# Saving the European Eel (*Anguilla anguilla*) from extinction, the role of conservation aquaculture.



Winston Churchill Memorial Trust Travelling Fellowship Dr.John Taylor 2011-2012

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## **INTRODUCTION**

The Winston Churchill Memorial Trust (WCMT) was established after his death by in 1965 with generous donations from members of the public wishing to express their gratitude for all that this great man had achieved for Great Britain.

Each year the WCMT awards over 100 Travelling Fellowships to ordinary British citizens from all walks of life in order that they can gain knowledge which will benefit others on their return. Their stated objective is:

"The advancement and propagation of education in any part of the world for the benefit of British citizens of all walks of life in such exclusively charitable manner that such education will make its recipients more effective in their life and work, whilst benefiting themselves and their communities, and ultimately the UK as a whole".

During this Fellowship I travelled to 8 countries in 3 continents flying over 27,000 miles on 13 flights; driving 2,800 miles by car visiting 3 universities, 3 fisheries research institutes and 4 commercial eel farms.

The knowledge obtained from the Fellowship will be used to support the Environment Agency's aim to return the European Eel stock to sustainable levels and will form a valuable part of its Eel Management Plans.

#### **1. BACKGROUND**

Stocks of elvers are known to be at an all time low across Europe and have plummeted to 1-5% of levels recorded 20 years ago. The species is now listed on the IUCN red list and is a priority species on the UK Biodiversity Action Plans. Most scientists and conservationists accept that the species is now outside safe biological limits and unless urgent action is taken to halt its decline, the European eel may be on an irreversible path to extinction.



Life cycle of the European Eel

European eels are thought to spawn in the Sargasso Sea and the young larvae (Leptocephali) can take up to 2 years to drift on the ocean currents towards European shores. On reaching coastal areas they metamorphose into glass eels and then become pigmented as elvers when they migrate up river systems. The young eel takes between 6-20 years to reach maturity at which time they migrate as "silver eels" out to the Sargasso Sea, spawn at depths of up to 1000m and then die.

The reasons for the rapid decline include habitat destruction, creation of barriers to migration, pollution, changing ocean temperatures and over-fishing. In order to return the eel to self-sustaining levels the causes for the decline must be reversed and exploitation reduced to as close to zero as possible. However there are fears that current abundance may be below minimum viable populations and some experts believe that even if all impacts are removed, it may not be enough to allow the eel to recover.

The European Commission has initiated an Eel Recovery Plan (Council Regulation No1100/2007) aiming return the European eel stock to more sustainable levels, requiring each member state to produce a set of Eel Management Plans. Part of the proposed management measures to address the decline includes the use of cultured eels in restocking programmes to increase silver eel escapement.

The Regulation requires that at least 35% of the commercial catch of eels of less than 120mm is made available for restocking in 2009, rising to 60% by 2013. However, at present there remains a paucity of information regarding the efficacy of stocking with most evaluations based on the contribution of stocking to commercial fisheries.

Stocking or translocation can be used to provide access to previously unattainable areas but the main advantage of artificial rearing relies on removal of natural bottlenecks for survival in the wild coupled with minimising domestication impacts to achieve an overall increase in life time fitness.

Most of the market for eel consumption lies in the Far East with a substantial input from the European continent. At present the production of eel in aquaculture relies totally on the capture and on-growing of elvers. If the market is to continue then the reliance on wild caught elvers must reduce in the near future. Methods for breeding eels in captivity must be an integral part of reducing pressure on wild elvers and subsequently halting their path to extinction.

#### 2. AIMS OF THE FELLOWSHIP

The overall aim of this Fellowship is:

"To discover best practice aquaculture, marking/tagging, eel stocking and spawning procedures which can be used to develop a conservation strategy to make a significant contribution to restore the endangered European eel."

The project will focus on gaining information and first hand experience in 3 key areas:

#### 2.1 Commercial eel culture

Although a significant proportion of elver resources lies in UK territories (River Severn, River Parrot), almost all of this is exported to Europe or the Far East for stocking or direct consumption. There are no commercial eel farms in the UK mainly as the eel is not considered an important food source.

Consequently, the most technologically developed culture methods are found in mainland Europe in countries such as Holland, Denmark and Sweden. The on-growing techniques for captured elvers are now so refined that survival rates to hardy juveniles in excess of 90% are regularly achieved.

It is imperative that this can be replicated in a conservation captive breeding scheme in order to make the best use of a scarce and dwindling resource. The most important aspects of eel culture that need to be discovered include:

- Transportation and handling techniques
- Holding and culture facility design and operation
- First feeding and weaning of captured elvers onto commercial diet
- Stock movement and grading
- Disease prevention and treatment
- Bio security

#### 2.2 Stocking and monitoring

The practice of capturing young recently migrating elvers and translocating them to more productive areas has been carried out for many decades. However in the past 20 years stocking has employed the use of aquaculture to obtain high survival rates to hardy juveniles (where natural mortality in the wild will be high). These young eels have then been stocked into productive water bodies with a survival rate to silver eel thought to be much greater than captured and translocated elvers.

However, virtually all of the stocking carried out has been to support commercial fisheries as opposed to conservation or restoration initiatives. It would appear that stocking of cultured

juveniles can contribute significantly to increased silver eel output for fisheries but this has rarely been demonstrated in scientifically robust programmes.

Thus there is a sizeable gap in our knowledge base in terms of properly evaluated stocking programmes that measure the effectiveness of stocking eels at various life stages Although stocking must also be evaluated economically in terms of cost/benefit it is believed that using aquaculture to overcome natural bottlenecks to survival will yield the best return.

There is an urgent requirement to investigate which stocking practices produce the best results, in terms of survival and contribution to future generations? Consideration needs to be given to the following:

- time of stocking
- density
- frequency
- life stage
- habitat yield (rivers/lakes)

Unless eels are to be stocked in an area completely devoid of any natural populations then the stocking programme must use a marking or tagging method to identify stocked eels and calculate their survival. Several European countries evaluate the efficiency of stocking by recapturing adult silver eels during migration and correlating their numbers with stocking effort. However this does rely on predicting accurate ages of migration and can take 6-20 years to yield results.

Short term studies that look at survival 1-2 years after stocking can employ a variety of existing methods such as using physical tags (microtags) detected by passing the fish over a metal detector or several dyes/stains that adhere to various tissues in the body. The techniques for carrying out some of these tagging methods are not widely known and experience of this would be invaluable.

#### 2.3 Breeding eels in captivity

At present it is not possible to artificially spawn the European eel and produce viable larvae that will yield offspring for commercial farming. If this could be achieved then it would remove one of the biggest pressures on wild elvers which, at the moment, need to be captured during migration into rivers to supply commercial eel farms.

Some breakthroughs have been made in recent years notably led by the Technical University of Denmark (DTU) in Kolding. Here researches have managed to induce adult eels to spawn in captivity and have succeeded in rearing larvae to 18 days. However the larvae could not be weaned onto artificial diet and perished after absorbing their yolk sac.

In Japan, researchers have recently successfully bred the Japanese eel (*Anguilla japonica*) so that the first artificially reared eels are now producing viable offspring themselves. Similar work is being carried out by the Fisheries Research Institute in Keelung, Taiwan. Although these workers recognise that the programme is still far from commercially viable it is evidence that they have advanced techniques and this may be vital to work carried out on the European eel.

Similarly, research is underway in Auckland and Dunedin in New Zealand to induce spawning and rear larvae of the New Zealand Short Fin Eel (*Anguilla australis*). At the University of Massachusettes, Boston, USA, American Eels (*Anguilla rostrata*) have been successfully induced to spawn and have produce larvae surviving up to 3 weeks.

By visiting countries that have successfully spawned eels species it was my aim to gain experience in the following techniques:

- Hormonal induction of gametogenisis and ovulation
- Assessment of egg quality at final maturation
- Assessment of sperm quality
- Fertilisation and assessment of viability
- Egg incubation

Using the knowledge gained at these breeding centres it is proposed to set up a collaborative programme in the UK to look at aspects of eel breeding that require further study.

#### 3. FELLOWSHIP ITINARY

The Fellowship had to be divided into two phases due to the nature of the timing of elver migration and adult spawning. Also, as the available knowledge was spread over Europe, Asia, New Zealand and the USA it was not practical to undertake this project with "one journey".

Phase One was carried out in April 2011 to coincide with European eel migration and an intense period for commercial eel farmers. The following itinerary outlines the countries and destinations visited:

#### PHASE 1

Places and Countries visited	Meetings attended/Institutes
	visited
11 <sup>th</sup> April – fly from Heathrow to	
Amsterdam, Holland.	
$12^{\text{th}}$ April – travel from Amsterdam to	Visit Nijvis BV – Commercial eel farm
Nijmegen	
13 <sup>th</sup> April – travel from Nimegen to Dronten	
14 <sup>th</sup> April	Visit Eel Farm selco – Commercial eel
14 April	farm
15 <sup>th</sup> April – return to Amsterdam and fly	
to Stockholm Sweden	
18 <sup>th</sup> April travel from Stcokholm to	Meeting with Hakan Wickstrom of the
Drottingholm	Swedish Board of Fisheries at the
	Institute of Freshwater Research. Discuss
	stocking techniques for eels.
18 <sup>th</sup> April Travel to Alvkarleby 19 <sup>th</sup> April	Visit Swedish board of Fisheries
19 April	Alvkarlerby salmon farm. Meeting with
	Erik Petersson.
19 <sup>th</sup> April travel from Alvkarleby to	
Stockholm	
20 <sup>th</sup> April travel from Stockholm to	Visit Scandanavian Silver Eel with
Helsinborg	Hakan Wickstrom of the Swedish
	Fisheries Board. Visit eel farm and
a oth the state of	discuss stocking and marking techniques
20 <sup>th</sup> April travel from Helsinborg to	
Copenhagen, Denmark. 24 <sup>th</sup> April- travel to Stege	Visit Eel Farm Jupiter – commercial eel
24 April- traver to Stege	farm
26 <sup>th</sup> April – North Copenhagen	Visit Jonna Tomkiewicz at the Technical
	University of Denmark. Discuss eel
	spawning and the PROEEL project and
4	possible collaboration.
27 <sup>th</sup> April – travel from Copenhagen to	Meeting with Michael Pedersen of the
Silkeborg	Technical University of Denmark to
	discuss eel tagging and marking
27 <sup>th</sup> April – travel from Silkeborg to	techniques.
Hanstholm	
28 <sup>th</sup> April	Visit Royal Danish Aquaculture – very
1	large commercial eel farm
28 <sup>th</sup> April – travel from Hanstholm to	
Copenhagen	
29 <sup>th</sup> April – return to UK	

Phase two was carried out in January and February 2012 to coincide with the spawning period of the Japanese, New Zealand short fin and American eels:

PHASE 2	
Countries and places visited	Meetings attended/ institutes
_	visited
17 <sup>th</sup> January – Fly from Heathrow to	
Hong Kong	
18 <sup>th</sup> January – fly from Hong Kong to	
Taipei, Taiwan	
19 <sup>th</sup> January – travel from Taipei to	
Taichung	
20 <sup>th</sup> January – travel from Taichung to Lu Kang	Visit Freshwater Research Institute and meet with Assistant Director Chen Guan- Ru. Visit endangered species breeding
	centre and take part in practical session inducing spawning in Japanese Eels.
21 <sup>st</sup> January – travel to Taipei and fly back to Hong Kong	
23 <sup>rd</sup> January – Fly from Hong Kong to Auckland via Sydney	
26 <sup>th</sup> January – travel to Warkworth, N Auckland.	Visit Mahuragi Technical Institute. Meet with Paul Decker and David Cooper.
	View endangered species captive
	breeding programmes and discuss
	holding, spawning and larval rearing of NZ short fin eels.
27 <sup>th</sup> January – fly from Auckland to Nelson, South Island NZ	
30 <sup>th</sup> January – drive from Nelson to Dunedin	
1 <sup>st</sup> and 2 <sup>nd</sup> February – Dunedin	Visit Dr Mark Lokman at the University of Otago. View eel research facilities, meet with research students. Gain practical experience in final maturation, stripping, fertilisation and egg viability assessment of NZ shortfin eels.
2 <sup>nd</sup> February – drive from Dunedin to Christchurch	
$3^{rd}$ February – fly from Christchurch to	
Auckland then from Auckland to Boston via Los Angeles	
7 <sup>th</sup> and 8 <sup>th</sup> February – drive from Boston	Visit Professors Kenneth Oliviera and
City to Dartmouth Massachusettes	Whitney Hable of the University of Massachusettes. View research facilities for eel breeding and take part in inducing and stripping American eels. Also gain experience in egg biopsy for viability assessment.
8 <sup>th</sup> February – return to Boston and fly	
back to UK	

#### 4. FELLOWSHIP FINDINGS

#### PHASE 1

#### **4.1 EEL AQUACULTURE**

The Netherlands and Denmark are the biggest net exporters of eels within Europe accounting for more than half of the 9000 tonnes annually exported. Consequently, they have some of the most advanced eel farming methods employing finely managed water re-circulation systems. However, each farm may employ the use of slightly different techniques and equipment which are worth evaluating for effectiveness at producing good quality eel, cost of running and ease of operation and maintenance.

Each of the farms visited produce between 150-900 tonnes of eels exported within Europe annually. Most farms import glass eels from a variety of sources throughout Europe depending upon their market. English and Welsh elvers, exported to the continent by Peter Woods of UK Glass Eels, are prized for their excellent condition due to the careful dip net fishing techniques and handling and transport conditions. French glass eels, on the other had have a reputation for being heavily damaged and exhibiting high mortalities although they tend to be directly supplied to market for immediate consumption.

#### 4.1.1 Transport of Glass eels

Eels can absorb oxygen through their skin as well as their gills so can be transported in very small volumes of water as long as they are kept cool.

Polystyrene boxes of 35 X 44 X 10cm deep with 300-400mls water are routinely used to transport 1kg (3-4000 glass eels) over a 24 hour period. A small beaker of ice is usually placed in one corner of the box to keep temperatures from rising. It is expected to obtain 97% survival during transport to eel farms.

#### 4.1.2 First Feeding and Weaning

Glass eels are transferred to GRP culture tanks after temperature equilibration. Rearing tanks can be troughs, circular or square but are usually 0.5 to  $1m^3$  in volume.

All rearing vessels tend to have an inward facing "lip" at the top of the tank wall. This is to prevent the glass eels/elvers from escaping; they are perfectly capable of crawling up a steep moist surface. (See Fig 1).



Figure 1. 2m diameter tank with recently weaned elvers, notice tank lip.

At present the only reliable method of weaning glass eels to ensure high survival is the use of frozen cod roe as a first feed. Weaning tanks are stocked with 30-40,000 glass eels per  $m^3$  and are fed at a rate of 1/2kg of cod roe per kg of fish. All juvenile tanks must have a self cleaning screen to avoid water outlets from blocking with elvers/glass eels.

Commercial fine pellet food is gradually introduced in paste form over the next few weeks. After 3-4 weeks some fish have a noticeable growth advantage and can be "graded" out and fed solely on commercial diet. The smaller elvers are put back onto cod roe and the process is repeated until all elvers are weaned and graded by about 3 months.

All tanks have a "resting basket" or net just under the surface of the water, the young eels appear to prefer this area to feed. It is usual to expect 95% survival to this stage.

#### 4.1.3 On- growing

When successfully weaned onto dry crumb commercial diet, young elvers are transferred to larger tanks. A variety of circular and square tanks can be used but rectangular troughs and tanks are preferred for later life stages.

A 2m diameter tank of 1 m<sup>3</sup> volume can comfortably hold 80,000 elvers.



Figure 2. Elvers in resting basket in 2m diameter tank at Scandanavian Silver Eel. Note the automatic feeder with commercial dry diet above the tank

Older eels (1-2 years of age) tend to be kept in larger rectangular tanks measuring up to 10m x 3m x 0.7m deep. These tanks can hold 40,000 eels up to 100g average weight.

High density rearing has a peculiar propensity to produce predominantly male fish (80%). Females are usually distinguished by the fact that they will reach over 200g in 18 months to two years whereas males never exceed 180g.

There are several commercial feed producers but generally they recommend to feed older juveniles at a rate of 1% of their body weight per day. A mixture of hand and automatic feeding is used but the farmer relies on the automatic feeder to deliver the vast majority of the food. Some producers prefer to use demand feeders where the eels can be seen almost climbing over each other to touch a swinging pendulum to release feed into the tank (see Figure 3)



#### Figure 3. 1 year old eels activating a demand feeder at Jupiter Eel Farm in Stege, Denmark

During the on-growing process fish must be sorted into their various size classes (grading) every 3-4 months in order to increase feeding efficiency and reduce cannibalism. This process is highly mechanised involving fish pumps which transfer fish to a grading platform.



**Figure 5. Grading machine** 

Figure 6. Close up of grading machine

#### 4.1.4 System requirements

Although extensive eel farms exist in countries like Italy, Spain, Greece and Portugal (mainly due to the warm climate), virtually all of the European production is carried out using highly technical water re-circulation systems.

These systems can produce a phenomenal amount of eels in a relatively small area but the water quality parameters must be very tightly managed. Temperature is critical and must be maintained at 25°C in order to obtain high survival of weaned elvers and ensure optimal growth rates.

Oxygen is usually maintained at 90% + and 8.0mg l<sup>-1</sup>, all farms have oxygen monitoring equipment linked to alarms and automatic oxygen injection if the saturation falls below 60-70%.

All re-circulation systems must have several basic components that maintain water quality by removing solid waste, reducing ammonia levels, sterilising water to remove pathogens and maintaining optimal oxygen concentrations.

The components of the recirculation systems on the commercial farms visited can be described as follows:

- 1. **Mechanical filtration** This is usually a drum filter (rotating, self-cleaning drum with a fine mesh screen) with a 40 $\mu$ m screen. This filter takes out most of the particulate matter such as uneaten food and fish faeces. The drum filter also helps to reduce the proliferation of gill parasites such as *dactylogyrus*.
- 2. **Biological filtration** Biological filters usually take the form of large tanks filled with various types of inert media with a high surface area. The media are colonised by bacteria which convert ammonia (toxic breakdown product from food protein digestion) into nitrite and less harmful nitrate.

For example, Jupiter Eel in Stege, Denmark uses a series of fluidised beds containing kaldness media, upwelling static biofilters with biorings and a trickle filter to reduce  $C0^2$  build up. The size and operation of the filtration plant varies between farms due to the intensity of rearing operation and personal choice. However, as a general rule fluidised beds take 8kg of food perm m<sup>3</sup> and upwelling filters 2kg per m<sup>3</sup>.

- **3.** UV sterilisation All water re-circulating through various filters then passes through and ultraviolet filter which reduces the level of any circulating harmful pathogens.
- **4. Oxygen monitoring** All farms have oxygen probes in rearing tanks continually monitoring oxygen levels. These are linked to telemetered alarm systems and automatic oxygen injection should the levels fall below the desired amount.
- **5.** Temperature control The system must be maintained at 25 °C so buildings are usually well insulated and heated.



Figure 7. Fluidised bed biofilters at JupiterFigure 8. Drum filters at Royal DanishEel, Stege, Denmark.Aquaculture, Hanstholm, Denmark

#### 4.2 Disease control and biosecurity

It is in the interest of the farmer to maintain a well balanced system with good water quality which should avoid many of the bacterial, fungal and parasitic infections common to high density fish rearing.

However, attitudes vary widely in the control of some diseases and to the extent of biosecurity needed for transfer between sites and into the wild. For example Nijvis believe that exposure to known pathogens is a good thing so they limit biosecurity measures on site. Some European farms are actually known to favour the practice of exposing juveniles to the Eel Herpes Virus (EHV) so that losses occur at the relatively inexpensive stage while the eel develops immunity. However, a sister company of Nijvis, in Germany, has actively developed its own vaccine for EHV.

In Sweden the situation is very different. Scandanavian Silver Eel are required to quarantine imported glass eels for a period of 10 weeks so that they can be tested and certified as disease free before they are released into the wild.

#### 4.3 Marking/Tagging and stocking

#### 4.3.1 Marking/tagging

In order to optimise stocking projects and add scientific certainty to the results most researchers use some form of marking eels before stocking. The exception to this is where eels are stocked into barren environments or where the life history patterns are well know so that correlations between stocking of juveniles and emigration of silver eels can be estimated.

In Sweden, Hakan Wickstrom of the Swedish fishery Board insists that all his glass eels for stocking are marked with Strontium Chloride. Glass eels or elvers are bathed in a solution of Strontium Chloride for 24 hours, the chemical is absorbed in the otolith and causes a stain like a growth ring. This can be identified using special microscopy techniques. This process can be

carried out more than once allowing different batches to be marked ( for example different ages at stocking or different stocking locations).

Michael Pederssen of the DTU in Silkeborg has been stocking eels for many years and has perfected methods of tagging eels with coded wire tags (CWT) sometimes known as microtags. Anaesthetised eels are injected with a tiny piece (1mm) of coded steel wire in the dorsal musculature about mid-way along the body. This method has the advantage that eels can be detected by a scanner and do not need to be killed to read the tag, unless different batches are needed. Using this technique Michael has been able to tag eels down to 3g and maintain a 95% tag retention.

#### 4.3.2 Stocking

During a meeting with Hakan Wickstrom and William Dekker at the Swedish Fishery Board research station in Drottingholm, Sweden, they made the following comments regarding stocking:

- All glass eels stocked are marked with Sr Cl2
- The sooner eels are stocked the better to avoid domestication and uneven sex ratios
- Stock from as local a source as possible to include any possible local adaptation and avoid disease
- Sweden stocks between 750kg -1 ton of glass eels annually imported from France but only from areas using dip net capture
- 2 million 1 g eels are used for stocking mainly because they have strict quarantine for 8-10 weeks hence they reach 1 g.
- Stocking definitely correlates with increased silver eel output
- 60% of recaptured eels from Baltic lakes are stocked.
- Virgin lakes will give a very rapid response to stocking due to utilisation of unutilised resources, very quickly, possibly results in 3 or 4 years. However the output will decrease over time to a stable level
- There is little evidence of the eels spawning anywhere but the Sargasso and so the "panmixia theory" (one single stock) is the most likely explanation. Therefore in theory the genetic origin of eels for stocking is immaterial. There also appears to be no solid evidence of any form of imprinting on migration routes.

Dr. Curt Gelin and Richard Fordham of Scandanavian Silver Eel have stocked 27 million eels since 1987. At present the stock approximately 2.3 million annually in Sweden and 300,000 in Finland. They have shown that increases in silver eel escapement correlate nicely with tons of elvers stocked.

Prior to stocking they recommend reducing the system temperature to that of the receiving water over two days. If Sr  $Cl_2$  is used as a marker they recommend waiting at least 1 month if a second mark is needed. Elvers are best stocked from a boat about 5m from the shore, reedy areas provide good cover.

#### 4.4 Induced maturation and spawning of eels

Most of the knowledge gained in this area in was attained in Phase Two of this study tour apart from a visit to the Technical University of Denmark in Copenhagen to meet Dr Jonna Tomkiewicz in April 2011. Jonna is the leader of PROEEL, a collaborative European project involving academic, industry, conservation and government partners to investigate the techniques for successful spawning and larval rearing of the European Eel.

This section will be described by species

#### 4.4.1 European Eel

At the DTU in Copenhagen work has been underway since 2009 to examine the spawning protocols for the European eel. A small amount of research is carried out at the DTU but the main breeding programme is maintained at Lyksvad eel farm, near Kolding, Denmark.

The collaborative project has been able to successfully produce larvae of up to 22 days post hatching, which to date remains the best achieved. Notable achievements include:

- Producing viable eggs by using hormonal treatments. Males are brought to spermiation using human chronic gonadotrophin (hCG). Females are ripened with salmon pituitary extract (SPE).
- Successful fertilisation of eggs with fresh, cryopreserved and diluted sperm.
- Studies on assimilation of amino acids by larvae to elucidate suitable feed types.
- Manipulation of broodstock diets to improve quality and predictability of egg production.
- First feeding of larvae to 25 days enabling different rearing environments to be tested.

#### 4.4.2 Japanese Eel

It is important to note that one of the original aims of this study tour was to visit the Fisheries Research Agency in Yokohama, Japan where the most successful eel breeding has taken place. Japanese researchers have successfully reared second generation eels in captivity although they have not been able to do it on an economically viable scale.

Unfortunately, the Japanese Government changed their policy on research sharing just prior to the agreed visit and so it was not possible to visit their facilities.

However, the Freshwater Aquaculture Research Centre, part of the government Fisheries Research Institute, in Lu Kang, Taiwan also have been running breeding trials on the Japanese eel and were happy to host a visit.

Associate Director Chen Guan-Ru uses the following protocol for inducing spawning and fertilisation:

- Males and females injected with SPE weekly for 3 months starting in November
- 1 pituitary gland (kept in acetone) is used per 1kg of fish. Pituitary is ground up in saline and used at a rate of 1ml per fish.
- During handling and injection eels are anaesthetised using 500ppm of 2-phenoxyethanol or clove oil at 350-400ppm.
- Maturation condition is judged on final body weight compared to body weight pretreatment. When the BUI of females reached 110% twice the dose of SPE was given.

- 16-18hrs later the BUI when the BUI reaches 120% and injection of DHP (Dihydroxyprogesterone) at 4mg/kg.
- 16-18 hours after final DHP injection eels would be expected to spawn.
- Females are spawned naturally using 2-4 males per female in spawning tanks kept at 25°C using recirculation. Photoperiod is natural. Plankton nets are used to collect eggs on the overflow of the tank.
- Natural spawning is preferred but when stripping manually eggs and sperm are mixed dry then a small amount of seawater is added. After a few minutes the sea water is renewed.
- Eggs were incubated in static sea water in 100L conical tanks with aeration. Eggs hatched in 36 hrs and larvae remained on their yolk sac for a further 3 days.



Figure 10. Injecting a female Japanese eel with SPE at the Freshwater Aquaculture Research Centre, La Kung



Figure 11. Egg incubation and larval rearing tanks at the Freshwater Aquaculture Research Centre, La Kung

#### 4.4.3 New Zealand short fin eel

The Mahurangi Technical Institute (MTI) in Warkworth (1 hour North of Auckland) is a Fisheries and Aquaculture training and research centre also running courses in Marine Engineering. The centre carries out captive breeding trials for many local endangered species but specialises in breeding NZ eels.

Paul Decker and David Cooper of the MTI have a novel approach to inducing spawning relying on a more natural approach:

- Eels are kept in a salt water recirculation system with biofiltration and protein skimmer, maintained at 25 °C.
- They believe that eels can be brought to maturation by use of photoperiod and temperature manipulation
- Based on condition and timing 1 HCG injection is given
- 8 hrs later fish is ready to strip and fertilise using the dry method.

Using this method researchers at the MTI believe they can produce egg batches of high viability virtually all year round. Eggs are incubated in conical vats with a small flow through and a "banjo" type filter. Hatching usually occurs in 3 days.



Figure 12. Paul Decker, Director of MTI with a mature NZ long fin eel

The MTI also employ a novel technique for rearing juveniles after yolk sac absorption. As young eel fry are extremely delicate and lack the capability to swim strongly for some time. They are prone to becoming washed against tank outflow filters and becoming damaged or dying.

To solve this problem the MTI have developed a fry rearing channel where the water is circulated in a loop past a long central overflow pipe. The pipe has a long fine mesh screen and widens in the direction of the water flow. The effect of this is to speed the fry passage up as the channels narrows safely carrying past the mesh screens without becoming damaged.



# Figure 13. David Cooper, Special Projects Manager with MTI. Note the black broodstock spawning inducement tanks in the foreground and the small white conical larval rearing tanks.

At Otago University in Dunedin (South Island NZ), University lecturer and researcher Dr Mark Lokman has been studying eel spawning for some time. He learned his trade studying with Japanese researchers and now has two PhD students investigating optimal techniques for producing viable eggs of the NZ short fin eel.

Mark uses the following protocol adapted from Japanese workers:

- Females were given weekly injections of acetone dried Chinook salmon pituitary homogenate (SPH) were administered at a dosage rate of 5mg per kg.
- Males were anaesthetised in 0.3% benzocaine and given weekly intra muscular injections of hCG at 250IU.
- Female body weight was measured every three days
- After a 5% increase in body weight was observed ovarian samples were collected by cannulation and examined under a low power microscope.
- Final maturation and ovulation were induced once germinal vesicle migration and cytoplasmic clearing were obvious. An SPH injection was given at 10mg per kg then, after 22-24 hrs a final injection of DHP was administered at 10mg per kg over the whole length of the abdomen (3 sites).
- After 16 hrs further cannulation procedures were performed to determine developmental stages of ova. At this stage a decision was made to manually strip the eggs from the female or wait another hour.
- Males were stripped manually with sperm stored on ice for up to 3 hours.
- Sperm % motility and quality was checked by activating a sperm sample under the microscope (X40 objective) with SW and observing the number of sperm actively moving and the length of time of motility.
- Females were stripped manually into a glass bowl and mixed dry with sperm
- A small amount of salt water was added to the mixture and the eggs were fertilised within two minutes
- Fertilised eggs were incubated in a conical incubator with static salt water aerated at the base of the cone.





Figure 14.

Figure 15.



Figure 16.



Figure 17.



Figure 18.



Figure 19.

Dr. Mark Lokman's fish breeding research lab at the Department of Zoology, Otago University, Dunedin, New Zealand.

Figure 15. Using a cannula to extract eggs from a mature, induced female NZ short fin eel. Note the hugely swollen abdomen full of eggs.

Figure 16. Manually extracting sperm, collected in a disposable plastic pipette from several anaesthetised mature male NZ short fin eels.

Figure 17. Matt (right) and myself manually stripping eggs from a ripe female NZ short fin eel.

Figure 18. Dr Mark Lokman checking the motility and quality of freshly collected NZ short fin eel sperm.

Figure 19. Fertilised egg incubator with air tube and aquarium heater to maintain temperature at 25°C. Note the mass of clear neutrally buoyant eggs.

#### 4.4.4 American eel

The final visit of the fellowship was to the University of Massachusettes in Dartmouth, 50 miles south of Boston, USA. Two days were spent with University Professors Kenneth Oliveira and Whitney Hable and their research student discussing the inducement of spawning in the American eel and observing and taking part in practical demonstrations.

Kenneth and Whitney have developed successful methods for producing viable eggs and are primarily concerned with the effects of contaminants on eel eggs and larvae. Their methodology for producing viable eggs is similar to others with subtle changes:

- Males mature in freshwater with weekly injections of hCG at 50 IU/ eel for up to 10 weeks.
- Females were matured in sea water and given weekly injections of SPE at 10mg/fish in 0.5ml saline. Injections were given weekly for up to 12 weeks and body Mass Index was calculated weekly.
- When female BMI was >105% coupled with body shape changes eggs were sample by aspiration biopsy and measured under a low power microscope to assess oocyte maturation.
- Females were considered for final maturation when the eggs showed cortical clearing, oil droplet condensing, migration of germinal vesicle and were in excess of 800µm in diameter.
- When considered ready females were given an initial final SPE injection of 10mg and then the following day were induced with a DHP injection at 2µg/g of body mass. The injection was spread throughout the abdomen over both sides.
- Males were stripped under light anaesthesia and sperm motility was assessed. Fresh sperm was stored in vials on ice.
- Females were lightly anaesthetised and manually stripped into a plastic beaker.
- Eggs and sperm were mixed dry and sperm was activated with filtered sea water.
- Fertilisation success and egg development was monitored by hourly sampling of eggs to observe cell divisions.





Figure 21.



Figure 22.



Figure 23.



Figure 25.

Figure 24.



Figure 26.





Figure 27.

Figure 28.

Figure 21. Dr Kenneth Oliveira's Freshwater Research Laboratory where the male eels were matured.

Figure 22. Marine Research Station affiliated with UMASS where the female eels were matured in salt water.

Figure 23. DHP injection in a female American eel to induce final maturation and ovulation.

Figure 24. Aspiration biopsy using a 16 gauge needle to assess oocyte maturation

Figure 25. Mature oocyte showing germinal vesicle migration and condensed lipid droplets (courtesy of Dr Kenneth Oliveira)

Figure 26. Dr Kenneth Oliveira manually stripping eggs from an induced American eel

Figure 27. Fertilised egg after 2<sup>nd</sup> cell division, 1 hour post fertilisation. (Courtesy of Dr Kenneth Oliveira)

Figure 28. Fertilised egg 8 hours post fertilisation showing many cell divisions. (Courtesy of Dr Kenneth Oliveira).

### **FELLOWSHIP CONCLUSIONS**

As a result of the research, meetings and investigations carried out during this fellowship the following is a summary of the key learning points:

- 1. Eels can be cultured in captivity in precisely maintained recirculation systems to obtain a 95% survival to 3 months.
- 2. Cod roe is the best feed for weaning of early caught juvenile eels.
- 3. Elvers can be transported in minimal water in small polystyrene boxes as long as they are kept cool.
- 4. Elvers for stocking can be marked with dyes that stain their otoliths or tagged with a small coded wire tag.
- 5. Elvers cultured for 1-3months generally give better returns than those stocked as freshly caught glass eels.
- 6. Stocking in to shallow lakes or slow flowing productive river environments produce the best results.
- 7. Stocking definitely correlates with increased silver eel output.
- 8. Adult eels can be kept healthy in captive environments and induced to spawn.
- 9. Eggs and sperm of good quality can be produced by careful administration of the correct programme of hormone injections.
- 10. Incubation of fertilised eggs can achieve a high survival to hatching.
- 11. As yet survival of larvae of European eels after yolk sac absorption has not been achieved
- 12. Discovering the preferred food types for weaning eel larvae is critical to ensuring the production of viable offspring.

This report will disseminated to a wider technical audience in the Environment Agency and will be made available for other Government Fisheries Agencyie, Fisheries Trusts and Academic Institutes. Based on the findings of the initial Phase of this Fellowship a presentation has been already given to students at Swansea University studying the MSc in Aquaculture and an experimental eels stocking project has been initiated in Llangorse Lake near Brecon, Wales.

In addition to this I plan to give a further presentation on the findings of Phase Two at the Institute of Fisheries Management's annual conference in Cardiff. It is also hoped that The Environment Agency aquaculture experts will collaborate with various European partners on the PROEEL project to close the life cycle of the European Eel in captivity.

In order to ensure the long term sustainability of European eel populations the overall objective of restoring river habitats, water quality and river connectivity (from source to sea) must be of paramount importance.

However, care must be taken not to ignore the short term threats to population viability whilst focussing on long-term aims. Restoring optimal environments is likely to take several decades which may be preceded by eel numbers falling below minimum viable population levels. Therefore short term measures such as reducing exploitation are also vitally important.

Although the reasons for the drastic decline in elver recruitment of European eel have not been completely identified it is clear that either the survival of spawning stock or their progeny is far lower than is needed to sustain a healthy, viable population.

The use of artificial culture and stocking to increase survival of juvenile eels in the wild and subsequently migrating silver eels to the Sargasso sea can only aid to reduce the impact of known and unknown threats. Stocking is known to increase silver eel output and has been shown to make

a significant contribution to increasing catches of commercial fisheries therefore it can equally be well employed as a conservation initiative.

The successful production of juvenile eels in captivity appears to be some years away from producing results that can mean a cessation of exploitation of wild elvers and the continuation of a valuable, historically and culturally important eel farming industry. However, rapid progressis being made as the collaborative efforts of industry, governments and academia escalate to solve the biological mysteries of this intriguing animal.

Captive breeding is often criticised as a poor compromise or a last resort, an admission of failure of our ability to control our impacts on the environment. Whilst mankind may have been guilty of short term greed over the last two centuries and ignoring the implications for future generations, it is unrealistic to expect that we can turn back the clock to the pristine environments of the pre agricultural and industrial revolution days.

The arrogance of the view of some "academic conservationists" who would prefer no interference from man is self-defeating. Practically every major food source important to man is highly managed through captive breeding and genetic manipulation yet we still have a romantic view about some fish stocks, that we can somehow keep harvesting from the revered "wild" stock with no regard for the balance of nature.

Natural populations produce offspring in the numbers needed to survive the unpredictability of their environment and natural predators in the food chain to ensure that the species continues in a self sustaining manner. The heavy burden of unnatural levels of predation and environmental impacts caused by man is often considered as unnecessary and totally avoidable, yet in reality it can never be removed, merely reduced. Highly fecund animals such as many fish species are often assumed to be producing exploitable surpluses, as with the elvers. However, this exploitable surplus is natures "insurance policy" to ensure that the population continues as any individuals chance of surviving is extremely low due to chance factors (this is why the eel produces 3 million eggs when each breeding pair only needs to produce two to keep the population stable). This equilibrium cannot be maintained by the impacts of an ever increasing population.

However, agricultural and industrial practices can be improved and more sustainable methods of harvesting wild stocks can be developed. We can only achieve these aims through careful management of this planet and its resources and the realisation that we are part of evolution and if this means we must put something back to compensate for what we take out, then so be it.