the development of the industry in the UK. Changes in research priorities, however, meant that in February 2003 Seafish announced that direct funding of aquaculture research and the management of the Marine Farming Unit at Ardtoe no longer made the best use of their limited levy resources. Efforts were made to ensure the research facility was kept open and the Scottish Association for Marine Science (SAMS) formed SAMS Ardtoe Ltd. to take over its management and operation in November 2003.

Defra policy objectives and involvement with industry

Defra has worked to encourage the development of a diverse, competitive and sustainable finfish farming industry. The intention has been to provide information that enables producers to make informed business decisions about establishing and developing fish farming operations:

There has always been consideration of future development of the industry with an element of the supported scientific research directed towards investigating new fish species and cultivation techniques that show promise. For example, Defra has commissioned research to develop practical and cost effective techniques for halibut broodstock management and hatchery operations. Research has also been funded to address the major technical constraints in hatchery operations for turbot, cod and Dover sole.

In order that the fish farming industry is able to comply with any forthcoming fish welfare codes, additional work has focussed on providing information that will help refine management techniques in order to increase production efficiency without sacrificing fish welfare or product quality.

It is Defra policy to make available the results of the research it funds, and final project reports are posted on the Defra website at:

http://www2.defra.gov.uk/research/project_data/default.asp .

To foster collaborative research, technology transfer and the development of a competitive aquaculture industry, a five-year LINK Aquaculture Programme was started in 1996. Defra and SEERAD predecessors (MAFF, Ministry of Agriculture Fisheries and Food, and SOAEFD, Scottish Office Agriculture, Environment and Fisheries Department), along with NERC (National Environment Research Council) played a significant role by providing up to 50% of the costs of individual projects with the balance coming from industry. Very often industry provided "in kind" contributions through access to the facilities and materials needed for the research to take place. Delegates from various associations that represent different parts of the industry contributed to the panel that considered project applications and decided which should be funded.

At present, research and development priorities are set in consultation with the industry through the stakeholder forum 'Committee for Aquaculture Research and Development' (CARD). Defra, through the committee, has allocated approximately £1 million of research funds over a 5 year period to collaborative R&D with the aquaculture industry. This programme follows on in the same vein as the LINK programme, and provides a forum within which the industry can publicise their research priorities.

Acknowledgements

The authors would like to thank the various contractors who provided the information and illustrations in this report. Particular thanks are extended to Dr Craig Burton, of Seafish, for contributing additional photographs of the Ardtoe facility.

Part 1.1 Technical feasibility – the production cycle

1. Broodstock

Details of the following projects are included in this section:

FC0102 Techniques for the cultivation of Dover sole (Cefas - Conwy)

FC0103 Development of cultivation techniques for halibut (SFIA - Ardtoe)

FC0104 Halibut egg and larval quality (SFIA - Ardtoe)

FC0107 Environmental control of halibut broodstock and rearing procedures for feeding larvae (SFIA - Ardtoe)

FC0904 (*FC0113*) Spawning of flatfish in captivity - sex pheromones and reproduction in flatfish broodstocks (University of East Anglia, Norwich)

The provision of a reliable supply of fertilized eggs of good quality is fundamental to finfish cultivation and yet the factors that contribute to this are imperfectly understood. Obtaining a captive broodstock of wild caught fish is just the first step on a long road to achieving this end. Natural spawning of broodstock fish held in captivity may occur given suitable conditions and husbandry, but for some species this is not the norm and provides an unpredictable source of material on which to base a commercial operation. For these species, viable gametes (eggs and sperm) are handstripped from the broodstock and artificial fertilisations carried out *in-vitro*. The quality of gametes obtained this way varies considerably, but in general continuity of supply of high quality material can be ensured by careful management of the stripping schedules. For species such as turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*) this is the way that the farming industry operates. Dover sole (*Solea solea*), on the other hand, is a particularly difficult species to strip, but fortunately will spawn naturally in relatively small tanks and fertilized eggs can then be collected from the tank water.

Figure 1.1. Halibut broodstock: an adult halibut being manoeuvred on to a table placed in a broodstock tank to allow gametes to be collected by hand stripping.

Initial work with a new species focuses on establishing conditions necessary for an adequate supply of fertilized eggs, but once that has been accomplished the centre of attention of the research generally shifts to clarifying the requirements of larvae and juveniles. The comparatively large resources needed to replicate experimental treatments of broodstock fish means that investigations with broodstock have been carried out rarely and therefore the work funded by Defra/MAFF has been a valuable contribution to knowledge in this field.

Work undertaken since 1990 has been concerned primarily with factors that affect the spawning of two species, Atlantic halibut and Dover sole. Halibut was considered to have the best immediate potential for the UK marine farming industry, and research into the endocrine control of reproduction in flatfish was applied to this species in particular. Work with Dover sole was needed to support more generic research on improving juvenile quality and reducing dependence on live feeds in marine fish culture. The research focussed on an evaluation of the effects of various husbandry factors on egg production, but included studies that sought to improve understanding of the underlying endocrinological and behavioural mechanisms involved in the control of reproduction.

1.1 Atlantic halibut

In the wild, Atlantic halibut spawn in cold water at very great depths off the coasts of northern Atlantic countries (from Norway, through Iceland to the eastern coast of Canada). The spawning season is from February until April and the females are batch-spawners, each releasing from 3 to 8 batches of eggs during the season.

In the 1980s work focussed on capturing wild broodstock and establishing them in the experimental facilities at Ardtoe and in commercial hatcheries. This was a major exercise partly funded by the British Halibut Association (BHA) and involved several different periods of collection; by the end of the decade stocks of mature Atlantic halibut had been set up with individuals captured off Iceland, Greenland, Faeroe and Shetland. The first Atlantic halibut juveniles were produced from their gametes in 1990. Defra then funded work to investigate the best methods of maintaining these broodstock (FC0103 and FC0104). This involved determining holding conditions and diets that would support good survival of adult fish and the production of plenty of high quality gametes throughout the year.

As a result of the Defra funded project work, along with data supplied by Norwegian researchers, it was established that close temperature control of broodstock holding tanks, particularly during the spawning season, was essential for the production of fertile eggs. It was shown that water temperature needed to be maintained below 6.5°C, requiring the use of water chillers. In a broodstock temperature experiment run over three spawning seasons, conclusive differences were found between the spawning performances of halibut receiving chilled as opposed to ambient temperature seawater. Both the total yield and the viability of eggs were improved when water temperature was reduced year-round and chilled to a constant 6°C during spawning.

It was also shown that the spawning season could be greatly extended by manipulating the maturation cycle using photoperiod control. It was found necessary to retain stocks on an increasing photoperiod until day-length reached 16 hours in order for spawning to proceed normally. Reliable egg production for more than six months a year was initiated by establishing different tanks of broodstock under ambient, delayed and advanced (phase shifted) photoperiods.

As well as optimising egg production by manipulating temperature and day length, the effect of other physical parameters, such as flow rates and bottom substrata were also tested. Early work also established reliable protocols for "stripping" eggs and sperm (milt) for subsequent artificial fertilisation. It was recognised that social hierarchies between individual halibut developed within tanks and that these interactions needed careful management.

A separate experiment demonstrated that it was possible to increase the stocking density of broodstock halibut from 2.5 kg m^{-2} (3 pairs per tank) to >5 kg m^{-2} (6 pairs per tank), without impairing the quantity or viability of eggs obtained. No significant differences were seen between density treatments in any of the recorded parameters relating to spawning (timing of spawning, length of spawning season, egg yield, egg quality, fertilisation rates). Fish held at the higher density consumed approximately twice as much food per unit weight as those in the low-density treatment over the 21 month period of the experiment, yet they did not increase in weight. Halibut held at the lower density did increase in weight significantly, despite their lower food intake. The finding that spawning performance was not impaired in the high density treatment could be valuable in terms of devising more cost-effective broodstock groupings for commercial halibut production.

1.2 Dover sole

Dover sole spawn in the wild from March until May in coastal waters around the UK. Natural spawnings of captive Dover sole at the Cefas Conwy Laboratory occurred over the same period, at water temperatures from about 9°C to 13°C. The fish were fed live, natural foods such as worms or shellfish that ensured good broodstock condition and reasonable egg quality, but even so spawning was intermittent and batches of eggs were frequently poorly fertilized (<50%). Attention was paid to the behaviour of the fish to see if the tank environment affected the spawning process.

Overnight video-recording of the broodstocks revealed that Dover sole form pairs when spawning. During four nights, six separate pairings were observed, all within 3 hours of the start of the dark period. Initially the males were more

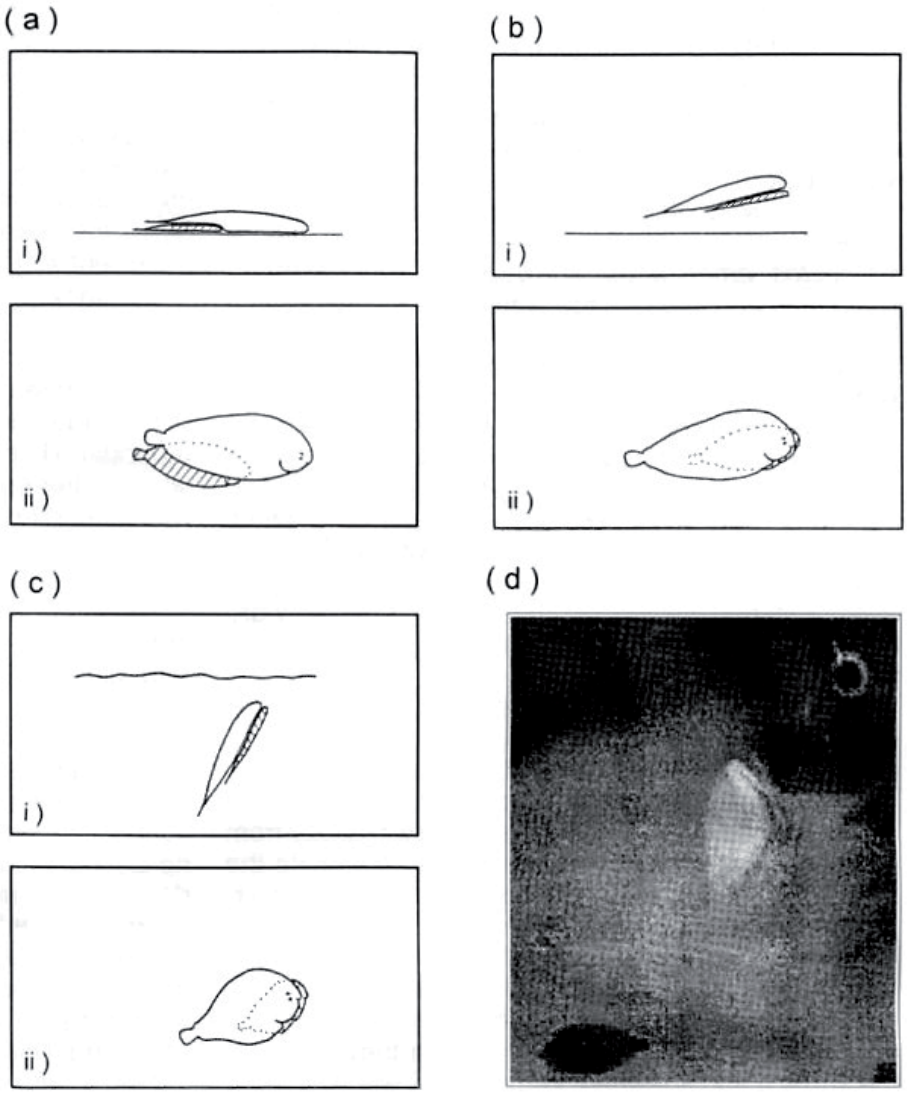

Figure 1.2. Dover sole pairing at spawning: diagrams (i) side view, (ii) view from above. a) A small male (hatched) moves under the female b) The pair swim towards the surface, heads close together, gonadal pores adjacent; c) The male under the female maintain a head-up position near the surface; d) A frame from a video record looking down on a pair; the back of the female, and the anterior edge of the male, are at the surface. Another darker fish rests on the floor of the tank at bottom left and a standpipe in the tank is visible top right.

active than the females and in turn approached individual females resting on the tank floor. If a female was receptive the male moved underneath her into a position where their genital pores were adjacent. The pair then swam in unison towards the water surface and there they remained together for about a minute, maintaining a 'head-up' position just beneath the surface. It was presumed that this intimate behaviour was associated with release of eggs and sperm, and the proximity of the fish ought to have ensured high fertilisation rates. However, the relatively poor fertilisation rates obtained in captivity indicate that the observed pre-spawning behaviour and spawning were disrupted in some way, probably by the tank conditions provided. Tank size, stocking density and the presence of obstacles would affect spatial relationships and could have been of particular importance. No confirmation of this was obtained, but the detailed description of spawning behaviour in this species provided a sound basis for future investigations.

1.3 Sex pheromones and reproduction

It is not uncommon for marine fish to reproduce poorly in captivity; the problem has affected all marine flatfish species that have been investigated for potential cultivation in the UK: plaice, turbot, halibut, as well as sole. Project FC0904 was commissioned to assess whether this poor reproductive performance could be explained by the release of pheromones from maturing fish into the relatively small water volume of a tank disrupting normal reproduction. The research utilised plaice (*Pleuronectes platessa*) as a model flatfish species and the expertise developed at the Cefas Lowestoft Laboratory to detect and quantify levels of steroid sex hormones in the fish and of pheromones released into water.

Concentrations of reproductive hormones in the plasma of plaice caught on North Sea spawning grounds were shown to fall as much as one hundred fold following capture and transfer to tanks in the laboratory. The consistency of the milt also changed from being light and runny in sea-caught fish, to being thick and viscous in captive fish. In an attempt to understand the phenomenon, a number of experiments were conducted to test if hormone concentrations in male plaice in the laboratory would change if the fish were exposed to various flow rates of clean sea water or water from tanks holding males or females. None of the experiments gave any indication that steroid levels in male plaice were being suppressed by pheromones.

Following these results the emphasis of the work on male plaice shifted towards understanding which hormones were involved in regulating milt fluidity. Plaice were treated with an implant containing gonadotrophin-releasing hormone analogue (GnRHa). GnRHa is an analogue of the natural hormone that triggers a cascade of reproductive hormones in the body. GnRHa releases gonadotrophin from the pituitary gland, which in turn stimulates steroid production in the gonads. In implant studies, GnRHa is released slowly from an implanted pellet and this can maintain the hormone cascade over many days.

In captive male plaice, GnRHa treatment led to levels of reproductive steroids and properties of milt that were similar to those found in wild spawning fish. In turbot, however, GnRHa had no significant effect on steroid concentrations or on milt properties and so in the absence of any measurable endpoints, work with turbot was discontinued.

GnRHa trials with halibut proved particularly successful; the concentrations of a hormone, 17α 20β dihydroxy-progesterone were enhanced by GnRHa and, more importantly, improved the quality of the sperm and the fluidity of the milt. The most appropriate dose rate of GnRHa was found to be 25 µg GnRHa kg^{-1}. The technique also proved useful for prolonging production of good quality sperm at the end of the spawning season.

The work indicated that maintaining some species of marine finfish in tanks leads to a down-regulation of the hormone cascade and reproductive system. In some species normal levels can be restored by treatment with GnRHa, but the reasons why the concentrations of reproductive steroids are suppressed were not identified. The results did demonstrate that suppression of reproductive hormones was probably not caused by the accumulation of pheromones in tank water.

2. Larvae rearing

Details of the following projects are included in this section:

FC0102 Techniques for cultivation of Dover sole (Cefas - Conwy)

FC0103 Development of cultivation techniques for halibut (SFIA - Ardtoe)

FC0104 Halibut egg and larval quality (SFIA - Ardtoe)

FC0106 Digestive physiology of juvenile Dover sole (University of Wales, Bangor)

FC0107 Environmental control of halibut broodstock and rearing procedures for feeding larvae (SFIA - Ardtoe)

FC0108 Further studies into the digestive physiology of juvenile sole (University of Wales, Bangor)

FC0905 (*FC0115*) Halibut egg and early larval rearing (SFIA - Ardtoe)

FC0913 (*LINK - FIN22*) Rearing protocols for Atlantic halibut larvae during transition from endogenous to exogenous nutrition. (SFIA - Ardtoe)

2.1 Egg and yolk-sac larvae stages

As well as developing methods of holding halibut broodstock, work under FC0103 and FC0104 also established the technical feasibility of fertilising halibut eggs, incubating them and producing fry. In 1993 UK hatcheries produced approximately 500 halibut fry, a major achievement at that time. The number surviving through to the juvenile stage however represented only a fraction of 1% of the eggs fertilized. Despite advances, halibut fry losses during the early life stages were very high, restricting the commercial viability of the industry.

Research project FC0905 was commissioned specifically to address these problems of persistent high mortality rates, in UK hatcheries, during the egg and pre-feeding larvae stages. In particular, the prolonged rearing phase of the pelagic yolk-sac larva was typified by UK survival rates of around 10% or lower, compared to reported average survivals of *circa* 60% in Norway. The different conditions prevailing at UK and Norwegian sites, indicated that inferior ambient water quality (the physical and chemical attributes) could have had a major bearing on the performance of UK hatcheries. Also there was a tendency to recycle hatchery water for purposes of temperature control in the UK and this was thought to represent an additional water quality challenge to the delicate early developmental stages. Rearing experiments with eggs and yolk-sac larvae were conducted on a pilot-commercial scale using animals and facilities of the Seafish Marine Farming Unit, Ardtoe.

2.2 Tank configuration

Before this work was undertaken UK hatcheries had employed a design of halibut egg incubator which used seawater of 35.5‰ salinity to maintain the buoyancy of the eggs. The research showed that changing from 'elevated salinity' to an 'upwelling' design, similar to the type used in Norway, resulted in an increase in mean survival rate from 56.0% to 76.8%. In this design a steady flow of water at ambient salinity from the base of the tank kept the eggs in suspension in the water column. It was found that separation of viable fertilized eggs from unfertilized eggs could be reliably achieved by salt dosing. The flow of water to the eggs in the upwelling incubator would be turned off and a layer of high salinity water (40‰) introduced forming a saline 'plug' at the base of the incubator. Dead eggs were not buoyant in the high salinity water while viable eggs floated at the interface, so the bulk of the high salinity water containing the dead eggs could then be drained away. The upwelling flow would then be restored to resuspend the viable eggs. Stocking density could be safely increased to *circa* 800 eggs litre^{-1} under this configuration, compared to 210 eggs litre^{-1} previously. It was also found that by reducing the diameter of the water inlet from 100 mm to 15 mm the water inflow rate could be reduced from 2 litre min^{-1} to less than 1 litre min^{-1} without the eggs settling out. A simple recycled water circuit, without nitrification filter, was found to be satisfactory for egg incubation; there was no advantage in using flow-

to-waste water for this developmental stage allowing water chilling to be more cost-effective. The research showed that if UK hatcheries adopted these relatively straightforward procedural changes it would make a considerable difference to the numbers of yolk-sac larvae that survived.

At the outset of Project FC0905, occasional high survival rates were obtained with the 400 litre tanks used for rearing halibut yolk-sac larvae in the UK, however the variability between runs was high and mean values were much lower than the published figures from Norwegian hatcheries. Accordingly, an important objective of the project was to investigate whether rearing performance could be improved by altering the physical configuration of the tanks to more closely resemble the larger volume Norwegian systems.

The work compared 400 litre tanks with 1150 litre tanks. Under the initial 1150 litre tank configuration, the mean survival rate of yolk-sac larvae to 220 degree-days post-hatch was 13.4%. Following the success of studies with the egg incubation tanks, it was found that successive reductions in diameter of the water inlet from 100 mm to 15 mm improved the upwelling flow in the tanks and survival was increased. The best mean survival in a replicated experimental series was 48.3%, for 1150 litre tanks equipped with 15 mm inlets (n = 4 replicates). Even with a reduced inlet size the 400 litre tanks performed less well than the 1150 litre tanks in another series of replicated comparisons, the mean survival rates being 18.3% and 34.1% respectively (n = 4 replicates). All tanks displayed high inter-batch variability in survival, although this was most pronounced in 400 litre tanks supplied with flow-to-waste water.

It was found preferable to stock tanks with late-stage embryos (pre-hatch) rather than newly hatched larvae (mean survival rates for yolk-sac larvae of 41.9% and 28.1% respectively). There was no evidence for a negative effect of egg stocking density on the survival of yolk-sac larvae, for densities up to 25 litre^{-1}.

These technical advances in yolk-sac system design were further developed under FC0913. The research demonstrated that damage to larvae was reduced by having a more stable salinity regime in the tanks during the yolk-sac phase, together with a low salinity surface layer. This layer reduced larval interaction with the water surface and resulted in a more even distribution of larvae within the tank. Damage to larval sensory systems, in particular neuromasts (mechanoreceptors) was reduced, increasing the number of larvae able to feed. An optimum transfer protocol was developed (Annex 3), which specified the regime of temperature increase through the developmental stages and its adoption reduced mortalities and deformities recorded at the first-feeding stage.

Figure 2.1. A 1150 litre tank for halibut yolk-sac larvae: the scale can be appreciated from the relative size of a fluorescent lamp unit suspended from the ceiling, and visible about halfway up the tank.

2.3 Egg quality assessment

A valuable outcome of work was the development of indicators of egg quality. For most fish species, blastomere size and shape are uniform during normal cleavage. However, abnormal blastomere morphology had been observed in a range of studies and it was proposed that abnormalities indicate low egg viability and could

provide a predictive tool for egg quality assessment. In order to investigate the relationship between blastomere morphology and fish egg survival more closely, incubation experiments were carried out with eggs of Atlantic halibut. This work was carried out under a NERC supported studentship at the Institute of Aquaculture, University of Stirling (IOA), using Defra-funded facilities and material. Eggs were individually assessed at the 8-cell stage for 5 blastomere characteristics and incubated to hatch in multiwell plates. For data pooled from 13 egg batches, the mean score for each blastomere characteristic showed a significant positive linear regression with the number of eggs hatched. Multiple regression analysis, incorporating all five blastomere characteristics, demonstrated a high degree of correlation between the independent variables. While the experimental procedure that was used for halibut eggs would be too laborious for routine hatchery application, the work clearly showed that subjective observations of blastomere morphology provides a useful early assessment of egg viability.

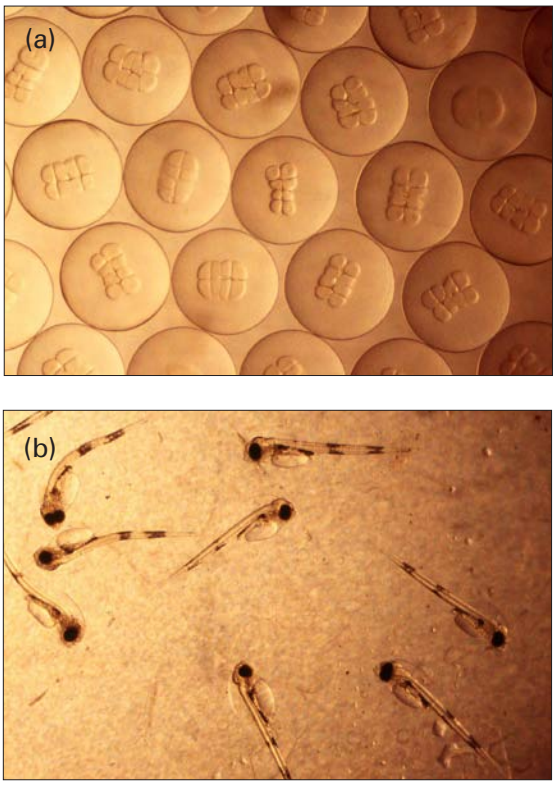

Figure 2.2. (a) Fertilized halibut eggs showing embryos during early cleavage; most are at the eight cell stage. The individual blastomeres of many embryos are not particularly regular in size or shape, indicating these eggs were not good quality; (b) halibut yolk sac larvae about a month after hatching.

2.4 First-feeding

When FC0107 began in 1993, the majority of farmed halibut in the UK and elsewhere were reared with an element of "natural" marine zooplankton in their diet. Indeed, rearing methods that depended on the use of harvested zooplankton formed the basis of Norwegian halibut output at that time. The approach had been pioneered by research biologists who took advantage of the high seasonal plankton productivity encountered in Norway. As Norwegian researchers transferred to the commercial sector, the use of marine zooplankton became central to large-scale halibut production in their country. However, this zooplankton-dependent approach contrasted with the prevailing "intensive" hatchery rearing methods being used globally for the production of other marine fish species. The limitations of zooplankton-usage, such as the unpredictability of copepod productivity and difficulty of maintaining good hygiene were appreciated by UK operators who were experienced in intensive rearing practices for turbot, Dover sole and plaice that involved the production of live prey (rotifers, *Brachionus* sp. and brine shrimp, *Artemia* sp.) within the hatchery. Accordingly, the agreed goal was to develop a suitable intensive rearing protocol for halibut juvenile production, while evaluating the apparent nutritional advantages of marine zooplankton.

The nutritional superiority of marine copepods over *Artemia* was evident from their biochemical composition and this was borne out by results on metamorphosis traits and histological structure (gut, retina) of halibut. However, copepod cultivation trials demonstrated the difficulty of reliably producing sufficient quantities of calanoid copepods as feed for halibut larvae without resorting to low density fish culture in very large water volumes. This finding reinforced the requirement to establish a reliable *Artemia*-based production method for halibut. The essential fatty acid composition of fish larvae could be altered *via* different *Artemia* enrichments

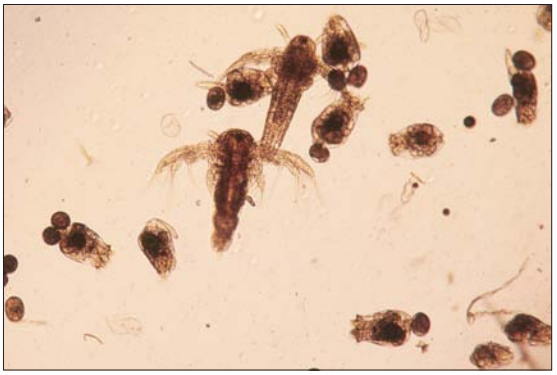

Figure 2.3. Rotifers and *Artemia*: cultured rotifers and larger *Artemia* metanauplii. Some of the adult female rotifers are carrying external parthenogenetic eggs.

and the efficacy of a range of enrichment methods was evaluated in terms of larval rearing success. Experiments were undertaken feeding enriched *Artemia* to the larvae in different combinations for up to 40 or 60 days, however, irrespective of diet, the metamorphosis characteristics of the fish such as eye migration and pigment distribution remained predominantly abnormal.

By 1998 more than 140,000 halibut juveniles were produced in the UK, however variation in survival through the critical early stages remained a significant bottleneck for reliable commercial production. A particular problem was successful transfer from the yolk-sac stage to exogenous feeding, with high, apparently batch independent, variation in the proportion of yolk-sac larvae able to initiate a first-feeding response. For this reason the multidisciplinary, LINK funded project, FC0913, was initiated with a range of commercial and academic partners to investigate the factors affecting successful commencement of first-feeding.

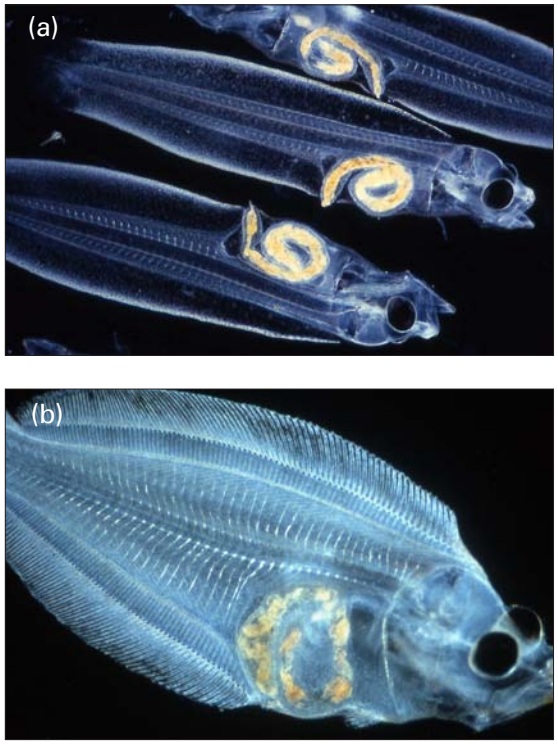

Figure 2.4. (a) Feeding halibut larvae, with partially digested copepods visible in the gut. An *Artemia* metanauplius, also used as a live food organism is visible on the extreme left of the picture between two larvae. (b) Metamorphosing halibut larva, at an early stage of metamorphosis showing a flattened body, and the left hand eye just visible on the upper surface (right hand side) of the head.

These included The British Marine Finfish Association (BMFA), Scottish Association for Marine Science (SAMS) Dunstaffnage, the University of Glasgow, as well as SFIA Ardtoe.

As mentioned earlier, the study led to a successful protocol that reduced damage (both due to injury and pathogens) to larvae at the yolk-sac stage and during the stress of transfer to feeding tanks, and maximised the number of larvae able to feed. The first-feeding stage was also studied, with particular emphasis on the use of "green water" when planktonic microalgae were added to encourage feeding. Three species of microalgae (*Nannochloropsis* sp., *Isochrysis* sp. and *Pavlova lutheri*) were used in studies of the first-feeding stage. Larvae fed and survived better when *Nannochloropsis* was used. Higher algal densities than had been used in the past produced better survival and growth. This did not appear to result from a strong chemosensory stimulus effect, nor was it a nutritional effect, but was likely to be due to the physical presence of the algae (inert particles could be successfully substituted for *Nannochloropsis*).

2.5 Stabilising the microbial environment

It was recognised that the variable egg hatch rates and survivals during the different stages of the halibut production cycle were likely to be influenced by the microbes the developing eggs and larvae were exposed to, as well as other physical and biological factors. Work, initiated by the BMFA, was followed up under FC0913 and showed that it was particular types of bacteria present, rather than numbers present *per se*, that had a significant effect on halibut larval survival.

It was shown that functionally similar bacteria colonised the guts of first-feeding larvae, particularly *Vibrio* spp.. *Vibrio splendidus*, which apparently originated from the live feed, was found proliferating within the guts of larvae reared at three separate UK hatcheries. Genetically and phenotypically similar *V. salmonicida*-like organisms were also isolated from the guts of older animals from all hatcheries surveyed, suggesting these organisms are well-adapted to survival in the gut of halibut.

Rearing trials suggested that colonisation of the guts of yolk-sac larvae by bacteria was a more random process. In replicated trials, 400 litre yolk sac rearing tanks were coupled to a recycle circuit and performance was compared with tanks receiving a conventional flow-to-waste supply, both with and without the addition of antibiotics. Mean survival rates were highest in tanks receiving recycled water (45%), or antibiotic-treated water (53%). Larvae

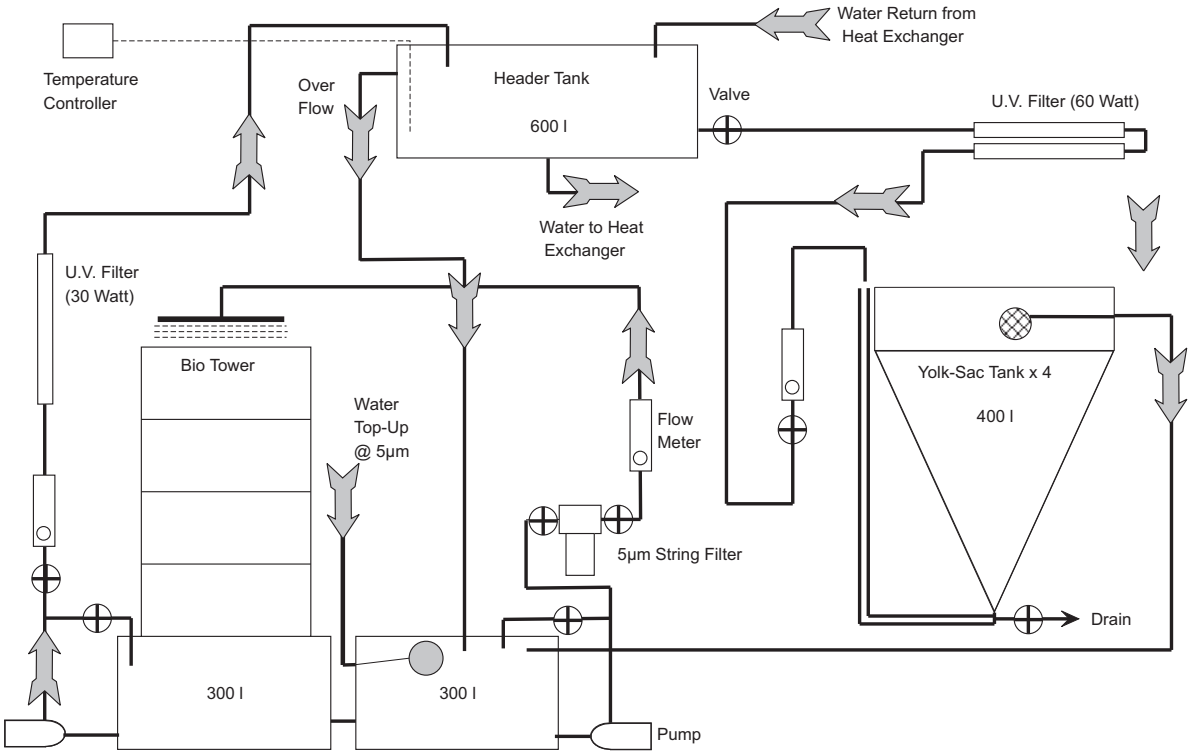

Figure 2.5. Recycled water circuit: schematic representation of the system designed to hold yolk sac halibut larvae in 400 litre tanks. Figure taken from MAFF Research Contract FC0905, Final Project Report, September 1998.

that had received antibiotics contained very low numbers of gut-associated bacteria (<10 larva^{-1}), while those reared in recycled water contained the highest numbers of bacteria (6-7 x 10^3 larva^{-1}). Prefeeding yolk-sac larvae, under recycled water conditions, also had high numbers of bacteria. It was shown that the gut flora of larvae in the recycled water system was much more stable than in the flow-to-waste system with comparable numbers of genetically similar *Pseudoalteromonas* spp. isolated from larvae reared under the recycled treatment regime in temporally replicated trials. The implication is that rearing larvae in recirculated water systems results in more stable rearing conditions, both physically and microbiologically, and leads to more predictable survivals overall.

Further work investigated the toxicity of more than 20 bacterial isolates originally isolated from halibut rearing systems on yolk-sac larvae. It was shown that the majority of these organisms were benign, with very little effect on development or survival, even following exposure to large numbers of these bacteria for more than 20 days (> 10^6 ml^{-1}). However the major pathogen *V. anguillarum* was shown to be lethal to halibut larvae. Work was also initiated under FC0913 to investigate the potential applications of probiotics in Atlantic halibut larviculture. Bacteria isolated from halibut and turbot rearing systems were screened *in-vitro* for production of substances that inhibited the growth of potential pathogens. Three candidate organisms from the *in-vitro* screen, two *Carnobacteria* spp. and a *Pseudoalteromonas* sp. were tested for their ability to protect Atlantic halibut yolk sac larvae against an *in-vivo* challenge by *V. anguillarum*. It was shown that these organisms were not able to protect larvae under the conditions tested. Consequently it was suggested that better success may be achieved using the probiotic approach during the feeding stages, which offer greater opportunity to introduce potential probionts into the larval gut through their diet.

3. Weaning

Details of the following projects are included in this section:

FC0106 Digestive physiology of juvenile Dover sole (University of Wales, Bangor)

FC0108 Further studies into the digestive physiology of juvenile sole (University of Wales, Bangor)

FC0910 (*FC0123*) Optimisation of formulated diets for marine fish larvae (University of Wales, Bangor)

FC0911 Optimisation of formulated diets for marine fish larvae (Cefas - Conwy)

In fish culture "weaning" is the term used for the phase of the rearing process when the fish are transferred from a diet of live food such as *Artemia* to an artificial, inert diet. It is a critical stage when the mortalities can be high if the conditions are not appropriate. Factors such as the size, developmental stage and condition of the fish, the composition and particle size of the diet, and tank hygiene and water quality, all play a part in determining survival. Weaning larval stages of marine fish has met with variable success, but most species of marine fish can readily be weaned after metamorphosis. The sole has been an exception to this, partly because of its sensitivity to the flavour of food, but perhaps also because it metamorphoses at a relatively small size before its digestive system is fully developed. Sole was chosen as the target species for this work because of growing interest in the commercial farming of this species and also because of its availability and the ease with which its larvae could be reared.

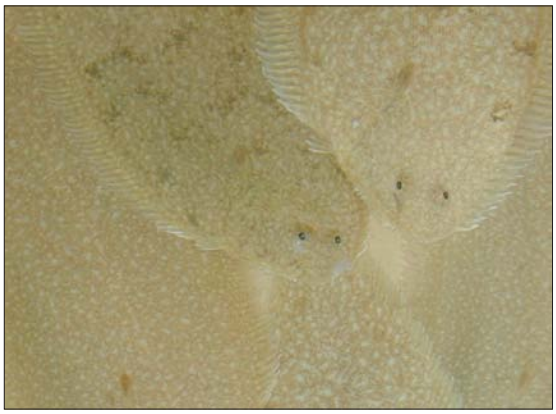

Figure 3.1. Dover sole juveniles: normally pigmented, fully weaned fish.

3.1 Post metamorphosis rearing

Work on weaning to artificial diets was carried out with metamorphosed Dover sole at the Cefas Conwy Laboratory as part of project FC0106. The suitability of a range of weaning diets of different composition was tested for metamorphosed, 30 mm long fish; the most interesting finding was a positive correlation between the proportion of hydrolysed fishmeal in the diet and survival over the weaning period. This suggested that diet digestibility, as well as palatability, was of vital importance for the successful weaning of juvenile sole.

For those groups of fish which had been successfully weaned, an average growth rate of 25 mm month^{-1} could be achieved with some diets. This growth rate compared favourably with an estimated maximum growth rate of 30 mm month^{-1} for sole fed live food. These growth rates were similar to those of wild populations and were maintained over a period of 18 months and survival was 100%.

The knowledge gained in project FC0106 contributed to the design of novel feeds for Dover sole in a collaborative project (FC0108) with the Norwegian Herring Oil and Meal Research Institute (SSF). The company had developed a novel agglomeration process that enabled high concentrations of soluble protein to be included in small diameter particles without loss of particle stability. Weaning trials were conducted with small post-metamorphosis sole of 14 mm (42 mg) and results were very encouraging with over 90% survival and growth equivalent to that obtained with live food. This led directly to the production of first commercially available diet on which juvenile sole could be reliably weaned with minimal mortalities.

These agglomerated diets were not suitable for larval sole, but their use allowed a considerable reduction in the size at which post-metamorphosis juveniles could be weaned and this substantially alleviated the requirement for live food during rearing. The improvements that have been made to weaning diets used to rear sole have enabled further exploration of commercial rearing possibilities for *Solea solea* and the related *Solea senegalensis*, but other problems, such as relatively slow growth of *S. solea* and outbreaks of disease amongst some stocks of reared juvenile *S. senegalensis*, have discouraged industry from pursuing anything more than pilot production of these species for the time being.

3.2 Weaning during the larval stages

Work was also undertaken as part of project FC0106 to explore the suitability of microencapsulated diets for larvae as a total replacement for live feed. At the time, the marine fish-farming industry was very dependent on commercial supplies of *Artemia* cysts from managed wild populations. There had been recent shortfalls in the global supply of *Artemia* cysts following the worst harvest on record from the Great Salt Lakes, Utah, USA. In the long-term, development of artificial diets for larvae would mean that production of fish could be independent of an *Artemia* supply. Initial work with microencapsulated diets (MED) was not encouraging; sole larvae did not feed well on the inert particles, and although staining the microparticles red improved uptake, once ingested they passed along the gut intact. It seemed that first-feeding sole larvae were not producing enough digestive enzymes of the right type. Published information indicated that the presence of microalgae in the tank water would have a stimulatory effect on digestive enzyme production and so the benefits of the 'green water' technique were investigated with sole. Addition of algae however did not improve larval sole growth rates. It was concluded that first-feeding larvae relied on enzymes present in live prey for digestion, and were unable to digest artificial diets because the enzymes were lacking.

The ability to synthesise endogenous digestive enzymes such as trypsin developed quickly as the larvae grew. Ten days after hatching they were able to produce digestive enzymes after ingesting food. An interesting finding was that this was a response to gut fullness rather than the biochemical attributes of the diet. It was shown that even inert polystyrene spheres would stimulate enzyme production.

During these studies information gathered on growth, food intake and oxygen consumption of a cohort of larvae revealed changes in the digestive strategy during development. An observed decrease in the daily weight-specific ration was accompanied by an increase in gross assimilation efficiency; this finding has important implications for the assessment of digestibility and the required energetic content of an artificial diet when a new one is being formulated.

The dependence of first feeding sole larvae on the presence of digestive enzymes in their prey suggested that larvae may be able to utilize artificial feed if digestive enzymes could be incorporated in the formulation; project FC0108 explored this hypothesis. Attempts to make MED containing enzymes using spray-dried particles were successful, but particle sizes larger than 100 µm, which were required for sole larvae, could not be formed without entrapping microscopic air bubbles. After larvae ingested the diet air accumulated in the gut and affected larval buoyancy. Instead, particles were produced by a protein cross-linking method that allowed the production of a wider range of sizes and without the entrapment of air. The stability of the protein cross-linked particles in water was very good. Different mixtures of active digestive enzymes (pancreatic proteases) were included in the formulation. The protein cross-linking process was found to reduce the activity of the enzymes by 60%, but this was considered acceptable. These diets were ingested by first-feeding larvae and particles that included enzymes were shown to break down in the gut, however, feeding trials revealed that they did not produce satisfactory growth. It appeared that other differences between live food and microparticles, such as shape, movement, smell and taste were important in stimulating larvae to feed adequately.

3.2.1 Further studies

The development of commercial formulated feeds for marine fish larvae had been changing rapidly during the years immediately preceding the start of project FC0910. The current status of larval diet production was reviewed as part of the project and a selection of new experimental diets that were being produced by commercial companies were obtained for testing.

Diets from eight feed companies were tested against live-feed controls in feeding trials carried out with sole larvae at 10-days post-hatch (DPH). The diet formulations gave high survival rates in all treatments, but live-feeds still produced superior growth during the early developmental stages. None of the artificial diets tested was shown to be completely suitable as a replacement for live-feed at so

early a stage in larval development; a means of reducing the quantities of *Artemia* needed during larval rearing was an important goal of the work and so alternative approaches were explored.

Further advances in the technology involved in the preparation of inert microdiets of a size suitable for larvae led to major breakthroughs in terms of increasing the bioavailability of nutrients and the digestibility of the diets. Examples of these new diets included high quality partially buoyant diets for the sea bass/bream industry. Several of these new commercial preparations were obtained and tested.

The availability of these diets allowed comparison of growth and survival of larvae weaned onto inert particulate diets at different ages (14 and 49 DPH). The control treatment (49 DPH) represented the standard hatchery practice at the time. The inert diets used were a partially buoyant particulate diet and a negatively buoyant weaning diet. The partially buoyant diet was designed to remain suspended in the water column so that the action of the water current and aeration kept the particles available to pelagic larvae. The results showed that weaning onto an inert diet at 14 DPH reduced the growth and survival of sole larvae compared to later weaning.

Although the earlier video trials had indicated that 14 DPH sole larvae would strike at inert feeds, evidence from other work indicated that the larval gut structure was not fully developed until 21 DPH. It therefore seemed likely that larvae younger than 21 DPH still lacked sufficient digestive enzymes necessary to break down these diets, although partially buoyant inert diets stimulated appropriate behavioural responses.

Further studies demonstrated that sole could be weaned on to inert diets at 21 DPH, but also that there could be disadvantages to this early weaning if inappropriate particle sizes were used. Sole larvae aged 21 DPH were offered either an SSF agglomerated feed (consisting of particles 100-300 µm in diameter) or an experimental weaning diet produced by INVE which was a distinct rod shaped particle of about 400 µm by 700 µm (slightly larger than enriched *Artemia* metanauplii). A further group of larvae were weaned at 49 DPH onto a mixture of the two diets. The trial was run from 21 DPH until 63 DPH; at 63 DPH, once all groups were weaned, there were no significant differences between treatments in terms of survival. The growth results showed that there was no difference in size between the sole weaned onto the agglomerated diet at 21 DPH or onto the mixture at 49DPH, however sole weaned on to the rod shaped diet as larvae at 21 DPH showed a significantly greater size variation than the other groups. Although early weaning may be achieved if appropriate diets are used, the use of inappropriate diets too early could have serious implications in terms of commercial juvenile production. The development of size variation within cohorts increases the necessity to grade fish during rearing, a stressful as well as time consuming operation. Once such size variation is established it compromises the ease of selection of diets with appropriate particle sizes and that may lead to cannibalism in some species or at least facilitate the establishment of dominance hierarchies.

Quantification of behavioural aspects of feeding facilitates interpretation of comparative diet trials, providing information on actual food intake rather than just what was provided. Within studies FC0910 and FC0911 three video techniques (transmitted light silhouette, reflected light silhouette and dark field) were applied to allow feeding behaviour of fish larvae to be recorded in different situations. A new fluorescent spectrophotometric technique was also developed to quantify ingestion of feed by incorporating fluorescent tracer particles in the diet.

3.2.2 Alternative ways of reducing the use of *Artemia*

Around the time that the project FC0911 began, new culture techniques for live-feeds were being adopted by the industry. These allowed maintenance of self-sustaining populations of rotifers in hatcheries to be more reliable and production to be increased 100-fold. In the past the small size of rotifers and difficulty producing a consistent quality product meant that rotifers were only considered suitable as a live food for the earliest stages of fish larval development; as soon as possible they would be followed by a long phase of *Artemia* feeding. Improved rotifer production techniques however enabled commercial practice to shift towards a longer period of rotifer feeding, followed by a short *Artemia* phase before weaning the fish. The project research work therefore concentrated on assessing how best to wean fish from *Artemia* to an inert diet as early as possible as a means of reducing *Artemia* use.

Weaning trials demonstrated that it was possible to wean the fish early and yet avoid increased size variability by 'co-feeding' the inert diet with *Artemia* at the start of weaning. Sole were weaned from *Artemia* onto inert particulate diets either as larvae at 21 DPH, or as 56 DPH juveniles (a week later than standard practice). Groups were also co-fed the inert diet together with a diminishing amount of *Artemia* from 21 DPH until 56 DPH, after which no more *Artemia* was introduced. Sole fed only *Artemia* and those co-fed the mixture of *Artemia* and inert diet did not differ in size significantly. Sole weaned at 21 DPH showed significantly

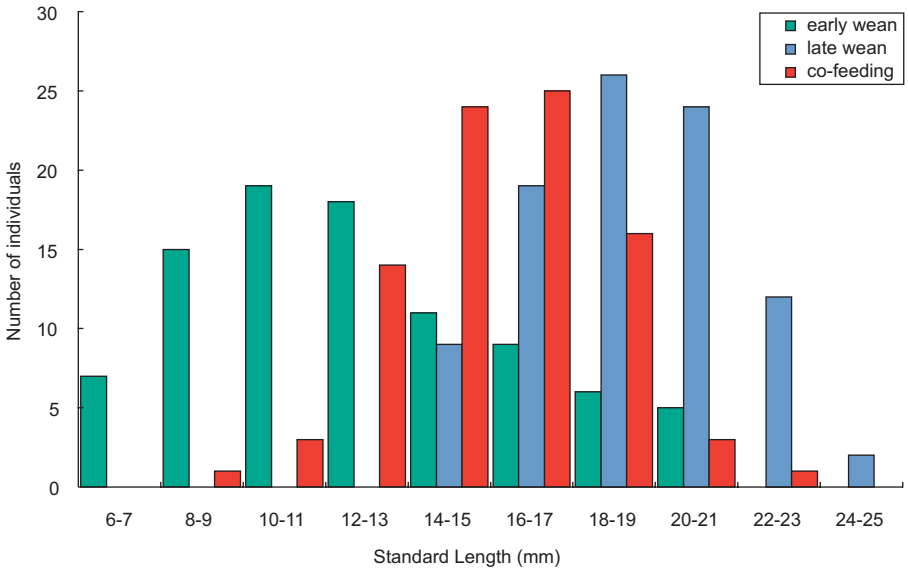

Figure 3.2. The effect of co-feeding: size variation of *Solea solea* weaned from *Artemia* using different dietary regimes; the number of individuals in different size classes when measured at 75 days post-hatch. Co-feeding *Artemia* and the inert diet avoided the increased size variability associated with early weaning. Figure redrawn from MAFF Research Contract FC0911, Final Project Report, March 2001.

higher size variability than the other two treatments (Figure 3.2). Final survival rates (at 75 DPH) were much higher for those fish reared using the co-feeding regime than for those sole weaned at 21 DPH (84% and ~55% respectively), although it was close to 100% for fish weaned at 56 DPH.

It would appear therefore that the use of a co-feeding regime would be a valuable option to help ensure production of juvenile sole of consistent size range and quality while reducing the total quantities of *Artemia* required during the rearing cycle.

Part 1.2 Technical feasibility – quality

4. Morphology

The following project is addressed in this section:

FC0107 Environmental control of halibut broodstock and rearing procedures for feeding larvae (SFIA - Ardtoe)

Abnormal metamorphosis, particularly with regard to pigmentation patterns, characterised much of the early flatfish rearing. The problems might occur intermittently, but in general, with a species such as the turbot, the frequency of occurrence lessened as husbandry practice became more standardised. There had been little advance in understanding the causes of the problem when the work with halibut began.

Halibut larvae that successfully reached the live feeding stage, but subsequently suffered high mortality rates, often exhibited abnormal metamorphosis characteristics such as incomplete pigmentation or pigmentation on both ocular and blind sides (ambi-colouration) as well as partial or failed eye migration. These traits were considered to be exacerbated by conditions that prevailed in UK hatcheries at the time; specifically these were relatively mild ambient water temperatures for spawning and possible nutritional deficiencies arising from the use of cultured live prey.

The effects of dietary and environmental factors on the metamorphosis of halibut larvae were investigated in a series of experiments within project FC0107. Small-scale rearing systems were developed, enabling replicated groups of halibut larvae to be maintained under experimental conditions until the completion of metamorphosis. Standardised morphological and physiological criteria for assessing the quality of the larvae were also established. These were objective indices to record the extent of pigmentation or the position of the eyes, for example. Dietary studies sought to refine the techniques for preparation and presentation of *Artemia*, and to nutritionally evaluate this prey species relative to natural marine zooplankton. The research effort on marine zooplankton was complemented by participation in an EU Concerted Action (AIR3-CT94-2094).

An experiment revealed important physiological, as well as morphological differences between halibut fed

Figure 4.1. Juvenile halibut: metamorphosed fish being hand fed. The normal pigmentation pattern of the fish camouflages them quite effectively against the tank floor, but malpigmented individuals, which are largely white on their dorsal surface, show plainly.

on copepods and those fed on *Artemia* that had been enriched with the commercial products Super Selco™ and Algamac 2000™. In this experiment, replicate groups of halibut larvae were fed either the calanoid copepod *Eurytemora velox* or enriched *Artemia* metanauplii. Only *circa* 10% of *Artemia*-fed group exhibited the correct pigment distribution, compared to 60% of the group reared on copepods. Specific growth rates and indices of eye migration did not differ significantly between diet treatments. Histological examination of livers and intestines was consistent with lipid from copepods being better assimilated than lipid from *Artemia* metanauplii, during the first half of the live feeding phase and furthermore the retinas of halibut reared on enriched *Artemia* contained significantly fewer rod cells, indicative of a lack of retinal membrane DHA.

Results of industry-sponsored research demonstrated that there was a positive relationship between successful eye migration in halibut larvae and their growth rate during the early larval phase. The incidence of ambi-colouration was also seen to be associated with eye migration. It was therefore concluded that the abnormal traits observed in earlier diet experiments within this project resulted from low growth rates. The adoption of higher feeding rates during the larval stages, by consistently feeding to satiation, enabled better eye migration to be achieved, but despite this no method of consistently obtaining normal pigmentation was identified.

Published information indicated that light could affect skin pigmentation of flatfish and a further series of experiments was conducted to determine whether pigment expression could be stimulated in *Artemia*-fed halibut by exposure to short wavelength light sources. Significant light-dependent differences were found in the survival and growth rates of larvae which were attributed to variation in prey intake under the different light regimes. However, pigmentation characteristics remained abnormal, irrespective of light source and it was concluded that light manipulation did not offer a means of inducing pigmentation during metamorphosis.

5. Growth and sex

The following projects are included in this section:

FC0120 Sex ratios in Dover sole (Cefas - Conwy)

FC0901 Environmental influences on the sex of cultured marine flatfish (Cefas - Conwy & Weymouth)

FC0903 *(FC0112)* Sex control in halibut and turbot (Cefas - Conwy & Weymouth)

Juvenile fish grow quickly, but once they become sexually mature the associated gonad development leads naturally to a reduced somatic growth rate. It would therefore be beneficial in a fish farming context to have ways of controlling sexual maturation in stocks being reared for sale so that growth rate could be enhanced. Growth rate suppression associated with maturation occurs in both sexes, but in some farmed species (e.g. rainbow trout) the male matures before market size is reached; the female matures at a size that is larger than generally required for sale. Rearing all-female groups of fish avoids valuable tank space being taken up by slower growing males and the technique has been applied to great advantage within the UK trout farming industry for over two decades. The same approach offers considerable promise for use with marine flatfish.

The economic viability of farming flatfish in the UK depends to some extent on the fish attaining a size suitable for harvesting within three growing seasons. Maintenance of a high growth rate throughout this period is important for the farmer, but needs to be achieved at relatively low cost; raising water temperature to realize this, for example, would not always be a financially viable option. The sexes of both turbot and halibut, as with rainbow trout, show substantial disparity in growth rate during the period between the onset of male and female maturation. They were therefore considered suitable candidates for all-female production, provided the requirements for producing all-female stock were relatively straightforward to implement.

Project FC0112 (subsequently FC0903) was commissioned to assess the technical feasibility of single sex production for turbot. The work was a co-operative study with a commercial turbot-hatchery, which provided gametes, larval rearing, tank space and other facilities.

An unforeseen complication became apparent early in the work when it was discovered that male predominance occurred within groups of hatchery reared Dover sole being produced for other work. The proportion of males was frequently found to be in excess of 60% of the population. Published reports showed that in a number of other species the sex ratio of hatchery-reared fish could be heavily skewed towards male predominance. The reasons for this were not fully understood, but male predominance in the flatfish species, *Platichthys olivaceus* (Japanese flounder), and in sea bass, *Dicentrarchus labrax,* was associated with rearing at temperatures at the upper end of their normal thermal range. There was also increasing published evidence that endocrine disrupting chemicals in the environment could affect the phenotypic sex of fish. Such information suggested that the functional sex of species such as sole, turbot and halibut might be affected by similar environmental influences. If this were so, it would have confounded the studies on genetic sex control and would have significant consequences for hatchery reared fish in general. This led to work to investigate the effect of environmental factors on sex determination in these species (projects FC0120 and FC0901).

5.1 Sex control in halibut and turbot

In many species, sex determination is controlled primarily by sex determining genes on the sex chromosomes and two predominating models are recognised. In one the female is the homogametic sex (XX) and the male is heterogametic (XY) while in the other the female is heterogametic (WZ) and the male homogametic (ZZ). With these sex determining systems normal mating leads to 50:50 sex ratios. Less common, alternative, sex determining mechanisms exist that involve minor sex genes on other chromosomes. In species with such systems different individual pair matings may yield batches of progeny that display varying skewed sex ratios due to different combinations of these minor sex genes in the offspring.

The principal objective of project FC0903 was to identify the sex determining system in turbot so that methods could be devised for the production of all-female broods. The approach chosen was to make inferences about the nature of the sex determining mechanism from the

sex ratios of normal and gynogenetic fish and of their progeny from crosses utilising sperm from sex-inversed fish. Gynogenetic fish have only maternal genetic material and sex-inversed fish in this instance were genetic females, hormonally treated to produce sperm. These studies required protocols for the induction of diploid gynogenesis and sex inversion to be established at the outset.

Turbot was the target species initially, but because of increasing interest in farming halibut in the UK the scope of the project was extended to include halibut as well. Halibut show a growth differential between the sexes that is even greater than that of turbot. In halibut the growth of males slows as they mature after their first summer in sea cages, whereas the females reach a harvest weight of 3-4 kg before maturation affects their growth rate. Advances were made in developing the methodology during the project, however the project was terminated early when it became clear that technical problems and commercial priorities would not allow the final stage of the work to be completed within the time-frame set for the project.

5.1.1 Turbot

The sex ratios of batches of reared 'normal' turbot obtained from commercial hatcheries were consistently found to be 1(male):1(female). This was indicative that a simple genetic system determines sex; one of the two common sex determining systems could operate.

The first part of the work to identify the sex determining system was to develop a means of producing gynogenetic individuals. If batches of gynogens are all-female this would be indicative of the XX-XY system whereas if at least 50% are male it would suggest the WZ-ZZ system. Meiotic gynogenesis was used for this work as it normally leads to better embryo survival than mitotic gynogenesis, but a consequence of this is that recombination during meiosis can lead to a variable proportion of males in species in which the female is heterogametic.

Previous work had shown that turbot eggs could be activated by exposure to halibut sperm. Activation with halibut sperm was considered to produce haploid gynogenetic individuals, without a genetic contribution from the male because the resultant embryos displayed typical haploid features (relatively small eyes and a short thickened body). A protocol was developed to restore the diploid state following activation by exposing the eggs to a low temperature shock. Three minutes after the eggs were activated at 12°C they were immersed in water at approximately 0.5°C for 1 hour and then returned to 12°C. These embryos developed with a normal appearance, confirming their gynogenetic status. The success of this procedure was found to be highly variable and depended on securing good quality eggs, obtained within a few hours of ovulation and displaying high fertilisation rates and symmetrical early cleavages.

Relatively large scale induction of diploid gynogenesis was achieved four times during the life of the project, producing batches of 20,000 to 250,000 eggs. Two batches were reared through the larval stages. Although survival was low (< 1%) sufficient fish were reared for an estimate of sex ratio and for on-growing to maturity. The

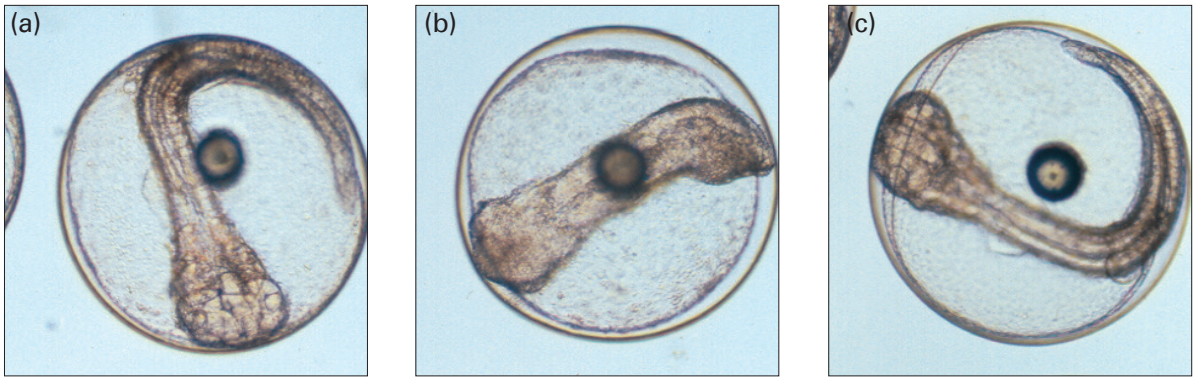

Figure. 5.1. Turbot embryos: A comparison of (a) normal, (b) haploid and (c) putative diploid gynogenetic turbot embryos 4 days after fertilisation (1 day before hatching). Note the typical shorter and stouter features of the haploid and the very strong resemblance of the putative diploid gynogen to the normal embryo.

first of these batches, reared on the Isle of Man, had a high proportion of females (90%), the second, reared at the Conwy laboratory, was lower (69%). The presence of a variable proportion of males amongst the gynogens could be explained by recombination during meiosis if turbot possessed the WZ-ZZ system (female heterogamy). Further evidence for this was sought by progeny testing.

Methods were developed to induce sex inversion (by masculinising the gynogens); the most effective treatment identified was to feed juvenile fish a diet containing methyl-testosterone at a concentration of 1 mg kg^{-1} of food over a period of 400 day-degrees. Treatment began after the fish had been weaned from live feed to an inert diet (starting at approximately 800-1000 day-degrees post-hatch).

Once the masculinised gynogenetic fish matured, their sperm was used to fertilize eggs from normal females. The sex of progeny from such a cross provides information on the sex determining system. If the system were of the XX type gynogenetic fish would also be XX and genetically female; crossing masculinised gynogens with normal females would give rise to all-female progeny. Several crosses using different masculinised gynogens were carried out so that the sex ratios of the offspring could be checked, but only one group of progeny survived to metamorphosis. The cross produced a group of fish in which the sex ratio was skewed towards a predominance of females (65%), but that was considerably lower than expected if turbot possessed the simple XY sex determining system. Unfortunately time constraints meant that no further progeny testing was possible within the lifetime of the project and repeated tests would have been necessary to make an assessment of the variation between individuals.

The results provided evidence that sex determination in turbot is not the simple XY system. The sex ratio of progeny from the cross of a normal female and a masculinised gynogen was consistent with the ZW system, but further progeny testing would be needed to clarify whether this is really the sex determining system in turbot. Other results from the work were not wholly consistent with the heterogametic female model and other systems involving minor sex genes could also have given a similar outcome.

5.1.2 Halibut

Developing protocols to induce diploid gynogenesis in halibut eggs proved more challenging than for turbot. Experiments using heterologous sperm demonstrated that it was possible to activate halibut eggs with either turbot or cod sperm, but there was little morphological difference between the resultant embryos and normal diploids and they failed to hatch as viable larvae. This was taken to indicate that the embryos had probably received a genetic contribution from the sperm and were therefore not true gynogens. The uncertain genetic status of these embryos led the researchers to instead use ultraviolet (UV) irradiated sperm to activate the eggs. UV irradiation, at the correct dose, destroys the functionality of DNA in sperm without completely disrupting its motility and ability to penetrate the micropyle of the egg. Having established an appropriate treatment to provide a means of activating the eggs, they then found that cold-shock was not a reliable method of inducing diploidy for halibut; using a hydrostatic pressure shock appeared to be a more suitable technique. Preliminary findings suggested that a workable protocol would involve exposing activated eggs to a pressure of 600 kg cm^{-2} for 5 minutes, starting less than 15 minutes after activation. The time involved establishing these procedures and the difficulties associated with rearing viable halibut gynogens meant that unfortunately there was no opportunity to produce progeny for sex-ratio determination before the project ended.

The project did generate workable protocols for inducing gynogenesis and producing sex-inversed turbot and halibut; it also went some way to establishing that turbot do not have a sex determining system of the XX-XY type. Comparable work involving both species continues in Spain, Norway and Canada and results from FC0903 have been made available to scientists involved in these international projects. By using molecular techniques now available it should soon be possible to clarify the uncertainties that remain over sex determination in these species.

5.2 Environmental influences on the sex of flatfish

In the mid 1990s male predominance was found to be a characteristic of batches of Dover sole reared at the Cefas Conwy Laboratory. Sex ratios of 2 males : 1 female or 3 males : 1 female were common, with exceptional batches consisting entirely of males. Anecdotal reports indicated that such occurrences were not confined to sole at Conwy, for example sea-bass reared in commercial hatcheries in southern Europe were reported to be, on average, 70% male.

A survey of published literature and preliminary experimental work suggested that skewed sex ratios in Dover sole could be caused during the early weaning stage by effects of routine husbandry practices such as the relatively high temperatures, artificial diets or high stocking densities, used in intensive rearing.

Experiments were undertaken to investigate whether deliberate manipulation of these factors could be shown

to affect phenotypic sex in Dover sole. Despite a series of experiments with fish of different ages no effect of elevated rearing temperatures during larval development, weaning or the early juvenile growth phase could be demonstrated. The influence of diet was tested by using rotifers instead of *Artemia* during the larval and early juvenile stages as well as by delaying weaning onto artificial diets until the fish were 40 mm long. These changes had no significant effect on sex ratios either. An experiment on stocking density showed no effect, but did give an indication that passing the incoming hatchery water over activated carbon, which would be expected to remove low molecular weight, water soluble substances, might reduce the predominance of males.

One of the objectives of the project was to obtain information about the normal timing of gonad development to enable the period of differentiation leading to a recognisable testis or ovary to be established. It proved difficult to obtain sufficient samples of ovarian tissue from normally reared fish because of the predominance of males and it was also difficult to identify primordial gonad tissue in histological material from late larvae and newly metamorphosed sole. Relatively few samples were obtained during the first four months of development in which phenotypic sex was unambiguous. The earliest indications of gonads differentiating were in samples from fish aged between 80 and 100 DPH when the sole were between 23 mm and 26 mm standard length.

The low numbers of females in batches of reared fish indicated that there could be some disruption to the endocrine system controlling differentiation. However an element of the work provided confirmation that sole were at least sensitive to oestradiol, and that the skewed ratios were not a result of a lack of oestrogen receptors in the tissues. Genetically normal, metamorphosed sole were treated with 10 µg l^{-1} 17β-oestradiol in the rearing water for 2 hours daily between day 50 and day 115 post-hatch. The proportion of females in the treated group was 35% whereas in the untreated control group it was only 5%.

In a separate piece of work using turbot produced as part of project FC0903, low growth rates during the larval stages, independent of temperature, were found to be associated with an increase in the proportion of males. Gynogenetic fish reared at Conwy, under standard conditions were graded during early growth; when sampled 6 months later, the group that had grown fastest before the initial grading were found to be 79% female, compared with 58% of the group that had been slower growing. The sex ratio of the faster growing group was much as expected for gynogenetic turbot, but the explanation for the higher proportion of males in the group that initially grew more slowly remains unclear.

At the end of the work it was apparent that features of standard rearing protocols routinely used for Dover sole husbandry, such as temperature and diet did not directly contribute to the phenomenon of male predominance in hatchery reared sole stocks, but the causes underlying skewed sex ratios were not clarified. A single observation made at the end of the study, that passing the hatchery seawater over activated carbon before supplying the tank was associated with a greater proportion of females than usual was not followed up, but could indicate that small molecular weight substances dissolved in the hatchery sea water contributed to the problem; further work would be required to confirm this.

6. Stress tolerance

Details of the following projects are included in this section:

FC0902 *(FC0109)* Quality of hatchery reared flatfish (Cefas - Conwy)

FC0906 *(FC0116)* Dietary lipids and cold tolerance in juvenile flatfish (University of Liverpool)

Commissioning of Project FC0902 was stimulated by widespread concern in the fish farming industry over variable performance of juvenile marine fish supplied by hatcheries. Variability, in terms of criteria such as growth, survival and susceptibility to disease had been reported both between hatcheries and different batches within hatcheries. Previous research had provided evidence of an association between rearing conditions during larval development and subsequent performance. This included weaning success in turbot and the growth rate of juvenile lemon sole, both of which had been linked to nutritional factors during earlier developmental stages.

One aspect of an evaluation of the feasibility of stock enhancement undertaken at the Conwy Laboratory was to consider options for releasing reared fish into the sea. Fish would normally become available for release in the autumn, but information on low-temperature tolerance was needed to ensure that such a strategy would not have a detrimental effect on survival. This led to experimental work that revealed that the response of reared 50-70 mm juveniles to a standard simulated winter temperature regime was markedly different in two different years. Subsequent work demonstrated that this difference was probably attributable to a change in feeding protocols for the larval stages between the two years. The use of *Artemia* enriched with (n-3) HUFAs as a larval feed during the second year produced a marked increase in the low temperature tolerance of the juveniles about 4 months later.

Growth and survival are the more usual parameters by which quality of fish is assessed, but this observation importantly indicated that nutritional (and other) factors during early development might have a marked, but perhaps subtle, effect on performance much later in life. This led to project FC0116 being commissioned from a research group at Liverpool University to complement FC0902, working with the Conwy investigators and using the material generated by that work.

The principal objectives of these two projects were to determine the effect of larval diet quality on the performance of the fish measured in terms of various aspects of commercial importance. These included the effectiveness of the transfer from live to formulated feeds (weaning), juvenile growth rates and tolerance to low temperatures. Subsidiary aims were to determine the effect of nutritional factors on the response of fish to stressors other than extreme temperature, to explore the involvement of specific fatty acids known to be of importance in marine fish, and to determine whether there were specific periods during development when a poor quality diet was particularly likely to lead to the fish becoming susceptible to environmental stressors. Dover sole was the principal species used for this work because of the background information already obtained, but in addition, a single experiment was carried out with turbot. The aim of this particular experiment was to determine whether juvenile turbot from hatcheries that had adopted different rearing methodologies displayed differences in their stress responses in a similar way to sole, but none was demonstrated.

As the sole were at an age when they were feeding on a live- rather than a formulated-feed, their diet was manipulated by using standard reference lipid emulsions to influence the composition of *Artemia*. These emulsions contained equal quantities of lipid, but the quantity of HUFA was controlled and this enabled the nutritional quality of the *Artemia* to be varied in relation to lipid quality not quantity. In particular the levels of the HUFAs docosahexanoic acid (DHA or 20:5 (n-3)) and eicosapentanoic acid (EPA or 22:6 (n-3)) were controlled. Various attributes of the fish reared on these diets were assessed including growth, survival and tolerance to stress. The latter was assessed by both a short-term (<24 h) exposure to extreme environmental conditions (mainly a combination of low temperature and salinity) and a more prolonged, but less severe exposure to low temperature that simulated winter sea conditions (Table 6.1).

The work confirmed that variations in thermal tolerance of juvenile sole were attributable to the lipid quality of the diets offered during the live-food stages. These variations were manifest even when there were no effects on other important performance characteristics such as survival and

Table 6.1. Fatty acid composition of enriched *Artemia* used to investigate the effect of larval diet on juvenile quality. The composition is recorded as mg fatty acid methyl esters (FAME) g^{-1} dry wt of newly hatched (NH), and enriched *Artemia* (AZ, zero HUFA-enriched; AH, high HUFA-enriched) and the enrichment emulsions ('zero' and 'high' HUFA) used. From MAFF Research Contract FC0902, Final Project Report, March 2000.

	Utah *Artemia*			Enrichment emulsions	
FAME	**NH**	**AZ**	**AH**	**Zero HUFA**	**High HUFA**
14:0	6.75	30.82	6.25	61.34	6.49
16:0	20.55	23.52	17.83	35.50	32.26
18:0	8.99	9.23	7.74	10.22	7.8
16:1(n-9)	0	0	0	0	10.31
16:1(n-7)	5.67	4.84	5.13	0	0
18:1(n-9)	29.17	33.89	25.99	23.24	34.01
18:1(n-7)	14.81	13.61	13.69	0	0
20:1(n-9)	1.00	1.05	1.25	0	1.24
18:2(n-6)	9.20	14.50	8.62	17.30	16.17
18:3(n-3)	62.33	44.29	31.06	1.61	2.24
18:4(n-3)	19.55	11.50	7.45	0	2.91
20:2(n-6)	0.38	0.34	0.51	0	0
20:3(n-3)	3.67	2.79	2.12	0	0
20:4(n-6)	0.69	0.89	2.84	0	2.94
20:5(n-3)	3.48	3.53	11.06	0	10.56
22:5(n-3)	0	0.19	1.70	0	1.69
22:6(n-3)	0	1.45	18.60	0	33.10
Σ HUFA (n-3)	7.15	7.96	33.48	0	45.35
DHA/EPA	0	0.41	1.68	0	3.13

growth rates. There were no differences in response of the different diet groups to abrupt exposure to low salinity or high light intensity, but low-HUFA fish were considerably more susceptible to low oxygen levels than high-HUFA fish. This indicated that nutritional deprivation with regard to lipid quality during early development might have had a general effect on 'hardiness' rather than merely influencing a specific characteristic such as thermal tolerance.

There was a marked difference in response to short-term stress tests at the time the different diets were being administered, however, once the dietary deficiency had been removed the differences in response disappeared within four weeks. The use of *short-term* stress tests as indicators of juvenile quality, common in the shrimp industry, may therefore be misleading in sole. Even when these showed no difference between treatment groups, a persistent difference in stress tolerance could be demonstrated after a further 4-6 months by a less severe, but more prolonged challenge.

Such demonstration of a long-lasting effect of larval diet quality on thermal tolerance was not achieved consistently when the work was repeated with different groups of fish. This lack of reproducibility appeared to be attributable to the temperature conditions prevailing during the two months prior to the challenge. Only limited support for this suggestion was obtained from an experiment in which different rates of acclimatisation over this two month period were tested; the results proved inconclusive because of low mortality rates among all the groups. Some circumstantial evidence, however, did indicate that dietary induced differences in stress tolerance existed even when the standard challenge failed to demonstrate it.

The experiments revealed an apparent low dependence of Dover sole on a dietary source of DHA, provided adequate quantities of EPA were available. It was not clear from the research whether the low dependence was because EPA could be substituted for DHA and nevertheless ensure normal cellular function, or alternatively, because sole biosynthesise DHA from EPA at a rate sufficient to avoid impairment of normal function. The researchers suggested that the apparent lack of dependence on dietary DHA, compared to requirements of other marine fish in culture, could be a reason for Dover sole being relatively easy to rear through their larval stages.

7. Behaviour

Details of the following projects are included in this section:

FC0102 Techniques for the cultivation of Dover sole (Cefas - Conwy)

FC0902 *(FC0109)* Quality of hatchery reared flatfish (Cefas - Conwy)

Research to establish the requirements for producing juvenile fish that would be fit for release as part of stock enhancement exercises not only explored stress tolerance, but also assessed differences in behaviour of hatchery reared and wild fish in order to evaluate whether survival would be compromised by rearing in a hatchery environment. Laboratory experiments carried out with Dover sole under FC0102 showed early on that cryptic behaviour was influenced by the hatchery environment. The rate of colour adaptation was reduced by holding fish on backgrounds of constant colour, and experience appeared to accelerate the rate of burying in a sandy substratum. Hunger was shown to be a major determinant of activity levels so the rapid establishment of feeding after release to the wild would be important in reducing dispersion and vulnerability to predation. Fish released into mesocosms fed within 6 hours, but the normal nocturnal feeding pattern was less marked among hatchery fish than amongst those reared in semi-natural environments

The work was followed up within FC0902, a project more specifically concerned with effect of the nature of the diet in the hatchery on the quality of reared fish and this work was complemented by participation in an EU AIR2 project, Evaluation of Stock Enhancement of Marine Flatfish (AIR2-94-1732).

As described in the previous section, evidence was obtained that a diet deficient in DHA would adversely affect the sensory and nervous systems of marine fish. This suggested that brain function could be compromised as a consequence. An experiment carried out to examine learning and memory in sole reared on diets of different DHA content showed juveniles could be conditioned using a feeding stimulant (betaine) to show a touch response to either a black or white rod when rewarded with food. The fish then had to choose between the colour to which they had been trained and the non-trained colour. On completion of learning, the fish were tested again up to 24 days later, during which time there was no reinforcement of the training. Sole showed a significant memory that was unaffected by the duration of the period. The experiment demonstrated that neither learning nor memory were significantly affected by a DHA-deficient rearing diet, and showed that juvenile sole can learn a conditioned response using visual clues for discrimination that can be retained for several weeks.

Various behavioural studies were carried out to compare hatchery-reared and wild sole. There was no difference in motivation to bury between reared and wild fish, or in selection of a sandy substratum in preference to a hard substratum, indicating that burying behaviour is innate. However ability to bury did improve with practice; there were significant differences between the proportion of cover achieved by wild sole, naive hatchery-reared sole, and hatchery-reared fish that had been given previous experience of sand. The proportion of cover achieved increased after hatchery-reared sole were kept on sand and fish tested after they had been kept on sand for 30 days achieved the same cover as the wild sole (Figure 7.1).

There were differences between wild and reared sole in their choice of light or dark substrata although this may have been due to a preferred light intensity rather than the colour *per se*. Adaptation of sole skin colour to match the substratum colour took some time to complete. The change to the point that the relative darkness of the skin closely resembled that of the substratum took less than a week, but complete adaptation of hue and shade was more prolonged, taking 4 and 12 weeks respectively. The effect of colour adaptation on vulnerability to predation was examined experimentally, but the result was not-significant

Reared sole showed an innate response to a model predator that was in accord with wild fish, the reactive distance was affected by whether the fish were buried or not. Reared sole also showed an innate signalling behaviour by raising the black pigmented pectoral fin on the ocular side when exposed to a predation threat. This was considered to be a behavioural defence against predation as it mimics the black first dorsal fin of the venomous weever fish.

Reared turbot and wild turbot were also compared. Reared fish did not eat as large a size range of prey as wild fish, resulting in a lower weight of stomach contents. An increase in feeding rate with experience

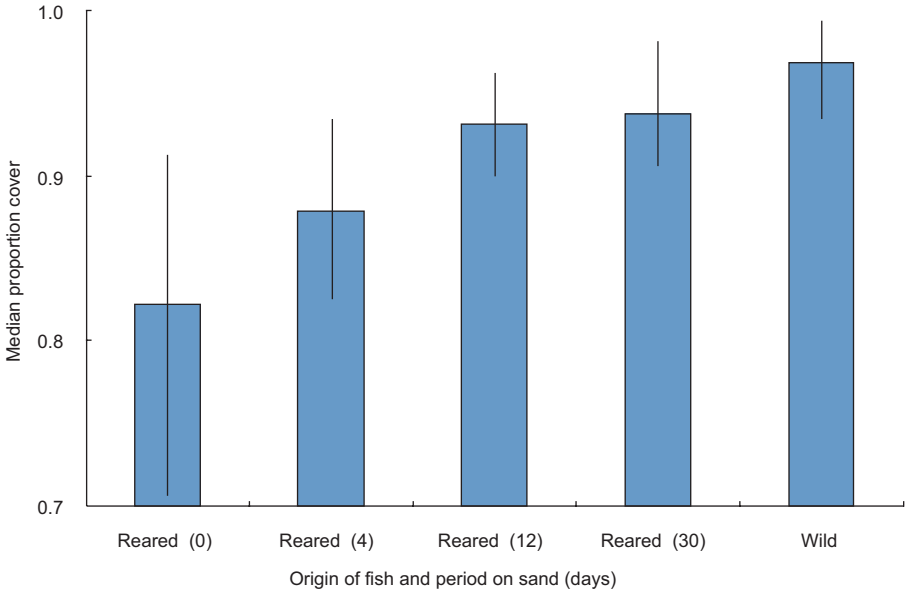

Figure 7.1. Proportion of cover achieved by sole: Reared fish tested after various periods of maintenance on sand compared to wild caught individuals. Values are medians with 95% confidence limits. Figure redrawn from Ellis *et al.*, 1997.

was recorded, due to an increase in motivation to attack and in efficiencies of prey recognition and capture. These behavioural parameters increased to the level of the wild turbot within 9 days experience of natural prey; a slow creep style of pursuit of shrimp was innate to reared turbot. A mesocosm experiment illustrated that reared turbot previously conditioned to natural prey foraged more effectively than naive turbot. Selection of natural prey was not innate to reared turbot; they differed from wild fish by selecting pellets in preference to shrimp and a memory for objects with familiar pellet-like visual characteristics resulted in attacks on stones.

Reared turbot differed from wild fish in body shape; they were heavier at a given length than wild, due to a greater body width and body depth. Abnormalities in the lateral line morphology were also identified in a high proportion (96%) of reared fish. Although these morphological abnormalities would be of little significance for turbot on-grown in intensive culture, the ability of fish reared in hatcheries for stock enhancement, to detect and respond effectively to predators and prey after release may be affected by their differences in mechano-reception and locomotory performance.

It is apparent that certain behaviours are innate to reared fish, however behavioural deficits of reared fish were identified in feeding and burial efficiency and these were potentially detrimental to survival after release. The efficiency of such behaviours improves with experience implying that conditioning fish to sand and natural prey before release would improve performance. However, the hatchery environment not only results in differences in behaviour, but also generates differences in morphology and physiology that will not be alleviated by conditioning. The work demonstrated that quality of reared fish will fundamentally affect performance and behaviour, not only for stock enhancement but also for intensive culture.

8. Technical feasibility - uptake of the results of the research

The major investment in research on new species has been directed towards Atlantic halibut, a valuable cold-water fish suited to conditions on the west coast of Scotland where it was considered to provide a valuable diversification opportunity for the existing salmon industry. The research described has contributed significantly to the pool of knowledge on the culture requirements of the early developmental stages of this very challenging species.

In the hatchery, there has been a great improvement in the success of rearing methods based on the brine shrimp, *Artemia*. Although the research findings have confirmed that natural marine zooplankton provides more nutritionally-complete prey organisms for halibut larvae, wild-caught zooplankton carries with it a disease risk which is not acceptable for modern aquaculture production. As an alternative, *Artemia*-based rearing protocols have been devised that enable high halibut survival rates to be regularly achieved. Approximately 85% of established feeding larvae can be reared successfully to weaning. The research led to a better understanding of energetic requirements of juvenile fish; consequently the typical growth performance of *Artemia*-fed halibut has improved substantially over the lifetime of the projects. The use of improved rearing procedures has also been associated with better eye migration characteristics in the juveniles. Commercial hatcheries have obtained increased egg yield and viability by supplying temperature-controlled water to their broodstocks. The benefits accrued from providing temperature control have been boosted by the more recent availability of a commercially-produced, formulated broodstock diet.

During the course of this research, the halibut farming sector in the UK developed significantly to the stage where it established increasingly reliable juvenile production systems, with an output of some 500,000 weaned fish in 2003, compared to less than 500 in 1993. Much of the work reported here was done in conjunction with industry partners, thus results were disseminated quickly and new and improved methodologies were rapidly implemented. By 2003 more than 200 tonnes of Atlantic halibut were being produced in the UK and four commercial hatcheries were operating, all using technologies developed and refined under these and allied research programmes in the UK and Norway. The level of reproducibility required for commercial practice was not fully achieved however, consequently industry uptake in the UK has been less than anticipated and industry involvement has diminished. Since 2003 one of the major aquaculture companies in Scotland, which accounted for approximately 70% of Scottish production in that year, has relocated its halibut operations to Norway and two hatcheries have transferred to cod production. Cod is a species in which interest has been heightened in the UK by the increase in market value brought about by declining wild catches, making it a more attractive species than halibut for the time being. Rearing cod is more straightforward than halibut; hatchery methods can be based on those already developed for species such as turbot and on-growing can make equally good use of salmon rearing facilities such as sea-cages.

The work on halibut benefited from the close association between researchers and industry representatives, in terms of planning, monitoring the work programmes and disseminating results. This was achieved through the Halibut Scientific Steering Group and by direct liaison with members of the BMFA. The BMFA now hold annual technical workshops, providing an important forum for updating commercial growers on recent research findings and for establishing relevant industry research priorities. These workshops were started by the Marine Farming Unit at Ardtoe, as a result of funding from Defra and SFIA.

In many ways project FC0913 offered a good example of how diverse funding organisations and institutes were able to maximise research effort by combining resources to solve particular problems. Bringing together industry, microbiologists, physiologists and ethologists to tackle the challenges provided insight and practicable solutions. For example, the role of algae, which is added to first-feeding tanks to achieve reliable survivals, is now better understood and that has meant that cheaper, inert particles can be substituted. The requirement for algae was a significant cost for marine finfish hatcheries, with specialist facilities required for the production of algae on a year round basis. The addition of algae to tanks at the time of first feeding may even have a detrimental effect on tank hygiene and the finding that cheap, inert particles can potentially be substituted for algae, will be of major benefit to the industry generally. A further change in hatchery routine that arose directly from specialist research and provided immediate benefits to commercial hatcheries was the identification of a successful method of enhancing and prolonging milt production by use of GnRHa implants; this followed endocrine studies on spawning of wild and captive broodstock fish.

Work on Dover sole was undertaken largely in the context of evaluating the feasibility of enhancing natural stocks with reared fish. The long-established problem of weaning juveniles from live food to a commercially-acceptable formulated feed was solved largely through studies of feed digestibility. This was done so that the fish could be readily grown to the required release size, but the experimental results enabled the design of a novel

agglomerated feed customised for sole and its success rekindled an interest in intensive farming of sole in the UK and elsewhere. Since the project work on sole diets was completed the agglomerated weaning diets developed by SSF have become commercially available and are now widely used by both research and commercial organisations engaged in continuing R&D work with this species. The diets have also been generally adopted for weaning post-metamorphosis fish by several companies in southern Europe who are working with *Solea senegalensis* culture.

The other main thrust of the work on sole focussed on the survival potential of reared fish in a natural habitat. Evidence of morphological, behavioural and physiological deficiencies that would prejudice the survival of reared fish in the sea was produced. This information has been presented at international fora and published widely and its acceptance by the scientific community means it is now being incorporated into the planning of stock enhancement exercises for marine fish throughout the world. In a farming environment, the studies of the effect of nutritional factors during early development showed that poor performance of juvenile fish may be influenced by their relatively distant nutritional history and, conversely, that the full impact of nutritional deficiencies may not be evident in short-term trials.

Maximising the growth rate of fish is a key economic target in fish farming and controlling sexual maturation may make a significant contribution to this end. For this it is essential to know the way sex is determined, not only to allow sex to be manipulated, but to understand why in some species, including sole, hatchery-reared fish may be male dominated. The research undertaken revealed this to be a complex problem and while significant progress was made much remains to be done.

Various publications arising from these projects provide more details of the research than it has proved possible to include here and are listed in the following section.

The research that Defra has funded to solve problems of technical feasibility has underpinned the development of marine finfish farming in the UK. Over the years that the Seafish MFU, Ardtoe was in operation the research and development that was undertaken there made a significant contribution to the advances and to technology transfer to the industry. Aside from the direct gains that were made in halibut rearing, a further important benefit of the Defra-funded work was to enhance the UK's ability to carry out good quality scientific research on halibut and other fish species relevant to the developing finfish farming industry. This was evident not only in facilities, staff and livestock at the Ardtoe MFU, but in the increasing number of collaborating researchers who made use of those assets. Ardtoe's long-term collaborative link with the IOA at the University of Stirling was particularly important in this respect, and provided valuable expert input in the fields of fish reproduction and nutrition. Several of the research students who completed their training with projects at Ardtoe have since made further personal contributions to the industry by going on to take up positions with marine fish farming companies. More recently, the UK research effort has expanded to include the topics of immunology, parasitology, endocrinology and microbiology, all of which incorporated practical work at Ardtoe during the period of Seafish's tenure. Despite the Unit's closure, the publications, students and know-how that have come from the research funded there provide an invaluable legacy that remains a major resource from which the marine finfish farming industry can draw in future.

9. Technical feasibility - publications arising from the research

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Part 2 Sustainability

For finfish farming to continue to develop as an environmentally sustainable industry, various elements of current aquaculture practice require attention. Defra has supported work in this area with defined projects that addressed particular problems.

One of the most pressing issues being considered is better use of available protein sources for diets so that fisheries resources, which are exploited for the production of high quality fish meal, are used more efficiently for the production of carnivorous fish on fish farms. Substitution of a proportion of fish meal by vegetable protein is one method that is being explored.

Other areas of research that have been supported relate to managing the environmental impacts of intensive aquaculture. One has sought to develop an objective means of improving the selection of sites for cage based fish farm operations in sea-lochs through modelling water flows. Better understanding of water flow in a location enables cage sites to be selected so their environmental impact can be minimised, and production efficiency maximised. For tank-based farms effluent can be minimised and the consumption of non-renewable resources reduced through optimising water use by partial water recycling. A project to optimise recirculation systems for rearing the nursery stages halibut production was supported as part of FC0912, but the recommendations on the best use of recirculation technology, based on the data generated, are applicable to many aquaculture situations.

Numerous projects that seek to minimise the environmental impact of disease outbreaks and disease treatments, frequently of relevance to the salmonid cultivation industry, are not included in this particular review. However, one in this field is of some relevance: the project explored methods of improving the survival of wrasse used as cleaner fish to control parasitic lice amongst salmon, which both reduced the exploitation of the wild populations of wrasse and fostered a reduction in the use of chemicals for lice treatment.

Aquaculture can be made environmentally sustainable, but the industry also needs to ensure that its practices are acceptable to the public if it is to prosper. Fish welfare is an important consideration and it needs to be shown that husbandry practices do not adversely affect the fish. Work has been supported that developed techniques to assess whether cultured fish are stressed because of the density at which they are held. The quality of aquaculture products is also of critical importance to the public. The products will not be acceptable in the marketplace if not of the highest standard. Defra has supported work in this area too, with research to evaluate the antioxidant role of carotenoid pigment in salmonid diets and to understand the causes of flesh taints in cultured trout.

10. Efficient use of fish meal in aquaculture diets

Details of the following projects are included in this section:

FC0108 Further studies on the digestive physiology of juvenile Dover sole (Cefas - Conwy)

FC0930 Substitution of fish meal with vegetable protein in cod diets (SAMS - Ardtoe)

FC0931 Laboratory assessment of samples involved in substitution of fish meal with vegetable proteins in cod diets (SAMS - Ardtoe)

Most farmed fish species are carnivorous and have a requirement for high quality protein in their diets. Formulated feeds used for fish production, in general, have comprised more than 80% fish meal. Substituting vegetable protein, which can be readily obtained from agricultural crops, for a proportion of the fish meal in such aquaculture diets reduces the quantity of fish from the capture fisheries needed for feed production. In the past, vegetable protein preparations have not been widely used in diets for carnivorous fish because of the presence of anti-nutritional factors (ANFs), but now most of these can be removed by improvements in processing. The amino acid profile of vegetable proteins also requires supplementation to ensure a suitable dietary intake for the fish.

Work carried out in project FC0108 addressed the optimisation of on-growing diets for sole and turbot. Based on availability, cost and chemical composition, soya bean meal (SBM) was selected as the most suitable alternative to fish meal as a source of protein. While processed forms of soya have been routinely used in the formulation of commercial diets for salmonids, their use in marine fish diets had received little attention when this work began. Experiments with one year old sole and turbot demonstrated that 25% of the fish meal protein could be replaced with soya protein without any significant change in growth, though at higher levels growth was reduced due to a combination of lower intake and inefficient usage of the amino acid supplements required to maintain a balanced dietary amino acid profile.

Work with cod under FC0930 indicated a similar level of replacement could be achieved with diets for that species. The trial was conducted by SAMS Ardtoe in conjunction with the UK Association of Fish Meal Manufacturers (UKAFMM) and four fish feed companies: SFF, EWOS, BioMar and LAXA. The project tested the effect of partially replacing fish meal with different levels of a vegetable protein containing minimal anti-nutritional factors and reduced carbohydrate. Three levels of inclusion of full fat soya protein (12, 24 and 36%) were compared. Fish growth and the quantity of feed needed to produce a unit weight gain (feed conversion) were measured. The trial demonstrated some reduction in growth and feed conversion in fish fed with 36% vegetable protein, but no significant effects were detected at the 12 or 24% replacement levels.

Project FC0931 expanded on the work of FC0930 with an examination of the effects on muscle, liver and bone samples, essential fatty acids and pathology of cod fed the experimental diets. Inclusion of 12% full-fat soya led to a decrease in the apparent digestibility coefficients (ADCs) for fat, protein and starch as well as in the retention efficiencies for both protein and energy, but there were no further statistically significant changes with increasing levels of substitution.

Inclusion of soya in the diet did affect the zinc content of bone and the content increased significantly with increasing levels of soya. Calcium and phosphorus, however, were not affected in the same way. All diet treatments led to livers with favourable EPA and DHA profiles that were similar to those of the diets, but linoleic acid (LA) levels in the liver were higher. If such diets were used to rear cod commercially the LA levels would be sufficiently high to preclude their livers being used for cod liver oil production and this might affect the financial viability of the enterprise. The fatty acid composition of muscle was less affected by the diet; EPA and DHA were selectively incorporated into the muscle, but the increase of LA in the muscle was less than the increase in dietary content. Over all, the inclusion of full-fat soya in the diets did not have a marked effect on the whole body composition or nutrition (Figure 10.1).

Fish fed full-fat soya at 36% substitution showed mild posterior gut inflammation. The severity was significantly less than has been reported in Atlantic salmon fed similarly substituted diets, however, these effects could influence performance. The researchers considered that the effects of full-fat soya on the immune system should be examined

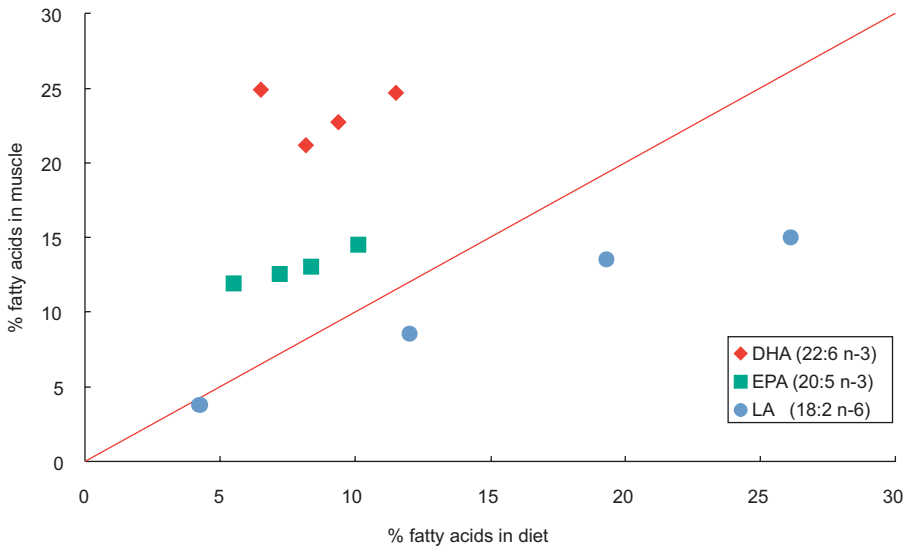

Figure 10.1. Changes in fatty acid content of muscle of cod fed on diets containing soya meal. Incorporation of both eicosapentanoic acid (EPA) and docosapentanoic acid (DHA), measured as a percentage of total fatty acids in cod muscle, was greater than the percentage in the soya meal diet. Linoleic acid (LA) on the other hand was not incorporated into the muscle to the same extent as the increase in dietary content. Points are means of duplicate analyses. Figure redrawn from Defra Research Project FC0931, Final Project Report, February 2005.

further. They felt that even though a diet with substituted raw materials could be utilized effectively by cod, there was sufficient effect on the fish for them to recommend a cost-benefit analysis be undertaken on the use of whole soya as a protein and oil source.

This research has contributed to the search for sustainable feed production for marine finfish species which is so important given the expanding nature of the fish farming sector. Expansion is likely to bring changes to north temperate areas from what is at present primarily salmon farming to a greater emphasis on alternative marine finfish species in the near future. This area of growth is likely to be promoted by declining wild stocks of marine finfish species and would place further pressure on supplies of fish meal. The results should be of particular benefit to UK fish farmers; the economics of cod farming are looking promising, but profitability is marginal, in part, because of the high cost of fish meal based feed.

11. Managing the environmental impacts of aquaculture

The following projects are included in this section:

FC0105 Optimal model formation for marine ecosystems (University of Strathclyde)

FC0110 Winter survival in wrasse (Dunstaffnage Marine Laboratory)

FC0114 A tool for the evaluation of sea-loch nutrient loading (University of Strathclyde)

FC0912 *(LINK – FIN 19)* A new recirculation system for rearing juvenile halibut using novel technology from the tropical marine fish industry (Gatty Marine Laboratory, University of St Andrews)

11.1 Modelling nutrient flux from marine fish farms

Sea-lochs provide a sheltered, marine environment in a fjord-like system that have been adopted by mariculture enterprises such as salmon and mussel farming. In the west coast sea-lochs of Scotland, salmon farming in particular developed rapidly in the 1980s and 1990s and is now a multi-million pound industry. However, there has been concern that nitrogenous excretion from high densities of caged fish may have deleterious effects on the ecosystems of these partially land locked bodies of water.

The development of fish farming needs to be managed in a sustainable fashion, both economically and environmentally. As the industry began to expand into coastal waters only limited information was available about the potential impact of salmon farming on the marine environment, or how potentially adverse impacts could be mitigated. To address these problems Defra part-funded research that was aimed at modelling nutrient flow in sea-lochs (FC0105 and FC0114). One of the objectives of this research was to develop predictive models that could be used to aid management and site selection for fish farms in sea-lochs chosen as sites for salmon mariculture.

The work undertaken was a collaborative exercise between FRS Marine Laboratory in Aberdeen and the University of Strathclyde. A strategic simulation sea-loch ecosystem model was developed, which integrated tidal flushing with chemical and biological fluxes. Initially, they used a data set assembled from a long term collaborative study of Loch Linnhe to develop the model, they then validated it by testing its predictions against real observations from five contrasting Scottish and Irish lochs. The model incorporated all the major physical and biological elements of the system, but was still simple enough to allow unambiguous identification of the mechanisms underlying its behaviour.

The vertical structure of the sea-loch was modelled using a three layer structure, assuming constant depths for each layer, while the biota were divided into 3 groups - phytoplankton, zooplankton and carnivores. The pelagic food-web in a sea-loch is relatively straightforward. A phytoplankton group, often dominated (at any given time of year) by a single phytoplankton species, is grazed by zooplankton, again frequently dominated by a single species; the zooplankton, in turn, are eaten by carnivores (jellyfish in this food-web). It was assumed that nitrogen was the limiting nutrient in primary production while carbon was the limiting factor in the growth of zooplankton and carnivores and therefore the model described the circulation of these quantities around the system.

When the model was configured to represent a generic west coast sea-loch, results indicated that sea-lochs should not be regarded as self-contained biological mesocosms. Tidally driven flushing of dissolved nutrients and suspended phytoplankton, together with immigration of herbivorous and carnivorous zooplankton had overriding influences on the productivity of the system. However, in different seasons of the year, a sea-loch basin could apparently behave as either a sink or a source of dissolved inorganic nitrogen, depending on the rates of biological processes. The behaviour of the system as a whole was shown to be analogous to a laboratory chemostat culture, importing nutrients when the internal concentration fell below that of the sea, and exporting if the internal concentration exceeded the external concentration.

The researchers used their model to make predictions about the effects of adding very large quantities of nutrients (particularly nitrogen) into the system, such as

Figure 11.1. Salmon cages, Scottish west coast.

might be associated with a large fish farm in a sea-loch. They based their hypothetical system on a known sea-loch, Loch Creran, with a 'worst case scenario' modification of tidal flow with an order of magnitude reduction. Even under these extreme conditions, the model predicted that phytoplankton growth would be limited by light and grazing pressure, not by nutrient availability. Thus the model predicted nitrogen enrichment associated with fish farms would have little or no effect in these systems, provided that grazing pressure was not affected. Sediment processes were relatively insignificant in the modelled system and the majority of nitrogen flux in and out of the basin was found to be from the sea.

11.1.1 Predictive tool development

The model developed in FC0105 was not intended to be suitable for routine use as a management tool to assist with the assessment of fish farm applications. It needed an experienced researcher familiar with its structure and function to configure it for any particular loch. The original model also needed far more data to characterise a loch system than would commonly be available. For this reason a follow-up project, FC0114, was commissioned by Defra to turn the original model into a management tool. The objective of this project was achieved and a 'user friendly' implementation of the sea-loch ecosystem model was developed to run on an *IBM* PC (or compatible). It was suitable for use by environmental managers for risk and impact assessment, with particular reference to mariculture enterprises and to facilitate fish-farming impact studies, an additional nutrient supply term was added that simulated the nutrient flux from such an enterprise.

11.2 Developing a recirculation system for water re-use

Work on optimisation of recirculation systems for halibut production, specifically for rearing the nursery stages as part of FC0912, was undertaken as a LINK project (FIN 19). The work brought together the expertise of Marine Harvest McConnel, the Tropical Marine Centre and the Gatty Marine Laboratory of the University of St Andrews. The research investigated how technology that had been developed for the tropical ornamental marine fish industry could be applied to intensive rearing of halibut juveniles.

A marine recirculation aquaculture system for the production of juvenile halibut was designed and developed. The environmental operating parameters were optimised by running trials that compared the results from the experimental recirculation systems with those of a more conventional through-flow set-up. The project highlighted a series of issues that required further research in order to improve the system. The most important was heightened aggression between individual fish as stocking density increased. This led to eye damage and subsequent detrimental effects on fish growth, together with increased mortalities. It was recognised that this behavioural effect was influenced by a number of factors that included tank shape and design, the presence of shelves in the tank, stocking density, frequency of grading, the light and the feeding regimes. The conditions required for optimum tank design, environmental temperature and stocking density were successfully specified as a result of the project, but more work was needed in the areas of shelf design and the specification of the light and feeding regimes. Optimising on-growing conditions for larger fish was also considered to need further research in order to fully capitalize on the growth premium generated during juvenile production.

The project developed a very practicable system by employing a number of well established water filtration techniques and these elements were combined with significant benefit. The aim was a high-flow 'clear-water recirculation' as opposed to a low-flow 'brown-water recirculation' in order to maximise growth and performance during the nursery phase of halibut; this was largely achieved. Problems such as the risk of supersaturation in

high-flow systems were highlighted and resolved, but in highly stocked systems, first stage removal of suspended solids remained a challenge. Control of pH, required to maximise the potential for denitrification, could not be fully explored before the end of the project and was considered to need further work.

The rewards of applying such systems to commercial marine hatchery situations across a range of sites remain to be realised, but even so, the benefits of a controlled environment for growing sensitive marine fish were clear. A series of recommendations on the best use of the recirculation technology employed, based on the experimental data generated in the project, were subsequently made and full practical working guidelines were prepared as a handbook available to BMFA members.

11.3 Avoiding the use of polluting chemicals and protecting wild stocks

Three species of north European wrasse are commonly used to control sea lice infestations in Atlantic salmon farming, goldsinny, *Ctenolabrus rupestris* (L.), rock cook, *Centrolabrus exoletus* (L.) and corkwing wrasse, *Crenilabrus melops* (L.). This considerably reduces the requirement for chemical treatments. The effectiveness of wrasse as cleaner-fish has resulted in exploitation of natural wrasse stocks on the west coast of Scotland and there has been doubt whether natural stocks would be capable of sustaining such a fishery. One reason for continued exploitation of the wild stocks at this level has been high over-winter mortality of wrasse in cages, necessitating restocking in spring. In the wild, however, wrasse are observed to survive winter conditions at water depths comparable with those used by the salmon farming industry and so this project was commissioned to investigate the physiological determinants of wrasse survival and whether provision of refuges would improve survival rates and allow reduced exploitation of the natural stocks.

Short-term tolerance of low temperature and low salinity was examined for corkwing wrasse caught during the winter. Survival was much higher in winter-caught fish compared with summer-caught fish subjected to similar physico-chemical conditions. The ability of winter-caught corkwing to osmoregulate over a salinity range at 4°C was better than summer-caught fish and it was suggested that wrasse acclimated to winter water temperatures should be capable of surviving rapid decreases in water temperature and salinity. Poor survival was considered to result from rapid temperature reductions which occur in late autumn, when wrasse were not seasonally-acclimated to the lowest water temperatures.

The physico-chemical performance of three refuge designs were assessed and compared with rock scree areas known to be inhabited by wrasse during the winter. The influence of an adjacent sea-loch to the study area resulted in large and rapid fluctuations in water temperature and salinity associated with tidally-influenced freshwater runoff. Protection afforded against this large physico-chemical variation by a closed refuge design, was similar to that offered by rock scree habitats. The complexity of the refuge design was similar to the natural habitat and this was considered important in improving wrasse survival over winter. These results demonstrated that a relatively simple system could be adopted by the industry for improving wrasse winter survival.

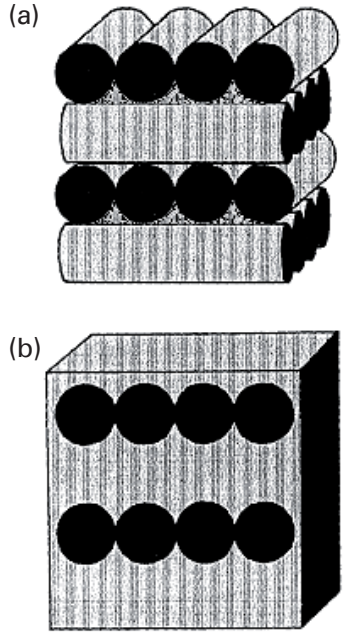

Figure 11.2. Experimental refuges for wrasse: constructed of either (a) open plastic piping, or (b) closed with limited access. Figure adapted from Defra Research Project FC0110, Final Report, March 1995.

12. Fish welfare and health

The following projects are included in this section:

FC0916 Endocrinological & behavioural measures of the welfare of farmed fish in relation to stocking density (Cefas – Weymouth)

AW1203 The effect of stocking density on the welfare of farmed rainbow trout (Cefas – Weymouth & IOA Stirling)

AW1204 Rainbow trout fin erosion - epidemiological analysis of prevalence, development, risk factors and effects on welfare (Cefas – Weymouth, IOA Stirling & University of Bristol)

AW1205 The interaction between water quality and welfare in farmed rainbow trout (IOA Stirling, Cefas – Weymouth & University of Bristol)

Project FC0916 aimed to provide tools with which the welfare status of fish could be objectively measured and to improve the understanding of stocking density effects on farmed fish species, as recommended by the Farm Animal Welfare Council (FAWC).

A non-invasive stress assay for rainbow trout was developed. The assay made use of the fact that the classic stress hormone, cortisol, is released from the gills and can be measured in the water (Figure 12.1). The research demonstrated that the amount of cortisol released was a valid measure of stress level – it responded rapidly (within 15 minutes) to a handling stress and was correlated with cortisol levels in the blood plasma and with the severity of the stress. The technique developed is a novel alternative to traditional blood sampling that is ideal for stress research, enabling samples to be taken without disturbing or handling the fish.

The non-invasive cortisol assay could be used straightforwardly in the laboratory, but it was also desirable to have a technique that could be used for welfare assessments on fish farms. Water cortisol concentrations are affected, not just by the stress response, but also by fish biomass and water flow rate. Loading densities and flow rates on farms are generally not known with sufficient accuracy to allow different systems and sites to be compared. However, these variables could be allowed for

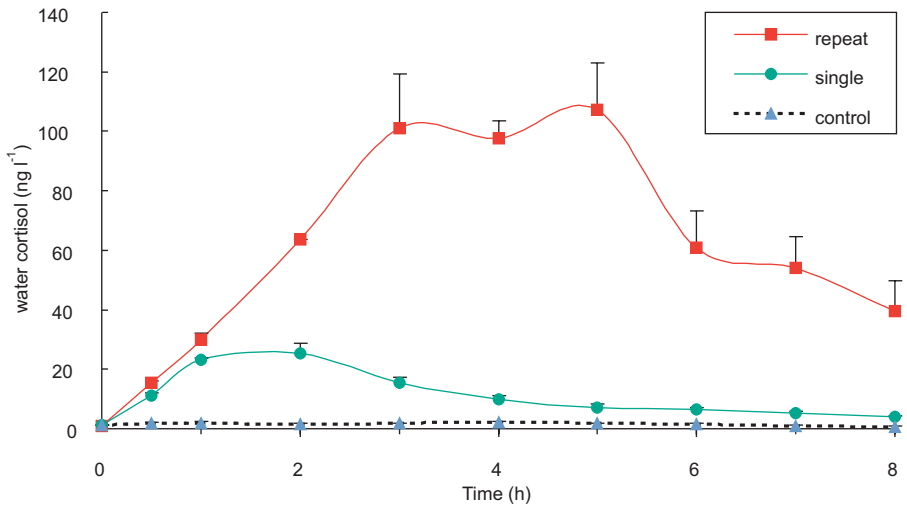

Figure 12.1. Cortisol changes in response to stress: mean concentration in water samples from rainbow trout tanks (± SE) (n=4 per treatment) after exposure to a repeated handling stress (at 0, 1 and 2 h), a single handling stress (at 0 h) or no stress (control). Figure redrawn from Defra Research Project AW1203, Final Report, June 2004.

if another compound, a physiological marker, unaffected by stress and released into the water in a consistent pattern, could be detected and measured. Melatonin (a hormone secreted at night) was identified as a candidate marker and proved to be suitable. It was shown to be released into the water, like cortisol, via the gills, but was not affected by stress and the pattern was consistent. Assaying both hormones in the water enables cortisol concentrations to be calculated with an allowance for the tank conditions, and so different situations may be reliably compared. The non-invasive cortisol and melatonin methodology was shown to work equally well for Atlantic salmon in seawater as for freshwater rainbow trout.

The complex issue of stocking levels for farmed trout has been highlighted as an area needing research to determine optimal densities that would not be prejudicial to health and welfare. Project AW1203 was a co-operative programme of research by Cefas and IOA, Stirling, and endorsed by the British Trout Association. The project involved a literature review, laboratory experiments and field sampling to examine the effects of density on trout welfare.

A series of laboratory experiments explored the effects of stocking density (10-120 kg m^{-3}) on trout welfare. Cortisol level and a suite of traditional health and condition indices were assessed in relation to changes in density, loading rate (the fish biomass per unit inflow), and water quality. A time series of population stress measurements using the non-invasive assay indicated that rainbow trout do not suffer from crowding stress. Most of the other welfare indicators such as red and white blood cell numbers, liver and body condition indices, as well as growth and mortality, showed no change with density; however, the severity of fin erosion did increase with increasing density and affected all fins.

Field sampling confirmed that higher densities are linked to increased fin erosion on commercial trout farms. Fin erosion was, however, greatly affected by other environmental and local farm factors. Further experimental work, on water current direction indicated that increased fin erosion was primarily due to behavioural processes rather than deterioration of water quality. This demonstrated that the relationship between stocking density and welfare is not straightforward - different welfare indicators respond in different ways to density and are also affected by factors other than density.

At present there is little objective information on the prevalence and severity of fin erosion on UK farms, whether it is a welfare issue, and how intensification of the industry affects its incidence. The main aim of project AW1204, which is in progress at the time this report is being prepared, is to identify the risk factors for fin erosion of rainbow trout held in UK production systems. It is planned to achieve this by an epidemiological approach assessing the condition of fins on farms.

An extensive literature review, undertaken as part of the project, illustrated that various husbandry and environmental factors interact to affect the severity of fin erosion. Despite this complexity however, a previous study of commercial rainbow trout farms in the United States of America demonstrated that just four key risk factors explained most of the between-farm variation.

The prevalence and severity of the condition, and variation between farms, will be documented during the first year of the project to give a preliminary indication of factors involved. This will be followed by a study to determine the period during the production cycle when fin erosion occurs and identify the key risk factors involved. Correlating fin erosion with other welfare indicators should allow an assessment to be made of whether fin erosion represents a major insult to trout welfare.

The project results will allow assessments to be made of the incidence of fin erosion within the UK rainbow trout industry and of the importance of fin erosion to welfare. Through the identification of key risk factors for fin erosion it should be possible to develop strategies to reduce its prevalence and severity and to facilitate incorporation of such assessments into farm assurance schemes.

There is increasing pressure on the regulatory authorities to legislate on fish welfare. The need to base legislation on sound data has been identified by FAWC and more recently by the European Parliament's Committee on Fisheries. To this end the fish farming industry is supporting research that addresses the need for scientific data on which to base regulation. Project AW1203 on stocking density demonstrated the potential important influence of water quality on welfare. Further research is required to resolve contradictions and gaps in the coverage of published literature and recommend limits for water quality parameters that will ensure good welfare.

Project AW1205 was planned to address this need and, like AW1204, is in progress at the time of compiling this review. The work is aimed at defining acceptable limits for water quality with regard to trout welfare, and at setting auditable welfare measures. The initial part of the project involves a series of focus groups with stakeholders to identify current indicators of welfare and farm visits to assess the capacity for monitoring and control of water quality over the entire industry.

Experimental work on specific welfare indicators will involve replicated systems using tanks linked in series, a set-up that provides robust data on the effect of deteriorating water quality independent of other influences on welfare. Information from these experiments will contribute to a large epidemiological study that will examine water quality and welfare indicators throughout the industry over the annual production cycle. The project will conclude with a stakeholders' workshop to discuss the identified risks to welfare from water quality and agree key auditable welfare measures. The work will ensure Defra will be in a strong position to advise and contribute to any recommendations or regulations that the Council of Europe may wish to introduce relating to water quality and trout welfare.

13. Ensuring product quality

The following projects are addressed in this section:

FC0908 *(FC0119) (LINK - SAL 04)* Physiological and biochemical roles of carotenoids in Atlantic salmon (IOA, University of Stirling)

FC0917 *(LINK - TRT 13)* Off-flavour problems in farmed trout: identification of causative organisms and development of management strategies (The Robert Gordon University)

13.1 The roles of carotenoids

The first objective of project FC0908 was to establish a model of nutritional vitamin E deficiency in Atlantic salmon post-smolts and determine if there were any synergistic antioxidant activities derived from the pigment, astaxanthin (AX).

Four experimental diets were formulated using casein as protein source and fish oil, which had been stripped of vitamin E (to a level of 4.4 mg kg^{-1}) as the lipid source were fed to duplicate groups of fish. The diets were either supplemented (100 mg kg^{-1}) or deficient (1.2 mg kg^{-1}) in vitamin E and each was also either supplemented (75 mg kg^{-1}) or deficient in AX (0 mg kg^{-1}). After 22 weeks the fish had increased in weight by more than 3.5 fold and there were no significant differences between final weights. The relatively low growth rates were attributed to the use of purified diets containing non-fish protein.

It had been hoped to generate classical overt symptoms of vitamin E deficiency, but no gross pathologies were evident in any of the fish sampled, neither were any histopathologies identified in heart, spleen or white muscle. In liver, lipoid liver degeneration, ranged from a trace to moderate severity in 17% of the fish sampled.

Fish fed vitamin E deficient diets had significantly lower vitamin E concentrations in all tissues tested compared to fish fed the supplemented diets. The most severe vitamin E depletion was in liver, where values were only 3% of those found in fish that had received the supplement. Interestingly the vitamin E levels in neural tissues of fish fed deficient diets fell to only 35 and 40% of those levels in fish fed the supplemented diets; this was in brain and eye respectively and indicated that physiological systems are in place to maintain levels, emphasising the importance of antioxidant and HUFA function in neural tissues.

In-vitro preparations of muscle microsomes from fish fed the four experimental diets indicated that both AX and vitamin E contributed antioxidant protection; formation of malondialdehyde, a secondary product of lipid peroxidation, was less in microsomes from fish fed the supplemented diets. In addition, the concentration of plasma 8-isoprostane, which reflects tissue oxidation, was elevated in fish fed the diets lacking AX whether supplemented or deficient in vitamin E. This suggested that AX in particular may prevent production of 8-isoprostane. Both these results confirmed that AX has a functional role as an antioxidant *in-vivo* and *in-vitro* and that it is not merely a flesh colorant.

A significant increase in fatty acid desaturation and elongation was observed in isolated hepatocyte preparations from fish fed diets deficient in vitamin E; to a lesser degree this was also observed for fish deprived of AX. The exact mechanism was unclear, but it was thought likely that loss of essential membrane HUFA may have led to an up-regulation of the fatty acid synthesis pathways. Similar results had been recorded in the mammalian literature before, but this effect had never been previously observed in fish; the work also provided the first record of the effect in intact cells as opposed to sub-cellular fractions.

The work had established a model of vitamin E deficiency in Atlantic salmon at a fixed dietary lipid level (16%), a further experiment examined the link between the level of polyunsaturated fatty acid intake and the development of vitamin E deficiency. Formulated feeds which contained dietary lipid at 20, 25, 30 or 35% were either supplemented with vitamin E (195 mg kg^{-1}) or left deficient in it (four diets ranging from approx. 20 to 32 mg kg^{-1}); these were fed to replicate groups of fish. At the termination of the trial, after 23 weeks, muscle vitamin E concentrations were highest in fish fed diets with the lowest lipid levels. Both muscle and liver total lipid content were higher in fish fed supplementary vitamin E than those fed diets deficient in vitamin E. There were significant differences in fatty acid desaturation and elongation in hepatocytes isolated from fish fed the various diets with activities inversely related

to dietary lipid level. A significant effect of vitamin E level on desaturation and elongation activities was also demonstrated in fish fed at the same lipid level. It is clear from these interrelationships that the effects of dietary lipid level and vitamin E concentrations on fish should not be studied in isolation.

The significance of the type of pigment in a diet to the quality of the fish product was investigated. An experiment was carried out to investigate the deposition of total carotenoid in the flesh and visual perception of colour when salmon were fed different dietary ratios of AX and cantaxanthin. The researchers experienced difficulties obtaining both unpigmented fish to set up the trial and commercially prepared diets of the required specification. These problems impinged on the work, but the results demonstrated that increasing dietary pigment (70 mg kg^{-1} to 100 mg kg^{-1} total for the combined pigment types) resulted in increased stepwise uptake of pigment into the plasma, but that this was not reflected in a similar increased deposition in the flesh.

A study was undertaken to identify possible alternative pigment sources to the chemically-synthesised AX and cantaxanthin currently used by the salmon industry. Five alternatives were compared:

1) Ecotone - a natural source of free AX from the yeast, *Phaffia rhodozyma*;
2) Krill meal - a natural source containing mostly esterified AX;
3) NatuRose - a naturally produced pigment containing mainly esterified AX from the microalga *Haematococus pluvialis*;
4) Venaxan Red - a food colorant isolated from capsicum and containing predominantly capsanthin and capsorubin in free and esterified form;
5) Carophyll Pink, a mixture of free AX isomers.

Commercial formulations were prepared with a total pigment concentration of 100 mg kg^{-1} of diet and these were fed for a period of 6 months. All of the pigment sources, with the exception of Venaxan Red, were associated with similar AX deposition (> 3 mg kg^{-1} flesh); inclusion of Venaxan Red achieved about half this level.

13.2 Off-flavour problems in farmed trout

In a study of the UK farmed trout industry (Project FC0917) the researchers found that musty/earthy taints in the flesh of farmed trout are caused mainly by geosmin (GSM) and to a lesser extent by 2-methylisoborneol (MIB). The most likely sources of GSM and MIB were shown to be the blue-green algae that grow in thick mats on rocks in rivers as well as on raceway walls. Blue-green algae need sunlight and nutrients (nitrate and phosphate) as well as suitable temperatures to grow. Optimal conditions tend to occur during the summer months in the UK and the studies revealed that this was the time when the highest levels of taint could be detected.

In flow-through farms the source of the taint was shown to be generally external to the farm and so there appeared to be little that a farm manager could do to prevent either the development of the algae or the arrival of musty/earthy taint compounds in the water entering the farm. Taints were shown to accumulate in trout flesh in less than 24 hours.

Depuration, to remove the taint from flesh, was found to be possible if the fish were kept in clean, taint-free water, but this could take several days depending on the original level of taint. Where GSM and MIB entered the farm from an external source it was impractical to eliminate them from the water and the only real solution for a farm manager to avoid marketing tainted fish was to avoid harvesting while the compounds were present.

It would be possible to employ some treatment methods to eliminate these compounds within a recirculation system. Several systems were considered including granular activated carbon, UV/ozone and UV/TiO_2 although the practicality and economics of such systems have yet to be evaluated for this application. Nevertheless if depuration is to be successful, trout do need to be placed in water free from taint compounds. In some locations this could be possible through the use of ground water.

Detecting the taint compounds by use of an analytical method, such as the researchers had at their disposal, would be neither affordable nor practicable for routine monitoring of trout on farms or at processors. The most powerful tool available to farm managers, especially for daily assessment of tainted fish entering a processor, was demonstrated to be robust quality control via a trained taste panel (organoleptic testing). The researchers compared the organoleptic analysis of trout by a group of experienced tasters with that of the laboratory-based analysis and found good correlation. However, a less well-defined taste panel demonstrated an inability to detect musty/earthy taint, highlighting the need for training and ring-testing where appropriate.

14. Sustainability - uptake of the results of the research

Finfish farming will progress through advances in production technology, but the nature of the development must be environmentally sustainable. Defra has supported work in areas that are seen as limiting sustainability; the research has examined aspects of current practice that need attention, sourcing solutions to problems and developing tools that will assist with the management of the industry; the topics are diverse. This has not been done in isolation; Defra has encouraged active involvement of fish farming businesses in this research through LINK Aquaculture projects and industry representation at CARD. Best practice is fostered widely within the industry by Defra promoting dissemination of research results through trade publications and meetings as well as through scientific journals and fora. For example, recommendations about the use of recirculation technology came from project FC0912 and were quickly made available as a handbook to BMFA members. Participation in LINK projects had the benefit that industrial partners were in a position to adopt new approaches as an immediate outcome of the work; feed manufacturers involved in FC0930 on fish meal replacement are continuing to pursue investigations into alternative sources of protein and oils for fish diets.

The environmental impact of fish farming has been of significant public concern. The development of recirculating land-based systems is a practicable solution for some species and locations, and provides a means of reducing water use and controlling effluent. Recirculation systems also possess an inherently improved biosecurity control over traditional open systems. A few recirculation systems are already in operation for marine finfish species in the UK. For some species better management of simpler technologies already in use may be more appropriate. Modelling studies of areas where salmon are farmed have contributed to both the effective management and regulation of these activities to the benefit of producers and other users of the environment. Many freshwater ecosystems are almost hydrodynamically-closed environments, and because primary productivity is nutrient-limited in such situations the paramount objective of the system manager is to restrict anthropogenic supply of nutrients. By contrast, a sea-loch is a hydrodynamically-open environment and the nutrient supply is of much less significance unless levels are low enough to alter the competitive balance between species. The key determinants of sea-loch behaviour were found to be irradiance and plankton grazing. This implies that a change in the background water turbidity can have a significant positive or negative impact on primary productivity. In this situation therefore the most serious potential threat a manager must combat is a reduction in the efficiency or population size of the grazers; understanding this allows a greater level of environmental protection and much better planning to ensure sustainable aquaculture production. The computer model developed in project FC0114 has been used effectively by government agencies to make assessments of the impact of planned cage farms in sea-lochs

The study of wrasse winter survival has led the various farms and companies involved to develop alternative methods of over-wintering the fish. The use of refuges has improved survival, but rather than holding wrasse in the relatively shallow water of cages they are either transferred to traps kept in deeper water or held in pump-ashore tanks. This not only helps to conserve stocks of wild wrasse, but is clearly the better option as far as their welfare is concerned

Public opinion demands that high standards of welfare are maintained under farm conditions. Robust data is required to make objective assessments of how fish production impinges on fish welfare. Understanding the effects of stocking density on fish held in tanks and cages is one of the priorities. The innovative assay for cortisol that was developed in FC0916 proved to be excellent for the purpose. The measurement of stress has enabled the discrimination between the relative importance of social factors and water quality in inducing stress. The cortisol assay is a research tool, but the information that the work is generating has wider application. Its relevance to the assessment of fish welfare means that its use will help with provision of guidance for codes of practice, quality assurance schemes and potential legislation on stocking practices to safeguard fish welfare. The non-invasive methodology also has research potential extending beyond cortisol to the measurement of melatonin (which is important in the timing of maturation and egg production) and other hormones.

Ensuring that the marketed product is of the highest quality is critical for the industry. Studies on the function of pigments in diets have provided evidence that supports the role of astaxanthin as a co-antioxidant with vitamin E in Atlantic salmon. The value of including substances that act as flesh colorants, in formulated feeds, has sometimes been questioned, but there are clearly benefits from astaxanthin as an antioxidant. Further work on the influence of pigments on the maintenance of flesh quality after culling has already been completed. Studies such as these have been of great value to the industry and further investigations of antioxidant effects are warranted, particularly in relation to their influence on egg quality

and its effect on larval viability, as well as in the quality of salmon fillets subjected to oxidative stress during the smoking process.

Although the work on off-flavour problems in farmed trout demonstrated that it would be difficult to avoid the occasional occurrence on farms, it also showed that good management would avoid the problem affecting the final product. Robust quality control can be achieved with trained taste panels and daily assessment for tainted fish is something that could be dealt with by a processor.

Fin-fish farming has only developed to a level at which it has made a significant contribution to food production during the last two to three decades. The industry is still young; continuing technical problems have commanded much of the research investment throughout the early years and Defra has provided support during this period. Now that production is more secure there is greater opportunity for industry to consider how it can best operate to ensure its future is sustainable. Defra, through its research programmes, has successfully encouraged collaboration between industry and academia. This brief summary of work undertaken since 1990 demonstrates the breadth of approach and has provided synopses of the valuable progress made. More details of the results of particular studies can be found in the various publications that have been prepared and are listed in the following section.

15. Sustainability - publications arising from the research

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ANNEX 1. Finfish cultivation research projects funded by MAFF/Defra, 1990-2004

Code	Title	Contractor/Funded Organisations	Start	End	Total cost	Summary Objectives
FC0102	Techniques for cultivation of dover sole	CEFAS - Conwy	Apr-90	Mar-93	£326,120	Develop procedures for the production of juvenile sole and assess their viability in the wild. Assess the feasibility and advantages of producing sterile fish for release in the sea.
FC0103	Development of cultivation techniques for halibut	SFIA - Ardtoe	Jul-90	Mar-95	£475,999	Determine for halibut the optimum larval rearing conditions and feed requirements to achieve good survival rates, as an input to the assessment of the potential for halibut farming.
FC0104	Halibut egg and larval quality	SFIA - Ardtoe	May-90	Mar-93	£229,000	Identify those factors such as broodstock diet, egg collection technology and environmental conditions which control Halibut egg and larval quality, with an aim to finding optimum husbandary methods.
FC0105	Optimal model formulation for marine ecosystems.	Univerisity of Strathclyde	Jul-91	Jun-95	£122,085	Describe and model seasonal variations in the nitrogen cycling of a sea loch by constructing a simulation model incorporating its major hydrological and biological features, in support of the SOAFD experimental programme.
FC0106	Digestive physiology of juvenile dover sole	University of Wales, Bangor	Oct-91	Sep-93	£62,459	Investigate the feeding and digestive physiology of juvenile sole to enable the development and testing of diets suitable for the rearing of sole under laboratory conditions.
FC0107	Environmental control of halibut broodstock and rearing procedures for feeding larvae	SFIA - Ardtoe	Apr-93	Mar-98	£575,400	Examine the effects of variations in diet and environmental conditions in rearing tanks on the spawning performance of halibut broodstocks and survival of first feeding larvae.
FC0108	Further studies on the digestive physiology of juvenile dover sole	University of Wales, Bangor	Oct-94	Mar-97	£79,126	Further experimental development of artificial feeds for larval and juvenile sole incorporating microencapsulated digestive enzymes. Related studies into digestive physiology of young fish will also continue.
FC0110	Winter survival in wrasse	Dunstaffnage Marine Laboratory	Dec-94	Mar-95	£5,518	Investigate the winter physiological state of wrasse, including the effect of temperature and salinity and identify methods to improve their survival in winter through the development of refuges.
FC0114	Development of a sea loch ecosystem management tool	Univerisity of Strathclyde	Jan-96	Dec-96	£29,820	Develop a user friendly model of sea loch ecosystems which can be used to predict the development of plankton and nutrient concentrations.

ANNEX 1. continued

Code	Title	Contractor/Funded Organisations	Start	End	Total cost	Summary Objectives
FC0120	Abnormal sex ratios in Dover sole	CEFAS - Conwy	Jun-96	Mar-98	£134,730	Explore the causes of observed male domination among reared Dover sole at Conwy and bring a greater focus to the nature of this problem. Review the evidence for the nature of sex determination in the sole and experimentally quantify the possible role of contaminants.
FC0901	Environmental influences on the sex of cultured marine flatfish	CEFAS - Conwy	Apr-98	Mar-01	£422,949	Examine the causes of observed male domination among cultured Dover sole and turbot and assess the relative importance of environmental influences during early development on subsequent sex ratios.
FC0902 (original code FC0109)	Quality of hatchery reared flat-fish.	CEFAS - Conwy	Apr-94	Mar-02	£260,454	Examine how rearing methodology including diet, affects the performance of juvenile flat fish (sole and turbot), including their growth and tolerance to stress.
FC0903 (original code FC0112)	Sex control in turbot.	CEFAS - Conwy	Apr-95	Mar-00	£577,592	Develop a method for the production of all-female turbot through the identification of the sex determining mechanism.
FC0904 (original code FC0113)	Spawning of flatfish in captivity - sex pheromones and reproduction in flatfish broodstocks	University of East Anglia	Jan-96	Jun-99	£60,451	Establish whether the poor reproductive performance of marine flatfish brood stocks, as shown by failure to spawn, low egg yield etc is caused by the release of pheromones, brought about by social factors e.g. overcrowding and competition.
FC0905 (original code FC0115)	Halibut egg and early larval rearing	SFIA - Ardtoe	Apr-95	Sep-98	£20,000	Investigate the main husbandry problems encountered in egg incubation and yolksac larval rearing, including water quality and temperature, and develop methods for viable commercial hatchery operation.
FC0906 (original code FC0116)	Dietary lipids and cold tolerance in juvenile flatfish	University of Liverpool	Oct-95	Jan-00	£56,653	Raise the level of resistance of juvenile flatfish to environmental stress by optimising larval production techniques.
FC0908 (original code FC0119)	Physiological and biochemical roles of carotenoids in Atlantic salmon (LINK SAL04)	University of Stirling - Institute of Aquaculture	Apr-98	Jul-99	£36,643	Examine the role which pigments play in the life cycle of salmon and trout, including the effect of Vitamin E on flesh pigmentation levels and lipid peroxidation.

ANNEX 1. continued

Code	Title	Contractor/Funded Organisations	Start	End	Total cost	Summary Objectives
FC0910 (original code FC0123)	Optimisation of formulated diet for marine fish larvae	University of Wales, Bangor	Mar-97	Mar-98	£26,803	Investigate the potential for replacing live feed with formulated microdiets for larval fish, including analysing larval feeding behaviour, and effects of colour and olfactory cues.
FC0911	Optimisation of formulated diets for marine fish larvae	CEFAS - Conwy	Apr-98	Mar-01	£227,276	Evaluate the effect that various diet characteristics, such as colour, olfactory cues etc, have on larval feeding behaviour and hence provide an understanding of how specific stimuli make food acceptable to fish or determine behaviour such as cannibalism.
FC0912	A new recirculation system for rearing juvenile halibut using novel technology from the tropical marine industry (FIN19)	University of St. Andrews - Gatty Marine Laboratory	Jul-99	Jun-03	£360,334	Design and develop a marine recirculation nursery system for the culture of juvenile halibut and provide information on the inter-relationship between biological filtration/chemical filtration and biomass to help define the optimal working parameters and limitations of such a system.
FC0913	Rearing protocols for Atlantic halibut larvae during transition from endogenous to exogenous nutrition. (FIN22)	SFIA - Ardtoe	Apr-99	Aug-03	£293,567	Through a better understanding of the behavioural competence and environmental tolerances of different development stages of halibut larvae, develop improved husbandry (rearing) protocols, including optimising the tank environment to promote prey-capture behaviour.
FC0914 (extension for FC0903)	The development of methods for the control of sex in turbot and halibut	CEFAS - Conwy	Apr-99	Mar-02	£116,250	Develop a method for the production of all-female turbot and halibut through the identification of the sex determining mechanism.
FC0916	Endocrinological & behavioural measures of the welfare of farmed fish in relation to stocking densities.	CEFAS - Weymouth	Apr-00	Mar-05	£402,296	Develop a non-intrusive and non-invasive technique to quantify levels of water-borne cortisol in order to assess stress levels on commercial trout farms and use this to determine acceptable maximum stocking densities for trout and other commercially produced fish.
FC0917	Off- flavour problems in farmed trout (TRT13)	The Robert Gordon University	Apr-00	Mar-03	£101,628	Identify the organisms that cause off-flavours (taints) in trout and investigate the biological and environmental mechanisms that promote them. Develop tools to predict early detection thus enabling taint mangement strategies to be developed.

ANNEX 1. continued

Code	Title	Contractor/Funded Organisations	Start	End	Total cost	Summary Objectives
FC0930	Substitution of fish meal with vegetable proteins in cod diets	Scottish Association for Marine Science	Jan-04	Mar-04	£15,000	This short term cod feeding trial is designed to test different levels of fish meal replacement with vegetable proteins with reduced carbohydrate and minimal anti-nutritional factors. Growth and feed conversion will be monitored.
FC0931	Laboratory assessment of samples involved in substitution of fish meal with vegetablre proteins in cod diets	Scottish Association for Marine Science	Oct-04	Feb-05	£7,075	Undertake the proximate analysis of fish feed, faeces, muscle, liver and bone samples collected for project FC0930 to obtain added value from the first study and determine the changes in fish body composition that occur when fish are fedsubstituted diets.
AW1203	The effect of stocking density on the welfare of farmed rainbow trout	CEFAS - Weymouth; University of Stirling - Institute of Aquaculture	Jan-00	Mar-04	£354,550	Develop and employ a novel, non-invasive technique to quantify levels of cortisol, released by fish into water. This wwill allow quantifiable measures of stress and performance to be made and be related to stocking densities.
AW1204	Rainbow trout fin erosion - epidemiological analysis of prevalence, development, risk factors and effects on welfare	CEFAS - Weymouth; University of Stirling - Institute of Aquaculture	Apr-04	Mar-08	£298,721	The main aim of this project is to identify the risk factors for fin erosion of rainbow trout held in UK production systems. This will be achieved by a field epidemiological approach assessing fin state on farms
AW1205	The interaction between water quality and welfare in farmed rainbow trout	CEFAS - Weymouth; University of Stirling - Institute of Aquaculture	Apr-04	Mar-07	£295,209	The project aims to define acceptable limits for water quality with regard to trout welfare and agree with stakeholders auditable welfare measures

Details of all Defra funded research final reports can be accessed online at: http://www.defra.gov.uk/research/project_data/Default.asp

ANNEX 2. Glossary of terms and acronyms used in the review

Activation	Initiation of a chain of events in an egg, by penetration of a spermatozoon, that is necessary for embryo development, but is independent of any genetic contribution from the sperm.
ADC	Apparent digestibility coefficient = 100 - (100 * I in diet * (N in faeces / I in faeces) / N in diet), where I = indicator concentration and N = nutrient concentration.
Ambi-colouration	An abnormal pigmentation pattern often found in hatchery-reared, metamorphosed flatfish where irregular patches of pigmented skin are present on the ventral surface and un-pigmented areas occur on the dorsal surface. It is often associated with incomplete metamorphosis.
ANFs	Anti-nutritional factors: compounds found in vegetable protein meal that can inhibit nutrient absorption in the gut.
Androgens	A group of steroid hormones; the principal male sex hormones (e.g. 11 keto-testosterone), synthesised chiefly by the testes. They influence the growth and function of male reproductive organs and the development and maintenance of male secondary sexual characteristics.
Anthropogenic	Produced or caused by man.
AX	Astaxanthin: a red-pink carotenoid pigment that is naturally present in crustacea; it can also be synthesised artificially.
BHA	British Halibut Association.
Biomass	Total weight of a particular species or of a collection of animal and/or plant species in a given area.
Blastodisc	The early embryo visualized as a disc of cells at one pole of the egg during cleavage (q.v.).
Blastomere	One of the many cells forming the blastodisc.
BMFA	British Marine Finfish Association: formerly the British Halibut Association.
Broodstock	A stock of adult fish held by a farm to provide fertilized or unfertilized eggs and sperm for production of juvenile fish.
Cantanxthin	A red-pink carotenoid pigment that is naturally present in some marine algae; it can also be synthesised artificially.
CARD	Committee for Aquaculture Research and Development.
Cefas	Centre for Environment, Fisheries and Aquaculture Science: an executive agency of Defra (formerly the Directorate of Fisheries Research (DFR) within MAFF).
Chemostat	An enclosed culture unit usually used for microbial research in which growth of the cultured organism can be limited and regulated by controlled additions of essential nutrients to the culture medium and by regular harvesting of the growing population.

Chromosomes	Structures within the cell nucleus, composed largely of DNA arranged into genes, which carry the hereditary information.
Cleavage	Subdivision of the fertilized egg following fertilisation.
Day-degrees	A unit of time that is used to make simple comparisons between growth stages because it provides some allowance for water temperature. It is calculated as the product of time in days and water temperature in degrees Celsius.
Defra	Department for Environment, Food and Rural Affairs: was created in June 2001 incorporating the Ministry of Agriculture, Fisheries and Food, (MAFF).
DHA	Docosahexanoic acid: a highly unsaturated fatty acid with 22 carbon atoms and six unsaturated bonds, important in the make up of nervous and visual tissues. The first of the unsaturated bonds is positioned adjacent to the third carbon atom from the end of the chain (n-3). A carnivorous fish such as turbot cannot synthesise this from shorter fatty acid molecules and needs to obtain it from the diet.
Diploid	Having paired homologous chromosomes in the nucleus; characteristic of almost all normal animal cells except gametes which are haploid (q.v.).
DNA	Deoxyribonucleic acid: a very large and complex chain molecule found in all living cells; the carrier of genetic information.
DPH	Days post-hatch (i.e. age in days since hatching)
Ecosystem	A community of interdependent plant and animal species together with their non-living environment that is distinct from neighbouring communities and relatively self-contained in terms of energy flow.
Embryo	The stage of development of a young fish (or other animal) while it is still contained within the egg.
Endocrine	Relating to hormones.
Environment	Collective term for the conditions in which fish live, used in this context to include water, oxygen, light, temperature, food, pollutants, other organisms etc
Enrichment	A term for the enhancement of the nutritional value of live feeds, usually to increase the HUFA (q.v.) content. Sometimes achieved by the use of particular species of microalgae, but frequently with commercially prepared suspensions of lipid droplets.
EPA	Eicosapentanoic acid: a highly unsaturated fatty acid with 20 carbon atoms and five unsaturated bonds, the first of which is positioned adjacent to the third carbon atom from the end of the chain (n-3). The ratio of EPA:DHA is important to the functioning of cell membranes.
ERE	Energy retention efficiency = ((E2 * W2)-(E1 * W1))/(FE * FI)*100, where W is weight (g), E is energy concentration in fish (MJ/kg), FE is energy concentration in feed (MJ/kg) and FI is feed intake

Ethology	The study of the behaviour of animals in their normal environment.
EU AIR project	A European Union funded project concerned with cooperative research by food and agriculture related industries.
EU Concerted Action	A European Union funded project that is largely concerned with the exchange of information between participants.
Exploitation	Removal of fish from a natural stock by fishing.
FAME	Fatty acid methyl esters: produced from the lipid extracted from tissue samples to allow the quantification of specific fatty acids in tissues.
FAWC	Farm Animal Welfare Council
Flatfish	Fish of the order Heterosmata, which undergo metamorphosis during early development to give the flattened adult form.
Fry	A general term used for young fish at stages from the time the yolk-sac is the primary source of nutrition, through independent feeding larvae, until metamorphosis.
Genotype	The genetic characteristics of an organism.
GnRHa	Gonadotrophin releasing hormone analogue: a synthesised chemical that has the active attributes of the natural brain hormone that stimulates the release of gonadotrophin.
Gonadotrophin	A hormone that controls reproductive activity by affecting production sex steroid hormones and gonad development.
GSM	Geosmin: a chemical that contributes to flesh taints of cultured fish.
Gynogen	An individual carrying only maternal genetic information inherited from the parent.
Gynogenesis	A process intended to limit inheritance of genetic information to that from the female parent.
Haploid	Having a single set of chromosomes (inherited from one parent) in the nucleus of each cell. Haploid embryos are not usually viable after the first few days' development
Heterogametic	Having non-identical paired sex chromosomes (e.g. in male salmonids, normally denoted XY).
Heterologous sperm	Sperm from a species that is different to the maternal parent and therefore not expected to contribute any genetic material to the embryo, but it used to activate the eggs.
Homogametic	Having identical paired sex chromosomes (e.g. in female salmonids, normally denoted XX).
Hormone	A chemical substance produced by endocrine glands in the body, usually released into the blood in minute amounts, and which induces a specific response in other tissues.

HUFA	Highly-unsaturated fatty acid.
Hydrolysed fish meal	Fish meal processed by chemical hydrolysis so that large protein molecules are partially broken down to produce smaller molecules such as peptides and amino acids. These can be more readily absorbed in the gut of young fish.
ICES	International Council for the Exploration of the Sea.
IOA	Institute of Aquaculture, at the University of Stirling.
Juvenile	A young fish that has the appearance of an adult, but is incapable of sexual reproduction.
LA	A fatty-acid with an 18 carbon atom chain and two unsaturated bonds that is common in terrestrial plant material. The first of the unsaturated bonds is positioned adjacent to the sixth carbon atom from the end of the chain (n-6). Many marine species are unable to convert this to the longer chain (n-3) HUFAs needed for the make up of cell membranes.
Larva	A young fish before it metamorphoses into the juvenile form.
LINK Aquaculture	Programme of co-operative research that ran from 1996 until 2001 with up to 50% of the costs of individual projects being provided by Defra (then MAFF), SOAEFD and NERC. The balance came from industry.
Lipid	A term for fats and oils, substances that are largely composed of fatty-acids.
Loading density	Stocking level of a fish holding facility, measured in relation to the volume of water flowing through it (for example: kg m^{-3} water h^{-1}).
MAFF	The former Ministry of Agriculture, Fisheries and Food; incorporated in June 2001 into Defra (q.v.).
Mariculture	The cultivation of marine organisms.
MDPE	Medium density polyethylene.
MED	Microencapsulated diet: a diet consisting of inert particles which are minute capsules that enclose and protect soluble nutrients in the diet from leaching in water, but are intended to breakdown and release them in the gut after ingestion.
Meiotic gynogenesis	Gynogenesis (q.v.) induced by doubling the single set of chromosomes in an egg during the final stage of meiosis that occurs immediately after activation (q.v.).
Mesocosm experiment	An experiment using a large enclosure in which communities are allowed to develop naturally and provide an environment similar to those that might be expected in the wild.
Metamorphosis	The rapid change from larva to miniature adult form (juvenile), which in flatfish results in the individual lying and swimming on one side of its laterally flattened body with both eyes on the uppermost side.

Metanauplius	A stage of development of a crustacean such as *Artemia* when the body begins to elongate and becomes segmented. It is during their early metanaupliar stages that *Artemia* start to filter-feed and can be enriched using suspensions of lipids before being fed to fish larvae.
Methyl testosterone	A synthetic male steroid hormone which stimulates development of sexual organs and secondary sexual characters.
MFU	Marine Farming Unit, at Ardtoe.
MIB	2-methylisoborneol: a chemical that contributes to flesh taints of cultured fish.
Mitotic gynogenesis	Gynogenesis (q.v.) induced by doubling the single set of chromosomes in an egg during the first mitotic division of the nuclear material that occurs when cell division begins.
Nauplius	The non-feeding newly hatched stage of development of a crustacean such as *Artemia* when the body is unsegmented and yolk reserves are reasonably abundant.
NERC	National Environment Research Council.
Neuromasts	Sensory organs composed of hair cells that are sensitive to vibration (mechanoreceptors).
Oestrogens	A group of steroid hormones: the principal female sex hormones (e.g. 17β oestradiol), synthesised chiefly in the ovary. They influence growth and function of female reproductive organs and the development and maintenance of female secondary sexual characteristics.
pH	A value, on a scale of 0-14, that gives a measure of the acidity or alkalinity of a medium such as water; a pH value of 7 denotes neutrality, a value less than 7 indicates the degree of acidity, and a value greater than 7, alkalinity.
Parthenogenetic	Characteristic of a type of reproduction where the egg develops into new individual without being fertilized.
Phenotype	Characteristics manifested by an organism and determined through the influence of environmental factors interacting with its genetic make-up.
Pheromone	A chemical released (usually in minute amounts) by an animal and which can be detected by, and acts as a signal to, another member of the same species.
Phytoplankton	Very small aquatic plants, mainly single-celled algae, which drift in fresh- or sea-water.
Plasma	Blood from which all the red blood cells have been removed (for example by centrifugation).
ppt	Parts per thousand (mg g^{-1}).
PRE	Protein retention efficiency = ((P2 * W2)-(P1 * W1))/(FP * FI)*100, where W is weight (g), P is protein concentration in fish (%), FP is protein concentration in feed (%) and FI is feed intake.

Probiotics	The deliberate use of benign species of bacteria (probionts) in the fish rearing environment; they are added to the diet or the water to inhibit growth of pathogenic species and reduce the risk of disease outbreaks.
R&D	Research and development.
Salmonid	A fish belonging to the family *Salmonidae*, which includes Atlantic salmon (*Salmo salar*), trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*).
SAMS	Scottish Association for Marine Science.
SFIA	Sea Fish Industry Authority: formerly the White Fish Authority (WFA), also known as Seafish.
SOAEFD	Scottish Office Agriculture, Environment and Fisheries Department.
SOS	School of Ocean Sciences, at the University of Wales, Bangor.
SSF	Acronym used by the Norwegian Herring Oil and Meal Research Institute, Bergen.
Stock enhancement	The release of hatchery reared animals in to the environment to supplement natural, wild stocks.
Stocking density	The quantity of fish in a holding facility, measured in relation to the size of the containment unit (for example: kg m^{-3}).
Triploid	Having three sets of identical chromosomes in the nucleus, rather than the usual two sets of a diploid (q.v.) organism.
UKAFMM	UK Association of Fish Meal Manufacturers.
UV	Ultra violet light: short wave radiation that disrupts DNA (q.v.).
WFA	White Fish Authority: now the Sea Fish Industry Authority (SFIA).
Yolk-sac larvae	Young fish after hatching and when still dependent on yolk as the primary source of nutrition.
Zooplankton	Very small aquatic animals, such as copepods and larval stages of larger animals, which drift almost passively in sea- or fresh-water.

ANNEX 3. Halibut egg and yolk-sac larvae handling procedures, reproduced from the Final Report of project FC0905

1. Egg handling and incubation procedures

1.1. Egg fertilisation

Eggs are stripped from the female broodstock into 2 litre polyethylene jugs, and milt is stripped from the males into 500 ml polyethylene beakers. The eggs and milt are then carried in an insulated, light-proof container to the egg handing room, where fertilisation will take place. A quantity of milt from 2 males is added to a 5 litre bowl of UV-sterilised sea water at 6.0°C, 35 parts per thousand (ppt) and mixed. The eggs are then added to the bowl and left for 20 minutes to allow fertilisation. Batches of eggs from different females are treated separately. During the fertilisation period samples of the milt are checked under the microscope for motility. The fertilised eggs are then rinsed well, and transferred to a static 80 litre MDPE hopper, filled with UV-sterilised sea water at 6.0°C and 35.5 ppt. Air and water temperature in the egg handling room are maintained at 6.0°C, by means of an air chiller.

1.2. Egg examination

At 16 hours post-fertilisation a sample of floating eggs is examined under the binocular microscope for assessment of percentage fertilisation and morphology. At this time the eggs should have reached the 3rd division, or eight cell stage. Quality is assessed on the basis of chorion appearance, egg shape, symmetry of dividing cells, and appearance of cells and cytoplasm. Only batches of eggs showing high (70%+) percentage fertilisation and reasonable cell symmetry are selected for incubation, and are counted and transferred to the incubation unit.

1.3. Egg counting

Floating eggs are skimmed off the surface of the static hopper and poured gently into a large nylon sieve supported in a bucket of clean sea water (6.0°C, 35.5 ppt). The sieve is then removed and surface water allowed to drain off the collected eggs. The eggs are then poured into a jug of sea water tared on an electric balance and the weight noted. A small sample of eggs is weighed and counted, in order to calculate the number of eggs per gram, this being used to calculate total number of eggs available for stocking.

1.4. Incubator stocking

It is estimated that each of the 80 litre incubators has the capacity to hold up to 1 kg eggs (50-60,000) eggs. Eggs are transferred to the filled incubators directly after the post-fertilisation assessment and, being negatively buoyant, the upwelling flows have to be adjusted to maintain the eggs well dispersed in the water column, whilst avoiding excessive turbulence. Different egg batches have varying degrees of buoyancy, therefore flows must be adjusted accordingly and cannot be standardised. Flow rates must also be adjusted in response to changes in the salinity of the ambient sea water supply.

1.5. Daily husbandry

Water temperature is monitored daily and used to calculate age in day degrees. Halibut eggs start to hatch at approximately 80 day-degrees post fertilisation i.e. 13 days in incubation and thus the age of differing egg batches is important when future rearing stage are to be considered i.e. yolk-sac tank stocking.

Removal of dead eggs is achieved by the introduction of 7 litres of high salinity water (40 ppt) to the bottom of the incubator via the inlet pipe - the regular flow having been turned off. The high salinity water creates a halocline on which the live eggs rest, but through which dead eggs sink. These are now drained off through the drain valve into a bucket along with most of the high salinity water. The total drop out is weighed and the number of dead eggs calculated from a count of number per gram. This enables a record of eggs remaining in incubation to be maintained.

1.6. Egg disinfection and transfer to yolk sac tanks

At 65-70 day degrees post fertilisation the eggs are disinfected to remove surface bacterial contamination. To facilitate collection of eggs for this procedure, they are floated at the water surface by the addition of enough concentrated brine (260 ppt) to to raise the incubator salinity to 35-35.6 ppt. The eggs are skimmed off the surface and collected in a sieve, which is then immersed for 1 minute in a 1:250 dilution of the disinfectant "Kick Start". Following disinfection the eggs are rinsed and weighed in a tared jug of UV-sterilised sea water, in order to calculate number available. They are then transferred to a yolk sac tank in the same jug.

2. Yolk-sac larvae rearing procedures

2.1. General precautions

Halibut larvae are fragile and are sensitive to mechanical stress and changes in environmental conditions, thus management of the yolk sac tanks requires great caution. Operations and observations are carried out under very dim lighting and are kept to a minimum, so as to cause the least disturbance to the larvae. The tanks are held in darkness when not being operated on.

2.2. Daily husbandry

Tanks are fitted with individual flow meters and the rate of inflow is checked daily. Newly stocked eggs generally exhibit negative buoyancy and flow rate must be adjusted to retain the eggs in the water column. Immediately before and during hatch, the eggs/larvae generally become positively buoyant, and flow rate is reduced to avoid entraining material on the outflow screen. After hatching is completed, the inflow is switched off for a period of hours, to let egg shell debris and dead eggs sink to the bottom, from where they are drained off.

Flow rates were set to maintain a supportive upwelling effect without excessive turbulence. Through trials, the flow settings for the 1,150 litre and 400 litre yolk sac tank were set to 1.5 litre min^{-1} and 0.8 litre min^{-1} respectively.

Water temperature and salinity, maintained at 5.5-6°C and 33.0 ppt, are recorded daily using a WTW LF 196 microprocessor conductivity meter. At the same time, the vertical position and concentration of the larvae in the tank was subjectively assessed, with the aid of a torch. For this purpose, the water column is divided into horizontal zones: five zones for 1,150 litre, four for 400 litre tanks (see Figure A.1).

Depending on the purpose of individual trials, water analyses are carried out at varying intervals, to quantify levels of ammonia, nitrite and nitrate, using a Hach Model DR/2000 spectrophotometer. Total bacterial counts on marine agar plates also provide a basic measure of microbial loading. More detailed bacterial sampling is carried out for specific rearing experiments.

Dead larvae are not drained off routinely, as the necessary interruptions to flow are thought to be detrimental, and there is a possibility of removing live larvae situated low in the tank. However, if large amounts of dead material have accumulated around the inlet, and the surviving larvae were positioned well clear, the flow is stopped and the detritus quickly drained off.

2.3. Removal and inspection of "first-feeding" larvae

Prior to the removal of the larvae for first feeding, the yolk sac tank water temperature is increased by 1°C per day increments from circa 170 – 180 day degrees, until it matches that of the first feeding tank, this normally being 10°-10.5°C in 1998.

During 1998, it was standard practice to retain larvae in the yolk sac tanks until 220-230 day-degrees post hatch, although several batches were held in the tanks until 270 day degrees. At this stage, larvae are already at the water surface, or can be encouraged to swim up by removing the lid and supplying low level room illumination. If an accurate survival count is required, larvae are counted into 3 litre plastic jugs; otherwise they may be scooped into a larger container, such as a 10 litre plastic bucket. At this time, an assessment of morphological development is made, by examination of 20 individuals per tank. Developmental abnormalities may be observed on the jaw, liver, gall bladder and eyes. Fish are assessed primarily on the basis of jaw formation, liver development and the presence of a gall bladder, although incidence of oedema and spinal deformities are also noted. From this assessment, the number of larvae potentially able to feed, or regarded as viable, is calculated.

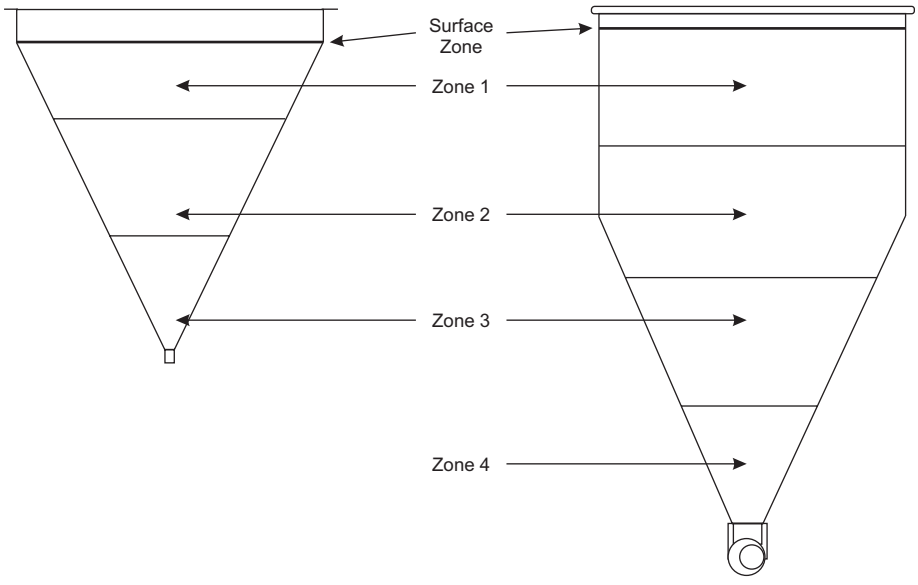

Figure A.1. Illustration of zones used to assess vertical position of yolk-sac larvae in 400 litre and 1,150 litre rearing tanks.

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