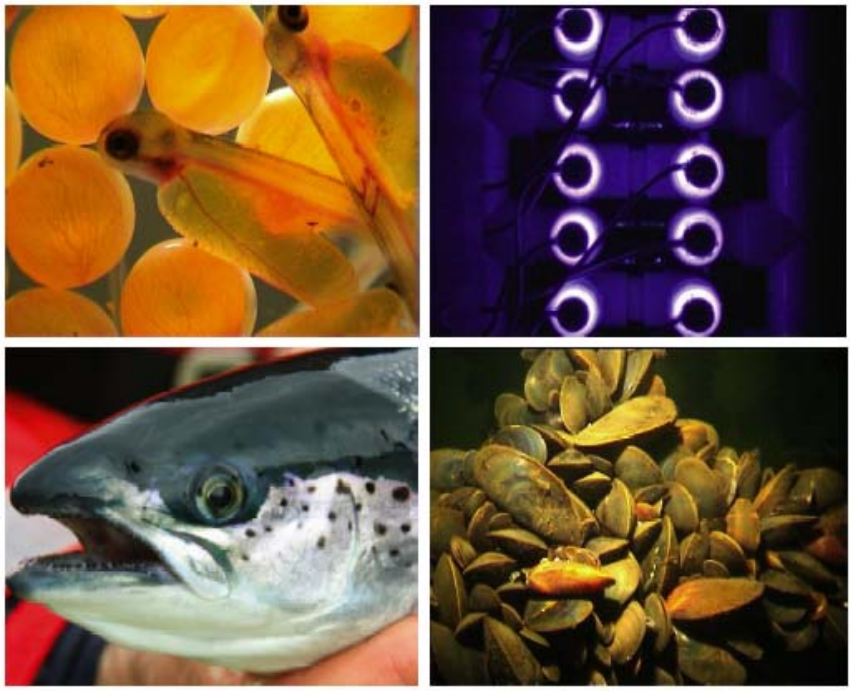
Scottish Aquaculture Research Forum

Sarf063-02

Acquisition Of A Hake Broodstock



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Viking Fish Farms Ltd

Ardtoe Marine Laboratory

Research Report

Acquisition of a hake broodstock
SARF063-02

Prepared by Jim Treasurer, Tim Atack

Funded by

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Abstract

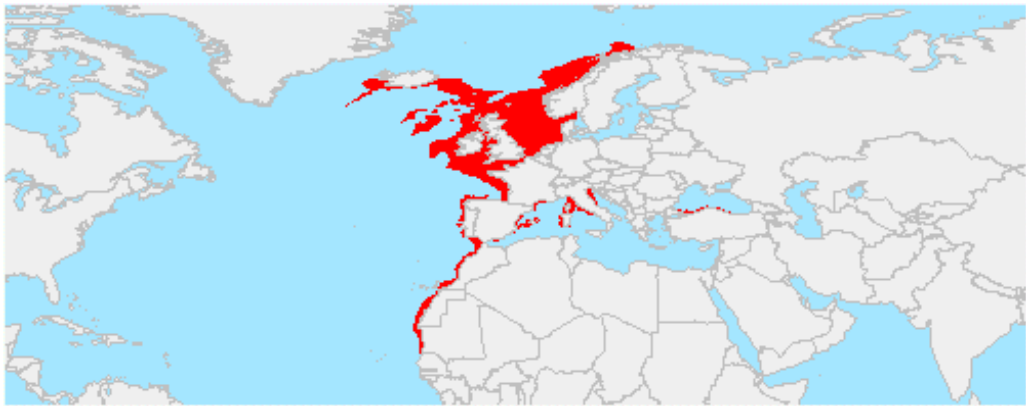
There is a major demand for hake species in Europe including European hake *Merluccius merluccius*. There are around 1,000,000 tonnes of *Merluccius* spp captured globally per annum taken in all fisheries and it is considered that all of the major fishery species are heavily exploited. The European hake *M. merluccius* accounts for approximately 10% of this global catch with around 100,000 tonnes landed per annum, with the catch having remained stable for some time. These catch volumes are unlikely to increase under current management arrangements in the near future despite EU recovery plans for the European hake. The fishery has to be managed rigorously and the species looks a prime candidate for aquaculture. Consequently several research groups have expressed an interest in farming this species. However, a first step is the capture and domestication of a broodstock population. The objective of the current project was to capture live hake and to domesticate the fish to use for rearing trials in Scotland. Hake were captured by trawl in the Firth of Clyde and the Inner Hebrides on the west coast of Scotland. 19 hake were captured alive but died overnight on board a survey vessel, while a further 29 hake were captured later, transported to the laboratory and discharged to a holding tank, but did not survive overnight. Other capture methods were tried such as long lining and angling but no hake were captured. Large baited fish traps were deployed in the sound of Arisaig, west Scotland, and also off northwest Scotland and Shetland, but no hake were captured. Hake were caught in three research surveys but no hake were identified running with eggs and it was therefore not possible to collect viable eggs. The opportunistic collection of hake eggs remains the most probable method of establishing a captive stock of hake.

Biological parameters were measured monthly in hake captured off the island of Canna over a 15 month period. Further samples obtained from the North Sea and west coast of Scotland were used to examine the reproductive biology of hake. This included a description of all stages of ovarian development, calculation of the gonadsomatic index, egg diameter and fecundity. Otoliths and genetic samples were collected and will be utilised further for age analyses and genetic examination of North Sea and west coast stocks respectively. The hake is an interesting species for aquaculture showing a wide distribution from the eastern Mediterranean to northern Norway. It has a higher growth rate than assessed historically and the species has highly adaptive reproductive strategies enabling potential egg production in any month.

1. Introduction

There is a major demand for hake species in Europe and around 1,000,000 tonnes of *Merluccius* spp are captured globally per annum taken in all fisheries (Lloris, Matallanas and Oliver, 2005). All of the major hake fishery species are heavily exploited. The European hake *M. merluccius* accounts for approximately 10% of this global catch with around 100,000 tonnes landed per annum, with the catch having remained stable for some time. These catch volumes are unlikely to increase under current management arrangements in the near future despite EU recovery plans for the European hake. The European hake *Merluccius merluccius* belongs to the family Merlucciidae and it is found from north Africa to Norway and eastwards into the Mediterranean and Black Sea (Alheit and Pitcher, 1995) (Fig. 1). These at first may appear to be very different habitats in terms of water temperature. However, in each region, the hake inhabit depths where the water temperature remains in the region of 9-13^{o}C. This temperature preference therefore makes it a potentially interesting species for culture in the surface waters of northern Europe. With this objective a multinational project, coordinated from Norway, was submitted to the EC in in 2007/8 for funding under the "research for the benefit of SMEs" scheme. Unfortunately this proposal was rejected, with one of the reasons given that it was too ambitious within the timescale restrictions on such projects, especially as it was recognised that a large part of this time would be required to capture and establish the necessary broodstocks.

Fig. 1 Distribution of European hake (FAO)



Given the latter constraint, groups in Norway, Spain and Scotland have made various attempts in the last two years to capture and establish hake breeding stocks. The main problem is that hake are present through most of their lifespan at depths of 150-300 m (Alheit and Pitcher, 1995), and are therefore subject to gas supersaturation problems such as exophthalmia and over-inflated swim bladders when brought to the water surface. Immediate puncturing of the swim bladder can reduce the mortality level, but the fish inevitably do not survive for longer than 24 hours. Even bringing the fish to the surface more slowly seems to have little impact on survival rates, perhaps due to the longer term stress involved in such slow retrieval. As far as is known only one European group, in Vigo in Spain, has managed to establish a very small stock of captive hake.

The establishment of captive stocks in the potential partner countries would clearly greatly facilitate obtaining the European research funding required to develop the technology for hake culture. The purpose of the current project is to develop breeding stocks of hake in Scotland. This would enable Scottish RTD and SME groups to become meaningful partners in such international collaborative projects on hake, and therefore ensure that Scottish SMEs are in a prime position to commercially exploit the technology so developed. The current project could be the key to securing larger scale funding for a more comprehensive project that may lead to new production opportunities for the aquaculture industry in Scotland.

The main objectives of the project were:

- To investigate shallow water fishing methods for the capture and safe recovery of potential broodstock hake
- To establish and manage a hake broodstock for the UK aquaculture industry
- To undertake preliminary broodstock handling, nutrition and spawning trials with the new stocks
- To undertake some initial larval rearing trials

Various methods to capture hake were utilised and trialled including trawl, lines and angling, and using experimental fish traps. The cooperation of Marine Scotland, SAMS and the UMBSMillport were also sought in using the facilities on their research vessels. Latterly the Scottish Fishermens' Federation was asked if their members could help with egg collection and provision of biological material. Attempts were also made to strip hake eggs from fish caught at sea during the spawning period. While no live hake were obtained on the west coast a sample of hake was taken at monthly intervals where possible through one year from the Canna area to determine the spawning season, GSI, HSI, size and age distribution, and the annual cycle of maturation. These hake samples together with other hake caught in the North Sea and west coast in research surveys were used to study the reproductive biology of hake in Scottish waters, obtaining information on spawning period, and egg production.

2. Landings of hake in the UK

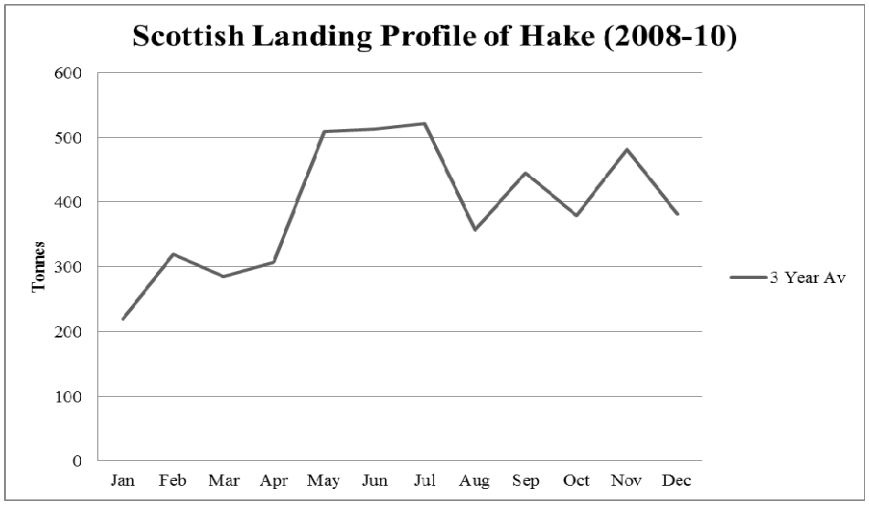
The distribution of hake has changed considerably in recent years and whitefish fishermen in Scotland are now catching large quantities of hake in all areas and especially in the North Sea (Seafood Scotland pers. comm., 2012). The landings of hake in Scotland were 1500 tonnes in 2005 and this increased to 5700 tonnes in 2009 with a first sale value of £10.5 million. This increase has been rapid and so has created a challenge for the seafood industry in Scotland in marketing the species and in increasing consumer awareness. Most of the hake landed are therefore sent to European markets where there is a high demand.

3. Trends in hake capture in the UK and the distribution of landings by area

The three year average for hake landings is shown in Fig. 2 (Seafood Scotland pers. comm., 2012) and landings increased from January to 500 tonnes per month from May to July with fluctuations in catches to the end of the year. A range of fishing methods is used for hake or

hake are commonly caught incidentally with other gadoids. The fishing techniques include lines with baited hooks, midwater trawling, and demersal gill nets.

Fig. 2. Annual pattern of hake landings (Seafood Scotland pers. comm., 2012)



4. Trawl capture of hake

The challenge was to collect these deep water fish in good condition. Preliminary trawls in the Firth of Clyde indicated that the swimbladder was normally everted during lifting the net from 60 metres depth. In the current study trawl samples were also taken from the edge of the continental shelf at water depths of around 600 m so the opportunities to capture life fish from these deeper water locations were difficult.

4.1. Trawl

4.1. Trawl Fishing in the Firth of Clyde

Millport Marine Laboratory

The UMBSM research vessel R.V. Aora carried out exploratory fishing from September 2010 using a slow towed net to try to locate the distribution of hake (Duncan Fraser, pers. comm., 2010). A tarpaulin sheet was fitted to a trawl net to reduce damage during trawling. Fishing took place on several days in the Skelmorlie area, mainly between Largs and Weymss Bay. Many prawns were caught and a few cod but no hake were captured. The UMBSM was asked to carry out further fishing for hake in the Firth of Clyde. Further trawls were carried out on:

20/9/2010. Trawl fished off Bute in a slow haul for 1 ½ hours Only 1 hake was captured; it did not survive.

7/10/2010. Fishing opposite the village of Skelmorlie, between Largs and Weymss Bay. Three tows were carried out at a depth of 70 m, about 1.5 m above the seabed. Large quantities of prawns were captured and 1 cod.

4.2. Trawl catches from Loch Nevis and the Inner Hebrides

Fishermen in Mallaig were approached as hake had been captured previously in Loch Nevis. However, most boats working in this area were fishing for prawns. One fisherman, John MacLean, caught fish near Mallaig, Skye and the Inner Hebrides in the first trawls but no hake were caught. Subsequently hake were captured over 6 days in the Sound of Sleat at around 120 metres depth. Most of the hake were around 20 cm length. Some of the fish were transferred to a tank on board the boat but none recovered. Mr MacLean suggested that fish may be more capable of surviving at lower water temperatures. The Rebecca Janeen was another boat fishing from Mallaig that provided samples of 60 to 100 hake over a 15 month period for examination.

Fig. 3. Monthly samples of hake were obtained from the Rebecca Janeen (courtesy of skipper Robert Summers and crew)

4.3. Trawl catches from the Aberdeen and the Minch area

Hake were sampled from research survey cruises by Marine Scotland from the North Sea and west coast in February 2012. No live hake were obtained. A total of 18 hake was sampled from 60 km NE of Peterhead and 30 hake from the Minch. The fish were measured, genetic samples were taken, and the stage of sexual maturation was assessed and also egg production.

5. Angling

5.1. Ardnamurchan

Fishing was carried out over two days from a local prawn boat in an area south of Rum and near Glenuig, but no fish were caught.

5.2. Loch Etive

Ronnie Campbell Ltd, boat hire, reported that hake had been caught in Loch Etive two years ago but no hake had been caught since that time. He therefore suggested fishing a deep water area close inshore 10 miles south of Oban where hake might be present.

5.3. Hake angling south of Oban

Several anglers helped with a boat trip on 9 and 10th July 2011 to fish overnight in deep water 20 miles south of Oban. The area was selected using an echosounder on the boundary of deep and shallow water where bait fishes are known to collect. Fishing on the seabed at a depth of 150 m commenced at 6 pm in daylight and continued through the night until dawn. The bait used was silver spinners with mackerel or squid. The environmental conditions were: an incoming tide, cloud cover and clear areas, wind at Force 4, water temperature on the surface was 12°C.

As dusk approached, around 9.30 pm, fishing was carried out progressively in shallower water from 70 m to the surface. The lures used were silver spinners with mackerel attached to the hook. Light lures were also attached to the trace near the bait as an attractant. Other lures were standard squid and spinner lures that were held against the light for a few minutes to glow in the dark and then fixed to the line. No fish were captured in darkness. The anglers considered that fish were only present on the bottom and the lack of catches was not a function of darkness.
Five species of fish were captured (Fig. 4):

Lesser spotted dogfish 5 fish
Spur dog 3 individuals, a rare species
Skate 1
Thornback ray 1
Blue ling 3 fish

All these fish were returned to the water.

This is the nearest deep water near Oban apart from Loch Etive and it is not known whether the lack of hake catches denotes that hake are not present there at that time of year, or are not found at any time in that area.

Fig. 4. Fishing south of Oban with Ronnie Campbell Ltd, with the fish holding tank in the background.

6. Line fishing, Lochinver

There is a large line fishery for hake off the continental shelf in the Minch and on the west side of Lewis pursued by mainly Spanish boats and also Norwegian vessels. Processed line caught and gill net captured hake are landed at Lochinver and are handled by the fishing company Inverpeche. Initially contact was made to sample captured fish but the fish are processed at sea. A request was made to retain live line caught fish but it was reported that the bait lures are so large than only larger hake are captured and that there were no facilities on the boats to keep the fish alive before transport to shore (John Vincent, pers. comm., 2011).

7. Fish traps

This work was carried out in association with the fishing gear section of Marine Scotland working with Mr Jim Mair. Marine Scotland provided several traps on loan for fishing in the Sound of Arisaig and off north west Scotland in 2011 at depths from 15 to 80 metres in July and August, and independently off Shetland from July to September 2012. The traps were 1.5 m by 1 m by 1.5 m (Fig. 5) and were baited with freshly caught fish such as mackerel. The bait was highlighted with a light lure and also infused by injection with marine fish oil. No hake were captured.

Fig. 5. Fish trap used with bait and complex eye system to prevent escape (courtesy Jim Mair, Marine Scotland)

8. Egg collection

Both the CEFAS Lowestoft Laboratory and Marine Scotland Laboratory, Aberdeen, were asked if they could help in the collection of eggs and the capture of live fish during routine surveys and bins with instructions were provided for the stripping of eggs at sea, fertilisation and maintenance. Although fish were captured around the approximate spawning time, no eggs or live fish were obtained. Marine Scotland in particular tried to collect and fertilise hake eggs in two surveys in February 2012 by the research vessel Scotia in both a North Sea survey near Shetland (coordinates Latitude 6021.29 N, 0241.54 W and 6112.01 N, 0017.72 W) and west coast survey (coordinates 5924.83 N, 0625.16 W to 5813.96 N, 0557.99 W) of Scotland. They were able to provide material for reproduction, genetic and ageing studies but none of the fish measured was in spawning condition (ICES STAGE III maturity). The hake in the North Sea had spawned and were spent, and the fish on the west coast were immature or in the developing stage of maturation.

9. Sourcing hake from other locations and other countries

A number of fishermen and contacts were asked to catch hake at various locations: such as Weymouth (Mike Webb Aquarium Supplies), St Andrew's Aquarium (John Mace), Stranraer Aquarium, Steve Coates angling boat hire (Anstruther), and Keith Todd of AquaLogistix (Fife). No hake were captured in these areas.

The Carna Laboratory, University of Galway was also asked if hake could be sourced on the west coast of Ireland (R. Fitzgerald pers. comm., 2011) and the Killibegs Marine Aquarium in north west Ireland (Andrew Campbell, pers. comm.) but no hake were captured alive. IFREMER was also contacted in France (N. Picchi pers. comm., 2011). The Bergen Marine Institute reported that juvenile hake were captured readily off harbour walls but the water there was deep close inshore (A. Geffen, Institute of Marine Research).

10. Biological survey

As it proved difficult to capture live fish, it was agreed at a SARF review meeting to obtain information on the reproductive biology of hake including dates of spawning, gonadosomatic index, maturation, the annual spawning cycle, egg size and fecundity.

This was carried out in two surveys:

(a) Monthly sampling over 15 months of hake captured by fishermen using a prawn trawl boat in the Inner Hebrides, and

(b) Samples from research surveys by Marine Scotland in the North Sea and west coast of Scotland.

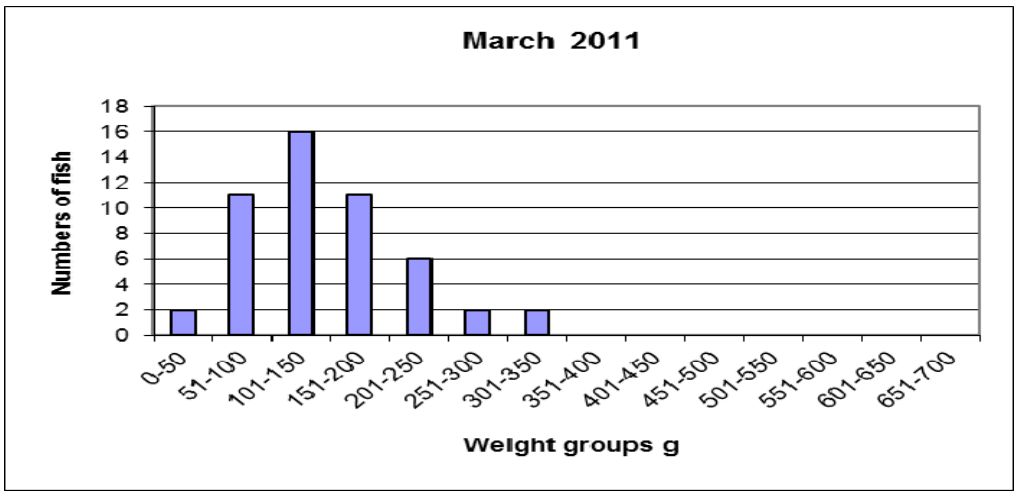
10.1. Sampling in the Inner Hebrides

Samples of 50 to 100 hake were captured near the sea bed with a trawl as a by-catch of fishing for prawns (Boat: Rebecca Janeen, skipper Michael Summers). Hake were examined monthly as far as possible. Fish were captured near the island of Canna in water of 120 metres depth. Fish were placed on ice and examined on landing at the port of Mallaig on the following day. The total lengths (to mm) and weights to 0.1 g of the fish were measured. The fish were dissected and the sex of each fish was identified. The gonad was removed and weighed to 0.01 grams, a sample of this was fixed in 10% formalin, and photographs were taken of the dissected fish. The liver was removed and weighed, as were the remaining viscera. The otoliths were removed for later age analysis by incision on the head with a scalpel behind the eyes. The otoliths were washed and stored in small envelopes for later examination.

10.1.1 Size distribution

The length of hake in monthly samples from March 2011 was in the range 200 to 290 mm and the weights for the same period were in the range from 50 g to 300 g (example, Fig. 6), with mean weights of to 162 g. This finding indicates that the majority of hake captured were juvenile immature fish. All the samples of age 1 year fish comprised 94% of catches and none was mature or maturing. Larger fish were captured in February and March 2012 with weight modes at 500 g, 800 g and 1000 g.

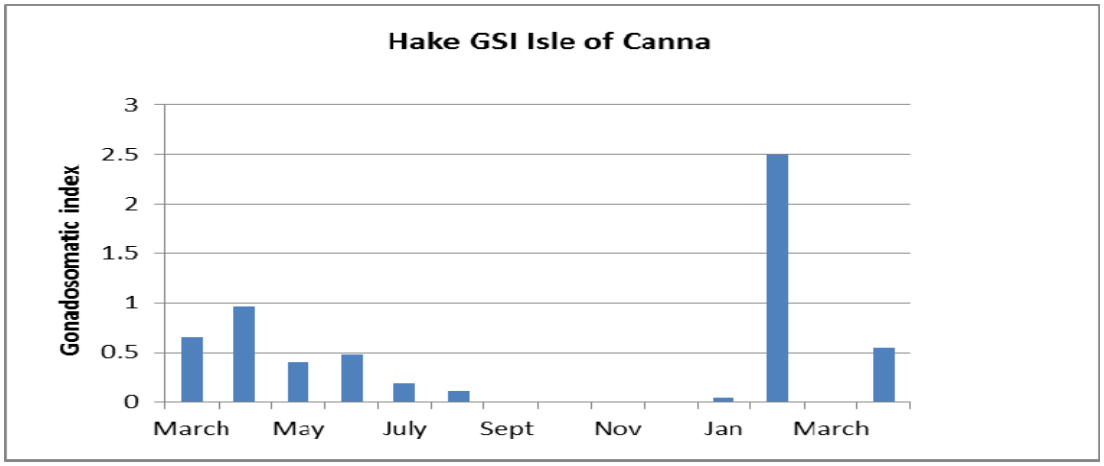
Fig. 6. An example of weight frequency distribution of hake catches by trawl in one month, showing hake of ca. 200 grams after 1 year.



The gonadosomatic index (GSI=(gonad W/eviscerated W)*100) of females declined from March and in all samples was less than 1 with the exception of the sample in February 2012 with a

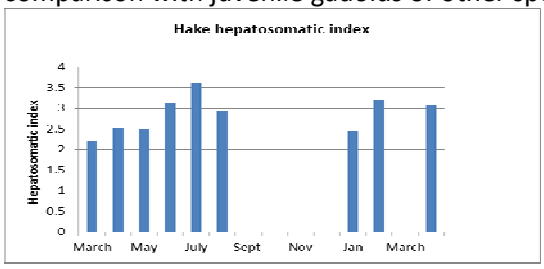
mean GSI of 2.5. The GSI of developing females varied from 3 to 8.7% and that of males from 2 to 6 %.

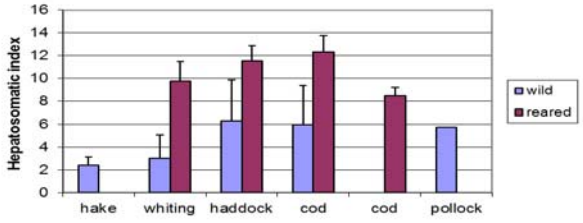
Fig. 7. The gonadasomatic indices of hake captured over one year on nursery grounds near the Isle of Canna (n=from 30 to 50 fish in each sample).



The hepatosomatic index (HSI=(liver W/eviscerated W)*100) increased from 2.2 in March to 3.6 in July and was 3.2 in spring 2012 (Fig. 8). This compares with mean HSI of 2.42 in juvenile hake captured by trawl in Loch Nevis, Mallaig, 3.02 in juvenile whiting, 6.31 in haddock and 5.96 in cod at the same location (Fig. 9).

Figs. 8 and 9. Mean hepatosomatic indices for hake captured near Canna (n=30 to 50), with a comparison with juvenile gadoids of other species captured in the same month.





10.1.2. Age and growth

The age of hake of 50 to 250 grams captured using prawn trawl near Canna was determined from examination of otoliths as being 1 year old and these fish had hatched in ca. spring 2010.

10.1.3. Diet

Some 47% of hake stomachs had exclusively fish content of various stages of digestion. The most common and identifiable species were sprats, herring and small hake. Some of the

stomachs were empty but contents may have been regurgitated during the recovery from the sea bed to the surface.

10.1.4. Conclusion

The prawn trawl either selected smaller hake or large hake were not present in the fishing areas and these could therefore be considered as nursery grounds for the fish. Some fish were transported to the hatchery alive (n=29) but did not survive overnight due to decompression illness and in some cases eversion of stomachs. This type of trawl gear used by the fishing boat gave little opportunity to collect larger adult hake and therefore few large fish were captured. It was therefore not possible to collect hake eggs from this source.

10.2. Hake samples from the North Sea and West coast surveys collected by Marine Scotland

An approach was made to the Scottish Fishermens' Federation and also to Marine Scotland to try to obtain eggs of hake fertilised at sea, and also to sample hake from both North Sea and west coast locations in Scotland to compare reproductive characteristics. Initially it was thought that health observers on commercial fishing vessels would be able to take samples but there were Health and Safety and Food Safety difficulties in carrying and using formalin aboard commercial vessels. Samples of hake of a range of size and maturation status were therefore taken during two research cruises by Marine Scotland in February 2012, from the North Sea survey near the Shetland Isles and in the north Minch area of the west coast.

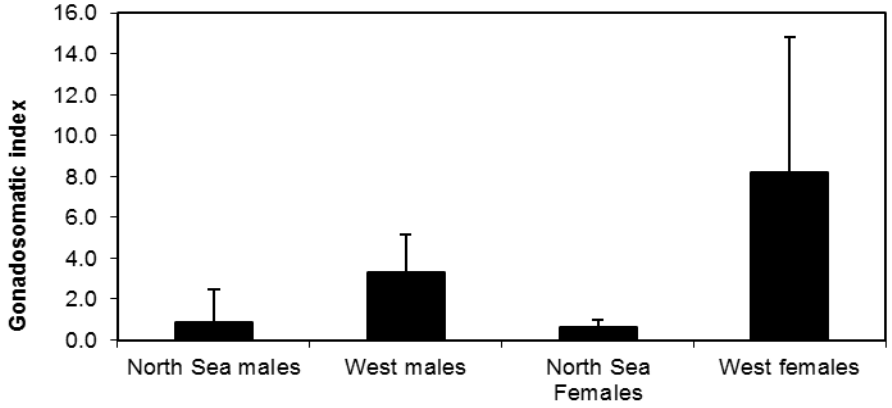
10.2.1. Age and size distribution

Hake in the length range 300 mm to 570 mm and 240 g to 1472 g weight were captured in the North Sea on 9/2/2012 and 10/2/2012, and hake of 190 to 900 mm length and 41 to 6275 g were sampled on the west coast on 21/2/2012.

10.2.2. Gonad samples and Gonadosomatic index

A range of maturation stages of both males and females was sampled and fixed in 10% neutral buffered formalin and processed for histological examination after cutting, embedding in wax, and staining with Haematoxylin and Eosin. The mean gonadosomatic index for males in the North Sea was 0.88 compared with 3.30 in the west (ANOVA $P<0.05$) (Fig. 10). The mean GSI value of females in the North Sea was 0.61 compared with 8.17 in the west (ANOVA $P<0.05$). This indicates that the fish in the North Sea survey had spawned, while the hake on the west coast had still to spawn. Although 22% (n=23) of females were mature on the west coast sample none of these was in the spawning stage of development and no free flowing eggs were obtained from the abdomen. The highest individual gonadosomatic index for males was 4.15 in the North Sea survey and 7.74 on the west and for females 1.52 in the North Sea and 24.42 in the west. Five of 23 females sampled on the west coast had GSI values in excess of 10%.

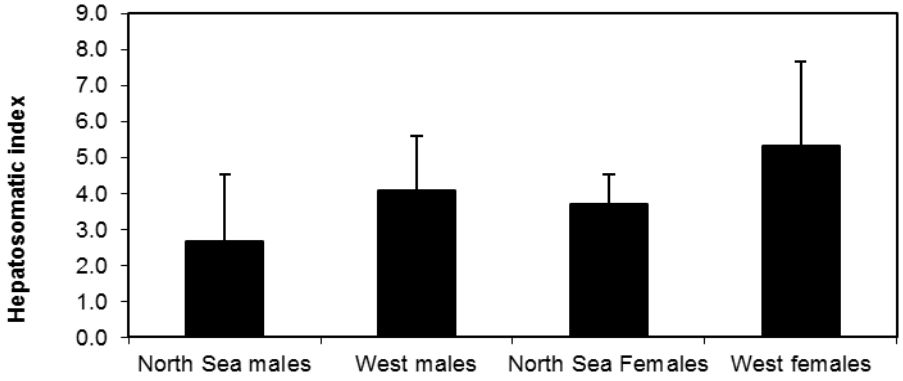
Fig. 10. Gonadosomatic indices of hake captured in the North Sea survey and west coast of Scotland, with SD.



10.2.3. Hepatosomatic indices

The mean hepatosomatic index (HSI) for males was 2.67 in the North Sea and 4.07 (ANOVA $P<0.05$) in the west and for females 3.71 and 5.31 (ANOVA $P<0.05$) respectively. These lower HSI values in the North Sea survey perhaps reflect lower body condition following spawning.

Fig. 11. Hepatosomatic indices for hake captured in the North Sea and west coast of Scotland; the SD is shown.



10.2.4. Gonad histology and stages of maturation

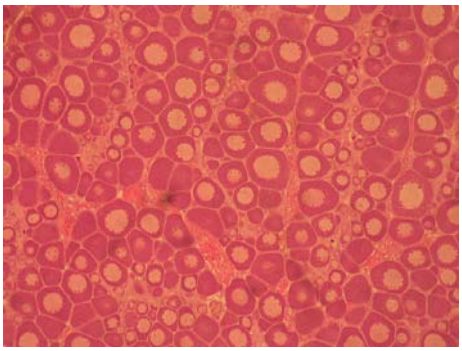
The visual stages of maturation used on the research vessel surveys was on the ICES scale, 1=immature, 2=developing, 3=mature, and 4=spent (Finlay Burns, pers. comm.). However, to accurately assess the development of oocytes using histological examination a modified form of a histological maturation scale after Rinchard (Rinchard and Kestemont, 1996) was used for the first time to classify hake reproductive development.

This is a 7 point scale representing:

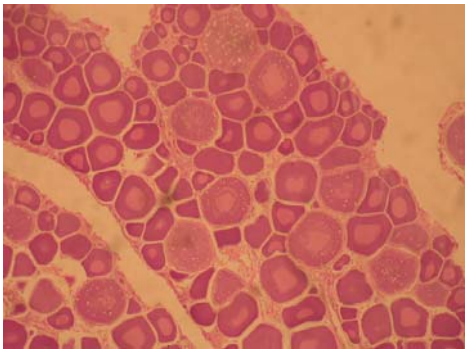
* 1=immature
* 2=start endogenous vitellogenesis
* 3=completion endogenous vitellogenesis
* 4=exogenous vitellogenesis
* 5=mature
* 6=intermediate spawning stage
* 7=post-spawning

Most developmental stages of hake ovaries were represented in the histological material from the two fishery research surveys in February 2012 and additional material of fish in the spawning and post spawning condition was obtained from an earlier fish survey from Suilisker and Flannan areas, west coast in May 2007 to complete all developmental stages (Appendix 1). A report on the stage of maturation of each ovary and measurements of the fish are shown in Appendix 2. All fish from the North Sea survey were classified as either previtellogenic with no vacuoles in the cytoplasm or with yolk vesicles on the periphery of the cytoplasm (Fig. 12). So recovery from spawning was advanced. Some of the ovaries from the west coast samples were in the final stage of maturation but not in spawning condition and were characterised with yolk globules and yolk vesicles on the periphery of the cytoplasm. Oocytes were of a range of diameters and developmental stages (Fig. 13) showing hake to be a batch spawner. Samples of ovaries from the earlier research survey in May 2007 showed hake ovaries in the spawning condition with oocytes in the final maturation condition (stage VI). In another ovary evacuated follicles were present and showing considerable disorganisation and atretic follicles in the post-spawning condition (stage VII).

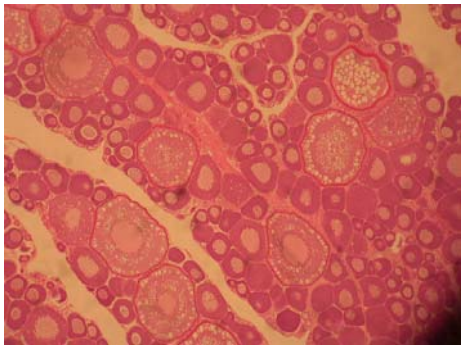
Fig. 12. The developmental stages of hake ovaries classified after Rinchard and Kestemont (1996). H&E staining.



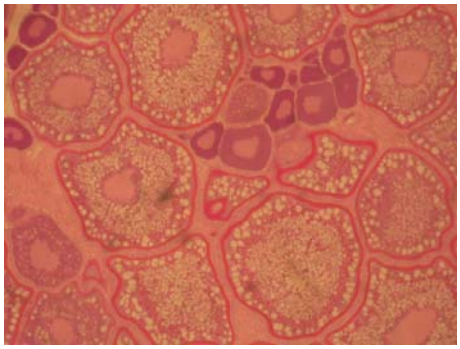
Stage I immature ovary x 100



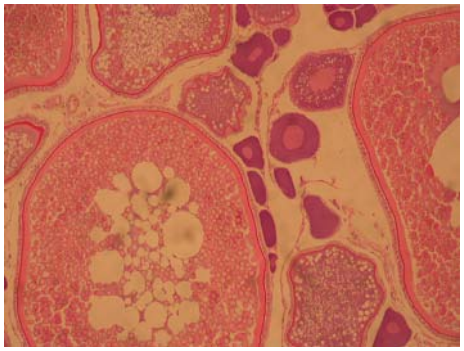
Stage II Developing x 100



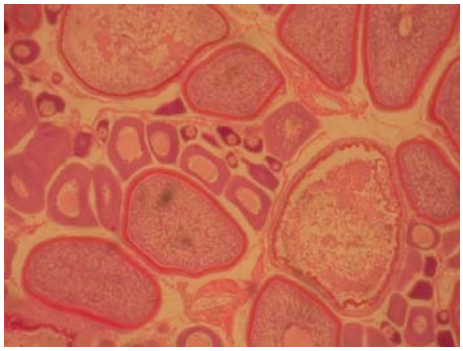
Stage III Developing x100



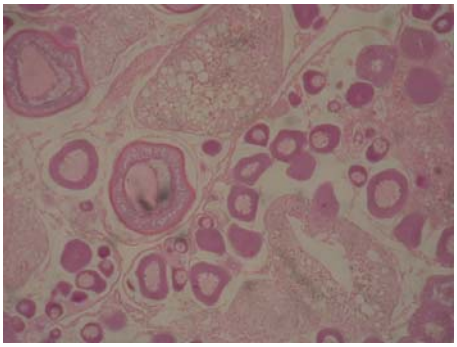
Stage IV Mature x100



Stage V Spawning x400

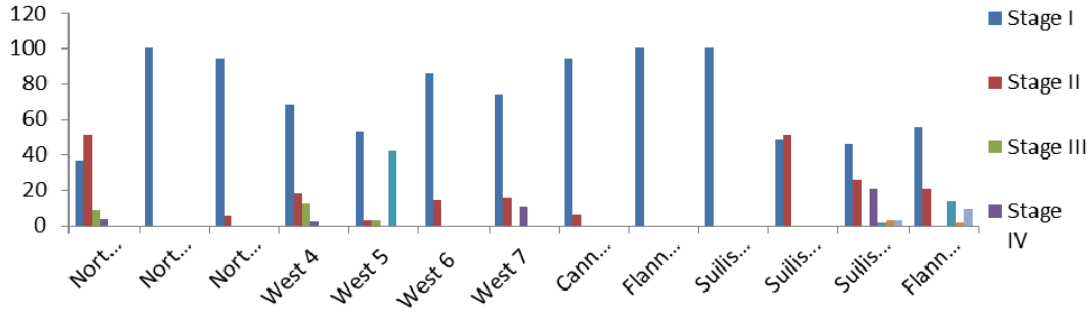


Stage VI Spawning/spent x100



Stage VII Spent/recovering

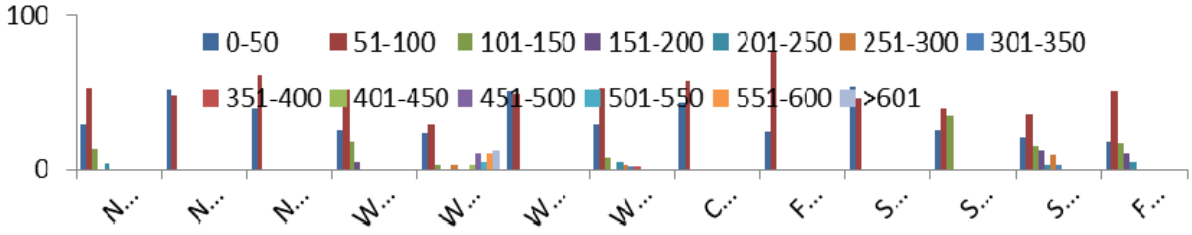
Fig. 13. % of oocytes in each developmental stage on the hake ovaries examined from the North Sea survey and west coast and from earlier material from west of Lewis in 2007 (Flannan and Suilisker grounds).



10.2.5. Size distribution of oocytes

The diameter and size distribution of the oocytes from the histological material in the various developmental stages is shown in Fig. 14. This confirms that the fish from the North Sea survey had spawned prior to sampling. The female ovaries from the west coast were in mature and pre-spawning condition and sequential smaller oocytes were present representing subsequent egg batches. Oocytes in the mature condition were in the diameter range 479-642 µm with a mean diameter of 556 µm+/-69 SD. In contrast diameter of oocytes in developmental stages 1, 2, 3 and 4 were 34+/-9 SD, 56+/-17, 105+/-8, and 249+/-54 µm respectively.

Fig. 14. Percentage of oocytes in each 50 µm diameter group from histological material of hake caught in the North Sea (N) and west coast (W) of Scotland, Canna (C) and from an earlier survey of the west coast Flannan (F) and Suilisker (S) grounds in 2007.



This can be compared with the diameter of oocytes measured from formalin fixed material and unprocessed, which gave a mean oocyte diameter of three fish in the mature stage of development of 900 µm +/- 10 SD which indicates that there was considerable oocyte shrinkage in the processed material from the same individuals. Published data report the diameter of spawned eggs to be around 1 mm (Murua and Motos, 2006).

10.2.6. Fecundity

On sampling hake the total weight of the ovary was recorded and then a section weighed and preserved for later egg counts. This was examined at x 40 magnification with a binocular microscope and a total count made of eggs and the fecundity (*F*) of individual fish estimated from

$$F = \frac{w \times n}{x}$$

Where *w* is the wet weight of all the eggs in the ovary, *n* is the number of eggs counted in the subsample and *x* is the wet weight of the subsample.

Ovaries in fish from the North Sea survey were not sufficiently developed to estimate individual fish egg production but seven fish from west coast samples were suitable to assess fecundity. The mean fish fecundity with Standard deviation (SD) and relative fecundity per gram (egg number/gram body weight) estimates for the seven fish were (Table 1):

Fish no.	Fish weight (g)	Fecundity X Million eggs	Fecundity SD Million	Relative fecundity X	Relative fecundity SD
1	3710	2.94	0.36	792	96
2	2330	1.34	0.28	574	120
3	962	0.42	0.12	433	119
4	790	0.63	0.06	794	79
5	3815	2.58	0.40	675	105
6	3715	2.74	0.34	737	91
7	2880	1.29	0.27	447	94

10.2.7. Age analyses

Otoliths of hake were removed and stored dry and examined later immersed in cedarwood oil and examined using reflected light on a dark background. The otoliths were too dense and opaque to distinguish annual growth rings. The otoliths were therefore fractured across the middle, embedded in plasticine, coated with cedarwood oil and examined using reflected light at x 40 magnification. The dark rings were counted and the distance between the rings measured to back-calculate length at age at earlier years. Pontual et al. (2006) noted that previous studies had underestimated the growth rates of hake by identifying too many growth rings. In the current study “false” annuli were also identified and were discounted and age at length confirmation was also undertaken by examining young of the year hake and following their growth monthly from length-frequency distributions from the Inner Hebrides. The otoliths in the current project will be examined at a later stage to produce growth curves for the species on the east and west coasts of Scotland.

10.2.8. Genetic samples

The Scottish Fishermens’ Federation was interested in comparing the genetics of hake stocks from the North Sea survey and west coast to determine if there is one or distinct spawning stocks. Genetic material was obtained by taking a 5 mm fin clip from the dorsal fin and fixing this in 90% ethanol. This material was obtained for 18 hake from the east coast, 37 fish from the

west coast and also reference samples were made available from Celtic Sea by the University of Galway and from samples from the Bay of Biscay. There has been no resource to process this material but the University of Bangor has been involved in a Fish Trace project and has offered to take the material and process it when resources become available.

11. Review of recent publications on hake capture and rearing

The hake is a very interesting species because of the wide distribution from the Aegean Sea to northern Norway, the high market demand in Europe, threats to the hake fishery, and a lack of basic information on this species in UK waters. Basic information on spawning of the hake in Scottish waters, and on ovarian development, gonadosomatic indices, egg sizes and fecundity are not available.

Iglesias et al. (2010) described the capture of adult hake in the Bay of Biscay using a trawl fitted with a liner bag. Several hundred hake were captured and a needle was used to deflate fish with over-inflated swimbladders. However, survival was low.

There has been a number of recent publications on the initial rearing of hake from eggs received from fish stripped by hand at sea in Norway (Bjelland, 2001; Bjelland and Skiftesvik, 2006). These studies enabled a description of larval development, although weaning of hake to the juvenile period has not been accomplished. Hatching occurred after 4 days at 50-60 degree days post fertilisation. The larvae were 3 mm on hatch and best survival was obtained at 12.7^{o}C with survival to yolk sac absorption highest at 14.5^{o}C.

Further, the growth rate of hake is much faster than predicted from historical fishery studies (Morales-Nin et al., 1998; Piñeiro and Saínza, 2003). Recent studies from tagged hake have shown that this previous work grossly over-estimated the age of fish as annuli were indistinct, and therefore accurate estimates of adult growth rates are not possible (Pontual et al., 2006; Iglesias, et al 2010). The hake is a fast growing fish with growth approximately twice that previously reported (ICES, 1993; Lucio et al., 2000) and is therefore a more attractive fish for rearing than previously thought. Courbin et al. (2007) suggested that the hake macrostructures do show structural patterns and that a reliable method of age estimation would be devised.

Tagging of hake has rarely been successful because of their deep water habitat and fragile nature (Lucio *et al.,* 2000b). Belloc (1935) recaptured one fish after 8 months and it had grown at a rate of 1.43cm month^{-1}. The growth rate of hake in the Bay of Biscay was estimated to be ca. 20 cm yr^{-1} from a tagging study (Pontual *et al*. 2003; 2006). This is high relative to most gadoid species.

The spawning period of most gadoid species in the wild and in captivity is predictable with most reproduction in the period February to March, for example cod, haddock, pollack, whiting and saithe. However, studies on reproduction in hake have been few compared with these commercial species and recent studies on hake reproduction have shown it to be untypical and unpredictable in terms of timing of egg production, with female hake found in all stages of maturation in any month of the year (Lannin *et al.,* 2005; Murua and Motos, 2006). It has been suggested that this prolonged spawning season and asynchronous oocyte development is an evolutionary 'bet-hedging' strategy to produce eggs at any season when environmental conditions are optimal to maximise larval survival (Lannin *et al.,* 2005; Pitcher and Alheit, 1995;).

A reproductive dormant period has also been suggested (Finlay Burns pers. Comm., 2012) Spawning appears to be all year round with a peak in activity in spring. As the spring advances the peak in spawning moves north along the continental shelf. Some evidence suggests that peak spawning off the south west coast of Ireland is from March to July (Coombs and Mitchell, 1982; Horstman, 1988; Fives *et al.,* 2001; Alvarez *et al.,* 2004; Murua and Motos, 2006; Murua et al., 2006). Spawning of hake was reported in the Celtic Sea along the continental shelf and on the slope from February to April-May (Poulard, 2001). Spawning in Iberian waters was slightly earlier and from December to May with a peak in February (Piñiero and Saínza, 2003). In contrast, mature adults were present in the Mediterranean throughout the year (Recasens et al., 1998). Continuous spawning was indicated from back-calculated hatch-date distribution data from the central Adriatic, with a peak in summer and a lesser peak in February-March (Arneri and Morales-Nin, 2000). The timing of maximum spawning may be governed by temperature as eggs of hake are frequently present when the sea surface temperature is 12 °C (Coombs and Mitchell, 1982; Alvarez *et al.,* 2004; Dransfeld *et al.,* 2004).

It appears that the population egg production depends on environmental conditions and the status of the spawning stock biomass (batch fecundity and spawning fraction) (Lannin *et al.,* 2005; Murua *et al.,* 2006). Hake are batch spawners but the annual fecundity is difficult to determine as the development of the oocytes is not synchronous. The peak in population batch fecundity in the Bay of Biscay was from January to April and was lower from May to October (Murua *et al.,* 2006). Potential individual fecundity was estimated between 100,000 and 350,000 off Portugal (Monteiro and Lima-Días, 1966) and the egg production in the current study is similar to these estimates. However, Miller and Loates (1997) estimated that in some larger fish individual fecundity could be as high as 2 million eggs. Although the ageing of hake from otoliths has been unreliable recent tagging studies suggest that maturation may occur as early as 1+ year in males and at 2+ years in females (Pontual *et al.,* 2003, 2006). Earlier studies suggested older ages at first maturation at around 3 to 4 years for males and 8 to 10 years for females! (Miller and Loates, 1997). Other reports have given 3 to 4 years as the age at maturation (Piñiero and Saínza, 2003) with males maturing earlier.

12. Discussion and conclusions

The current project has attempted to capture hake alive by a number of approaches and fishing gears and in collaboration with various institutions and research vessels in Scotland. While hake have been captured and sampled in a moribund condition only two batches of juvenile hake were caught and held overnight in tanks. A hypodermic needle was used in both instances to deflate fish with overinflated swimbladders. The fish were alive and held overnight in bins with circulating water and air supply but died within a few hours from trauma and physical damage. Bins for egg stripping and fertilisation of eggs at sea were distributed to Marine Scotland staff on North Sea and west coast fish surveys in February 2012, and also during fish trap sampling throughout 2012, but no hake in spawning condition were captured. In addition hake were examined in a 10 day trawl survey on the west coast and no hake with “running” ovaries were caught.

The collection of hake eggs may be the most promising method of obtaining material if it coincides with the timing of spawning but, given the time and cost involved, would require trained staff and opportunist collection. Opportunistic collection of hake eggs by Marine

Scotland staff on survey work remains the most likely way that a domesticated hake stock can be established. Night fishing with surface trawl gear may be an alternative option. Capture of fish by line fishing such as by Spanish boats off the north west coast may also be an option, but the support company did not consider this feasible given the lack of facilities on board and sailing time from Lochinver (Inverpeche, pers. comm., 2012).

The market for hake in Europe is still important and any opportunity to rear hake in the future would be of interest to increase the diversification of aquaculture species in the UK. Various European research groups have expressed an interest in generating a larger project on hake cultivation but the limiting factor continues to be the acquisition of broodstock. Within the UK catches of hake are increasing but there is a need to generate more interest in the species as much of the catch is exported (Seafood Scotland, pers. comm., 2012).

The current study has been able to access and analyse samples of hake from the North Sea, the island of Canna, the Minch, and Suilisker and Flannan to the west of Lewis. This material has enabled a biological examination of reproduction in hake, including maturation stages, egg size, Gonadosomatic index, and potential egg production in the North Sea and west coast waters of Scotland. Limited data are available on this species in Scotland due to the focus of fisheries research activity on other gadoid species such as cod and haddock. The current project has therefore offered an insight to the biology including reproduction of this increasingly important species in Scottish waters.

Acknowledgements

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13. References

Alheit, J., and T. J. Pitcher, editors. 1995. Hakes: biology, fisheries and markets. Chapman and Hall, London

Alvarez, P., Fives, J., Motos, L. Santos, M. (2004). Distribution and abundance of European hake *Merluccius merluccius* (L.), eggs and larvae in the North East Atlantic waters in 1995 and 1998 in relation to hydrographic conditions. *Journal of Planktonic Research.* 26 (6), 1-16.

Arneri, E. and Morales-Nin, B. (2000). Aspects of the early life history of European Hake from the central Adriatic. *Journal of Fish Biology.* 56, 1368-1380.

Belloc, G. (1935). Etude monographique du merlu *Merluccius merluccius* L., 8, 145–202.

Bjelland, R. (2001). European Hake, *Merluccius merluccius* (L. 1758), a new candidate for aquaculture? Rearing techniques, larval development and start feeding. Cand. Scient. Thesis, Department of Fisheries and Marine Biology, University of Bergen, Norway. 77 pp.

Bjelland, R.M. and Skiftesvik, A.B. (2006). Larval development in European Hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system. *Aquaculture Research.* 37, 1117-1129.

Coombs, S.H. and Mitchell, C.E. (1982). The Development Rate of Eggs and Larvae of the Hake, *Merluccius merluccius* (L) and their distribution to the west of the British Isles. *ICES Journal of Marine Science.* 40, 119-126.

Courbin, N., Fablet, R., Mellon, C., de Pontual, H. (2007). Are Hake otolith macrostructures randomly deposited? Insights from an unsupervised statistical and quantitative approach applied to Mediterranean hake otoliths. *ICES Journal of Marine Science.* 64(6), 1191-1201.

Dransfeld, L., Dwane, O., McCarney, C., Kelly, C.J., Danilowicz, B.,& Fives, J.M. (2004). Larval distribution of commercial fish species in waters around Ireland. *Irish Fisheries Investigations*. Marine Institute. 13.

Fives, J.M., Acevedo, S., Lloves, M., Whitaker, A., Robinson. M., King, P.A. (2001). The distribution and abundance of larval Mackerel, *Scomber scombrus* L., Horse Mackerel, *Trachurus trachurus* (L.), Hake, *Merluccius merluccius* (L.) and Blue Whiting, *Micromesistius poutassou* (Risso, 1826) in the Celtic Sea and West of Ireland during the years 1986, 1989 and 1992. *Fisheries Research.* 50, 17-26.

Horstman, R.D. (1988). An Investigation of larval and postlarval fish communities of the Celtic Sea, with special emphasis on the demographics of larval Mackerel (*Scomber scombrus*). Unpublished Ph.D. thesis. Department of Zoology, University College Galway, Ireland.

ICES. (1993). Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. *ICES Document CM*. 3.

Iglesias, J., Lago, M., Sanchez, F., Cal, R. (2010). Capture, transport and acclimatization to captivity of European hake, *Merluccius merluccius* L: preliminary data on feeding and growth. *Aquaculture Research* 41, 607-609.

Lannin, A., Rogan, E. and Connolly, P. (2005). A bet hedging strategy for hake enables maximum viable egg production. *ICES Document CM/Q*. 09.

Lloris, D., Matallanas, J. and Oliver, P. (2005). *Hakes of the World (Family Merlucciidae). An annotated and illustrated catalogue of hake species known to date. FAO Species Catalogue for Fishery Purposes.* 2.

Lucio, P., Murúa, H. and Santurtún M. (2000a). Growth and reproduction of Hake (*Merluccius merluccius*) in the Bay of Biscay during the period 1996–1997. *Ozeanografika.* 3, 325–354.

Lucio, P., Santurtun, M. and Quincoces, I. (2000b). Tagging experiments on Hake, Anglerfish and other species in the Bay of Biscay. *ICES CM/Z*: 09.

Miller, P.J. and Loates, M. J. (1997). Collins Pocket Guide: Fish of Britain and Europe. HarperCollins, London.

Monteiro, R. and Lima-Días, M. (1966). On some aspects of the ovary development in the Hake (*Merluccius merluccius* L.) of the Portuguese coast. *ICES CM/G* 5, 8.

Morales-Nin, B. and Aldebert, Y. (1997). Growth of juvenile *Merluccius merluccius* L. in the Gulf of Lions (NW Mediterranean) based on otolith microstrucure and length–frequency analysis. *Fisheries Research*. **30**: 77–85.

Morales-Nin, B., Torres, G.J., Lombarte, A., Recasens, L., (1998). Otolith growth and age estimation in the European hake. *Journal of Fish Biology.* 53, 1155–1168.

Murua, H. & Motos, L. (2006). Reproductive strategy and spawning activity of the European hake *Merluccius merluccius* (L.) in the Bay of Biscay. *Journal of Fish Biology*. 69, 1288 – 1303.

Murua, H., Lucio, P., Santurtún, M., Motos, L. (2006). Seasonal variation in egg production and batch fecundity of the European Hake *Merluccius merluccius* (L.). *Journal of Fish Biology*. 69, 1304-1316.

Piñeiro, C. and Saínza, M. (2003). Age estimation, growth and maturity of the European hake *Merluccius merluccius* (Linnaeus 1758)) from Iberian Atlantic waters. ICES Journal of Marine Science. 60 (5), 1086–1102.

Pitcher, T.J. and Alheit, J. (1995). What makes a hake? A review of the critical biological features that sustain global hake fisheries. *In:* Alheit, J. and Pitcher, T.J. *Hake: Biology, Fisheries and Markets.* Chapman and Hall, London.

Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., Groison, A.L. (2003). A pilot tagging experiment on European Hake (*Merluccius merluccius*): methodology and preliminary results. *ICES Journal of Marine Science*. 60, 1318–1327.

Pontual, H., Groison, A.L., Piñiero, C., Bertignac, M. (2006). Evidence of underestimation of European Hake in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. *ICES Journal of Marine Science.* 63(9), 1674-1681.

Poulard, J.C. (2001). Distribution of hake (*Merluccius merluccius*, Linnaeus, 1758) in the Bay of Biscay and the Celtic Sea from the analysis of French commercial data. *Fisheries Research* 50, 1-15.

Recasens, L., Lombarte, A., Morales-Nin, B., Torres, G.J. (1998). Spatiotemporal variation in the population structure of the European hake in the NW Mediterranean. *Journal of Fish Biology* 53, 387-401.

Rinchard, J. and Kestemont, P. (1996). Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. Journal of Fish Biology 49, 883-894.

14. Appendices

Appendix 1. Histopathology examination of hake gonadal material

Ovarian samples from February 2012

North Sea sample 1
Good organisation in the ovary with connective tissue supply between layers of oocytes. Oocytes developing with most at stage II in development.
North Sea 2
Many primary oocytes, all at stage I.
North Sea 3
All developing oocytes at stage I with many primary oocytes between the stage I oocytes.
West coast 4
Lot of stage III oocytes present, several weeks pre-spawning
West coast 5
Fish still to spawn. Many stage V hyaline oocytes present with a few stage I oocytes between these.
West coast 6
Recovering spent fish. The lamellae are open and loose with a lot of connective tissue.
West coast 7
No evidence of spawning. Many oocytes are stage IV and the remainder are mainly stage I.
West coast 8
Fish from Canna. Ovary probably developing for the first time with oocytes all at stage I.

15/5/2007 west coast survey, west of Lewis on Flannan and Suilisker grounds.
Scotia 1 Flannan
Looks like a virgin ovary with most oocytes at stage I and a few stage II oocytes
Scotia 2 Suilisker
Recovering fish. Oocytes all at stage I
Scotia 3 Suilisker
Possibly recovering spent fish with all oocyte at stage I and a few at stage II
Scotia 4 Suilisker
Ovary in a disorganised loose open condition, so there may have been some spawning and there are some atretic follicles. All oocyte developmental stages from II to IV are present.
Scotia 5 Flannan
A range of oocyte stages present with some at stage IV and V. The ovary is disorganised with many evacuated follicles. Spawning is almost complete but still some oocytes present. Several atretic oocytes present undergoing hypertrophy.

Appendix 2. Summary biological data of samples used for histological analyses of ovaries. N=North Sea survey (near Shetland); w=west coast; SC=Scotia west coast survey in 2007; these tows were on the Flannan and Suilisker grounds. GSI=gonadosomatic index. HSI=hepatosomatic index.

Fish no.	Date	Lt (mm)	Wt (g)	Sex	Devlp Stage	Gonad wt (g)	GSI	Liver wt (g)	HSI	Evisc wt (g)	Location
N1	09/02/12	570	1472	F	III	20.6	1.5	68.0	5.0	1358	North Sea
N2	09/02/12	400	604	F	I	2.6	0.5	23.7	4.3	551	North Sea
N3	09/02/12	420	609	F	I	4.2	0.8	23.0	4.1	558	North Sea
W4	21/02/12	590	1850	F	III	19.5	1.4	75.7	5.5	1370	west coast
W5	21/02/12	730	2598	F	V	241.0	11.0	103.3	4.7	2190	west coast
W6	21/02/12	610	1740	F	VI	7.9	0.5	66.4	4.4	1518	west coast
W7	21/02/12	620	1625	F	IV	26.4	1.8	70.5	4.9	1435	west coast
W8	11/04/12	488	817	F	VI	6.0	0.8	29.0	4.0	719	off Canna
SC1	10/05/07	470	734	F	I	5.0	0.8	26.0	4.0	653	Flannan
SC2	15/05/07	672	1886	F	I	14.0	0.8	98.0	5.7	1708	Suilisker
SC3	15/05/07	635	1968	F	I/II	16.0	0.9	92.0	5.4	1698	Suilisker
SC4	15/05/07	650	1708	F	IV	38.0	2.6	52.0	3.6	1460	Suilisker
SC5	10/05/07	820	3190	F	VI	68.4	2.3	64.0	2.1	3027	Flannan

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